





FINAL REPORT WIND SEA ALGAE WORKSHOP MARIBO, DENMARK APRIL 20-22, 2009

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Acknowledgments and Dedication



The Wind, Sea, and Algae Workshop (20-22 April 2009) in Maribo, Lolland, Demark, was organized by Dr. Jonathan Trent (Prof. UC Santa Cruz) and Dr. Susanne Trent (STP) with help from a team of science advisors: Dr. Maurice Averner (retired NASA US), Dr. Qiang Hu (Arizona State University, US), Dr. Lene Lange (Aalborg University, DK), and Dr. Peter Lindblad (Uppsala University, Sweden). Anders Muller and his colleagues at Baltic Sea Solutions (Bass) made local arrangements for the workshop and handled all logistics.

Lolland Municipality, Region Zealand, Nordic Energy Research and the Danish Ministry of Science, Technology and Innovation generously provided funding. We are particularly grateful to the mayor of Lolland Municipality Stig Vestergaard for his enthusiastic support of all aspects of the workshop.

We acknowledge and thank Miriam Ellis, Jennifer Kaehms, Marlowe Primack, Stefan Eckhard, Graham Akeson and the students of Los Altos High for their valuable assistance transcribing the lectures and to especially thank Miriam Ellis for also editing and correcting the English.

Finally, we want to dedicate this Proceedings volume to the memory of Maurice Averner, who died February 5, 2009. Mel played an indispensable role in planning and organizing the workshop. We acknowledge his inspirations about offshore algae cultivation, science, and life in general. His wit and good humor will be missed by all who knew him, but not forgotten.

To dare is to lose one's footing momentarily. Not to dare, is to lose oneself.

Søren Kierkegaard

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

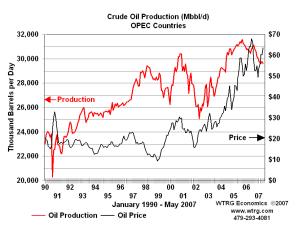
EXECUTIVE SUMMARY:

Microalgae offer great promise to contribute a significant portion of the renewable fuels... compatible with existing transportation fuel infrastructure (refining, distribution, and utilization).

- DOE National Algal Biofuels Technology Roadmap (Dec. 2008)

It has been known for decades that algae, in particular microalgae, have the potential to be used as a sustainable, carbon-neutral source of biofuels.¹ From 1978-1996 the US Department of Energy (DOE) investigated the use of microalgae as a source of oil as well as biomass, hydrogen, hydrocarbons, alcohols, carbohydrates, methane, and syngas. This DOE algae program (The Aquatic Species Program) ended in 1996 for three reasons: 1) the DOE predicted that the cost of petroleum would remain relatively flat for at least 20 years (1996-2016), 2) that algal biodiesel could not compete with such cheap petroleum prices supported by a highly subsidized corporate infrastructure, and 3) there were many formidable technical challenges associated with growing the large quantities of algae needed for fuels.

DOE was wrong about their predicted price of oil (Fig. 1). Within three years the price of oil began to rise and within ten years it was >5 times the "flat" value the DOE had predicted. By 2009, the global reserves of petroleum are reaching their previously predicted limits,² the largest remaining reserves of oil remain in politically unstable countries, and, most importantly, the increased burning of petroleum, impacting global climate, is of growing concern.³ Indeed, all things considered the price (in many senses) for the continued use of petroleum is too high. DOE's prediction about the "price" of



petroleum was wrong, could they also have been wrong about the economic and technical challenges preventing algae from becoming the much-needed replacement for petroleum?

Considerable efforts are now underway to determine if algae can indeed be cultivated in sufficient quantities and at prices that are relevant for biofuels. Nearly all of these efforts are focused on scaling and improving traditional algae cultivation methods on land, which use shallow ponds (raceways) or large photo-bioreactors (PBRs). There are some non-traditional efforts looking into growing and harvesting algae in natural lakes and in harvesting algae directly from the ocean. Progress is being made, but to date, none of these systems have demonstrated they will be able to reach the quantities

¹ http://www.nrel.gov/docs/legosti/fy98/24190.pdf

² http://www.hubbertpeak.com/hubbert/wwf1976/

³ http://www.pewclimate.org/brief/science-developments/June2009

Wind, Sea, Algae Workshop, Lolland, Denmark, April 2009

and economics-of-scale for algae to contribute significantly to biofuels. In addition, most of the landbased methods are problematic because they significantly impact the environment--changing natural ecologies and competing for agricultural land and water. Indeed, the amount of freshwater required for replacing evaporated water in raceways and for controlling temperature in closed bioreactors, is in itself prohibitive.

The goals of the Wind, Sea, and Algae workshop were to address the problems of large-scale algae cultivation on land by considering the possibilities of moving algae cultivation offshore into the ocean. More specifically, a multidisciplinary group of scientists and engineers from Universities, National Laboratories, and Industry, explored the idea of Offshore Membrane Enclosures for Growing Algae (OMEGA). Participants included experts on macro-algae (seaweeds), who provided background information from their offshore cultivation methods. We considered possibilities for ocean environments in general, and Lolland or more specifically the wind farms off the coast of Lolland, in particular. The program of the three-day workshop included lectures in the morning and breakout sessions in the afternoons. The groups discussed and debated, if offshore microalgae production is feasible, scalable, environmentally acceptable, and cost effective. Both the lectures and the breakout sessions were videotaped. This report includes transcripts of the lectures and discussions, selected and edited by the organizers. We hope this volume will help elucidate the challenges and opportunities of offshore algae cultivation and provide inspiration and guidance for future developments in this field in Lolland's community test facility and beyond.

OFFSHORE MEMBRANE ENCLOSURES FOR GROWING (OMEGA)

The Stone Age didn't end because we ran out of stones...

- Sheikh Yamani, Former OPEC oil minister, 2006

The goal of the workshop was to design an offshore algae cultivation system to economically grow copious amounts of algae in a way that improves the environment. Our focus was on the merits and drawbacks of the OMEGA system. The basic idea of OMEGA is to grow freshwater algae in plastic enclosures, filled with municipal wastewater, and in effect grow contained algal blooms, while tertiary treating the sewage released into the ocean.

OMEGAs are closed photo-bioreactors constructed primarily of inexpensive clear, flexible plastic with inserts of semi-permeable membranes, both gas-permeable and forward osmosis (FO) membranes. The OMEGAs are filled with municipal or agricultural wastewater that would normally be dumped into the

sea and contain sufficient nutrients to cause a bloom of algae. The wastewater in the OMEGAs is inoculated with freshwater algae that are well suited to the conditions in the OMEGA and that produce valuable products of some kind (e.g., biofuels, nutraceuticals, animal feed, etc.). The OMEGA module floats on the sea surface, getting light from the sun and getting stirred by wave action. The temperature of the algae culture inside OMEGAs is regulated and maintained by the temperature of the surrounding water. Gas-filled or water-filled bladders maintain the buoyancy and overall shape of the OMEGAs. The gas-filled bladder, filled with CO2, is separated from the algal culture by a gas-permeable membrane that allows the CO2 to diffuse into the growing culture. The water-filled bladders are used for buoyancy control and rigidity of the structure. There are small patches of forward osmosis (FO) membrane on the bottom of the plastic enclosure. These membranes retain the algal culture and the nutrients in the wastewater, but allow clean (tertiary-treated) water to slowly diffuse out into the surrounding seawater. During a typical algae incubation period (10-20 days), >75% of the water will be removed from the algae culture by the FO process. This process stimulates the growth of algae by concentrating nutrients and contributes to the dewatering of algae for harvesting.

At the appropriate time, the concentrated algae is harvested using buoyant cylinders that roll along the bottom of the module pushing the algae slurry out one end of the OMEGA module into a collection barge for further dewatering and processing. Note that freshwater algae are cultivated in OMEGAs and these algae cannot thrive in seawater. Therefore any algae lost during the harvesting process or that escape into the surrounding seawater for any other reason will not be able to compete with the marine species. The freshwater community inside OMEGAs also allows seawater to be used to remove the biofilms that form inside the enclosures with time.

An OMEGA farm consists of many OMEGA modules arranged with ample space between them. This allows exchange between the water and the atmosphere (gas and particulates), light to penetrate into the ocean below, and allows sea birds and marine mammals ample access. Both the individual OMEGA modules and the organization of these modules in a farm must withstand environmental forces (wind, waves, and mechanical stresses from hail, floating logs, birds, seals, boats, etc.). The number of OMEGA modules in a farm will depend on the location, shipping lanes, the amount of sewage to be processed, and the local capabilities and needs for processing, use, and distribution. In some places the modules will be anchored and in other places they will be free-floating and tracked by satellites or aircraft, while being tended by ships or barges.

Where should OMEGA farms be located? Ideally, an OMEGA farm will be established in marine (salty) water in the vicinity of a source of nutrient-rich freshwater (e.g., municipal wastewater outfall or near the mouth of a polluted river). They will also need to be in the vicinity of a source of CO_2 from, for example, a coastal power plant. These and other conditions at the location (temperatures, light, water clarity, frequency and severity of storms, geography, boat traffic, etc.) will dictate how the OMEGA farm will be configured and what algae will be suitable to cultivate. The harvested algae must be processed within a reasonable distance from where they are grown, where "reasonable" means economically accessible and environmentally acceptable. In principle, OMEGA could be deployed in any coastal zone in the vicinity of a city with an ocean outfall, with non-toxic wastewater, a CO2 source, and a processing facility. In fact, there are some other infrastructure issues with regard to access, ocean conditions, and moorings, which is why we came to Lolland.

Wind farms and OMEGA farms. Lolland has one of the largest offshore wind-turbine installations in the For OMEGA, this represents an offshore world. infrastructure with many attractive properties. Imagine an OMEGA farm distributed among the regularly spaced wind-turbine poles. The poles provide a physical anchor point for the OMEGA modules and the turbines provide local power. This power can be used for pumping water to and from the OMEGAs, it can be used for pumping air or CO2, and it can be used for pulling the OMEGAs underwater in bad weather. Power can also be used for providing artificial light for the OMEGA modules to extend the growth period of the algae. This



potential combination of OMEGA and wind farms could be a unique and distinguishing feature of Lolland.

How much biodiesel can an OMEGA farm produce?

It is estimated, based on land-based algae cultivation systems, that biodiesel production could be between 20.000 kg/ha/yr and 60.000 kg/ha/yr. Using the lower value (20.000 kg/ha/yr), we calculate it would take 3.550 ha or 35.5 sq. km of OMEGAs to match the total biodiesel produced in DK in 2005 (71.000.000 kg), which was almost exclusively produced from animal fat.⁴ The proposed Rødsand



II wind farm will be approximate 3500 ha or 35 sq. km,⁵ suggesting an OMEGA farm in that context has the potential to nearly double Denmark's (2005) biodiesel production. In 2007, the total petro-diesel use in all of DK was reportedly 2.750 x 10^6 liters. Using our conservative values for algae biodiesel production, this would require an area of 1.375 sq km, which is 39 time bigger than the area planned for the Rødsand II wind farm, but it is less than 0,4% of the are of the Baltic Sea.

In the WSA workshop we considered the possibility that offshore wind farms in Lolland could be the world's first OMEGA farm. An OMEGA farm, even on a demonstration or experimental scale, would require local assembly of materials, harvesting, processing, and distribution, which means development of local infrastructure for all of these activities. This translates into local jobs. We discussed the problems of access, infrastructure, maintenance, and processing. While there are many advantages to establishing OMEGA farms in the context of offshore wind farms, there are also some problems that

⁴ <u>http://www.danbio.info/Default.aspx?ID=47</u>

⁵ http://www.danmarks-vindmoelleforening.dk/fakta/pdf/P4

Wind, Sea, Algae Workshop, Lolland, Denmark, April 2009

would need to be addressed. These include problems with accessing the wind turbines if they are surrounded by OMEGAs, the drag forces on the turbine poles due to the attached OMEGAs, and the impact on boat traffic in general. There are also many fundamental questions remaining about the feasibility of OMEGA, many of which were discussed during the WSA workshop (see Appendix 1).

A CRITICAL ROLE FOR LOLLAND IN THE DEVELOPMENT OF SUSTAINABLE BIOFUELS FROM ALGAE:

New, but not true;

True, but not new.

New, but so what?

New, true, and important!

To determine the nature of an "algae roadmap" for Lolland, it is essential to identify the goals, the local constituents, and the stakeholders. There are a number of possible goals for Lolland, including products (e.g., algae biomass, biofuels, fodder, and nutraceuticals), algae processes (e.g., wastewater treatment, dead-zone remediation in rivers, CO2 sequestration), and algae industry (e.g., technology cultivation demonstrations, R&D, algae processing factories and green jobs). These categories are not mutually exclusive, and indeed, there may be different goals and different "algae roadmaps" for different parts of Lolland or at different times of the year, and for different purposes.

It is clear that there are many possibilities in Lolland for developing algae culturing facilities. In our workshop, we had intended to focus on the feasibility and scalability of developing an offshore microalgae cultivation system in association with offshore wind farms—existing or planned. The design we discussed, called OMEGA (Offshore Membrane Enclosure for Growing Algae) was much debated, but not particularly supported for the Baltic Sea both because it was argued that the available nutrients are too low at municipal wastewater outfalls and because the salinity gradient in the sea would not work well for the proposed osmotic dewatering process. There was more support and interest in developing onshore ponds for research purpose and cultivating either microalgae for biomass or biodiesel, but preferably macro-algae (seaweed) for biomass and other products. There were many people at the workshop, who suggested that seaweeds grown in traditional ways, near shore, or in less traditional ways on structures in association with wind farms is a important area for further research in Lolland. Lolland could contribute to existing seaweeds research on species such as *Ulva* and *Porphyra*, which are funded research programs (e.g., http://news.mongabay.com/bioenergy/2007/08/danish-researchers-look-at-seaweed-for.html). This seems to be a practical research track that could lead to

some additional biomass production and may be scalable to significant proportions if an offshore system was developed in association with the offshore wind farm (see Bela Buck lecture) or if Chinese systems are adapted to the Baltic (see GuangCe Wang lecture).

While the seaweed and aquaculture communities have significant support in DK and have ready potential for developing both onshore and offshore systems, the microalgae options, such as OMEGA, have no clear Danish advocates and hence there is no clear path to its development.

The vast majority of efforts in the US and elsewhere in the world have focused on growing algae in enormous shallow ponds or large bioreactors on land, although it is well known that there are formidable problems in scaling these land-based systems. Not the least of these problems will be due to competition with agriculture for land, water, and other critical resources; but there are also serious logistics problems to provide fertilizer and transporting algae. Most of these concerns can be addressed by moving algal cultivation offshore and we recommend that Lolland continue to investigate the Offshore Option.

THE PURPOSE OF THE WIND, SEA, AND ALGAE WORKSHOP:

Our first international workshop assembled a world-class group of scientists, engineers, economists, and policy-makers representing Universities, National Laboratories, and Industry to consider the question of offshore algae cultivation. During a three-day workshop, this multidisciplinary group explored and evaluated, discussed and debated, if offshore algae production is feasible, scalable, environmentally acceptable, and cost effective.

The purpose was to focus on the biological, engineering, environmental, and economic issues associated with offshore algae cultivation on a scale that would be relevant for biofuels, but not excluding other products. We discussed both microalgae and macroalgae cultivation. We considered possibilities for ocean environments in general, and Lolland or more specifically the wind farms of the coast of Lolland, in particular.

We succeeded in bringing together biologists, engineers, environmentalists, and ecologists. After the first workshop it is impossible to provide definitive recommendations about the path forward for Lolland. It is clear that follow up workshops will be valuable to convene to continue to explore in more depth the feasibility, scalability, and impact of establishing offshore algae farm in general and in the vicinity of Lolland in particular.

The transcripts and interpretations of the video of lectures and discussions in this report will help inform future workshops and elucidate the challenges and opportunities that lay ahead. There was no

consensus on methods, but there was universal agreement that the workshop was inspirational and the topic was worthy of further investigation.

A REAL	Scouting	Incubation* Business takeoff*	
	Key local considerations	Examples of critical environmental questions	
Environment	 How does this concept fit with the strategic plan for Lolland? How does it fit into Lolland's business/project "platforms"? Who are the organizational backers and blockers and how to manage them? What are the regulatory opportunities and obstacles? 	•What are the impacts of the local environment on OMEGA -How will an OMEGA farm cope with local weather and rough seas? •What are the environmental consequences of OMEGA? -Impact on natural environment? -Consequences of leakage?	
Economics Biology	 How does this fit with the Lolland's vision for the future? Is the new system compatible with 	 •What are the social-environmental implications of OMEG/ -How will it effect water use such as boating & fishing? •What are the policy and political environmental issues? •What are the implied OMEGA environmental impacts ? •What are the by-products of construction? •How will it be used when it is no longer useful for cultivating algae (cradle to cradle)? 	
Engineering	existing infrastructure and processes? Can "green" NGOs be brought in as supporters or "champions" of the concept?		
		Incubation & business takeoff not relevant at this early stage.	
	Scouting Validation	Incubation Business takeoff	
	Key local considerations:		
Environment		Incubation Business takeoff	
	Key local considerations: High-value vs. commodity markets (e.g., nutraceutical vs biofuels) Are these existing or new markets for Lolland and will current businesses in	Incubation Business takeoff Examples of critical economic questions •What are the economic requirements to initiate OMEGA? •What existing businesses will be stimulated? •What new businesses will be introduced locally? •How will the supply-chain begin? •What will be needed to reach economics of scale?	
Environment	Key local considerations: • High-value vs. commodity markets (e.g., nutraceutical vs biofuels) • Are these existing or new markets for Lolland and will current businesses in Lolland be enhanced or displaced? • What jobs will be created by the growth or development of markets (business models)? • What are the value propositions for local businesses?	Incubation Business takeoff Examples of critical economic questions •What are the economic requirements to initiate OMEGA? -What existing businesses will be stimulated? -What new businesses will be introduced locally? -How will the supply-chain begin?	
Environment	Key local considerations: • High-value vs. commodity markets (e.g., nutraceutical vs biofuels) • Are these existing or new markets for Lolland and will current businesses in Lolland be enhanced or displaced? • What jobs will be created by the growth or development of markets (business models)? • What are the value propositions for	Incubation Business takeoff Examples of critical economic questions •What are the economic requirements to initiate OMEGA? •What existing businesses will be stimulated? •What existing businesses will be introduced locally? •How will the supply-chain begin? •What will be needed to reach economics of scale? •What will be needed to reach economics of scale? •What is the potential economic impact of OMEGA? •Local impact?	
Environment Economics	Key local considerations: High-value vs. commodity markets (e.g., nutraceutical vs biofuels) Are these existing or new markets for Lolland be enhanced or displaced? What jobs will be created by the growth or development of markets (business models)? What are the value propositions for local businesses? What are the various expected current and future market sizes? What are the anticipated revenues in	Incubation Business takeoff Examples of critical economic questions •What are the economic requirements to initiate OMEGA? •What existing businesses will be stimulated? •What new businesses will be introduced locally? •How will the supply-chain begin? •What will be needed to reach economics of scale? •What will be needed to reach economics of scale? •What is the potential economic impact of OMEGA? -Local impact? •How will the OMEGA market develop?	

Executive Summary OMEGA Context: Anders Handlos Grauslund & Jonathan Trent

Wind, Sea, Algae Workshop, Lolland, Denmark, April 2009

AXXV	Scouting	Incubation Business takeoff	
	Key local considerations:	Examples of critical biological questions	
Environment Economics	 How is the technology related to the current bio businesses of Lolland? What are the potential synergies with agricultural or other methods? What aquaculture market could be developed? Will the organism be controllable and not impact the ecosystem? Are there regulatory issues with regard to the species cultured? 	 •What organisms would best be suited for an OMEGA demonstration project? - Locally adapted algae for biomass or oil? -High-value algae or other aquaculture species? •What species or mixtures of species would provide the best return on investment? •License strain? •What biological system is compatible with existing plans for Lolland? -Are there resident experts in the appropriate area of biology? 	
Biology	Can strain be inoculated directly in OMEGAs, or will "pre-growth" be required? – are these facilities available?		
ngineering		•How will Induction of oil/nutraceutical production be initiated	
		•Will growth cycle be sufficiently short in DK climate?	
	Scouting Validation	Incubation Business takeoff	
	Scouting Validation Key local considerations	Incubation Business takeoff Examples of critical engineering questions	
	Key local considerations		
nvironment	Key local considerations	Examples of critical engineering questions	
nvironment	Key local considerations What are the engineering milestones needed to deploy this technology? What existing engineering methods	Examples of critical engineering questions •What is the optimum size and shape of OMEGA modules? •What are the logistical engineering issues that must be	
(Key local considerations What are the engineering milestones needed to deploy this technology? What existing engineering methods can contribute to success? Are there local engineers or will new	Examples of critical engineering questions •What is the optimum size and shape of OMEGA modules? •What are the logistical engineering issues that must be addressed?	
evironment conomics	Key local considerations What are the engineering milestones what existing engineering methods can contribute to success? Are there local engineers or will new talent need to be imported? Is the new system compatible with engineering possibilities for the	Examples of critical engineering questions •What is the optimum size and shape of OMEGA modules? •What are the logistical engineering issues that must be addressed? •How will the filling, harvesting, recovery be done?	

Engineering

•Will OMEGAs be anchored in one place or "herded"?

-Instruments for monitoring growth and conditions?

•What will the system's energy requirements be?

•How will OMEGAs be instrumented? -GPS for tracking location?

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INTRODUCTION TO WORKSHOP, JONATHAN TRENT

I want to welcome you all here from the far corners of the Earth. We have an incredible group of people here from as far south as New Zealand and South Africa to as far north as Iceland, Norway, and Sweden; as far west as California and as far east as China. We have scientists and engineers from 18 different countries, representing among others: ecologists, marine engineers, phycologists, environmentalists, wastewater experts, civil engineers, mechanical engineers, politicians, philosophers, we even have rocket scientists.

To set the stage for our coming lectures and discussions, I want to give a brief historical perspective on the genesis of this workshop. It's started when Stig Vestergaard (Fig. 1), the mayor of Lolland, came to Santa Cruz, California to visit the City and the University and I was chosen to be one of the people to meet him. I think I was chosen to meet hi partly because I have a Danish wife, Susanne, and partly because I founded a grass-roots activity at NASA Ames called: "Global Research into Energy and the Environment at NASA" (GREEN). It did not take much



research to realize that I had an obvious connect to Stig and to Lolland—a community that is working so hard to make itself "green" by all measures. When I met Stig, he told me about a variety of exciting and innovative things that are going on here in Lolland and tomorrow morning I think he's going to show us some of these things. If you noticed the altitude of Lolland's landscape, you will realize that with very little rise in sea level nearly one third of Lolland will be submerged. No wonder Lollanders are so concerned about global warming-necessity is the mother of invention.

I remember how impressed I was by many things Stig told me, but it was when he told me about the offshore wind-farms that I was inspired.

I was inspired because I had been thinking for sometime about cultivating algae in enclosure in the ocean, but the big problem that often considered was infrastructure. How to organize, maintain, and harvest the algae enclosures, given the complexity of the ocean with its currents and winds and constant changes on so many different scales?

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No wonder, as soon as Stig mentioned the offshore windfarms, I had the epiphany that this could be the answer to the infrastructure

Wind, Sea Algae Workshop, Lolland, Denmark, April 2009

problem! I remember asking Stig if they were working on algae for biofuels and if they had considered growing algae offshore and I remember, he looked at me pensive without answering; either because he didn't understand my English or because it wasn't an area that were working on at the time. In any case, as I recall he said he'd look into it.

Some weeks later he contacted me through Anders Muller and asked me what I had in mind with regard to offshore algae and wind-farms. I confess, I had not thought about it much more, but I realized immediately that any scientific project of this magnitude needs a significant credibility check and I glibly answered that we needed to conduct an international workshop with experts from all over the world. Your sitting here tells the rest of the story...

PURPOSE OF THE WORKSHOP:

During the workshop, we will address two fundamental and closely related questions: 1) is it

Is it feasible and desirable to cultivate algae offshore for biofuels and other purposes?

feasible and desirable to cultivate algae offshore for biofuels and other purposes? and 2) what are the biological, engineering, environmental, and economic challenges that will need to be addressed to succeed in developing an offshore algae cultivation system?

Questions of feasibility and desirability are dependent on the biology, the engineering, the environmental impact, and the economics of such a proposed system. Similarly the questions about the biology, the engineering, the environment, and the economics are all inter-related.

What are the biological, engineering, environmental, and economic challenges?

For example, the algal species that is chosen to cultivate in such a system (biology), will determine the engineering of the enclosures, the enclosures will determine the environmental impact both in terms of their structure and the consequence of their failure, and all of these things will determine the economics (value of products, construction and

maintenance costs, offshore costs, environmental monitoring, clean up, etc.). In addition, there is policy as well as political and social issues related to uses of coastal waters and we will consider these issues in the category of "environment." I know there are way too many issues contained in these four categories (biology, engineering, environment, and economics) as they relate to offshore algae cultivation, but we will make a start in the next few days.

GOALS OF THE WORKSHOP:

We have assembled this diversity of talent from all over the world to use our collective intellect to reach a number of goals during our three-day workshop. Our first goal, is to

generate new ideas about the prospects of cultivating algae offshore, with emphasis on oilproducing microalgae and with lessons-learned from our colleagues growing macroalgae (seaweeds) offshore. Our second goal is to expand our networks of inspiring colleagues that will

hopefully become fruitful collaborators in the process of establishing offshore algae cultivation as a feasible and desirable field. Our third goal is to prepare a presentation about the out-come of our workshop for the local Ministers and

Main workshop goals:

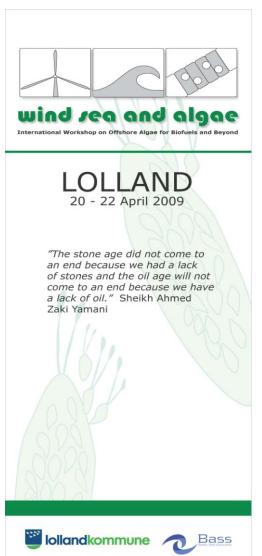
- * Generate new ideas about offshore algae
- * Meet potential collaborators
- * Prepare presentation for Wednesday
- * Gather material for our Workshop Publications

visitors who will join us on the last day. More generally, our ultimate goal is to communicate to future generations what we as a group thought would be the best way to use the oceans for producing sustainable fuel and food from algae. This goal will be reached by ultimately publishing a Proceedings volume that documents and describes our activities.

STRUCTURE OF THE WORKSHOP:

Because of the diversity of disciplines and cultures represented, we plan to have informative morning lectures to provide overviews and perspectives on topics of importance with regard to the biology, engineering, environment, and economics of offshore algae cultivation. In the afternoon we are going to focus on these four categories in small discussion groups, which will be video taped and will become the basis for the Proceedings volume that will be published.

The goal of the afternoon sessions will be to explore and expand what will be proposed by my team at NASA. In addition to the current concerns, we will also consider OMEGA in the context of a future world in which new coastal regions are flooded due to uncontrolled global warming. We will consider how OMEGA could contribute to "agro-aqua-culture" for food/energy in the flooded coastal plains—when offshore moves onshore. The exercise is meant to address the questions: What should we do to get ready for sea level rise? Should we be preparing to dig canals and build levies for OMEGA farms? Should we construct floating refineries and algae processing plants?



We will also discuss the role of OMEGA in dead zones remediation. Can OMEGA be used as a nutrient "sponge" as a prophylactic against wild algae blooms in coastal zones due to river run-off. Finally, we will focus on Lolland and the Baltic and consider how OMEGA applies to this region or not in the context of wind farms or other algae projects.

MOTIVATIONAL REMARKS ABOUT THE FRAMEWORK

I want to close by setting the tone for this workshop by referring to the three quotes that are written on the hanging banners. One of these quotes is from the former US vice president Al Gore, who said: "we face a genuine planetary emergency, we cannot just talk about it we have to act on it and we have to solve it urgently." I hope this sense of urgency is already in everyone's mind and that we do not have to convince anyone here that it is high time that we as a species that has reached such a huge population takes a more responsible role within the biosphere.

The second quote is from one of the founders of OPEC, Sheikh Ahmed Yamani, who answered a question about what will happen after we run out of oil by saying: "The Stone Age did not come to an end because of a lack of stones..." Indeed, the stone age

ended because technologies emerged that made stones obsolete. It is up to us to come up with new technologies that will make fossil fuels obsolete. I don't want to belabor this point because I think everybody in this room realizes the importance of coming up with good alternatives to fossil fuels, but it is important that we acknowledge that the fossil fuel industry has been developing for over 150 years and has reached annual revenues of over \$2 trillion. It appears that we have <20 years and preferably <10 years to develop viable alternatives and we will have to do this with the riches of our educations and our imaginations, rather than huge sums of money.

We must carefully consider the consequences of doing nothing, of continuing business-as-usual. We must ask ourselves if we are satisfied with our predicted legacy for our children, our children's children, and the children of all species. We must ask ourselves if we have confidence to act from our knowledge and our creativity to at least present to the world the possibilities of alternative futures. If we go deeply into ourselves, can we contribute significantly to the transition from fossil fuels to sustainable alternatives? I'm convinced we can if we are open and

enthusiastic, which brings us to the quote on the third banner. The US president, Harry Truman, once said: "There is no limit to what you can accomplish if you don't care who gets the credit." I have tried to set an example by putting a lot of ideas on the Internet and I hope everyone will follow suit. Please treat our human predicament with the urgency it deserves and for the next two days put aside your personal visions of personal grandeur and communicate honestly and openly.

Finally, I want to say (as an unofficial NASA scientist) that in September, 1962, the President of the US, John F. Kennedy said that we are going to put a person on the moon and at the time people said that this is impossible!

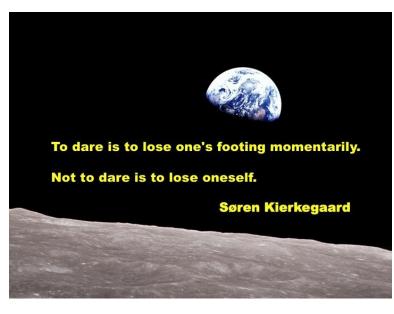
"We choose to go to the moon in this decade and do the other things, not because they are easy, but because they are hard, because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one which we intend to win..."

We did get to the moon and if we put our minds to it, we can grow algae in plastic bags in the ocean no matter how complex that seems to us now considering the oceans many dimensions, storms, tsunamis, and variability. For fossil energy, we have engineered oil platforms to stand in the North Sea and wind turbines to stand in the Baltic Sea.

I'm convinced if we truly realized the urgency of finding a sustainable alternative to fossil fuels, if we really believed that Stone Age will not end until we providing a better alternative to stones, and if we are willing to work together with the goal in mind and not our personal gain, then do any of you doubt that we could figure out how to put plastic enclosures into the ocean

in which we could grow copious amounts of algae?

One of the most iconic images from the Apollo missions to the moon, was the image of the Earthrise. It was that image that confirmed the metaphor of Buckminster Fuller's that we are on Spaceship Earth. The point Fuller was making however, was that we are riding on Spaceship Earth and we do NOT have the owner's manual. We are no longer passengers on Spaceship Earth, we are the crew...

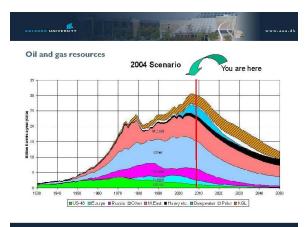


BIOTECH, BIOFUELS AND BIOREFINERIES, LENE LANGE



It's an honor and a pleasure to have this opportunity and I would like to say that I did come from Copenhagen University and I'm now at Aalborg University. I'm not going to take the time to tell you how good we are at Aalborg University or at the Copenhagen Institute for bioenergy and I will not use the opportunity to say how much we can do in the Danish Universities such as the technical University or Roskilde University, because there are so many universities in Denmark working on sustainability and sustainable technologies. We are very strong in bioenergy as we are in many of the renewables, but I will not talk about that. In my own field, I'm working on the discovery of new enzymes and new proteins for biomass conversion and for that matter, in the Copenhagen Institute of technology we see algae as just another feedstock—another source of biomass for our conversion systems. Biomass conversion has a lot of different products that serve a lot of different roles in a modern society. We will need a lot of different things from biomass and fuel is just one of them, because today we get so many different things from oil and natural gas—a whole spectrum of different things and we need to replace all of these with biomass... but that's not what I'm going to talk about. No, I'll use this opportunity to talk to you about the much broader picture, in the political sense, because I think the rest of the workshop will be much more on the technical part—I see we have many experts in the technical fields and I trust you'll do the technical part well, I'm sure.

I'm not going to try to convince anyone in the room here that our current predicament is serious. We are all already wondering how civilization is going to survive once the black gold runs out. We have the quote about the Stone Age ending not because we are running out of stones, but in fact we are running out of oil or at least affordable oil. I think we should take a moment to consider that we are the generation, here in this room, who used up all the accessible oil and natural gas and I think this is unethical. Denmark is a very striking example of this. We had a significant big deficit in the trade

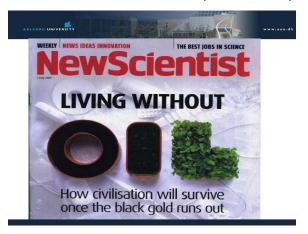


balance because we had to import all of our energy from the Middle East and elsewhere and then in my life time and most of your life times, we found the oil and natural gas in the North Sea and with that discovery we have been living happily ever after. Now, as a consequence of this discovery we have trade surpluses and a thriving economy, but we're using up all these oil reserves in the North Sea in one generation! I mean, don't we owe the world of the future to be at least the ones who will get the new technologies that will replace oil and natural gas at least introduced and that's what we are here for.

There is no discussion as to whether or not we're running out of oil and natural gas, we know these resources are not being replenished and we're using them at an incredible rate. We know they are going down in availability. We don't need all this discussion about if climate change is man-made or not, I don't really care.

We all know, we need solutions to a host of problems, which are related to CO2 production and climate change and if it's for one or the other reason, we still need solutions. I think it's not up to us to keep

describing what the problems are, let's not keep discussing what is the reason or who is to blame; let's get on with solutions--good and solid and robust solutions. We need to understand so many different fields and that's why these very broad based workshops are so important. I notice there is one field you didn't mention Jonathan and that's information technology (IT). I don't know if we have sufficient expertise here in IT, but Aalborg University is the biggest in Denmark in clean and green IT. There is so much in the IT part of this. I mean the modeling and forecasting and monitoring and so on...

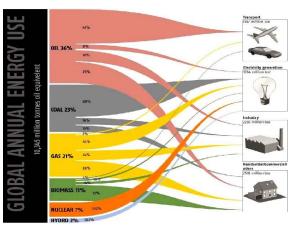


Okay, so we don't need to use time in discussing the above in this workshop because we have all understood that our global predicament is serious. We also don't need to discuss whether it's important to focus on fuel because we have the statistics telling us how much of the easily accessible oil (70%) is being used for transportation and in transportation we cannot use the windmills. Without windmills on the back of a car, we need to wait for fuel cells and hydrogen-powered cars-we are not there yet. In this intervening period we're going to have to find fuels for cars and for airplanes that will give us a sustainable solution for transport. The good part is that we know we can get the whole platform from biomass conversion. We can use biomass for making fuels as well as all of the other more costly products which we currently make from oil and natural gas, such as biochemicals and refined plastics, and so on. It is possible that someday we will get along with hydrogen and fuel cells and we won't need carbon-based fuels, fossil or alternative biofuels, but we need alternative fuels now for transport and later on when there is a hydrogen or fuel-cell based transportation system, we'll still have



The Challenge:

Feeding and fuelling the 9 billion -under climate change conditions



the biomass platform for all the other higher-value products. We already know we can do this with biomass. So, yes, we know we better get this started, because ultimately this is all about the real challenge.

The real challenge is very simple: feeding and fueling the nine billion. In other words, we're certain that we can change a lot of things in this world, but we will not be able to change radically before we have reached a global population of 9 billion. To feed the 9 billion, we are going to need to increase our agricultural yield by 55%, while you know what is happening to all the beautiful flatland, the best arable land for growing good agriculture, we are using it for roads and airports and cities and so on. In developing countries, this is what is happening. This fertile agricultural land is become the basis for social infrastructure, urbanization, and roads for transportation. This is putting further restrictions on useable lands.

So feeding and fueling the 9 billion is *the* challenge. It is not a discussion. This is the challenge and it will have to be done under climate change conditions, which means we cannot take historical regional agricultural practices for granted. The inevitable conclusion is that we cannot afford to proceed with business-as-usual—this is clearly not an option.

I was giving a keynote speech a couple of weeks ago in an EU conference about biosciences for business in Edinburgh and I was first on the stage and I thought that this sense of urgency that is so obvious to those of us here and I thought it would be obvious there also. For me, this is by now just a starting point, it's not a point of discussion, but I realized that all the speakers that came up to the podium after me, were all focused on how to make sure the chemical industry will survive in a competitive way for the next 20 years. In my mind, there is no question that the chemical industry will survive



during the next 20 years, but the challenge is to evolve the chemical industry so that it survives in a way that it is part of the new solutions. We are coming to a point where it is not just a matter of survival of businesses for 10 or 20 years, these businesses have to contribute not only to the bottom line but to actually contribute on the long time-horizon to a sustainable future that impacts above all the production of food and the availability of freshwater.

This means to me, full speed ahead for efficient technologies, that have a triple bottom line, which includes social and environmental and technical parts, but these parts have to be inclusive of each other to provide a 360° perspective. For example, the social dimensions of politics and security include not only the commons but the environmental dimensions in which we are part of the broader ecosystem—active in a local system, but embedded in a global system of biodiversity. In a 360° analysis, the challenge is essentially to take account of everything at once and it is an inherent condition.

It is based on thinking globally and acting locally that's an old saying but I think it's still very appropriate particularly for this meeting.

I would like to give my sincere acknowledgments and appreciation both to Lolland for its pro-active initiative that brought you all here this morning and for the next couple of days. I'm really impressed with what is happening in this part of Denmark, in this part of our global society. I want to also acknowledge the determination and charisma of Jonathan Trent—I'm impressed. Then of course, I



Think global -act Local!

-sincere acknowledgement and appreciation -of the Lolland proactive initiative -for the determination and charisma of Jonathan Trent -and for the many experts, who found their way to this part of the world

want to acknowledge all of you experts, who came here from far and wide and who are going to make this meeting a success I'm certain.

At this point, I need to mention the financial crisis because we hear people say: "no, but now we don't have time for the green part because now we're really busy with the financial things and that is the kind of thinking and tunnel vision that says you can only approach one thing at a time at best. But if you have this kind of starting point, you get it all wrong and we are lost. In fact, you all know that in the most important times in the world and in your life you have had to handle many different things at once. You were not allowed to approach your problems one at a time, but you tackled many of



investments world wide in new sustainable technologies is part of the solution-making new jobs and new hope

them at the same time and you will be better in doing that because you were actually forced to approach many problems at once. You will be better in solving one problem because you were actually were thinking in a more general and holistic way. That's the green challenge for feeding and fueling the 9 billion is actually help. Taking on such a challenge will actually help solve the world's financial crisis. It's a perspective that creates hope that we can actually meet the challenges and jobs that will do so. It's an option that suggests we can start from scratch and start with hope and get a lot of new things going.

Success will take unprecedented leadership and here I'm speaking about our local limitations in the European Union. The EU is dragging its feet in this. We have so many technologies, we have the infrastructure, and most important we have citizens who are ready and willing to act and to pay for it; who are ready to say "yeah, now let's do things different." We are stuck.

I worked for 20 years for Novo Nordisk and my boss, a lead scientist at Novo said in big headlines: "we are giving up on the EU." This is a Danish company working globally with 42% of the global market share



of enzymes, and they're saying that in second-generation biomass conversion Europe is not making up its mind and they are focusing on US, Brazil, and China. Those are the ones who are moving, but Europe is not getting anywhere.

On the other hand that's too negative, we Europeans do have a wonderful hub of renewable technologies. In Denmark, for example, in biomass conversion and in other types of renewable energy, we have a lot of things developing that are new and innovative technologies and we have the practice. We have demonstrated the ability to reduce to practice so many of these technologies and we have demonstrated that a significant part of our energy consumption in a modern society can be met by renewable sources. We really did introduce wind power to a level which has never been done before! Most people, even most of the early fans of wind power, had not really anticipated that it would be

possible for us to grow to the extent that we have. We are not only getting double-digit contributions to our total energy use from wind power, but it's a significant double-digit!

Here in the EU we have all this potential and all this good will and then some political reasons from some big special-interest groups that feel economically threatened, and that paralyzes progress. This is why I think only the small, local initiatives that demonstrate new technologies, are going to make the biggest differences. We need to show it's possible, because people and politicians will not change their minds and become supportive of these new technologies until they see things actually happening. It is possible for politicians to be the ones leading the way ahead particularly on a national and international level, but right now it's not moving fast enough and we have to show that it's possible.

We hear people saying: "this sounds very interesting but it's years until that's ready." This is what I hear about second-generation biomass. "It will take years..." and that means they don't intend to take it seriously. When people are saying: "oh, that sounds very interesting, but it will take years..." that means you're not being taken seriously. This is a kind of repressive tolerance of which we need to be aware and whenever we hear "it is years ahead..." we must firmly reply: "it is now!" And if we can get started, we can implement it in not that many years.

Once we start with the real implementation, it is really not going to be too many years to reach fruition. It will take innovative and out-of-the-box thinking, that will be cross-disciplinary and highly collaborative, involving world-experts in engineering and biology. I know how difficult this may be because I have seen how engineering and biology have been in separate silos that don't mix and don't talk to each other, let along collaborate, but it is clear to me that we need to get these groups together if we are to succeed. We need to rethink and get these things together. We must combine



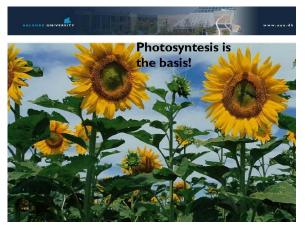
the best and the brightest from academia and industry with the detailed practical knowledge of locals in each region.

We know from biology that one-size does not fit all. Let's not get paralyzed by this knowledge, but it's a cruel fact. If you think, for example, that the west coast is the same as the east coast; no, it's not like that and biology is so complex, but we need to accept and build on this complexity. If we're going to articulate a vision for biofuels for the world or parts of the world, I think we have to consider algae as part of that vision. So we have to act locally, but we need to think globally and if we can make this work in this part of the world, it will be a stimulus and a basis for developments in other parts of the world; in different ways, different types of set ups, with different types of algae and technologies, but I believe it will be part and for many reasons.

There are many reasons I believe this, not the least of which is that it is based on photosynthesis--a fundamental part of nearly all eco-systems and the way we get the sun to work for us. The second thing is that it is not competing with agriculture and remember, we need to feed the 9 billion. This is

basic and our point of departure. Finally, we have the third generation biofuels produced directly from algae and photosynthetic bacteria and there are a lot of things going on in this area.

It's a little bit scary to see that if we go back to the research funded by the Department of Energy in the USA and they looked at algae, but they gave it up. They did a fantastic characterization of the wonderful oil you can get from algae, but then concluded it was not commercially feasible or economically viable and they said forget it. We must remember that it will still not be trivial to meet the many challenges and to be sure that it is feasible as well as commercially and economically viability and that it can be done in a reasonable period of time. But there are new fields for biomass production to



consider. We see biomass of macroalgae and what Jonathan is talking about with microalgae to actually culture them in the open sea. All these ideas are out there and we need to understand them and cross-fertilize our thinking and get things moving.

From a political perspective we can also ask why is the offshore algae so interesting? Basically for feeding the world in the context of climate change, it is a water crisis; it's not a food crisis *per se* it's a water crisis first. Climate change is making agriculture so difficult because there will be parts of the world now making a significant part of our production of food based on water which will not be there in the right amount at the right times for growing crops. This means farmers will soon not be able to harvest what they usually get. So we have a biomass in algae grown on the open sea, which is



not depending on water—this is a very very important part on what is being proposed. The other thing is that it doesn't put addition demand on the land use. We need all the flat land we can get in order to grow our food and the animal feed—animal feed is 70% of the global arable land so let's not forget about that. So no competing for growing food and feed is the most important reasons why this is such an interesting proposal. We also have to remember that algae can give much more than fuel. Fuel is a bulk product not a high-value product. People forget that all the time. Oh, the gasoline is getting so expensive and oil prices have to drop... But keep in mind that energy is bulk and energy is cheap compared to all the other products we can make from these building blocks. So we need to include higher value products as well and I'm sure I'm convinced our biorefineries thinking is the way to go. The algae is part of a biorefinery. Where we will make fuel and more higher value products which will together help us to keep the price down of the fuel so that its competitive. That's a very simple thinking but it's a necessity I don't think we'll have economic needs met if we only focus on the cheap energy part.

The people in the Far East in China and Japan and Thailand and so on they all know that algae is so valuable seen from a nutritional point of view and a health part. There are proteins and biochemicals the renewable carbon and the cell wall can be used as building block for building other biochemicals, food ingredients, nutraceuticals. I mean there are many different options and so much to do. In short, we need to go from bioenergy to biorefineries and I want to say one thing this morning. One pledge I'd like you all to take is: let us not waste our time during this workshop in discussing why we are so good and the other renewable techniques are not interesting. There are so many other renewable energy and sustainable biotech technology workshops that use half their time and it always what comes to the headlines in the newspapers is why this is not good but why my "hobby horse" is better and is the only one. If we do this, we get it all wrong, because when we need to switch to sustainable solutions and we need many solutions. We need really many different solutions not just one. so those other fighting sustainable technologies are our friends because we cannot produce everything we need from the algae. We will need other things as well. So let us not waste our time saying all the other ones are dummies and we are the only one that has seen the light and has the good ideas. So let us be sharp and focus on the sense of urgency and get on. We need to get coordinated, to demonstrate that it can be done, we need to convince the politicians that it's possible, we need people ready to invest.

This workshop could be part of such a paradigm shift and let's make sure that we do a good job, because if we disappoint if the technology do not meet expectations and become a catastrophe for whatever reasons, then we have a disappointment, which is the same as causing a backlash which will cost 20 more years before people get moving with this type of technology. Such as backlash is so costly and we don't have time for these types of serious backlashes. So in closing I would just like to say thank you so much for listening and I really look forward to next three days.

QUESTIONS:

Travis Liggett: I think you should consider using the term "spherical" vision instead of 360° because this problem is not flat.

Lene Lange: I like that comment so much! How come I didn't think of it? But when I think of 360° I think of it in all dimensions...

Jonathan Trent: Leave it to Travis... I have a question. Some people have suggested that the world cannot sustain 9 billion and that we should be focused on the number of people that are sustainable, like perhaps 4 billion. If this is true, then we are talking about feeding the nine billion but getting the numbers of people to a sustainable level.

Lene Lange: I think we have to convince people not to have so many children, but the only way to get to that point is to have a higher standard of living. So I think from 9 billion we'll go down, but I think it is really difficult to get the overall change in the worldview of people before we actually grow to 6, 7, or 8 and people say we won't turn around before 9 billion. If we could do it at the same time it would be wonderful because it is creating so much pressure. We need to be sure it will be a nice world to live in at the same time.

Several Fundamental Biological Aspects of Algal Biofuel Application

Presentation at

International Workshop on Offshore Algae Cultivation for Biofuels and Beyond

by

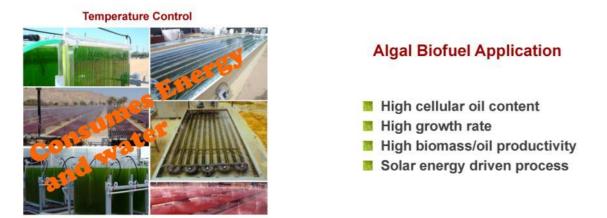
Qiang Hu

LABORATORY FOR ALGAE RESEARCH & BIOTECHNOLOGY Arizona State University

(Maribo, Denmark, 4/20-22/2009)



A combination of algae-based strategies [for biofuels] rests to a large extent on the promise that many algae can grow very fast and can contain high oil contents. As a result the algal mass production can give high biomass and oil productivity and since this process is driven by solar energy, this process is renewable and sustainable. However, this is not a fact. It is just a promise and potential. This potential will not be achieved unless we have an efficient engineering process and system. In order to achieve his promise and engineering system and process it can be very expensive and energy intensive.

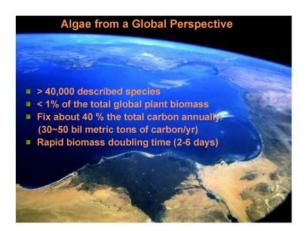


For instance, in order to achieve high growth rates in aquaculture, it requires mixing, which can be provided by different means like this rotating arm or the paddle wheel, or compressed-air aeration. In the case of the closed photobioreactor (PBR) different kinds of air pumps or water pumps are needed and all those processes require a lot of energy. To achieve high growth it is also critical to maintain the optimum temperature and of course we can control temperature by evaporative cooling or by submerging the bioreactors in the water (as illustrated in Fig). You can also use internal thermal exchangers like this panel for the bioreactors, but it is clear that to maintain temperature also requires a lot of energy and may waste a lot of water as well.

So the whole purpose of algae-based biofuels applications is to try to generate energy so therefore would like to have an energy output greater than energy input and ideally by several times, but right now there are so many energy input and there are only two algae outputs: algae oil and biomass residual. At this point we are not sure if we gain more energy or we spend more energy in this process.

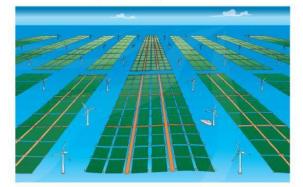
So for years we have been fighting and arguing about different approaches to grow algae on this limited piece of land in either closed photobioreactors or open systems, or how to control the temperature, or how to provide efficient aeration, but somehow we have ignored what is in front of us-the vast ocean and great To put algae in a global opportunities. perspective, the algae consists of less than 1% even less than 0.5% of global biomass, however this tiny biomass generates about 40% of our oxygen and removes about 40% of the total carbon dioxide. The small amount of algae in the

oceans are doing a great job. When it comes to provides unlimited amounts of water and surface area for algal growth, but also provides a natural means to agitate cultures and control temperatures. In some parts of the world you can certainly find parts of the ocean that have constant temperatures of about 20-30°C and can provide a very suitable mixing as well. Lolland has the world's largest offshore windmill facility and hopefully in the near future Lolland will also have a great algal offshore cultural facilities.



oceans are doing a great job. When it comes to the engineering process, the ocean not only

Offshore Algae Farming: a Stationary Mode



Here is one kind of design for an offshore algae system, which is in stationary mode and there's another concept of this kind, where there is a ship that acts like a shepherd, herding sheep for farming algae in the ocean, with a boat present functioning as a mobile oil refinery and oil tanker. Instead of my spending time to go into details about the technical designs of these systems, which is going to be the focus of the whole workshop, for the next 15 or 20 minutes, I want to talk to you more about the biological aspects, which relate to algal biofuel production. Since the audience here in this room has a very diverse

Offshore Algae Farming: a Mobile Mode



background and experience I think it may be helpful to facilitate our communication in the actions in the next couple of days. I'm going talk to you about what lipids and oils the algae produce; it may seem like a high school lecture, but perhaps not everyone understands all of these things at this point so I think it's important for us to talk about this at this point. Also, I'll talk about which species are the oleaginous algae—not every kind of algae produce oil. I'll talk about which are these algae and where and how oil is made and stored in the cell. Oil production follows a specific

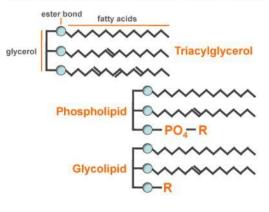
pathway and if oil is produced, we will consider under what conditions it is produced. If oil is not produced, why not? In other words, I'll discuss when and why algae make oil. I can tell you, normally algae don't make oil. I'll talk about what oil is made and what qualities of oil are made. Finally, I'll talk a little bit about what the photosynthetic response curve and what it means. Many times people who grow algae are struggling with these kinds of questions and these are important because they will affect us when we design a photobioreactor.

So what lipids do to algae produce? Algae produce very diverse kinds of lipids. When we talk about the crude lipids, it mainly consists of two categories: the non-acyl lipids and the acyl lipids. The non-acyl lipids are produced by the isoprenoid pathway and it includes the isoprenoids, carotenoids, or the phyto-tail of chlorophyll, this is a relatively small amount. The majority of lipid is the acyl-lipid, with attached glycerol these are glyco-lipids and this group is further divided into two categories: the polar lipids and the neutral lipids. The polar lipids are associated with the cell membrane and are a major constituent of biological membrane

Lipids Acyl Lipids (Vitacylglycerol, cholesterol) Polar lipids (MGDG, DGDG) Polar lipids (PA,PC,PE, PI) Non-acyl Lipids (isoprenoids, carotenoids, chlorophyll phyto-tail)

What Lipids/Oils Do Algae Produce?



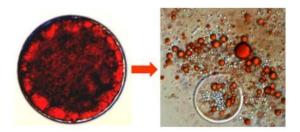


systems. The polar lipids are also divided into two sub-categories: glyco-lipids, namely the lipids bound to sugars of different kinds and the phospholipids, which is a different kind of species. The neutral lipids are mainly triacylglycerol, but algae also produced a little bit of cholesterol, which is different from animals where cholesterol is a major lipid.

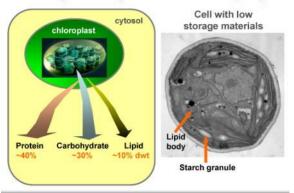
Under normal conditions algae only make polar lipids and make no or very little neutral lipids. This means that 99% of lipid is in the polar lipid category. However, when we talk about the algaebased biofuels, what is the feedstock? It is primarily triacylglycerol. Triacylglycerol is the preferred oil feedstock because of its chemical structure. Comparing triacylglycerol to the phospholipids and the glycolipids, you see that the triacylglycerol carbon backbone has three fatty acids attached to it and they can be easily hydrolyzed to release the free fatty acids or converted to hydrocarbon. These are the feedstock, but when it comes to phospholipids and glycolipids there is either one carbon bound to either the phosphor group or the sugar group. Therefore, on a mass basis if you have one pound or one kilo of tryacylglycerol you have one third more feedstock than you can get from one pound of phospholipids or glycolipids. This is why the goal is to get the maximum return of triacylglycerol rather than membrane-bound phospho or glycolipids, because these phopho groups and sugars cannot be used as a feedstock.

What are the oleaginous algae? Basically, any algae which can produce 20% or higher of tryacylglycerol. This is equivalent to oleaginous plants, which also produce 20% oil. What kind of algae can produce that much lipid? In this figure I show total lipid so if you look at cyanobacteria or blue-green algae they produce zero triacylglycerol because they are missing several genes involved in triacylglycerol biosynthesis or at least one critical gene. Also because cyanobacteria are a prokaryotic system and have no internal membrane system to separate triacylglycerol from the rest of the cell bodies. If you look at the marine algae there are many marine unicellular algae which can produce high amounts of triacylglyceryl-total lipid can go up to 50%. In particular, there are many diatoms, including both freshwater and marine species, which produce high amounts of triacylglycerol, similar to green algae. Each point on the Figure represents one specific species or isolate. The point I want to make here is that the production of oil is species specific. The oil content or the oil production potential is related to the species level and in many cases it's strain specific. Namely, if you have





Do Oleaginous Algae Produce Oil Anyway?



two algae, two Chlorella for instance, they look like brothers, but one somehow can produce or has the potential to produce up to 50% triacylglycerol and the other one will produce 50%

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

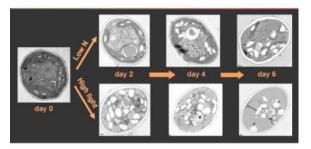
carbohydrate. Why is that? We don't know yet, but the reality is that oleaginous algae are species or strain specific. In other words, if someone shows you a great oil producing alga and you decide to try to find that kind, you may not be able to find it because the one you will find will be somehow different.

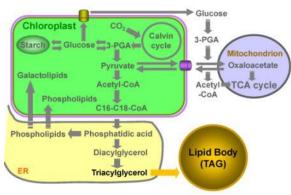
Do oleaginous algae produce oil anyway? The answer is no. If you look at a model of the cell and you look at the major organelle chloroplasts that's the primary place where products are formed in the cell. Using photosynthesis and carbon dioxide to produce organic compounds in the form of carbohydrates or sugars and therefore we start from sugar and go to different routes, either to produce protein, other carbohydrates, or lipid. if you look at the profile of these three major categories most of the carbon goes to the protein route: 40% on average. The carbohydrate route gets about 30% on average. The lipid route gets only about 10% of the carbon and in most cases algae only produce 5-7% lipid. So why is that? Almost all of the lipids are associated with the membrane system. In the cell you can see a few starch granules, which is a major carbon storage compound. You may also see a few oil droplets, which are primarily due to a scavenging process intended to gets rid of damage or modified membrane components, which are temporarily stores in the form of triacylglycerol. Under normal conditions, algae don't make triacylglycerol.

When algae start to make triacylglycerol? Even under very harsh conditions, we know very little about the mechanism of oil formation, but at least we know what are the physiological and

environmental conditions that can induce oil formation. Mainly we try to use high light or nutrient starvation to induce lipid formation. If we do a time course for lipid formation, we see that in the early stage a healthy cell makes very little or no triacylglycerol oil. Upon the stress induction they start to accumulate some type of storage compound either starch or neutral lipid. In the beginning, most of the storage compound is in the form of starch-not oil. Only when the stress persists or becomes more severe, then algae start to make more oil droplets at the expense of starch. This tells us the algae under normal conditions don't make oil and under stress conditions they prefer to make starch rather than oil. If you starve the cell for four days as soon as you enrich the culture with a nutrient, then they produced starch and no lipid formation. The reason is that to make triacylglycerol, to make lipid, is very expensive in terms of energy for the For instance, to take the carbon from cell. photosynthesis, such as a three-carbon sugar, we can ask how much energy it takes to form starch or to form triacylglycerol. To make one C16 fatty acid for instance, will consume 16 NADPH and 8 ATP, which means a lot of reducing power is

Starch or Triacylglycerol: Which is Preferred Storage Compound?





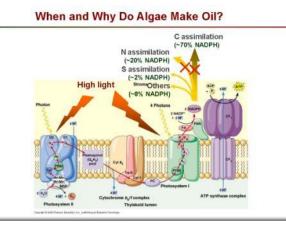


required. On the other hand if you make the starch, they use less than half of the NADPH. That's why from an economic perspective algae prefer to make starch rather than lipid.

Where and how is lipid made and stored? Now we understand at least the first part that the lipid is made in the chloroplast. The carbon dioxide goes to the Calvin cycle through photosynthesis to make a three-carbon sugar and then it can go to starch formation or it can make a pyruvate and acetyl CoA as a starting building block to make a fatty acid. When fatty acid is made then it acts to form acytl-CoA and thiol esters and is exported to the cytosol. These precursors end up in the cytoplasmic reticulum (one kind of organelle) and there they can either go through elongation or desaturation or otherwise different kinds of modifications, which includes assembling into the triacylglycerol with some of the precursors and can be sent back to the chloroplasts or other kinds of organelles to make membrane lipids. When triacylglycerol is formed it is made in the endoplasmic reticulum and it can give rise to the formation of lipid bodies. In the cytosol you can see a lot of lipid bodies, which store those triacylglycerols. This in the normal case for the majority of different types of algae, but still you can always find exceptions. For example in *Dunaliella*, the lipid bodies with triacylglycerol does not only form in cytosol, but also in the chloroplasts, in fact all those lipid bodies are not in the cytosol but are maintained inside the chloroplasts.

When and why do algae make oil? I said that under normal conditions, algae don't make oil because they don't want to use the reducing power from photosynthesis are used for more important purposes. In the case of a simple representation of photosynthetic electron transport chain. You see the photosystem 1 reaction center and the photosystem II complex and you see the ATP formation machinery. The photons strike the photo-reaction centers and that energy is used to split water and to generate electrons. The solar energy is actually stored in

finally end up forming reducing power in the form generated through this process, 70% of the reducing power is used to fix CO2, converting CO2 into sugar. You are left with 15 or 20% of those fixed electrons, which go to nitrogen assimilation and there is a little bit left for sulfate reduction and for other applications. That's how a majority of the fixed electrons go to carbon fixation. If you limit the cell's CO2 supply or their nitrogen supply, the electrons have no electron acceptor so all the electrons get stuck in the photoreaction system and all the electron-carrier intermediates get over-reduced. That causes problems. These problems also arise when you



the form of electrons, which go through all forms of electron transport intermediates and they finally end up forming reducing power in the form of NADPH. Once you have the NADPH and ATP

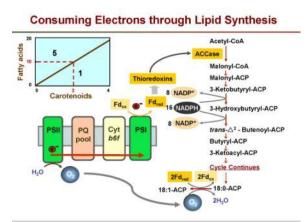
Consuming O₂ through Carotenogenesis

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

provide too much light for the photosynthetic machinery so the machineries splits too much water and those electrons get stuck in the electron transport chain. They form high-energy-potential intermediates and as a result in the photosystem II site the excess energy is somehow dissipated as heat, but a lot of energy may pass to molecular oxygen, which generates singlet oxygen species. This is one kind of reactive oxygen species, which is detrimental to biological compounds such as protein, lipid, DNA and so on. On the photosystem I side the reduce ferrodoxin is supposed to deliver the electron to form NADPH through this ferrodoxin-NADPH reductase but since the NADPH is in the reduced form it gets stuck there, the reduced ferrodoxin will pass the electron to molecular oxygen to generate a singlet oxygen species, which is further converted to hydrogen peroxide and hydroxyl radicals. so all those reactive oxygen species are detrimental.

Therefore, in order for the cell to survive in this kind of photo-oxidative stress condition, the cell has to somehow develop a protective mechanism. Either to get rid of oxygen or to get rid of those reactive oxygen species. I will show two mechanisms as to how the cell can deal with that stress. One is through the carotenoid biosynthesis pathway. There are several steps involved in carotenoid biosynthesis and to generate these electrons and to use these electrons can be delivered to the electron transport chain. In this case the electrons are sent to the quinone pool, which is a very important intermediate in the electron transport system. The electrons in this carotenoid biosynthesis function to reduce the proto-quinone pool and those proto-quinone in the pool can be somehow re-oxidized using an enzyme called PTOX or P-Tox-process terminal oxydase. That enzyme can reoxidize the proto-quinone pool and uses excess electrons to reduce molecular oxygen and generate water. through this mechanism we can reduce the concentration of oxygen close to the reaction center and is easily converted to reactive oxygen species. That's the mechanism to help cells cope with that kind of stress environment.

Another mechanism is through fatty acid biosynthesis. As I said, when ferrodoxin grabs the electrons and cannot pass them to NADPH, it can send the electron instead to thioredoxin, a water soluble electron carrier which is a signal molecule which can deliver the electron to activate the first enzymes involved in fatty acid biosynthesis. This is ACCS and when this enzyme is activated it triggers the entire fatty acid pathway. that is how these electrons can be utilize to use the very expensive reducing power to get rid of excess electrons. This pathway can be used to absorb



and drained a lot of electrons by utilizing a lot of reducing power. Through this process, it can also utilize oxygen and produce water. Thus through carotenoid biosynthesis and triacylglycerol the cell is able to get rid of those excess amounts of electrons produced under stress conditions.

By going this route, the cells used energy, but they also store more energy because they are storing more electrons. If you compare the energy density of triacylglycerol vs. starch you see that triacylglycerol stores more than twice as much energy compared to starch. However, through this route the cell will lose biomass because the cell takes a three-carbon compound, pyruvate, and produces one CO2 along the pathway, which means it loses one carbon. In other words, one third of the carbon fixed is lost through this process. That's why in the normal conditions algae are thin

fast growers, but once they start to accumulate starch, they get fat. in this case if you further stress the cell and the cells generate lipid it looks fat, but compared to the starch-rich it is actually smaller. That's because one third of the carbon is lost before it was in the form of starch and it is transformed into the form of carbon dioxide.

Sherwin Gormly: What you are saying is that you are going down in cell mass even though you are going up in lipid content.

Qiang Hu: Yes. You go up in lipid content and energy content, but you have lost some weight and some mass. This is triggered by high light or nutrient starvation. The question as to why the algae make oil is answered in part by the description above, as a way to get rid of excess electrons and detoxify oxygen singlets through carotenoid production, but another reason is that the stress stops reproduction. Since the light continues and therefore photosynthesis continues and carbon fixation continues, the cells are needing to story this somewhere. They are single

cells and they cannot export of partition this excess carbon in a different part of the cell body. The cell with its limited space has to deal with these storage compounds and the lipid which is extremely hydrophobic is very energy dense and rather than starch, which is more hydrophilic with the sugars and hydroxyl groups which have affinity to water. From a volume and energy perspective, storing triacylglycerol is the final strategy. However it is not preferred and the cells only make triacylglycerol when they have to do it.

Otherwise it is easier and faster to make starch.

That's why when you look at seaweed or even when you consider filamentous green algae they don't make lipid. Why? Because it's easier for those to make starch granules and if necessary they can export them to another cell or do cell division they can store those compounds. Still sound unicellular algae, like red algae, like Porphyra, they don't produce lipid even under stress conditions, because they developed another mechanism to secrete polysaccharides (sugars) into their growth medium and get rid of excess fixed carbon but still most algae don't have that mechanism so they have to somehow reorganize and re-manage their storage compounds.







Pyruvate

Acetyl-CoA

atty Acid (C18)

Triacylglycerol (37 kJ/g)

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Glyceraldedyde-3-P

Glucose-6-P

Glucos

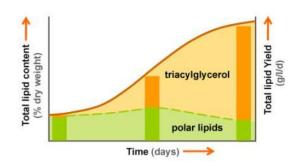
Starch (17 kJ/g)

'Weight Loss' by Converting Sugar/Starch to Lipid

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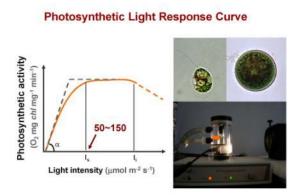
Oil content an oil quality: I have often heard from people that they have algae that can produce 50% oil, but 20% is not so bad. If the algae produce 20% it is not so bad because that means I may get some other value added products like protein or carbohydrate. As long as your primary goal of your process is to produce energy so it's a big concern because oil content is one thing and the quality of the oil is another. Under normal conditions algae, as I said, over time the lipid content can build up from a small percentage all the way to 50 or 60%. If you consider the lipid

Oil Content versus Oil Quality



composition it can be quite different. under normal condition algae produce 99% membranebound polar lipids and they're very difficult to handle, although polar lipids can have a high value, but since the sugar part is very sticky and gives the oil refining process more difficult. Therefore 99% of lipid in the form of polar or membrane lipids, which is in contrast to vegetable oils, like soy oil or sunflower seed oil. Those oils are 99% triacylglycerol, with only 1% and maximally 5% of those lipids are polar lipids, which means through what's called a degumming process these can be easily separated and still the majority of the compounds is triacylglycerol. When it comes to most algae 80- 99% of the lipids are membrane-bound lipids, which are regarded as gum or waste. However with algae, as the stress process proceeds both the content and the composition of the lipids changes. Over time, 60-80% of the total lipid may be in the form of triacylglycerol. This indicates that the quality of oil at this stage is higher, because it is easy to remove the 20% polar lipid.

Finally, I want to show a few slides about the lightresponse curve. When people design photobioreactors for algal cultures, they always worry about too much light. If you grow algae outdoors there is too much sun, which leads to photo-inhibition. If we look at the classical photosynthetic activity curve and measure the amount of oxygen evolved per unit chlorophyll, which indicates the number of cell, per minute as a function of light intensity. what you can see is that when the light is at a very low-level, increasing light intensity, increases oxygen evolution. The higher the light, the higher the

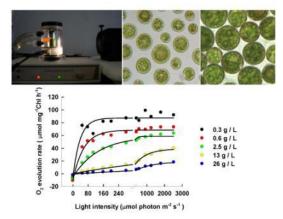


photosynthetic activity that takes place until you reach a certain level at which further increases in light no longer stimulates oxygen evolution—the system is saturated. If you further increase the light intensity, you see a reduction in photosynthetic activity, which is called photo-inhibition. What kind of light intensity can saturation the photosynthetic machinery? It's 50-150/200 umol. What the maximum level outdoors? Between 2000 and 2500 umol. So in order to saturate the photosynthetic machinery it only takes 1/10th or 1/20th of maximum solar energy, which is why people concluded that too much light is not good. What this light response curve really means is

what's going on with the single cell. How much light is required per cell to achieve maximum

performance. In this device you are measuring a response in a very dilute culture because you do not want to see the effects of shading.

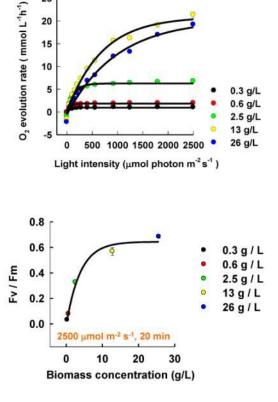
When it comes to algal cultures, we are not talking about a single cell we are talking about a population--many cells together. If you look in the measuring chamber you see how dense the cells are. As you increase the cell concentration from 0.3 g/L to 26.0 g/L, we see that already at a very low cell density the population quickly reaches saturation at 50 to 100 umol. If you increase your cell concentration to 2.5 g/L, which is still a quite



diluted culture, and the light saturated doesn't occur until you get to over 1000 umol. This is because of mutual shading and if you look at the absolute oxygen evolving from this system, the higher the cell concentration there is less oxygen produced--not because of photo inhibition, but because mutual shading causes light limitation. so the higher the cell concentrated for each individual cells they received much less light so that's why they perform less and generate less oxygen. The point I want to make here is that high cell concentration you don't see light saturation even at 2000 umol. This is with a mixing rate as fast as possible, with a light path length here of one-centimeter diameter.

25

For mass culture you're talking about population, not single cells, but per culture, per unit area or per culture volume. Using oxygen evolution per liter of culture per time. That goes totally opposite. Here you see at low cell concentration vou produce overall much less oxygen if you maintain the culture of 13 g/L for instance. At that concentration you reach a maximum oxygen production, which means maximum biomass productivity. If you further increase the cell concentration it starts changing. At 26 g/L your overall oxygen production is reduced because in that situation cells are extremely light limited. Although you provide 2500 umol, maximum solar energy, the cell is still in fact light limited situation. When you talk about a mass culture in the design of a photobioreactors the light is not an issue; the more light, the better as long as you know how to manage it. If you see a photo inhibition like in this case under normal conditions, when the algae are healthy and the photosynthetic machinery is fully functional, this



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parameter will give a value of 0.6 to 0.8, indicating photosynthetic activity is strong. When this value is reduced it just indicates photo-inhibition. In these experiments you can see that if you exposure algae to 2500 umol light for 20 minutes with cell concentration of 0.1 g/L there is severe photo-inhibition. If you increases cell concentration, which means increases mutual shading, the photo-inhibition is no longer an issue. The algae are perfectly healthy. Per cell they perform less because there's less light available but certainly there's no photo-inhibition. When you decide a photobioreactor for algal mass cultures you have to try to manipulate the population density to manipulate light availability per cell. The plan is to design a photobioreactor to maintain an ideal light path and to maintain vigorous mixing and the proper cell concentration so the culture enjoys maximum solar energy rather than shading your culture. When you cut off your light supply, then you certainly will reduce your yield because overall the light availability is decreased.

Thanks for your attention.

QUESTIONS:

Sherwin Gormly: One of the interesting things we are looking at for engineering is whether we should try to optimize for oil or biomass production.

Qiang Hu: If your final goal is to obtain oil-rich biomass with high quality oil, certainly you would like to harvest your algae at the end of your culture period. However, if your goal is to produce just biomass that may contain high protein for instance, then you can harvest your algae at the end of the exponential growth phase that's when it gives you maximum overall yield. When you first convert proteins into starch and carbohydrate into lipid that's why when people estimate or predict the oil yield that can be achieved from algal mass culture they think as high as the biomass which contains high carbohydrate, but it's not true because there is a loss of a lot of biomass through the conversion.

Jonathan Trent: When the cells are making lots of triacylglycerol and they are storing it, how quickly can they reutilize it if they find themselves in a condition that was not longer stressful? Do we know enough about the genetic pathways involved to genetically turn on the triacylglycerol pathway?

Qiang Hu: If you convert your culture condition to favorable condition then the algae start to use the stored lipid for growth and metabolism. However, the purpose of algae storing lipid is not to prepare for the situation in which the conditions become better. They make oil to survive during the stress. cannot survive

Jonathan Trent: How quickly does the lipid go away, when the cells are out of stress conditions?

Qiang Hu: Pretty quickly. After a couple of cell divisions, the lipid gets diluted away. Division is faster than the rate at which the cells will utilize the storage of lipids. After a few generations the cells dilute triacylglycerol.

With regard to the question of genetic pathways. There are many pathways and it appears that we understand them, but in fact we do not. The question is if we should spend more time and money on understanding those pathways and how they are regulated. It is very difficult to do genetic engineering because we do not know what we are doing. You can simply overexpress a couple of genes in the pathway or turn off other pathways in a hope to re-direct your carbon flow to a desirable pathway, but in many cases if not all this approach backfires and you get a negative result. We try to enhance oil production, but in the end you get less and those algae or mutants prove to be extremely sensitive to their environments because of the genetic modification at this stage. We don't understand what is going on in the cell with so many pathways that are interactive and interdependent. You screw up one and you certainly will effect other pathways...

Travis Liggett: I'm the only other Federal Employee here from the US government and whatever Jonathan said about not being associated with NASA applies to me as well. I'm here as a citizen of the earth, not as a citizen of the US... I'd like to suggest in one word, we have microalgae, we have macroalgae, what we're talking about here is "mega-algae" really big cells. I'm the designer of all the OMEGA bags that exist to-date... I want to present this as a vocabulary word...

Qiang Hu, I'd also like to ask you about your assumption in your plots, is the light in umoles/m2/sec, is that a binary—sunlight or not or have you explored the impact of different wavelengths.

Qiang Hu: Different wavelengths are less critical, because as long as the photon is in the range of 400 to 700 nm the algae can utilize all the photons. While shorter wavelengths, like 400 have more energy, but this doesn't matter because the excess energy will dissipate as heat. Even if you use red light which has a minimum energy, it is still able to drive photosynthesis—namely to split water and to produce electrons. The excess energy in that photon is dissipated as heat.

Anthony Heijenga: It sounds to me that to make this a commercial success one would need to synchronize the cultures on a massive scale because if you don't you will lose productivity because only a small fraction of the cells may be in the right condition for harvesting. Is there a trigger to use to cause stress by a simple method or would you do something and do the harvesting at night or in the morning when the sun is just coming up?

Qiang Hu: As long as you grow algae and go through a daily light-dark cycle, the cell division will take place at night and you may see the majority of cells doing that at night. With an algal mass culture at any given circumstance there is a mixture of cells at different stages of life cycle. In the exponential growth phase, you will see that the majority of cells are smaller and contain more proteins—enzyme machinery to do metabolism and cell division, but still even at ht early stage you will see lots of fat cells. In the late, stationary, phase you will see the majority of cells at the other extreme storing lipids and here too you will see young cells actively swimming. It's a mixed population, but there are different percentages of the different stages present. When it comes to the question of harvesting. It's hard to say when it's best to harvest. On the other hand the respiration at night will consume up to 30% of the carbon fixed during the day, so from this standpoint it is best to harvest the algae in the evening. However, since the lipid conversion mainly takes place during the night, the starch, which is a transient storage product, is transformed at night, so the best time to harvest is in the morning. Therefore the quality of your biomass may be higher if you harvest in the morning, but the reality is that if you have a

commercial production of algae mass culture then your harvesting is all day long all year around—continuously. You have no break time, anytime is harvesting.

George Oyler: In the photo inhibition curves and photobioreactor design, an additional parameter that appears to be important is the light-dark cycling of the algae and that seems to have an affect in shifting the photo inhibition curve. This is particularly important because if you have 5-10 second cycling it actually decreases the biomass, but if you have a light dark cycle of 100s or 10s of milliseconds it improves the biomass yield even at higher light and I wonder if you would like to comment on that?

Qiang Hu: It is true that when you look at the photosynthetic mechanism when photosystem I or system II at the moment a photosystem captures that photon that reaction Center is closed. If at that moment you deliver another photon it is not available for the machinery to utilize that photon. In this case if you have one single cell in the system at that given moment when the algae is saturated by the photon delivered to cell if you give it more light, it is a waste. That is why if you provide intermittent light supplied in works better. When scientists did these experiments back in the 1950s they used a rotating disc with openings to produce intermittent light and they found out that the efficiency is as high as continuous light. This demonstrated that the algae were not using all of the light from continuous illumination. This proved that the algae do not need continuous light but it is only true when a culture is at the very low sell density. This means there is no shading, but our culture is a population and the cells are moving constantly. Therefore at any given time even if your light is continuous illumination the individual cells always receive light intermittently. You do not need to provide flashing light.

Keep in mind that the light reaction of photosynthesis takes place in small fractions of time--in the nanosecond range. There is no mechanical way to provide the light cycle at that rate. Thus, first, it is not necessary and second, it is impossible. If you provide light continuously for each cell they will receive light intermittently anyway because of shading and mixing and because of the light path of your photobioreactor.

Raman Saravanane: I have a small clarification to ask. You have shown the effects of light intensity but my question is what are the effects of the presence of reagents like heavy metals and other toxic elements in the non-homogeneous mixture which is the medium for growing algae in the actual field? What I mean is if you use sewage for the growth medium, the inhibition may be due to other contents?

Qiang Hu: You mean inhibitors the besides light? Yes you are right. photo inhibition is a relative term. The photo inhibition caused by an excess of light depends on the conditions. If your culture conditions are perfect and optimized and the threshold before you reach excess light can be very high. Under other conditions that are not favorable for growth than the threshold is decreased. For example, in winter, low temperature causes photo inhibition at much lower light levels. The same thing occurs if you starve the cells for nitrogen. nitrogen starvation will induce photo inhibition. This means that even at low light you will see the light inhibition because there is a lack of nutrients and the cells cannot handle the excess reducing power and those reducing power become too much because light becomes too much.

Kirsten Olrik: your curves are based on batch cultures and you mentioned continuous cultures, but I don't understand the relationship between the data you showed and the kind of cultures you

are describing. there are many ways that you can treat your cultures to give them light and nutrients and would you please clarify what you mean in your experiments?

Qiang Hu: The lipid and oil production and algae growth are mutually exclusive. I mean the algae either produce high and lipid content or they grow fast. you cannot have algae that are growing fast and at the same time making lots of oil. That means in order to harvest algae with a high oil content you'd better use batch cultures. That way you harvest all when the algae population reaches 30 or 40% lipid. there are different ways to manage the production. For example, you can have a first stage to generate biomass as much as you can and not worry about the oil content, but the algae grow fast and that can be a continuous process. You can add more nutrients and dilute the culture to manipulate the environment so the algae continuously produce biomass. At some point, you can change the culture condition or transfer a culture from one system to another system to starve them for nitrogen or give them highlight to create a stress and the cells stop cell division and start lipid production.

Some people said that it is not true if I starve the cells and give them high light, my growth curves still increase. That's because although the cell stops division the cell number stays the same but when you measure dry weight the cells increase because they are increasing in volume. This is because the CO2 continues to be fixed and day produce sugars or oil so the cell mass goes up. It is not because they continue to sell division. Please understand, this stress biology is a tool, but the consequence of stress is death or deterioration of the organism, not only algae, but any living organism. The point of the protective mechanisms is that they prevent the cells from dying quickly. the pigments and lipid production are ploys that allow the cells to survive longer. When you count the cells the cell number will decrease with time under stress conditions. It is not that these protective mechanisms allow the cells to behave normally. It is just like when we get stressed we produce white hairs. Before I came here I dyed my hair because of stress.

Robert Baertsch: Why oil and not starch?

Qiang Hu: Because starch occupies too much space. Starch is sugar and there are so many hydroxyl groups, which can have high affinity with water. The energy content of oil is much higher it has a higher energy density. By converting starch to oil the cells reduce their mass...

Bio-Fuel and CO2 Capture by Micro-Algae

Ami Ben-Amotz

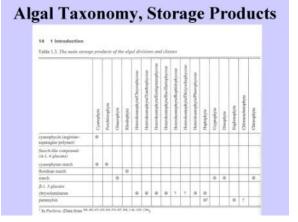
Wind, Sea and Algae April 20-22, 2009 Maribo, Denmark



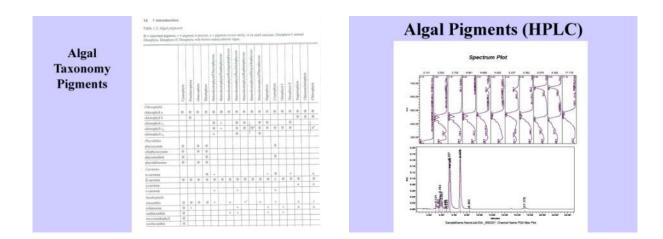
Thank you. I arrived from Japan yesterday. The Japanese way to start a lecture is with an apology like the American way is to start with a joke. My apology is that I have many issues and I will try to cover many aspects of this complex subject, which means I will have to be fairly superficial and if you want to go into more depth, it will have to be in our discussions later. I will not go into the detail of the biochemistry that Dr. Hu presented. I thank Dr. Hu, very much for giving such a good introduction. Now I will go directly into what I want to present to you. Microalgae classification:

There are many known species of micro algae as was mentioned. The different species are divided into 11 classes based on either taxonomy or their biochemistry. The best-known species are the chlorophytes or green algae. Most of the other algae are not as well known even though they are found in the oceans and seas, brackish and freshwater, and they are distributed globally.

The biochemical bases for algae classification are based on storage products and pigments. We are familiar with plant storage products such as starch, but many algae, like the diatoms, contain chrysolaminaran, which is beta-1,3-glucan, not alpha-1,4-[glucan] [higher plant starch]. There are other storage products, such as the *Euglena* type [paramylon] and the cyanophyte [cyanophycin]. The presence of these diverse storage products is one Hicroalgae



definitive way to classify algae, but another way is by using diagnostic pigments.



We use various pigments to classify algae. As you know, the chlorophtye or green algae have chlorophylls A and B, like any other plant, but most of the other algae have only chlorophyll A, no chlorophyll B, and possibly chlorophylls C and D, as well as many other pigments and carotinoids. We use HPLC to separate pigment extracts and spectrophotometry to analyze the pigment spectra, which gives us an accurate way to identify the different classes of algae present in a sample. Most people do

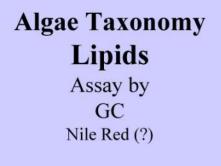
not use this laborious procedure, but use the more classical methods [microscopy], although it is not as accurate.

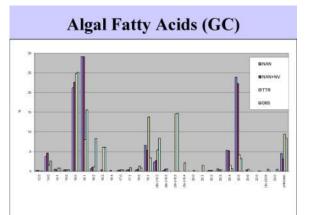
Another biochemical approach to identify algae is to use their lipids. This is done using a number of different methods. Many people now use the Nile Red method, but it should be noted that this method is not very specific and therefore not very accurate.

The actual way to know the lipids in the algae is to do gas chromatography (GC) and to analyze the different fatty acids or the fat.

Most algae, like higher plants, have lipids with carbon chains of between 14- and 18-carbon chain lengths. Only a few have long fatty acid carbon chains like EPA, DHA or what you call the Omega-3-fatty acids. There are only one or two classes, like the diatoms and the Eustigmatophytes that have Omega-3-fatty acids, which is why this is another good way of identifying the algae.

There are algae classification methods using proteins and amino acids, although there is not so much information about this yet. We know that some algae are like higher plants and have proteins composed of amino acids like soybean proteins, but many other algae have other amino acids, which may be essential amino acids. Unfortunately, we don't have very





Algal Taxonomy Protein, Amino Acids & *Molecular Biology*

much information about this yet, but I hope now with the expanded interest in algae, we will soon get this important information.

Finally, I should mention algae classification by molecular biology, which has been developing over the last ten years. This is a nice method, but from what I have observed there have been no real breakthroughs in this area yet.

Most large-scale facilities for cultivating microalgae are in the Far East, primarily Taiwan, Japan, and now China. All of these facilities use mixotrophic cultivation methods, which means they are using both light and organic matter to grow algae. This allows the pond for the culture to be deep, typically about one meter deep. The ponds are circular or raceways and they have developed very effective mixing systems. These mixotrophic cultivation facilities may be very large and effective for growing algae. Unfortunately, the experts in these facilities do not come to our scientific meetings. In the last ten years, you rarely see Japanese or Taiwanese algae experts coming from these companies to our meetings. I believe the reason is that they consider the details of their methods confidential information and they want to keep it confidential. We are trying to convince them to share their information to the people like us, interested in using algae for bioenergy for the good of the world, rather than just business.



Far East Chlorella and Spirulina

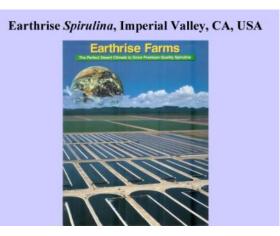


From the companies that do share their information, we know there are many different successful designs for algae pond facilities, which mainly grow *Chlorella* and *Spirulina*.

One company called Earthrise from Imperial Valley, California, is frequently represented at the scientific meetings by Amha Belay [Sr. VP and CTO Earthrise Nutritionals]. Amha comes to almost all the meetings and although he doesn't share much information, he does show many nice photos. Earthrise is a Japanese-owned company and as I mentioned, the Japanese consider algae cultivation information confidential.

This is better than the company Cyanotech, which is a very nice company in Kailua-Kona in Hawaii. We know they use cool underground water and seawater to grow *Spirulina* and *Haematococcus*, but the people in this company do not come to our meetings or at least it's been about ten years since I've seen the man in charge of Cyanotech at a conference.

A company phased-out a few years ago, called Microbio Resources in Imperial Valley CA, was run by the late Prof. Oswald and his students from Berkeley, was one of the best facilities for growing algae in CA. It was taken over by Carbon Capture Corp., and now it has transferred to another company from the University of San Diego, but I don't know who is currently responsible for these facilities. I hope to see them in the next conferences.





Microbio Resources, Imperial Valley, Dunaliella, Calipatria, CA, USA





Cognis, Whayalla, Australia Large Scale *Dunaliella* Cultivation



There are a few unique open ponds systems in Australia, where they do not build algae ponds, but rather they do what can be called "natural production." One of these systems is called Cognis in Whayalla, Australia not far from Adelaide [South Australia], where they spray algae into natural ponds from airplanes and grow *Dunaliella* on a large scale. They have developed an interesting system for harvesting a low biomass, but it is a carefully guarded



secret. We suspect they are using electro-magnetic separation, but I will not go into detail here.

Another Australian system is in Hutt Lagoon not far from Perth. This cultivation facility used to be run by Dr. Borowitzka. She has now retired to become a nun or a priest, probably because she was working with algae too long. She said to me: "Ami, you'll be next."

A very well known algae production center in Israel is called Algatech, which uses large-scale commercial photobioreactors (PBRs) to cultivate *Haematococcus* for astaxanthin—a high-value product. The facility is



interesting because they use long glass tubes for cultivation and end up with a powder of *Haematococcus*.

All of the facilities I mentioned today are producing "health food" or natural products, which are sold mainly in the East. As you may know the Japanese and people in the East believe in the healthy benefits of algae—so there is a stable market there. In the west, algae are mainly used for aquaculture. The company Naread, for example, produces *Nannochloropsis* on a commercial scale that provides Omega-3 fatty acids (EPA and DHA) to fish or fish larvae in aquaculture systems.



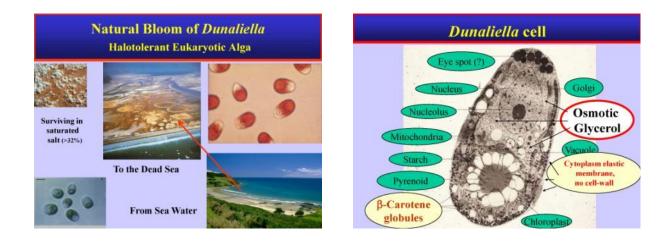
In summary, the point I'm making is that all current algae cultivation companies are producing high value and high priced products, which does not include commodity-priced products like biofuels. The question about whether microalgae can be used for biofuels arose some years ago and was the basis for my entering the field.

Microalgae and biofuels:

I first started considering how we could use algae for energy in 1975, during the first energy crisis.

The case study was the alga *Dunaliella*, but at that time, it was not for oil, TAG, or TAG glycerol, but for glycerol. I don't know why in 1975 nobody considered oil from algae, but the focus at that time was on carbohydrate.

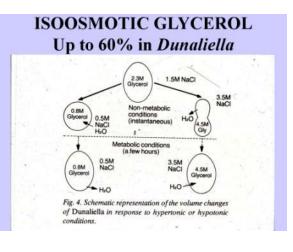


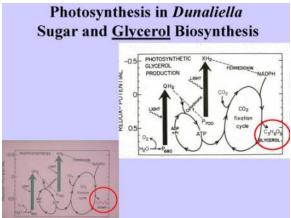


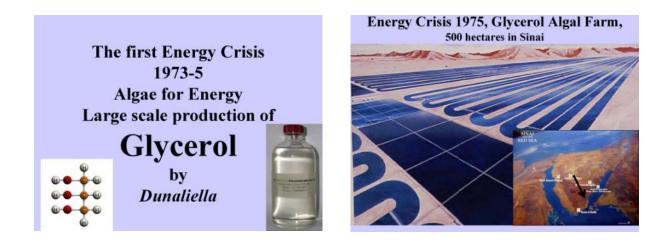
Dunaliella grows at high salt concentrations from seawater or in salt marshes like those found in Australia, by accumulating a lot of glycerol in the cell.

The glycerol content of the cell is proportional to the salt in the environment. In the Dead Sea or the Hutt Lagoon, which are 32% salt, the algae accumulate over 60% glycerin in the cell—60%. This is an osmotic adaptation for the algae in which increasing salt concentrations correspond to increasing glycerol—at 3.5M NaCl the algae are practically filled with glycerol [4.5M].

Dunaliella can use photosynthesis to make sugar or to make glycerol and the conversion of glycerol to useable carbohydrate is easier than the conversion that Dr. Hu mentioned from lipid to carbohydrate. The conversion is either in one way or another, but the algae reduce their starch and accumulate glycerol and we end up with a box full of glycerol.







In 1975, *Dunaliella* was considered a very good source of biofuels and it was decided to grow *Dunaliella* south of Sinai on 500 hectares in a wadi—an open area close to the sea. With these plans made, but before we got started, the price of oil started to go down and the project lost feasibility.

We had calculated based on construction and production costs that we needed at least \$100/barrel to be feasible for that project and when the price of oil went down below \$40/barrel, we had to stop. The fact is that the real problem was we didn't really know how to grow algae in the desert, under the sun, in seawater, on such a large scale and even though the imagination was very nice, I was lucky not to be involved in that project because if it had happened, I'm sure I would not be here now.

So when the oil price went down the project in the desert was impossible, we had time to go back to the university and study. We were lucky that we had more than ten years to study in the lab--how to grow algae and how to grow *Dunaliella* and many critical issues were considered and explored.



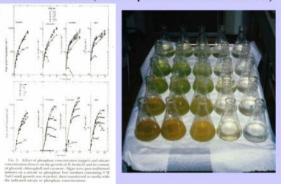
Algae & Oil Price 1975 oil price dropped down Dunaliella Project was stopped

Until then Search for High Value Algal Products Study!

At that time, most of the biology research was done at the Weizmann Institute, which is a university. We formed a *Dunaliella* club of about 40 students, 5 or 10 professors, and we all focused only on *Dunaliella*. You can imagine how much work was done over ten years on one organism to identify the conditions for growth and scale up. We didn't do single-phase experiments, but we did at least 2x2 or 3x3 phase experiments, which means we studied light and nitrate or temperature and another factor.

It took a long time to identify the conditions needed to grow the algae and the conditions necessary for scaling up outdoors. For example, we considered nitrate and phosphate under different condition and light under different conditions and salt and light under different conditions. What salt concentration? What light intensity? How to control the lipid? How to control the carbohydrate?

It takes a long time and when you come to the end and you think you know everything then you come to the GMO or mutant, which is a different story. In fact, when it comes to GMOs, even though we spent 15 years on GMOs, as Dr. Hu mentioned before, algae that are GMOs are not so strong when it comes to growing them out doors—even if we take a strain that grows very good in lab culture and we put it outdoors, it's relatively sensitive. So we have to wait The Weizmann Institute Long Term Dunaliella Research (nutrient optimization studies of 2x2 & 3x3)





The Weizmann Institute Long Term Dunaliella Research (outdoor biotechnology)



with the GMOs even though we did a lot of work with GMOs at that time.

Our first step growing *Dunaliella* out-doors was to set up cultures on the roof at the Institute. Over a period of seven years we made micro-ponds on the roof with the late Prof. Avon and we made many models of ponds with various mixing flows, depths, and many other aspects.

Eventually, the paddle-wheel shallow pond emerged as the best model and the information we got from this small micro-pond was used for scaling up. In this model system, which we studied for a long time, we used a small paddle wheel, and we monitored with a pH electrode and a screen, measuring a few parameters, and it was a good model system to go for the next step.

In the next step we determined that the key issues we need for growing marine algae are the seawater, light, carbonation and pH, culture mixing/waves (I instead call it waves of mixing), technology/engineering, which is essential, predators control (we're still behind here), and finally applied phycology experience, which is essential-you have to study the algae for a long time to get a feel for the algae and to understand what effects them. To do scale up biotechnology or what is called "intensive cultivation" I had many engineers follow along with me in these studies and there were many failures and successes. A few

The Weizmann Institute Long Term Dunaliella Research (outdoor biotechnology)

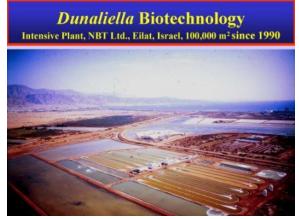


Scale-Up Key Issues Seawater Light Carbonation & pH Culture Mixing (waves) Technology (engineering) Predators Control Applied Phycology Experience



examples of engineering failures are related to the questions: what should be the shape of the pond and how should it be mixed. With regard to the shape, should it be a U-shape, an ellipsoid, round, and so on? What kind of mixing should we use—airlift, bubbling, pumping, etc.?

The airlift was very effective way to move the water and produced good mixing, but the algae did not grow! The algae died. *Dunaliella* is very sensitive to tension force so the algae break down. So the airlift is very nice, but it doesn't work for certain species of algae. What about bubbling? Air bubbling is nice, but it is not a good way to mix the algae to get the algae moving. Figure x shows an example of an airlift system that was constructed underground--\$1M



under the ground, eventually, I don't want to make the long-story short, but let's make it shorter, here we are today, I show you the facility [slide/Fig], which is 10 hectares surrounded by salt ponds with 25% of the area dedicated to recycling the salt and there are many reasons for recycling the salt because it is one of the problems of cultivation in this area.

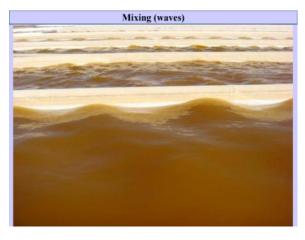
The scale-up model for cultivation involves ponds that go from 3m² to 3,500m² or 3x 500 m², which is an operation pond. All the ponds are raceways with one paddle or two paddle depending on the engineer, what kind of raceway and what size and what kind of paddle wheel, and how many and what size? There are many engineers here and I've given lectures before just about paddlewheels—how many paddle wheels we have here, I think I've counted 25 different paddles, as each engineer comes with a different idea and then we put a different paddlewheel-6-blades, 9-blades, 8-blades; diameter 1 meter, short, rotating at 12 rpm, 20 rpm, locations many possibilities... I still don't know what is the best paddle wheel per pond. We know how many paddlewheels per pond but still don't know what is the best design for the paddle.

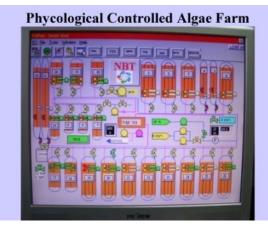


A pond with *Dunalliela* looks very good when the pond is orange, which indicates that it is making betacarotene at high concentrations.

I want to emphasize the importance of mixing. I think wave mixing is one of the key issues. There are also issues of the light, the flow, and the issue about how much light per unit time, but the wave system is the best system for mixing. We know this from ten years of studies. We get much higher productivity using the wave system compared to flat velocity (we call it laminar velocity). Most of the operational ponds use laminar velocities of 20 cm/sec., but this is not as important as the wave mixing and the distance between the waves [wavelength]. We will need to study this as it applies to systems that are offshore.

The whole system is fully controlled; the whole biology is controlled and fully controlled. So the number of employees we have today compared to at the beginning is much lower. Today we only have 16 people working on three shifts a day, which means only 4 people per shift and it's all because all the biology is here. I will not go into the details about the biology that's here, but the ponds are computer controlled, the pump, the paddlewheel, and the pH, is on this control. The harvesting, the transfer, everything, which means we just push a button and the algae goes from here to there and water goes from here to there and that's essential for large scale cultivation.





<section-header>

We started with many ideas about how to separate the algae from the water: sedimentation, centrifugation, or flocculation. You can see a variety of methods here for dewatering.

Without going into details, after a long time, we finally concluded the best system was a Westfhalia continuous clarifier centrifuge (SA1) which work at about 15m³/hr and we have four centrifuges or 60m³/hr that will give us about 1.5 ponds/day to harvest. We are limited by the centrifugation. These are very expensive centrifuges—stainless steel; the same type used for milk and the same process for CIP (cleaning in place). There is a lot of technology here.

The spray dryer we use is from Anhydro, DK, and it has been working for almost 30 years. During this whole time, we have had nothing to change. It works with a specific in-temperature and outtemperature, so we can get the right feed. Basically, this process uses the conditions similar to milk power. In other words, if you know how to make milk powder, you know how to make algae powder. After spray drying, the powder we make of *Dunaliella* is high in beta-carotene and has about 5% moisture.

The powder is spect'd and shipped and made into capsules in Japan. In Israel, we make the paste, the powder, we bag the powder, we ship the raw powder, and the Japanese make the final product as a capsule of *Dunaliella* beta-carotene.









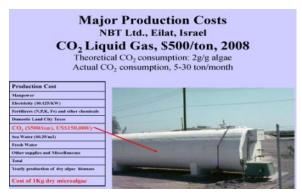
Our *Dunaliella* product has been sold in Japan since 1990 and the total market in Japan is over \$1B. It is sold mainly by half a million sales agents in Japan and many more in the Far East and it sells very well. It is a very expensive product and there is a very good market for it. Most of the money goes to the Japanese and not the Israelis.

I am the chief scientist for both a Japanese company [Nikken Sohonsha Co and the Israeli company [Nature Beta Technologies (NBT)]]. I requested the Japanese to share the know-how with people now interested in bioenergy because we think (unlike the *Chlorella* people) it is important that we give the know-how for growing algae to people wanting to make algae biofuels. The information I share with you now is approved by the Japanese to help make biofuels or bioenergy from algae.

The major production costs in NBT in Eilat, Israel, just to mention a few, are:

CO2, which is about \$500 per ton and we are the second biggest customer after Coca Cola, in Israel. Coca Cola uses two containers a day [see Fig], and we use one container like this ever two weeks. It is a lot





of CO2 and if you look at levels of CO2 during the summer most of it goes back into the atmosphere

because of low solubility—we know how to dissolve CO2, but at 40°C in the summer it is almost impossible and you can see it bubbling out. So CO2 is a major expense.

Major Production Costs NBT Ltd. Eilat, Israel Seawater, \$0.25/m³

CO₂ Input & Loss Summer (>30°C) CO₂ consumption: >10g/g Actual summer consumption: 30 ton/month

Additional Costs: Filtration & Chlorination



not far from the sea, because we have to buy the seawater from the Israeli salt industry or the water authority and we pay about twenty-five cents per cubic meter. So seawater is expensive when you do it onshore.

Seawater is also very expensive, even though we are

Centrifuge and power is very expensive as well and these are part of the major cost. The subterfuge by itself and the running cost is very expensive.





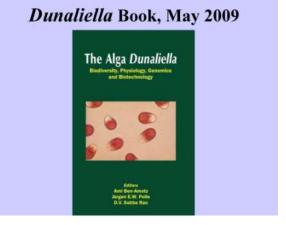
Fertilizer of course is expensive, we use mainly nitrate as people know, nitrate is expensive it is a fertilized and when you buy it in tons it is an expensive product.

	NBT Ltd. Eilat	
		Potassium Nitrate
Production Cost		POD ORADE
Manpower		1
Electricity (\$0.125/KW)	(~31.0/Kg)	5
Fertilizers (N, P, K, Fe) and other- chemicals USS 36,000/y		- Contraction
Domestic Land City Taxes		
roduction Cost monor ketricity (S0.125/KW) refilters (X, P, K, Pe) and other- emicals USS 36,0005/ medic Lad Cly Taxe 2 ₃ (5501bus) as Water her supplies and Miscellaneous fail atly production of 4r ₂ aguer kinamas	ALAN Hada Corrections 2	
Fresh Water		
Other supplies and Miscellancous	1	
Total	1	
Yearly production of dry algae biomass	1	
Yearly production of dry algae biomass Cost of 1Kg dry microalgae	-	E

So the total cost of production by NBT that I was allowed to disclose for the raw material is \$17 per kilogram and you can have the presentation so you can see the numbers in more detail. This is the cost of production for the Japanese and they are selling it for about 200 times more so they make a lot of money.

	Dunaliella NBT Ltd., Eilat, 2008
	Cost in USS/year
Manpower	500,000 (20 workers)
Electricity (\$0.125/KW)	180,000
Fertilizers (N.P.K, Fe) and other chemicals	36,000
Domestic Land City Taxes	50,000
CO ₂ (5509/ton)	150.000
Sea Water (50.25 m3)	200,000
Fresh Water	20.000
Other supplies and Miscellaneous	30,000
Total	1.166,000
Yearly production of dry algae biomass	70 tens (2g/m2/day)
Cost of 1Kg dry microalgae	\$17.00/kg
Market Price	S4,000/kg algal AFDA (β-Carstene Bealth Fool) Total sale ~\$100 million/year

The information about *Dunaliella* will be summarized in a book that is about 500 pages long and published in May 2009. The book is containing everything and is written by the very best contributors from all over the world. This will give you much more information, if you're interested in *Dunaliella*.



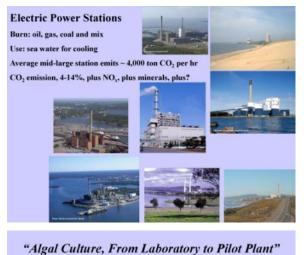
So the questions we raised a long time ago about how to reduce the cost of production we began to address by collaborating with the Israeli electric company. We considered the possibility of using seawater from the cooling system from the power plant and CO2 gas from the flue gas, which is 4-14% CO2 plus NOX plus minerals. Our power plant looks like the one I saw

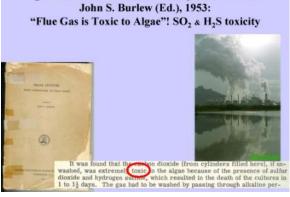
How to reduce the Cost of Algae Production?

here in Copenhagen and we on looking into using a waste from the power plant.

The Israel Electric power plant:

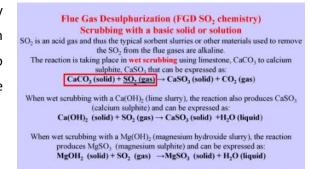
Historically, if you look at reports about using flue gas for growing algae, when we started, it was written that the flue gas is toxic. It's true that until 1995 most of the power plants discarded flue gas into the atmosphere containing a lot of SO₂,.





Today however, many power plants moved to what is called FGD [Flue Gas Desulphurization], which scrubs the SO₂ using a sophisticated engineering system.

Actually the chemistry is quite simple because they are just converting calcium carbonate to calcium sulfate—that is, they are transforming lime into gypsum. While the chemistry is quite simple, the engineering is quite difficult.



Some FGD systems go a step further and oxidize CaSO₃ (calcium sulphite) to produce marketable CaSO₄.2H₂O (gypsum): CaSO₃ (solid) + 1/2 O₂ (gas) + 2H₂O (liquid) - <u>CaSO₄.2H₂O (solid)</u>





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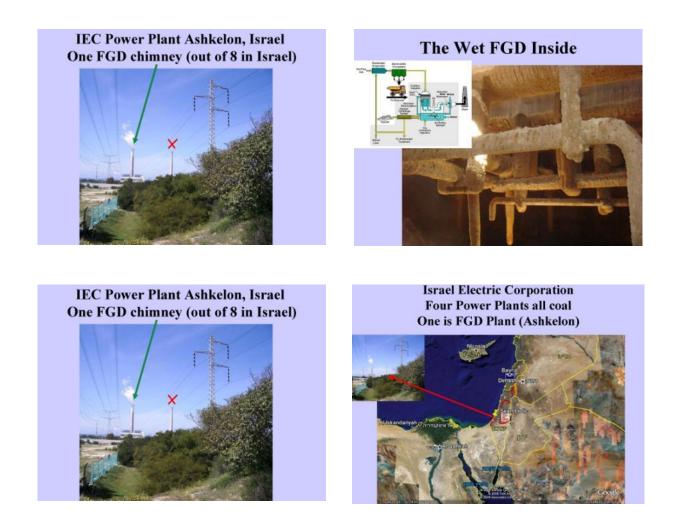
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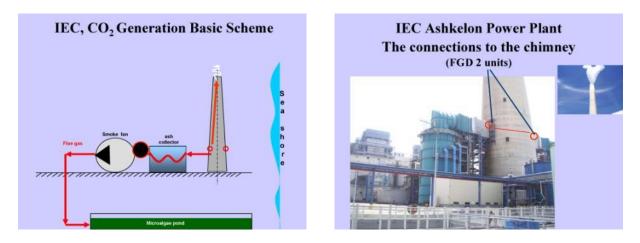
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Flue Gas Desulphurization (FGD, SO₂ Scrubbing)

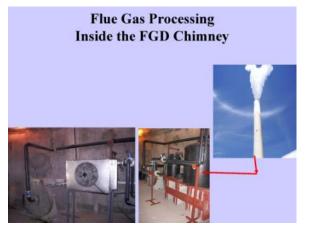
FGD is the current state-of-the art technology used for removing sulfurdioxide (SO₂) from the exhaust flue gases in power plants that burn coal or oil to generate the steam for the steam turbines that drive their electricity generators

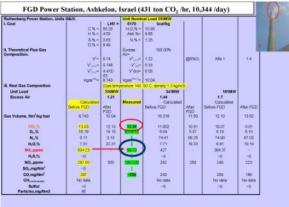


We found a power plant in Israel that has FGD in one of its two flue stacks. Power plants in Israel burn only coal and get the coal from different countries. The coal is very important for quality of the flue gas.



We took the flue gas from the stack by adding a flange and a pipe that leads the gas through a cooling treatment, some condensation and filtration, and then ran it into the algae pond. Not so difficult or high tech and now you see each step.



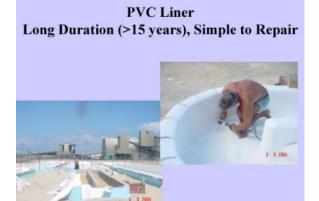


The flue gas starts with 500 SO2 ppm which contain CO_2 13% and 60 ppm NOx, and CO and the question is whether this is good for growing algae or not?

We reconstructed experimental ponds at the power plant that were very similar to what we made in Eilat.

Ponds on Construction





FGD Gas From the Chimney to the Algae Ponds

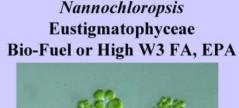


PVC liner which is very simple to assemble and simple to take care of and maintain. We filled the pond with the seawater the power plant uses for cooling on its way back to the sea. The power plant uses a lot of seawater, 450000 m3/hr, for cooling and we could get the cooling water for no charge.

The water is pumped from the sea using a very large pump and to protect their pipes the water is filtered and chlorinated. We take the water after this processing, which makes the water much cleaner. Basically, they are doing everything needed for the algae.

We built a pilot system of 1000 m² about 100 m from the chimney and this was started in 2005 and 2006. We now already have four years of experience growing different algae and all of them grew very well to high densities and very high concentrations. If you compare CO2 with flue gas, you see that flue gas is about 40% better. We did not know at the beginning what was the reason for this, but now we know quite well the major reason is that most people

Power Plant Seawater Filtered & Chlorinated





put soil extract to provide many minerals and the flue gas supplies these minerals. So it is not only the CO2 and the nitrogen in the flue gas, it is not only the literals is also a low pH. The pH comes from the FDG that produces sulfuric acid. This means the flue gas, which is a mist, has pH 2. This low pH dissolves the minerals and makes the algae very happy--I talk about algae being "happy," which demonstrates that I have become too involved with algae.

There is still some question if the nitrogen is enough in the flue gas. We use the nitrogen as is, but we also supply some fertilizer on top of that. We started growing *Nannochloropsis*, which is a Eustigmatophyte, because it's an easy growing alga and it is high in fatty acids.





As you can see we started in a laboratory, a classical way, and then outdoors in half Dome ponds, and eventually into the pot. We were surprised to see that the algae grew much better than we had ever seen. Compared to how we grew them on what is called pure CO2. At this point we are bubbling the flue gas into the pond without compression, which does not give us a significant head. You have to think about this for the future if we want to do engineering to compress the gas. There are many questions that are still open. But the algae grow very well even just using simple bubbling.

We've tested not only *Nannochloropsis*, but also all these other algae including diatoms and all of them grew very well in this flue gas. Since gas is actually a very good source and it's coming at a very low cost. The productivity that we have gotten so far is around 20 g/m2 per day, which in my opinion and according to the basic calculations is close to theoretical maximum under our conditions, which is about 25 g/m2 per day. If you consider any plant, like sugarcane or corn, you usually get about 5 g/m2 per day,

so we or about four times higher. Therefore the efficiency is very good if you could make it all year round. In our system, algae have been growing continuously and consistently all year round. With no problem from predators; no contamination--people say you have contamination, but there is no contamination if you have a good biology.

Based on this success, at the end of 2009, we are going to move to 5 hectares at the same vicinity of the power plant. The design of the new plant is like the smaller one, but it will be larger and focused on the production of fatty acids and it will be a model for bioenergy.

How can we make more efficient harvesting? In our process, harvesting the algae means removing the water to the level of 15% solids, before we dry them. The question is how to get to 15% solids?

We have tried many kinds of centrifuges, clarifiers, basket centrifuges, decanters, but which one is best depends on the algal species, it depends on the cell wall, on the density of cells, the diameter of each cell and so on. I cannot say which centrifuge is the best one for which species, we have many different alternatives. The basket and decanter centrifuges are used for yeast and the yeast model is very good for harvesting algae.





Flocculation is possible, auto flocculation, or coflocculation, and sedimentation are all possible. In NBT in Eilat, we use flocculation to get the algae concentrated for the centrifugation before spray drying.



In some cases, we use auto-flocculation, which the algae do by themselves.



Summary of Savings for the power plant:

If I take all these parameters including what's published, and assume we get free CO2, free seawater, low-cost harvesting, then we can lower the price of algae production to \$.34/kg. At this price, we are not so far from bioenergy—not to close to what we need but not too far. You will be able to find this in the presentation so I will not go into the details.

Microalgae Production Cost NBT <i>Dunaliella</i> Plant versus Seambiotic/IEC FGD Plant (10 Hectares Plant)			
	Dunaliella NBT Ltd., Eilat	Seambiotic/IEC Plant (estimated)	
		n USS/year	
Manpower	Sign.jom (20 wirelaces)	120,000 (8 workers)	
Elacreticity (90.125/KW) & residual energy	180,000	30,000	
Fertilizers (N.P.K, Fe) and other chemicals	36,000	36,000	
Departie Land Taxes	50,000	10,000	
co _t	150.000	5,000	
Saa Water	200.000	5,000	
Fresh Water	20;000	10,000	
Other supplies and Miscellancous	30,000	20,000	
Total	1.166.099	236,090	
Yearly production of dry algae biomass	70 tues (2g/m2/day)	700 tons (20g/m2/day)	
Cost per 1Kg dry microalgae	\$17.00	\$0.34	
Market Price	S4,000 β-Carotene Health Food	For Bio-Fuel cost should be below S0.5/kg algal dw	

How to take an algal paste and make the oil? As Dr. Hu mentioned it is not so simple. There are many questions, the cell wall, the lipid composition, and many other questions: to dry or not to dry before extraction? If you do Bligh and Dyer, which is the classical extraction of lipids by methanol-chloroform,

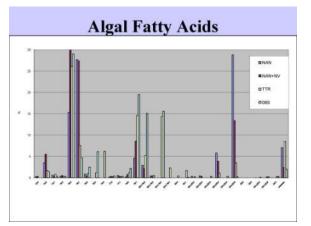
most of the algae would not be extracted—*Dunalliela* yes, but not Nannochloropsis. Should we break the cell wall? How to break the cell wall? Should we use the Japanese way? There are still many questions.

To answer some of these questions, we have given dry algal material to many companies and laboratories around the world. The goal is to let people do trial extractions and study the process of making algal biodiesel. NREL (AI Darzins from NREL is here), tested *Nannochloropsis* and you can see in the Figure that *Nannochloropsis* can yield up to 37% lipid compared to *Tetraselmis* 23% and *Nannochloris* 17%. The important observation from NREL was that the

NREL radiced herewable therey La				A selection and the selection of the 1.1 Systems office of the sign of the second selection
Lpc	contant of three sp	pecies of microalgae	based on dry weight	(DW)
Lipid content (w/dw biomass):	Ohio	rotom:Methanol (2:1)	Hexane Isopropanol (3-2)
Extraction conditions	40°C / 500 pci	70°C / 1600 pci	100PC / 1500 psi	70°C / 1500 pti
Nannochioropolis sp.	25.25 ± 0.15	29.83 ± 0.01	33.33 ± ND	15.15 ± 0.51
Tetraselmis chuil	14.28 ± 0.17		16.93 ± 0.055	
Nannochloris sp.	9.81 ± 0.41		13.08 ± 0.20	8
Lipid com	ant of three species	of microalgae base	d on ash free dry weig	nt (AFDW)
Lipid content (w/ach free dw biomass):	Chip	roform:Methanol (2:1)	Hexane isopropanol (3:2)
Extraction conditions	40°C / 600 pai	70°C / 1500 pa	100°C / 1500 pc	70°C / 1600 psi
Nannoohioropsis sp.	28.61 ± 0.19	33.68 ± 0.01	37.83 ± ND	17.11 ± 0.58
Tetraselmis chuł	19.80 ± 0.24	. (23.47 ± 0.08	
Nannochioris ad	1244+0.62	. N	16.69 ± 0.25	23

efficiency of extraction depends on the temperature and the pressure. I appreciate this work by NREL very much because they did it very well and showed that simple extraction will not work. They did extractions with high pressure and high temperature to get the oil out.

In addition to optimizing extraction methods, we have to consider the fatty acid composition of the algae to choose the best algae for biodiesel. Usually we take *Nannochloropsis*, which has omega-3 fatty acid with a chain length of 25. Most green algae contain fatty acids with carbon chains between 16 and 18 carbons in length, but which one is the best?





We started with *Nannochloropsis* to produced biodiesel with the company Inventure. The biodiesel was extracted by Inventure by methanol HCl which is methanolic hydrolysis to get the oil directly to biodiesel from the algae, which is possible, but (now the but), if you look at the certificate of analysis, the cloud point is -2°C. In other words, it's a good biodiesel for warm climates, but it will never work as a jet fuel, it will freeze. So we have many questions, this is not good for jet fuel. What are you going to do?

The reason is not good for jet fuel is because we work with *Nannochloris*, which has a long chain fatty acid and in order to make it into jet fuel we have to reduce the length of the carbon chain. To address the problem of jet fuel, first we need a cell without a cell wall which is very important for harvesting and second we need a high content of TAG which have low chain length: 14-18 and better 16 with low unsaturation.

Algal Bio-Oil Specifications for Low Cloud Point Jet-Fuel

No Cell Wall High Content of TAG Lower Chain Length Fatty Acids Higher FA Saturation

The solution we found was by going back to *Dunaliella*. It's a green alga that contains mainly palmitic acids (C16:0) and oleic acid (C18:1). This is good, but it's still not good enough for jet fuel. Now for the first time, I am going to show you, that we develop a new way to make short chain length fatty acids in *Dunaliella*. We have lowered the chain length by a growth treatment that I cannot describe in detail because it is still not open information, but if you look at the composition here we got down the bulk of the fatty acids down to caprylic acid which has chain lengths of 8, 10, 12, 14, 16, 18, and most of it is saturated. So by some treatment of the algae, it's a chemical treatment, very simple actually, we can get *Dunaliella* that contains lipids that can be used for jet fuel. In the last year, we have accumulated 4-

5 tons of powdered this *Dunaliella* ready for extraction for jet fuel. We are now discussing to whom should we supplied it. There are many requests by Boeing, by NASA to get the material.

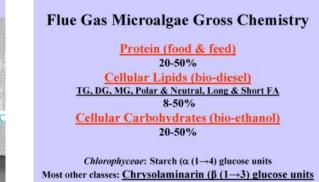


	ון: אריזח מקורית ך דיגום: 15/03/2009	ממפ': מקורר 00:00 תנאי שפירת חדוגמא וחחובלח		
	תאור בדיקח	יחידת מידח	תחום מותר	תוצאח
20011	חומצה קפרילית (C 8:0)	gr/100gr fat		12.8
20011	חומצה קפרית (C10:0)	gr/100gr fat		9.5
20011	חומצה לאורית (C12:0)	gr/100gr fat		0.5
20012	רומצה מיריסטית (C14: 0)	gr/100gr fat		1.24
20013	חומצה פלמיטית (C16: 0)	gr/100gr fat		17.56
20014	(C18:0) חומצה סטיארית	gr/100gr fat		1.75
20014	(C18: 1) חומצה אולאית	gr/100gr fat		37.2
20015	חומצה לינולאית (C18:2)	gr/100gr fat		14.9
20015	רומצה לינולנית (C18: 3)	gr/100gr fat		3.59
20000	שומן	גרם/100 גרם	לא נדרש בתקן	23.2

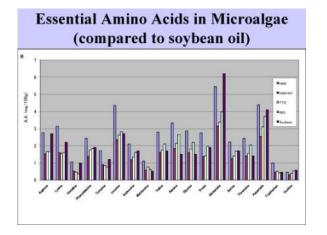
This is the first time we have made algae that have a shorter chain than palm oil. This is really a breakthrough in the process of growing algae for jet fuel. It is available at NBT, we call it *Dunaliella bardawil* and it's mainly in the form of triglycerides.

Seambiotic Algal Bio-Ethanol





As we requested, we made triglycerides, but short chain fatty acids. I'm not going to discuss the issue of ethanol of course you can do ethanol, but we have three issues that were mentioned before not only biodiesel, we have protein, lipid, and carbohydrate. We have to look at them all. And we have to remember that we can choose which we want not only the lipid. This raises many questions related to what we discussed before.





Concluding remarks:

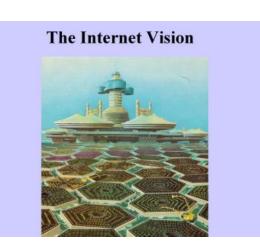
When it comes to making an economically viable algae system, don't forget to include the whole product. There are for example, the essential amino acids and the value of the proteins. The purging of the algae contains different amino acids. So we have the lipid and we have the essential amino acids in the proteins and these amino acids make the value of the algae much higher. If I assume the algae were 33% protein, 33% lipid, and 33% carbohydrate and we take the value of proteins with essential amino

acids, and lipid with short fatty acids, and finally the carbohydrate as ethanol, we get about \$1 per kilogram of algae in value. And that I think is where we stand today: \$1/kg of algae. We not only provide diesel, not only essential amino acids, not only ethanol, but take all together we have an economical system--we have a whole product.

If that could be in the ocean, I don't know, Jonathan Trent, maybe it could be done. I found an Internet view that is not so far from yours.

Acknowledgments:

I want to thank the Israeli electric company and people at NBT and *Seambiotic* and all of you for listening.





QUESTIONS:

Ron Pate: When you did the modification to get shorter carbon chains what was the yield you got?

Ami Ben-Amotz: The same. We have a good phycologist.

Travis Liggett: Do you know Christopher Hills and could you tell me where he fits into this hierarchy?

Ami Ben-Amotz: Christopher Hill is historical. I don't know why you want to know about Christopher Hill, it's not relevant to this discussion.

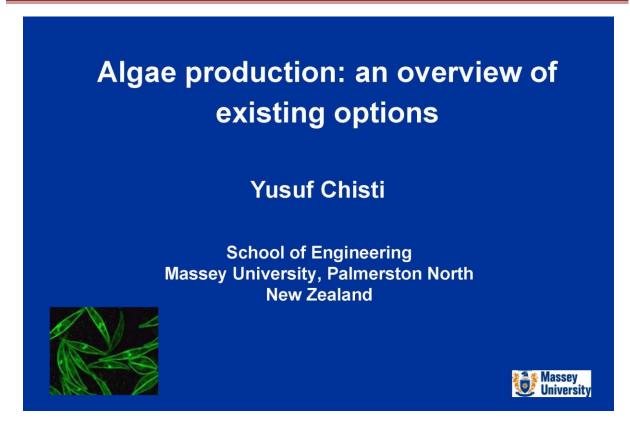
Travis: I'm living at his widow's house right now.

Ami Ben-Amotz: Christopher Hill was a nice story, but it's not relevant to this discussion. He was a guru, but it's not relevant here.

Ihab Farag: I noticed you are doing everything in open ponds. Do you have experience with photobioreactors or do you prefer open ponds to bioreactors?

Ami Ben-Amotz: You know that's a long question. We have been discussing this for the last 15 years: open pond vs. photobioreactors. I'm not going to discuss it I don't want to discuss it, but I said today if you look at commercial production anywhere commercial production of algae is open pond. There are only one or two photobioreactor companies making very high value products, which are fine if you are making astaxantin or a special food, but for low price protein and something else, it's not possible. We work on photobioreactors, but for large scale we need to use open ponds.

ALGAE PRODUCTION: AN OVERVIEW OF EXISTING OPTIONS, YUSUF CHISTI



Jonathan Trent: The next speaker is Yusuf Chisti. Who has come here all the way from New Zealand, and I invited him because one of my colleagues told me he gives a wonderful lecture about algae and also because I thought it would be valuable to have someone from the Southern hemisphere represented here. I would like to ask you to continue focusing your attention on the idea that what we are doing with these lectures is setting the groundwork for our afternoon discussions and so what we will be doing in the afternoon is focusing on how are we going to implement this and does it make sense to try to apply this to a system that would be offshore? That would perhaps solve some of the problems that we just heard exist still for harvesting and for land space and etc...

Yusuf Chisti: I will talk about algae production; microalgae, some of the existing options and how they might possibly lead to production in the sea.

I believe that microalgae are the only option for producing biofuels, renewable biofuels, as we will see in a minute.

We will be looking at a conceptual process for producing liquid fuels from microalgae. Of course to produce algal fuels we need to produce biomass somehow. So we'll look at options for large-scale production of the biomass.

We will look at some of the issues that effect productivity of algae in culture systems be it photobioreactors or open ponds. We will see some of the attributes of commercial algae; what characteristics we desire in them for a commercial process. We might have some time left to look at ways of improving energy prospects from algae.

I'll then summarize and conclude.

So the first thing: Why are algae—I believe—our only option for producing liquid fuels.

Let's take the case of biodiesel. A large economy such as the United States, requires something like 0.53 billion cubic meters of biodiesel to replace all transport fuels — all gasoline, all petrodiesel, and all kerosene jet fuel.

How we can possibly produce it? Well potentially we can get it from various oil crops, but here I have listed some oil crops and their annual oil

productivity. These are some of the best figures you have, the real productivity would be likely lower. These data are from the US Department of Agriculture.

In the third column, I have calculated the land area that will be required for that amount of oil, to be produced from a crop such as palm oil.

In this last column I have expressed the land area, as the percentage of the total cropping land area of the United States. So if you were to use palm oil, you would require 48 percent of all the existing cropping area in the country to grow palm oil to produce all the biodiesel requirements. You would have to starve yourself and of course forgo your cotton shirt and all that sort of thing. So I think this scenario is totally not feasible, if you want total replacement of petroleum products from biofuels.

Now this scenario changes totally if we look at microalgae. Now we calculate it is potentially possible to have those levels of oil yields per hectare from algae, assuming something like 20 % or 40 % dry weight oil in the biomass and those kinds of levels are possible. If we take those figures then we will require something like less than 10% of the cropping area of the United States to be able to satisfy most of the transport fuel demand; so it is a potentially feasible scenario. Even if the land area is not 8% of total cropland, but 16%; it is still a reasonable scenario compared to any other oil crop.



Outline

1. Microalgae - an only option for biofuels

3. Algal biomass production options

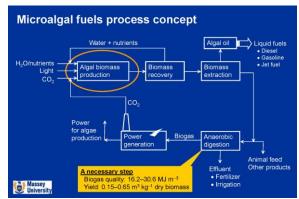
2. Algal fuels - a conceptual production process

Crop	Oil yield (L/ha)	Land area needed (M ha)	Percent of existing US cropping area	\succ
Corn	172	3,080	1,602	
Soybean 🧹	446	1,188	652	
Canola	1,190	446	244	
Jatropha 🔪	1,892	280	154	
Coconut	2,689	198	108	CLARK COLOR
Oil palm	5,950	80	48	
Microalgae 🔨	35,202	15.2	8	
Microalgae	70,405	7.6	4	

These numbers are based on biomass productivity, not oil productivity; biomass productivity of that level which I believe there is a tremendous amount of literature to back that value for photobioreactors: 160 tons or so of biomass per hectare per year.

All right, let's go on to see then what might a production process based on algae look like for making fuels.

Well, we will have to somehow produce the algal biomass that will require water and nutrients, these are usually agricultural fertilizers, nitrogen and phosphate fertilizers. Of course we have to have light for photosynthesis driven production, we have to have carbon dioxide. Once we produce the biomass we somehow have to be able to recover the biomass from the water, recycle the water with any residual nutrients to take out whatever value is left in there.



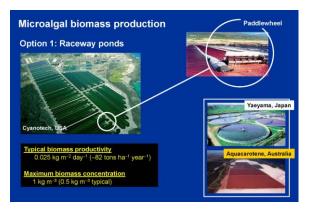
The biomass that we produce, somehow will need to be extracted of its oil. The algal oil then can be treated simply as crude oil, crude petroleum if you like, to produce diesel. Now I am not talking about biodiesel here, I am talking about a petrol diesel equivalent if you like. Not just triglycerides, everything; gasoline, jet fuel, all can potentially be produced from algal oil using existing petrochemical refining type of technology.

Now if your biomass contains something like 50% oil by weight, and you have taken out all that you are still left with a massive quantity of biomass. You need to be able to do something with it. Of course you can feed only so much to animals. I think you would have a lot of biomass if you were really trying to replace all petroleum production with algae. What I think we necessarily need to do is anaerobic digestion of the residual or most of the residual biomass for very important reasons. Anaerobic digestion recovers a lot of the nitrogen and phosphorous fertilizer that you put in initially. Production of nitrogen fertilizer is one of the most energy intensive processes that requires petroleum. Petroleum is what feeds us, the crops we grow, you supply them with nitrogen that we get from petroleum. So we need to be able to recover it, and recycle it twice to be able to have any net positive energy recovery from the whole process.

And of course what it does, in addition, is to provide us with biogas, a mixture of carbon dioxide and methane; and it provides us with sufficient biogas that we can run it in a power generation facility. To run this entire algal biomass production and recovery process, not drying. This is based on detailed energy analyses and so on; so you generate all the energy required for operation and therefore this process is fairly essential for the success of biofuel production, I believe.

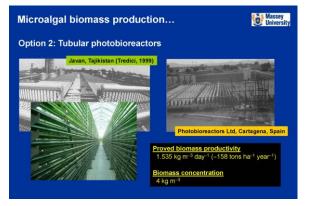
Now, to make this process happen we have to have some way of producing algal biomass. And how might we do that? Options of course are, you have seen the raceways, the open systems that have been around since the 1970s. They have been producing at the same level as they have been producing since the 1970s.

What those are, shallow ponds 25 to 30 centimeters deep, they can be deeper. A raceway is shaped like a racetrack. The flow is circulated at a sufficient velocity to keep the algae in suspension and to create a wave motion, if you like, to improve mass transfer and I believe even light-dark cycling. And that is a line of paddle wheels running along there. These systems; various variants; the open *Chlorella* ponds at this food *Chlorella* production facility in Japan, and beta-carotene open lagoon production, Australia. Of these open systems; raceways are the



best established and the most productive. Their productivity in the best conditions would be something like 82 tons per hectare per year, which is comparable to the productivity of sugar cane. The sugar cane biomass productivity is something like 95 dry tons per hectare per year, that sort of level. So there is nothing special about this. The biomass content in this broth is something like half a kilogram per cubic meter, fairly dilute broth, so to take out half a kilogram you need to process one cubic meter—one thousand liters—a massive quantity of broth to recover that amount of biomass—quite an effort there.

Other option of course is the use of tubular photobioreactors. These are transparent tubes of plastic or glass typically less than 10 centimeter in diameter, running, laid out flat on the ground, typically to capture sunlight, we want to maximize sunlight capture; that is what drives our photosynthesis and you could imagine this area of tubes called solar collector here, turned 90 degrees to give you another configuration of a photobioreactor shown there.



We have various other variants, you could have it indoors as opposed to outdoors and so on and so on. Some of the best productivity data reported for these systems is of the order of 158 tons per hectare per year. And this is absolutely consistent with the theory of photosynthesis, photosynthetic productivity and so on. I have done those calculations myself so I insist on this. The broth in these things have a biomass concentration of something like 4 kilograms per cubic meter which is something like 8 times what you would have in algal broth. The cost of downstream processing then is significantly reduced. You don't have to pump one cubic meter through your centrifuge to get a certain amount of biomass, you could do an eighth of that and get the same amount of biomass. Of course there are still other variants of photobioreactors. Vertical column bioreactors, something like 20 centimeter diameter of vertical glass or plastic columns, four meters tall at most. You have various, commonly used adaptation of it, if you like, these are polymer bags, thin flexible plastic, hanging in open or shaded areas to capture sunlight no more than 20 centimeter in diameter or so typically. Similar technology here again, there are various variants. The productivity of these systems is higher than in raceways even though the depth may



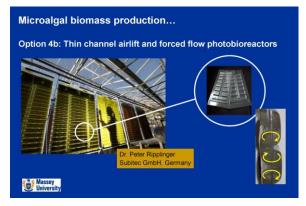
be 25 centimeters because of better mixing and better carbons dioxide supply and so on but the productivity is lower than in tubular photobioreactor because the surface to volume ratio is lower. You know 10 centimeter diameter versus 20 centimeter diameter.

And there are still other options for culturing algae. Thin channel airlift and forced circulation photobioreactors; and of course some start-up companies are attempting to use them. Very cheap to produce, thin plastic films like your plastic bags on a massive scale. Flow is circulated through various kinds of airlift devices or it could be mechanical devices. A very high surface to volume ratio, could be outdoors or indoors. Relatively inexpensive; high surface-to-volume ratio productivity and comparable to tubular photobioreactors.



But, I do not like using plastics, if I can help it, because it comes from petroleum. And we need to get away from petroleum totally and completely.

Other thin channel configurations here; you have a vast sheet of plastic, rigid plastic, not flexible anymore. Those are just plastic sheets pressed together to give you a narrow channel, the flow is circulated by airlift and mechanical action. And through these channels, compared to what you saw before, the flow is directed; the flow is such that you have movements, the circular movement that continually moves the fluid from the outer zone to the interior, so that the material that is in the dark zone is continuously being brought fairly rapidly to



the outside and back so that no material resides in the dark zone continuously for a long period.

And of course the story does not end there, you have other futuristic photobioreactor options if you like. And don't laugh at them. That looks like the head of a cobra to me, but then people have come up with this; all this slide is showing you is we can have photobioreactors to make use of the light 24 hours a day; that kind of configuration. Ok, it is laughable now but maybe not in the future.

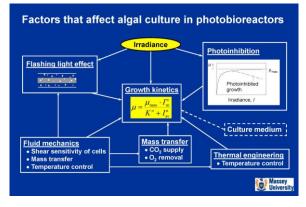


All right, now how can we possibly translate some of this technology into the sea? Well now you have deep clear ocean in many places like this. The ocean is quite clear; it is two or three meters deep. It is clear because it is nutrient deprived; algae can't grow in there. Now I am not suggesting you put nutrients in there but I am suggesting that you could possibly install your photobioreactor tubing and other flexible systems into here. With calm seas, even glass may be possible. Glass may actually be a better option, maybe a little more expensive but



then it will last for fifty years. It is possible to suppress growth of biofilms on the surface of the glass by coating for example with titanium oxide and other UV sensitive catalysts that will create free radicals that will prevent biofilm development on the surface of these things. So taking existing technology or inventing new technology, I think it is possible to look at some of these systems. And other than energy usage glass of course is a very basic and very safe product. It is renewable in a sense, of course all of this is renewable until the sun runs out then nothing is renewable. So those are some concepts there to look at. And now I am not going to discuss the details of how a photobioreactor works; I will go on to explain the issues that affect algal culture in photobioreactor and open systems.

What are those issues? First of all light level. And of course we need to know what the light level is inside a photobioreactor or in an open culture system, because light is the food that algae grow on, assuming that all other nutrients are available in excess. The growth rate, 2 here, depends in a specific way on the average light level that may be different for different algae, but we need that kind of relationship to be able to predict that a facility of this size will be able to produce this much biomass and therefore this much oil. Without that kind of



productivity you end up designing an airbus 380 and end up discovering that it can't lift it's own weight into air. You have to have those engineering analyses.

Of course it is not quite obvious from kinetics like this but there are issues of photo-inhibition. That is the specific growth rate increases with increasing irradiance in the photobioreactor, until a certain value and further increase in irradiance will reduce your growth rate. And those issues ought to be considered in the operation of photobioreactors with a certain dilution rate where you would have photoinhibition.

You have issues such as flashing light effect or I'll just call it simply light/dark cycling. In a photobioreactor or in a raceway with a high-density culture — half a gram or more per liter, you would always have depth that is dark. When it is dark there is no photosynthesis, when there is no photosynthesis there is not only no production but also algae are eating themselves. They are eating their biomass, and this actually reduces productivity. So you want to minimize the depth of the dark zone, and you also want to improve the movement between the light and dark zones, so that no algae are going to starve for a long period.

Of course all this light dark cycling for example is effected by fluid mechanics, turbulence and so on. Turbulence of course effects things like mass transfer, the ability to transfer your carbon dioxide from the gas phase into the liquid and the ability to remove oxygen produced by photosynthesis from the liquid to the gas phase, and that is important too because accumulation of oxygen inhibits photosynthesis, above a certain concentration. Of course too much turbulence is harmful just as it can blow off roofs from buildings and bring down trees in a storm and so on. So too much turbulence can destroy certain algal cells. So you have to have some turbulence but not excessive. And of course fluid mechanics also controls your ability to manage temperature which can be quite expensive in certain regions, but again those issues could be resolved if you were to place your culture systems in the ocean with a high thermal mass, a high thermal inertia.

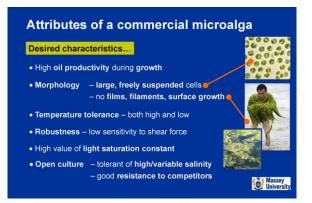
Now these culture systems that we had a look at, what kind of pricing do they come for? Raceways are about \$125,000 per hectare, 2008 figures. These are just estimates they could be off by 20% or so, some of you here may know better, I am an academic, not an industry person but those are the data that I estimate. Tubular photobioreactors are something like 8-10 times the raceway cost, but they are much more productive, but the higher productivity is not sufficient to compensate for the high cost. But then there are other issues to consider, I am just looking



at the capital costs, the downstream costs are processing the biomass. Because the biomass that comes out is more concentrated it might be possible to recover it at a lower cost compared to what would be possible from a dilute broth that comes out of an open system. These open, flexible, disposable plastic bags, this company which has the technology to produces algal biomass estimates costs which are comparable to the raceway systems, but the productivity I suspect is significantly higher in the vertical

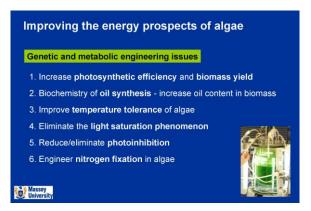
bags up here. Just because the surface area to volume ratio is higher. Vertical columns I believe are less expensive than tubular, but also have lower productivity. But a productivity that is better than the raceways because of higher ratio of surface area to volume. These thin channel airlift systems we do not have any information on cost, but I believe they are less expensive, these ones compared to those and likely more productive here than there.

Now what attributes do we look for in commercial algae for producing biofuels? What desired characteristics algae ought to have? Of course they have to have a high oil productivity during growth, but you heard that it doesn't go together and you have two-stage system then in which you maximize growth first and then you have a post harvest treatment of the biomass. And it is actually possible to do this post harvest biomass treatment in two or three days and change the lipid profile of the biomass, and the lipid content of the biomass. So of



course we want algae, large single cells well suspended, rather than this kind of growth that would be hard to manage in either a raceway or a photobioreactor. We want a high temperature tolerance, certainly in the photobioreactors, less so in the raceways. But again that problem could be eliminated, if we were to culture in the sea, because in a photobioreactor, temperature rises rapidly in the daytime unless you have a good cooling system. Of course you want an algae that is less sensitive to shear comparatively and you want to eliminate things like light saturation constant because light saturation limits the photosynthetic productivity for a given amount of light. And if you have an open culture system in the desert and so on, and no available freshwater to compensate for water loss by evaporation than you have to have algae that are highly tolerant to variable salinity. And an open system is relativity worse than its competitors. Now what options might we have for improving the prospects of producing oil from algae?

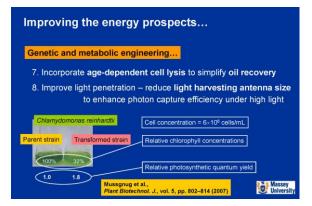
Well I actually think, a lot needs to be done about algal biology. Because that is your cell factory that is where all the production occurs it is all about algal metabolism. The rest of the culture system is just a supporting structure. We need to worry about the fundamentals. How can we influence the photosynthetic efficiency and biomass yield? And people are working on that, the fundamentals of biochemistry there and the biochemistry of lipid synthesis to be able to manipulate and modify it, and we have a long history of being able to do that.



Various other metabolites in plants, in bacteria, in fungi and so on there is an established history and industry based on this. We want to improve temperature tolerance for obvious reasons, we want to eliminate light saturation phenomenon, because that limits and places a lower ceiling on productivity. We want to eliminate photoinhibition and we want to engineer, if possible, nitrogen fixation in algae, because if you can do that you do not have to supply them with nitrogen fertilizers. And of course

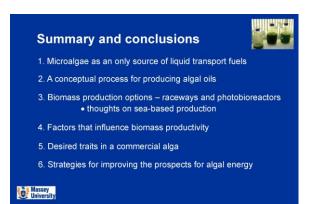
certain cyanobacterica are able to fix nitrogen. So again there problems of biochemistry here but it gives us something to think about.

We could have for example, for oil extraction purposes age-dependent cell lysis. Once the cell reaches a certain age it lyses . and then may be lipids just float to the surface. We want to improve light penetration in the photobioreactor or open culture channel and that is an important issue because the dark volume in a or raceway is not productive, in fact it is destructive to the algae. How can we do that? Again people have attempted this with the algae *Chlamydomas reinhardtii* strain, genetically modified strain contains the same concentration of cells, and



one is much greener than the other. Say the chlorophyll level is 100% here, the mutant only has 32% chlorophyll content. What that does if you have a photosynthetic productivity of a unit there, you have almost doubled the photosynthetic productivity in the second case. Because you are improving light penetration into your culture, more cells are able to produce at a higher level. Of course this data is published by these people and by several others—the earliest reports were from 1997.

At that point I'll summarize the points I covered. I believe microalgae are the only option, we do not have plants as options currently, unless we engineer them. We went through a conceptual process of producing algal oils. We saw some of the biomass production options, and how very speculatively they might be taken into the sea. We saw some of the factors that influenced biomass productivity and some desired traits we want in a commercial alga for producing oils, and some of the strategies for improving energy productivity of algae if you like.



That's the end of the story.

QUESTIONS:

Jonathan Trent: We are going to have a pretty tight schedule now. There is time for one question now and then we can have a lot of questions in the afternoon. Is there one question? Aaron you are raising your hand or not. Can you get a microphone to Aaron, please?

Aaron Baum: Are you, you talked about the mutants on reduced antenna are you aware of any work where they actually demonstrated any higher productivity from such mutants?

Yusuf Chisti: Well I haven't done any work on these mutants, but I have mentioned the journal article that has been published and they have actually had higher photosynthetic productivity. I have showed you a comparative value of 1 in one case and 1.8 in the other. These are photosynthetic efficiency.

Aaron Baum: Yes, I meant total productivity. I think no has demonstrated total higher productivity from a reduced antenna mutant.

Qiang Hu: The people who have made these antenna mutants have observed that they bleach within one or two days. When you see the color of these mutants, they are just pale and very sick organisms. They cannot compete with the wild-type and people who try to use these mutants in an open path or closed bioreactor systems observed they all died within a couple of days.

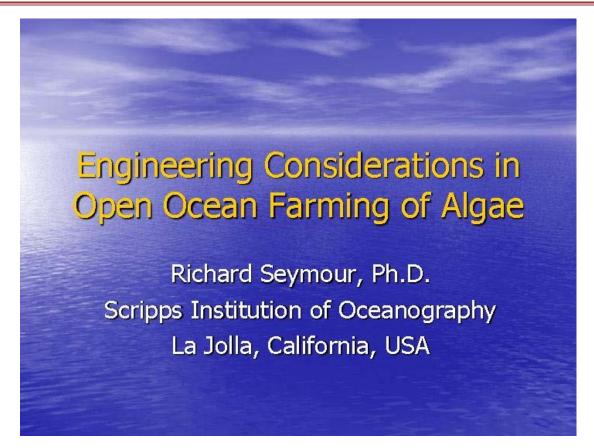
Jonathan Trent: Thanks.

Yusuf Chisti: I am not suggesting we are answering these questions today, I am suggesting people are investigating options for being able to do that. If something is not doable today it does not mean it cannot be done tomorrow. So don't just say no to anything.

Jonathan Trent: Why don't we wait for the last question, because we are going to have plenty of time in the afternoon and otherwise we are going to be late for lunch.

Jonathan Trent: All right fantastic!

ENGINEERING CONSIDERATIONS IN OPEN OCEAN FARMING OF ALGAE, RICHARD SEYMOUR



Jonathan Trent: The next speaker is Richard Seymour from Scripps institution of Oceanography. Dick Seymour was my mentor when I was in Oceanography, way back in prehistoric times for me, almost. He and I were at the Foundation for Ocean Research together; Dick was leading that foundation for a while, and I have asked him to give us a perspective on Ocean technologies. I know he worked in the oil industry for a while, as well as on designing floating breakwaters and other really innovative ideas. So, are you all set up to start?

Richard Seymour: Can everyone hear me? I'm going to try to make this presentation as much as I can independent of the configuration of an Ocean Farm. And I am going to concentrate on what I consider are the five major problems of farming in the open ocean. First of these is, of course, the very harsh environment; second, is that equipment breaks with great regularity in the ocean; third, is it is always a problem to try to stay in one place where you would like to be; the fourth is that there are very bad drivers in the ocean, just like on land;

The Big Engineering Problems in Open Ocean Farming

- 1. Wind, waves, currents and ice
- 2. Loss of Equipment because of
- Fatigue and Corrosion
- 3. Maintaining Position
- 4. Avoiding collisions
- 5. Knowing where the farm elements are if they are not where they ought to be

the fifth one is [under] the assumption that sooner or later some part of your farm is going to drift, and go someplace where you don't want it [to] and that you are going to have to know where that is and bring it back.

So, dealing with these, one at a time: Winds not only produce very large structural loads, or can produce very large structural loads, but they also generate waves, and waves can be extremely destructive. It is not possible to forecast, more than a few days in advance, the conditions under which you cannot do work at sea.. So this makes what would seem to be routine operations very difficult, because all of a sudden you are going to have to shut down and stop. Currents that change direction can cause very large motions of farm elements-- twisting, moving them in circles-- and this can be a problem,

1. Climate Extremes

- Winds are stronger but less variable than on land
- Storm waves can appear suddenly and be powerful enough to break almost anything
- Currents are more predictable, persistent, can reverse if tidally driven
- Even sea water will freeze when air is cold enough and it is splashed on your structure

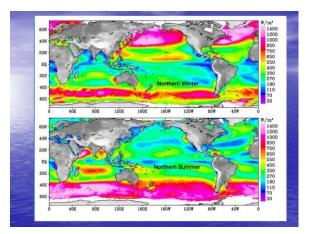
depending on how you want to load and unload the system.; and ice is very deadly.

So let's look first at winds. We do not have statistical data on winds offshore in very many places in the world, so we are dependent upon modeling almost completely. And since wind can be a major risk factor, we're assessing risk on the basis of models, which may or may not be very effective.

Considering Winds at Sea

- Ocean winds are very undersampled compared to land winds
- No reliable long term statistics (based on measurements) exist even for nearshore locations
- Risk is evaluated using modeled winds

Here is a diagram to show the seasonality of winds and these colors (the hotter colors) are higher stress levels near the surface of the ocean. You can see that the winds are stronger closer to the poles in either hemisphere, and certainly they are stronger in the winter than they are in the summer. One other interesting thing that this map points out is that, if you live in a place near the zero meridian, like here, then you have to look from one side of this map to the other in order to see what's going on at your place.



Waves are the best concentrator of solar radiant energy that we have naturally on earth. [It] Concentration can go up into factors of 1000 times of multiplication. So waves are, can be, and will be, highly energetic in any part of the ocean at some time. As indicated before, you are going to have a certain period of time, a percentage of time, where you simply cannot work. You have to stand by and wait until the waves go down before you can hitch up hoses, make a mooring and/or unmake a mooring. And the ability to reliably predict this kind of weather is only in terms of present knowledge--

Considering Waves at Sea Wave energy densities can be 10 to 100 times greater than wind

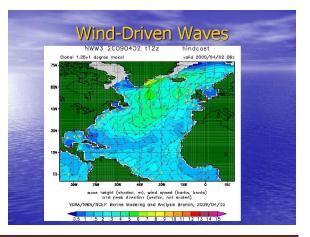
- Like high winds, large waves make normal farming operations at sea impossible
- Wave heights can be reliably forecast only about 3 days in advance
- Exceptionally large storms will produce waves big enough to severely damage (or destroy) floating assets

only in terms of a couple of days. One thing you can be sure of is that if you are in this business long enough, someday, waves will completely destroy your farm.

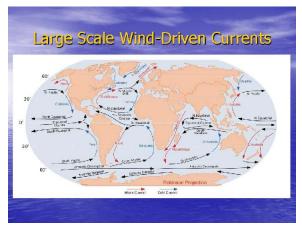
This is just a little reminder to get you in the mood for what's going to happen.

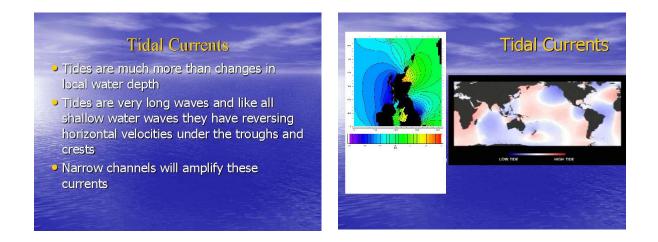


This is a seven-day forecast of waves in the north Atlantic; it's showing direction, while the color indicates magnitude. And if any of you are harboring some secret idea that you are going to hide out in the middle of the Sargasso Sea--forget it!



This is a standard text-book model for wind-driven currents, and what leads to your Sargasso Sea fantasy is the absence of any arrows out here, but that does not matter. These are the general circulation patterns in the ocean, wind-driven currents, and as long as you stay away from the red ones, the red arrows, things will not be too bad. We are able to design effectively to operate in the currents represented by the blue and black arrows in this picture.



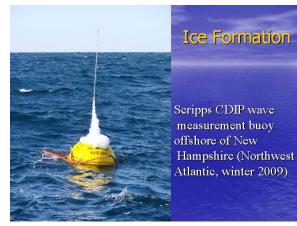


These pictures are just meant to indicate to you that tidal currents are caused by the fact that tides are really waves, and waves that move along the shore, i.e., "edge waves"-- and that a high tide is really the crest of a wave passing you and a low tide is really a trough passing you. It is not a question of the water going in and out, as we normally think of it, and in the right hand picture, you see the blue lows chasing the red highs around major ocean basins.

The Baltic, it happens, is the wrong size, the wrong shape, the wrong depth, to resonate at 12 or 24hour periods and therefore it has micro-tides. Not a problem here, but there are many places in the world, even a few hundred kilometers away from here, as you know, where some of the highest tides and strongest tidal currents in the world exist.

So the important thing is, if you are dealing with an area of the world in which you have tides, velocities are going to reverse. That means that [you] they are going to tend to twist structures around their moorings. And if you are anywhere near islands or other conditions that can make for narrow channels, you can have high amplification of these tidal currents and they can reach very dangerous velocities.

This is a picture of one of my wave buoys in the northwest Atlantic, this past winter. There were no icebergs anywhere around, just cold air; that's all it takes.



So the problem with ice is that salt spray from breaking waves will freeze, at very close to 0°C, and that layer upon layer can be built up on a structure, if you do not have occupants aboard to break the ice off and throw it back over the side. In fact, every couple of years there are shrimp boats that are lost, capsized, and sink, because the crew is not able to break off the ice from the rigging as fast as it forms. So for an untended structure in cold climates, you have to be very concerned about ice build-ups to the point where the structure is going to sink. And of course, it is fairly obvious that ice changes the

Consideration of the Ice Hazard

- Salt spray from breaking waves will freeze at air temperatures slightly below 0 C.
- On untended floating structures the ice load can cause either mooring failure or actual sinking of the structure
- Ice can mask markers, making collision by ships more likely
- Ice on antennas can hinder communication

appearance of the object and, as you'll see, might cover up some very important markers.

The most likely failure for any operation at sea is the failure of the mooring system. And these kinds of failures come about because the loading is cyclic, raising the probability of fatigue in metallic structures; furthermore, this fatigue failure is amplified by salt water. The answer to this problem is that you make the mooring system very, very redundant, so that if you have failures, you can still survive.

2. Failures of Moored Systems at Sea

- Fatigue (failure caused by many repeated cycles of load) is increased by salt water
- Small defects (inclusions) in metal parts corrode very rapidly in and near the ocean
- Risk is reduced by redundancy in mooring systems

This is an example of a local failure but turned out to be very critical. The failure is in the mooring system of one of my wave buoys and as a result, the wave buoy drifted away from its desired position.



This piece is from a very, very large mooring system for a very large oil platform. And I show you this image to indicate that size does not solve these problems; such failures can occur anywhere.

Even Oil Platform Moorings Fail



So let's think about the problem of trying to stay where we want to be, maintaining position. There are only three ways to do this, in general. The most common, is to construct a mooring system. a system of anchors and surface buoys that you use to attach or detach your structure. And the second most common means of moving things from one position to another, is of course simple towing-- the use of ocean tugs. To pull a load, in principle, the tug has enough power to maintain the structure in the position you want it to be, if you have a big enough tug or enough tugs and very deep pockets. I don't

3. Maintaining a Position

- Mooring (attaching to an anchoring system)
- Towing (using a tug to move or hold still in presence of wind, waves and currents)
- Dynamic Positioning (a self contained system to apply thrust vectors and maintain position within acceptable limits)

think the third system I put in here has any particular relationship to farming, but for completeness, there is the possibility of having thrusters on the structure itself and even autonomously maintaining position.

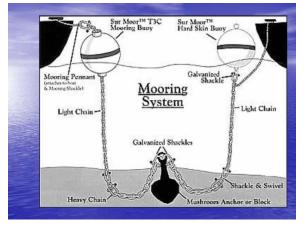
So, looking at the first of these ways to maintain position, and in my opinion, at the one that would be most likely used, you need to understand that anchors are not picked up. Anchors are put down permanently; very often they are suction piles or very, very large weights, in some cases. This redundancy requires multiple mooring lines and therefore multiple anchors and so all of the operations are done at the surface, attaching and un-attaching at some surface platform or buoy. Typically, the mooring lines are going to be slack; that is, they are not stretched tight in any direction,

Long Term Mooring Systems

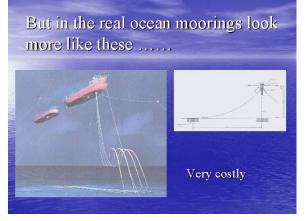
- Anchors are not ever recovered
- Multiple mooring lines are employed
- Attaching and unattaching is done at the surface with the mooring line supported by one or more buoys
- Typically, the mooring lines are slack allowing the floater to move in a large circle
- Therefore, moorings have to be well separated

which means that whatever you're going to attach to it is free to move around in a very large circle. Sailors call this "the watch circle." And the impact of that is that, if you have multiple elements, they have to be mounted fairly far apart, in order not to collide.

So if any of you from your childhood row boat experience think this is what a mooring system would look like, it doesn't.



In a real ocean, a mooring system would be much more inclined to look like this. So here you see the multiple surface buoys, the attachment point here and lines running off in all directions. If you can read the fine print on here, you'll see that there are actually eight lines used in establishing that mooring system.



I put the day-rate prices down here for ocean-going tugs, so you would get some idea about how much it would cost you a day to keep your platform in place. And I do not think I am going to say any more about that...



And amazingly, you can, in fact, lease this dynamic positioning system on the Internet, if you can afford it.



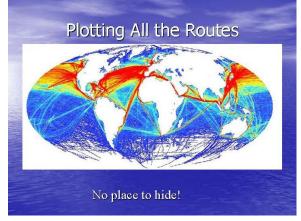
Lets go to the fourth problem, the problem of avoiding collisions. First question here: "Why is this a problem?" It looks like there are big holes here between the major routes.



But if we actually draw the important ports to the important ports' lines, we see that the holes between them get very much smaller.



But if you realistically plot all of the known drivers' lines for ships, there are no holes--none. And this plot does not even show fishing boats; these are just the tracks of commercial ships,



What do we do to solve this problem? One of the things is to make us more visible, with things like radar reflectors. These corner reflectors have the property of sending back radiated energy to the source directly. So, regardless of by what angle they're struck by the radar beam, they send the bounce back right to the source radar. This has the happy property of making you look much bigger than you really are. That way, this is an anti-stealth technology.





Obviously you can put up things that you can see in the day time and things you can see at night and this can even be arranged so that it spells out who you are in a simple code, to help the ship handlers' understand.

But I want you to look at this very carefully because, no matter what you did about these markers, this is the reality of ships at sea. If you start into this with the assumption that there are many eyeballs up there with binoculars just looking for something that they can avoid, you're wrong. But as hazardous as this is, it is not the real problem. Here is the real problem: fishing boats.



There are only three men on watch at night and one is in the engine room

They go where they want to go; they go where the fish are; they resent anything that anyone has put on a surface of the ocean that gets in their way or tangles their gear; they love to run into your stuff. So this is a major problem associated with collisions.



The fifth problem really has a very simple technology solution--it's expensive! I use this on my wave buoys all the time. There is a GPS on board; it knows where it is; it's set up so that when it moves far enough away from where it is supposed to be, it calls you on a satellite phone, at great cost, but it only calls you when it needs to, and every half an hour it tells you where it is, so you can get to it, sooner or later. So this is an expensive operation and it depends on how many of these we really need, to be safe.

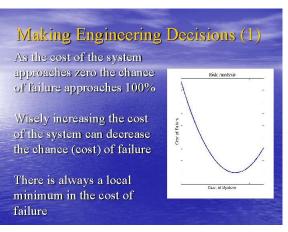
5. Tracking Drifting Farms

- If mooring fails or a ship drags it loose (most likely failure modes)
- Decision might be to recover as many assets as possible
- Requires that drifter know where it is and be able to communicate.
- Battery powered GPS and satellite phone is state of the art technology
- Arrange to call at intervals only when off station to reduce phone costs

But one of the things I would like to point out that you may not have thought about is the cost of doing business this way. Anytime you put a very large floating structure in the ocean and there is the risk that it can go adrift, there is a very real chance that weather conditions are such that it will be blown away from where you intended it to be, and that very large structure can end up in some nasty place.

For example, think about the entrance of some major harbor being blocked by hundreds and hundreds of big plastic bags that just lock themselves in between these two headlands and there are all these ships out there saying it is ten thousand dollars an hour and it's your liability and we will see you in court. So one thing that is really important to remember is the liability question associated with having very, very big things that you can't control.

OK, I'm just going to spend a few minutes on this and talk a little bit about risk-based engineering. So how do you design a system like this, which has many, many elements in it with different functions? You decide that you want to achieve the minimum configuration, SO you keep risk making improvements in the design and reducing the cost of failure. And the way you reduce the cost of failure is by saying it will be a longer time until it fails., You're really reducing the probability of failure, as long as the reduction in cost is greater than what you are adding to the cost of the system. This is a useful



exercise and eventually you will reach the minimum; that is the concept.

However, in this system it is very, very difficult to do this, because it's not one probability of failure; there are many, many, many probabilities that have to be dealt with and you are really dealing with the overall probability of failure of the system and so you have to-- when you make a change to the design, or improvement of the design-- you really have to consider its impact on all of these probabilities. So rather than this nice curve that I showed you in the last slide, it is really a very complex surface and the probabilities are highly intertwined.

So, in fact, for a system as complicated as this, this is a theoretical construct. You will never have enough information or enough money to really do this in a proper way.. [So] Things are designed the way they always were, component by component, and so you say I am going to design this component to last for one year, ten years, fifty years, whatever your choice is. And the result is that because of these interactions, for which you are failing to account, some of these elements are going to be too weak and many of them are going to be over-designed; that is simply the reality of a system as complicated as this! Making Engineering Decisions (2)

- $P_{\text{system}} = P_1 + P_2 + P_3 + \dots P_n$
- But P₁ depends upon the probability of big waves and P₂ depends on the probability of a collision, etc.
- So the probability of a system failure depends upon a matrix of individual probabilities

Making Engineering Decisions (3)

- There is never enough information to do this on complicated commercial systems
- There is never enough money to do this the right way
- Components are each designed to achieve an assumed service life
- The system will have weak links and some overdesigned parts

Thank you very much!

QUESTIONS:

Jonathan Trent: Wasn't that what a professor always has to say? Excellent presentation! There's time for questions. We should probably consider whether we should go on with the rest of the meeting!... No, I am kidding. Yes, Jake?

Jacob Davis: One comment and one question. I really appreciate the slide on making engineering decisions. I thought that you had some really profound observations and comments there, but I guess my question is on the suction-piled moorings. Can you -delve a little further into how that physically works and [what about] the differences between low depths and high depths for such a system?

Richard Seymour: Yes, absolutely. The assumption is that the sediment thickness at the bottom of the ocean is great enough to support the full length of this suction pile. And a suction pile is a piece of pipe with a closed cap and you simply start pumping water out of it and the pressure of the water on the outside drives it into the ground. So you don't pound on it with a pile driver, but you simply use reduced pressure on the inside to drive it. And it is very, very commonly used in the oil patch, and the Gulf of Mexico, for example. There is a lot, usually in deep ocean applications, there is plenty of sediment on the bottom to do that. That would not necessarily be true in shallow water closer to shore. But there are many types of anchors besides suction piles that would be effective in this, so you can design around whatever situation, including all rock--all rock with no sediment at all.

Jonathan Trent: I have a question and then we will have to go on. I have just been told that we're having a lunch speaker. So what we'll do is go get our lunches and then will bring them in here at some point. Is that all right? We're going to bring our lunches in here to eat? So there is a lunch speaker, so we have to move onto to the next speaker.

But I do have a question: In terms of the curve that you gave, which shows cost-per-risk issues, how does that work? In the oil industry, they are building these massive structures and they are costing a billion dollars for one of these platforms...

Richard Seymour: Oh, more than a billion dollars.

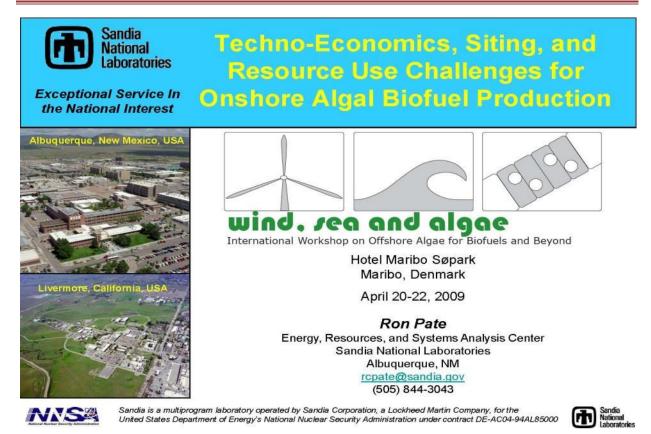
Jonathan Trent: Yes. So if you consider the investment versus the risk, do you think the kinds of problems that you were presenting could be compensated, if we were thinking in terms of the scale that the oil industry is thinking in terms of, or were you considering...

Richard Seymour: Did you see the pictures after the last hurricane, Katrina?

Jonathan Trent: Yes.

Richard Seymour: Some of those billion-dollar platforms that were on the beach or leaning over at 45 degrees had failures. Even with all the money they spent and with very, very sophisticated engineering, along comes a hurricane that is bigger than anything that we have seen before and they have a failure. That's why I say, if you live long enough, it will get you. It will.

TECHNO-ECONOMICCS, SITING AND RESOURCE USE CHALLENGES FOR ONSHORE ALGAL BIOFUEL PRODUCTION, RONALD PATE



Jonathan Trent: The next speaker is Ronald Pate from Sandia National Labs. I have heard Ron give a number of talks and he gives us THE BIG PICTURE. Ron is evaluating the whole problem of algae cultivation from a variety of different perspectives, including water, land, productivity, logistics, competition, and the prospects for producing biofuels. I'm sure he is going to give us a wonderful overview about problems and prospects.

Ronald Pate: Well thank you Jonathan for the opportunity to come here. Real quickly I have got way to many slides for the time between us and lunch so I am going to try and turn this sort of into a movie, and in fact I have pulled a few so anyway.

What I am doing is covering things at what I would call a higher systems level than from what you have seen thus far. Except for maybe the offshore engineering discussion, but it is looking from a national perspective at onshore production of algal biofuels with the focus on energy. So I will give a little bit of background on Sandia and why we care about biofuels. Some of the challenges associated with what we call the energy water nexus and then get into the promise and the challenges of algal biofuel, so having said that...

this is a quick snap shot of Sandia National laboratories, we are one of the DOE labs. Specially we are one of the nuclear weapons DOE labs. Which is different from say Al Darzins at NREL, which is an energy lab. So we have other programs, we have multiple program areas that are shown highlighted there. We started with nuclear weapons back in the days when the atomic bomb was built. We are an engineering laboratory as opposed to being a physics laboratory which is what Los Alamos and Lawrence Livermore are. So we have more of an engineering focus and we are a multiprogram we do a lot of other things now.

Biofuels fall into what we call the energy resources and non-proliferation area. Specially within that area is this: it is the fuel and water systems line of business, so our concerns here are energy security associated with transportation fuels and what else can we do besides petroleum. We are concerned a lot with water and water resources. And there is a tight coupling between water and energy and I'll talk about a little bit. This is a quick snap shot of why we care largely...

One is energy security and all of the things that we have already talked about, at least in different ways. We import a lot-- this is again centric to the united states, but it is a global problem. So we import lot of oil; which is a problem for our security. We are subject not only to the supply disruptions either man made but also the fact we are spending a lot of money to do this so it is a huge economic burden. And "Peak Oil" has been mentioned, we may be in it now it may be off a ways, it depends on supply demand dynamics but the point is, it is going to happen, and so we need to be doing things. We are seeing this increasing





Biofuels Interest & Motivation for Sandia

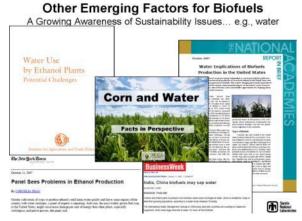
 Energy Security ... Heavy U.S. dependence on petroleum imports - Oil imports of ~10-M bbl/day (150+ B-gal/yr) two thirds for transportation fuels Subject to supply disruption from volatile regions - Represents \$400(+/-) B/yr burden on U.S. economy supports interests hostile to US - Increasing competition (China, India, etc.) & price volatility for limited global supplies - Inevitability of "Peak Oil" timing is uncertain, but long-term eventuality is not Desire for Reduced GHG Footprint Climate Change concerns make renewable biomass-based fuels attractive Potential for displacing fossil carbon fuels with more carbon-neutral fuels · Energy balance depends on systems and proces ses... not all good Energy-Water-Environment-Economy Interdependencies Need solutions to affordable & sustainable scale-up Need to ID best paths to avoid or minimize adverse impacts A Statut

concern about this green house gas foot print and the desire to somehow get away from the carbon emissions we've got now, so we need to be looking at that. And it is the energy-waterenvironment-economy nexus; all of these things are interdependent and you got to look at it as a big interdependent system or you miss the boat. If you are really focused on something in the weeds you can make mistakes.

One of the mistakes maybe corn ethanol, all right? This is an emerging factor for energy in general including an electric power supply but also biofuels which is a subset and that is it has an impact on water supply, particularly freshwater supply. So we need to be looking at things that can avoid those kinds of problems as we look at scaling up in a big way. The issue of water with biofuels is out there it is in the news, the National Academy of Sciences did a study a couple of years ago and so it's something that people are becoming more aware of.

We saw this slide already, I just put a few things on it. Food and feed versus fuel issue, that we want to try and avoid in the future. The only way you can avoid that in the future is stay clear of commodity crops that are also used for food and feed. And I would lump into that: sugar! I mean, the Brazilians with their sugar ethanol production ran into this problem years ago. So any kind of disengagement we can have with biofuels that gets us away from using ag land and using freshwater and using commodity crops is the way to go.

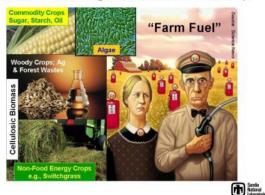
So we have this challenge: How do you scale up biofuel in a way that it is sustainable and by the way at least from the United States/DOE's perspective will have a major impact on fuel supplies. Because if you do this you could do something that is sustainable but it may only have some small market niche, well that does not help us.



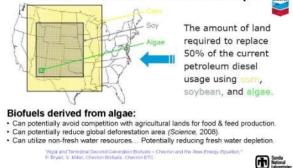
Other Emerging Factors for Biofuels More Sustainability Issues... food & feed vs. fuel



Biofuels Challenge: Sustainable Scale-Up

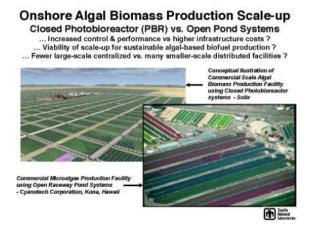


So the promise of algae. Everyone has talked about it. It has been hammered but I'll do it again. The productivity potential for algae, and again I'll stress that it is potential, it's not in practice yet, shows graphically here that you can do a lot with a whole lot less of a foot print. And that foot print is not just land it can be other things as well. Here is an example showing corn versus soy versus algae for displacing 50% of petroleum based diesel. And I can get into more detail later looking at this. The Promise of Algae: Potential for much more productive oil feedstock source for biofuels than conventional oil crops



So again the overall cartoon is we would like to be able to use non-fresh water and again I am thinking in terms of on shore here. So looking at brackish ground water, produced water from oil, gas and coal bed methane production, desal concentrate which is becoming something that is a waste stream. Ag waste water all these waste waters, the waste CO2 and the waste heat from industrial sources, all of this with either open closed or some kind of hybrid system, that will allow you to either use autotrophic production, using sunlight and photosynthesis or heterotrophic using organic carbon where you do not need the sunlight; either way to make fuel. And I want to point out something. Biodiesel has been talked about, green diesel has been talked about but the reality is there are techniques that are being developed. We are part of a DARPA team that is helping do this: to convert oils from any source, animal vegetable into aviation fuel, green diesel; its equivalent to petroleum based diesel and that's a done deal that is happening. It is a technology that works. So you don't have to be doing biodiesel the fact of the matter is the incremental cost of doing that is tiny compared to the feed stock cost. So the problem again is the feed stock, where are you going to get the oil? Which get us back to the algae. So we have seen this already, Solix...

The point is, one of the concepts for doing this on land if you are going to do it in a big centralized way, your closed versus open systems; the reality is it maybe some combination of those.



These are some really fundamental questions. Can it be done affordably? Can it be scaled up and/or replicated widely at smaller scales? So that the aggregate is a big deal and makes a significant difference in terms of fuel supply. Can it be done sustainably in terms of our resource use and utilization?

And I am going to get into one of the technoeconomic--what the status is of today as we know it? The best technologies and systems, pathways? Don't know, frankly. What are the key input resource issues? We'll talk about that.

The point is I am about to launch into the analysis that we have done along with NREL partners, a lot of this was done within the past year, over the past two years really. Looking at algae –part of our support for the DOE. And so it involves looking at this NEXUS here:

This is what we are calling Techno-Economic Modeling and Analysis, so it's looking at the economics, it's looking at the systems engineering and technology issues, and it is looking at policy. It is really that intersection that is the whole point, to support decision making. And we are not there yet, by the way, it is a work in progress.

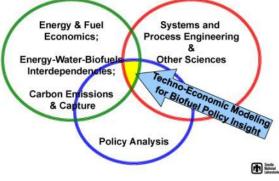
Fundamental Questions for Algal Biofuels from a National Energy Perspective

- Can it be made affordable?
- · Can it be scaled up and/or widely replicated?
- Can aggregate national capacity be built that provides sufficient biofuel volume to significantly displace petroleum-based fuels?
- Can it be done sustainably in terms of resource utilization and environmental impacts?
- · What is the current techno-economic status?
- · What are best technologies/systems/processes pathways?
- What are key input resource issues, resource demand consequences, and possible constraints for algal biofuel production scale-up?

Focus on Recent Work By Sandia, NREL, and Others on Techno-Economic Modeling & Analysis to Provide Insight and Guidance

- Assess technical performance & cost/benefit tradeoffs among different technologies, systems, and processes
- · Assess economic impact of R&D strategies & investments
- · Assess environmental impact of R&D strategies & investments
- Assess consequences & constraints of alternative pathways for algal feedstock, biofuels, & coproducts industry build-up
- Inform R&D and business development investment decisions
- · Inform policy decisions ... explore "what if " scenarios
- ... new work in-progress with only limited results
- ... lacks detailed enterprise-level project economics
- ... limited technical pathways and options considered 👔





But these are the goals, to be able to asses the technological and economic performance of a pathway you maybe taking based on what you know about that technology. And you need again to be taking the big system, the system of systems perspective and look at the economic impact of different technologies or strategies for research that could come into play. Look at the environmental impact and the strategies for investment based on that. And just play 'what if' games to be able to inform—not only research and development investment questions or issues—but also policy.

This is just a diagram to suggest that this is not a static problem; it is a dynamic interdependent problem. And for that reason we, at Sandia, have been using what is called system dynamics modeling as a mean of attacking a complex problem like this because it is very difficult to do it with other techniques. But the point is we've got-on the far left hand side we have all the inputs, including the algae itself, and for inputs we have all of the siting issues and the climate, and resource, you know, solar insulation and that kind of issue that comes into to play. Whether you are going to have to supply nutrients or whether it's

going to be available naturally with whatever water you are using, what kind of algae whether it is a GMO or otherwise. All the various multiple ways you could do the cultivation, different ways you could do the post processing, harvesting, de-watering, extraction, and then the bifurcation there to create either fuel or other things. And fuels can be anything from an oil-based fuel, the higher value, the higher energy density fuels to ethanol to biogas. And then the co-products. So all of these feedbacks really need be brought into play.

Own.

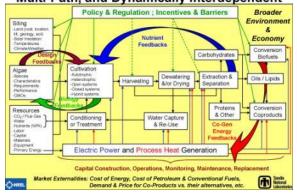
Purpose and Goals of Techno-Economic Modeling & Analysis

- Assess technical performance & cost/benefit tradeoffs among different technologies, systems, and processes
- Assess economic impact of R&D strategies & investments
- Assess environmental impact of R&D strategies & investments
- Assess consequences & constraints of alternative pathways for algal feedstock, biofuels, & coproducts industry build-up
- Inform R&D and business development investment decisions

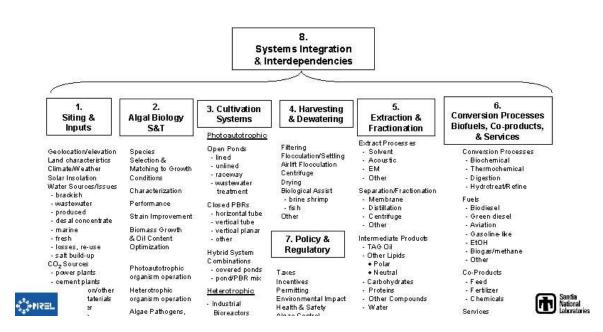
Carthe Sands Sectored

Inform policy decisions ... explore "what if " scenarios

Algal Biofuel Value Chain is Complex, Multi-Path, and Dynamically Interdependent



Elements and Issues for Techno-Economic Assessment of Algae Biomass Feedstock, Fuels, & Co-Products



These are some of the issues that we came up with, the key elements and issues that one needs to factor in when you are going to attack a problem like this. So this is sort of the frame work and you can imagine you could have a critical path with a whole bunch of different things there and then you could have multiple critical paths depending on what approaches you want to compare and contrast.

Complementary Levels and Approaches for Techno-Economic Assessment of Algae BioFuels & Co-Products

Higher-Level Dynamic "Meta-Systems" Modeling (Integrated Analysis Framework) Broad value-chain scope ... from resources & siting through production to end use Algae biofuel and co-products industry scale-up potential, resource use, constraints & impacts - Input resources, output flows, waste stream resource capture & reuse, co-generation - Integration with existing infrastructure - Required build-up of new infrastructure ... with time delays... with learning curves & improvement projections - Technical, economic, environmental, and policy issues Feedbacks and Multiple Sector Interdependencies ... can link to other models & analyses Static CAPEX & OPEX Calculations Process Flow Engineering Modeling Detailed process flow diagrams and data System and process equipment cost estimates Mass & energy balance calculations (ASPEN+) - Vendor/Supplier cost estimates Process GHG footprint (LCA) assessment Engineering and construction cost estimates - Engineering & construction estimates from industry - ICARUS cost estimate software (or equivalent) Geographic Information System Operations & Maintenance cost estimates (GIS) Analysis and Visualization · Discounted cash flow analysis · Land resources (characteristics, availability, etc.) Cost of biofuel feedstock production · Water resources (fresh, wastewater, other) · Cost (& offsets) of co-product feedstock production · Cost of biofuel production Solar resource (insolation) Climate/Weather/Temperature Conditions Cost (& offsets) of co-product production Water evaporation loss Carbon footprint cost accounting CO2 resources (point source emitters, pipelines) · Fuel processing, transport, storage infrastructure Other LCA Modeling Other infrastructure and environmental features GREET (Argonne National Lab) LEM (UC/Davis) Etc. Sandia National

So there are different ways also to do this modeling and analysis. This is just a snap shot. Again at the very high level we use systems dynamics—if you really know what you're doing and have a very detailed understanding of the systems and the processes; at what I would call the enterprise level you can use things like Aspen modeling, chemical process engineering modeling. So it depends on what you are doing.

Then there is the factor of what are the evaluation objectives. I just throw this up there to point out the way you make decisions with this sort of analysis depends on where you are trying to go. In the case of DOE wanting to invest in technology development it may be minimizing investment needed to reach the desired production volumes. So again it depends on what your goals are.

This is an analysis we did based on taking open literature information and some of our own internal work at NREL and Sandia and a few other University groups who were willing to share to look at what we think the state of the art is in terms of..., the state of the technology in terms of what is publicly out there. This does not include things that are being worked on by companies that keep everything very proprietary.

Must Establish Technical Evaluation Criteria and Objective Functions

Comparative T- E analysis results depend on metrics used ! ... for example:

- Minimize Capital Costs per unit of biofuel
 Minimize Operating Costs per unit of biofuel
 Cost \$/gal
- Maximize Biofuel Production Yield
- Minimize net GHG Footprint per unit of biofuel produced
- Maximize net Energy Balance
- Minimize net Water Usage
- · Minimize Land Footprint per unit of biofuel produced
- · Minimize Time Required to reach desired production volume
- Minimize Investment Needed to reach desired production

¢m.	volumes of algai reedstock, biofuels, and coproducts	Anthe Relation

Ami Ben-Amotz, has been....it is a wonderful thing to hear his presentations the last couple of years, because he has actually revealed a lot of detail from the perspective of somebody really doing this at large scale that is sort of sparse information in the overall environment. This is a sampling of the sources of information; Ami is in there as well. And this is kind of the outcome:

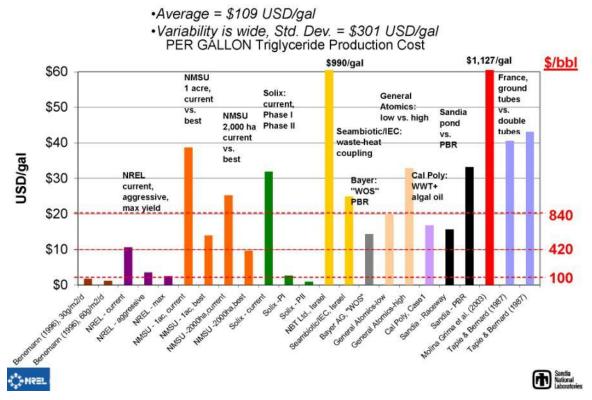
Selected Sources for Baseline Algal Biofuel Analysis and Comparative Cost Assessment

Bource	Authors	Year	Reference	
	Malt Ringer			
NREL	Bob Wallace	2006	Analysis completed for this exercise	
	Phil Pienkos		- 18 - 18	
NMSU	Meghan Starbuck			
NMSU	Pote Lammers		Analysis completed for this exercise	
Golia	Bryan Willson	2006	2nd Bundes-Algen-Stammtisch	
Seambiolics	Ami Ben-Amotz, Israel	2007-2008	Algae Biomass Summit	
Sendia	Ben Wu	2007	Analysis completed for this exercise	
Bayer	Ulrich Steiner	2008	European White Biotechnology Summit	
General Atomics	David Hazlebeck	2008	Algae Biomass Sommit	
California Polytechnic Institute	Tryg Lundquist	2968	Algae Biomass Summit	
	E. Molina Grima	2003	Biotechnol. Adr. (2003) 20:491-615	
	E. Belarbi			
University of Almeria	F. Fernandez			
	A. Medina			
	Y. Chisti			
Association pour la	P. Taple	-		
Recharche en Bioenergie	A. Bernant	1968	Biotech, Bioeng. (1988) 32:873-885	
University of California	John Benemann	1990	PETC Final Report	
conversely of california	William Osvald	1000		

Ch State

This is the range of US dollar cost per gallon for algal oil that came out of the analysis of this various range of sources.

Standardized Algal Oil Cost Comparison



The point is we have dollars per barrel over there in the red on the right hand side. You can see we kind of have a fundamental problem in being competitive; cost competitive right now, with petroleum based fuel. And I should say, the three order of magnitude spread here.. I think it is really more like we are in the 10 dollar to 100 dollar per gallon cost range. But then there are some outliers.

So the problem partly is there is a bunch of different assumptions and different levels of detail provided by these sources, so this just points that out. There is a lot of uncertainty in terms of the various categories you could break this down... so the price per gallon of the TAG oil for instance, the standard deviation is far higher than the average as you can see. Like wise for the facilities and the various operating costs. A couple of ways that were pointed out as being ways to get these costs down:

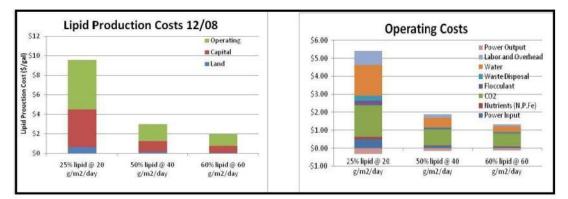
Example: CSU / Solix Projected Cost Reductions via Improved Systems and Processes Integration



Courtesy of Bryan Willson CSU/ Solix, if they commercialize today based on what they know, 33 dollars a gallon, for tag oil; but just by doing a really good job of systems engineering to integrate things, perhaps get a factor of ten drop in that almost immediately; without a lot of technical break through, so that is one approach.

Another approach is just to improve the performance of the algae production, productivity. And this is an example from NREL making that kind of projection based on improvement in productivity.

NREL Model Suggests* Major Cost Reductions Available by Increasing Biological Productivity



*NREL Spreadsheet model based on updated assessment of process design proposed by Benemann and Oswald.

Courtesy of Phil Pienkos from NREL



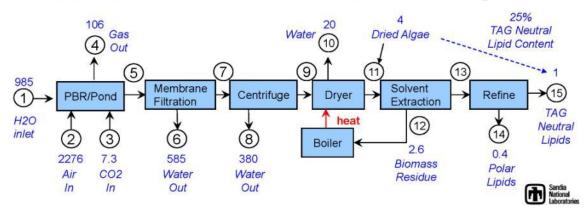
So we take a more detailed look at the systems and this is an analysis that Sandia did about 2 years ago and it is based on just getting a baseline--something put together. So this is just a value chain if you will, for producing algal biofuel. Not biofuel but crude oil; crude algal oil for biofuel feed stock. Either using a closed or open pond system and then running through the various processes. And there are a lot of assumptions and hand waves but this is just to point out some of the problem areas.

This is a mass flow for a potential scale up to 50 Mega-gallons per year. Assuming a productivity of about 3,000 gallons per acre 25% TAG content in the fuel and this is showing the mass balance for water content and CO2 when it is normalized to say one kilogram of tag oil or one pound.. whatever unit you... its unit less but it is normalized. So in other words it is going to take a lot of, it is going to take 985 units of mass of water on the input side to get that one unit of TAG on the output side. SO that is just so you understand this. The point is there is going to be significant water loss with an open pond system, there is going to be significant water loss for cooling if it is a closed system and you are doing active cooling.

O-NREL

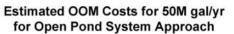
Preliminary Estimate of Algal Oil Production Mass Balance at Scale-Up to 50-Mgal/year

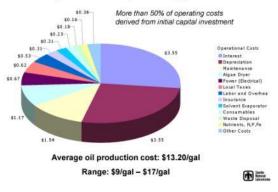
- Capture 75% carbon from 1 GW power plant in daylight
- 68 km² (16,796-Ac) to produce 50 million gallons/year of algal oil (TAG)
- Productivity ~2977 gal/Ac at 25% neutral lipid TAG content
- Significant evaporative water loss for cooling PBR ≥ 300:1 (H₂O:oil)
- Significant evaporative water loss with open ponds ≥ 300:1 (H₂O:oil)



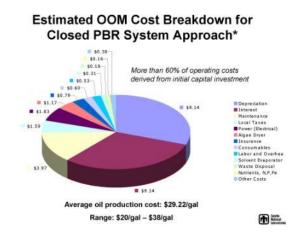
This is looking at the energy balance, and this simply points out some of the problem areas if you have to do cooling with a closed system. This particular model suggests that you are going to have to burn up 4 times the energy content of the oil you are going to get out. Probably not a way to go. Likewise, if you have to drying and you do not have enough feed back for heat control or process heat through the residue; you are going to be loosing a lot of your energy balance.

These are some rough numbers that we came up with for the open system approach; it ranges from about nine to 17 dollars a gallon, about twice that for the closed system approach.





There are some assumptions in here that make this, ...there is a lot of looseness..., we probably did not do enough enhancement with the productivity with the closed system, but at the same time we probably underestimated the cost of the closed system. So again these are to be used with caution, but this gives you a range based on some real detailed work we did.



Summary of Algal Oil Production Order-of-Magnitude (OOM) Cost Estimate

Basis

- Costs taken from Benemann and Oswald report (1996) and adjusted to 2008 dollars
- Balance of plant costs¹ estimated from Peters et al. (2002)² based on historical data from petrochemical industry
- Cost of land neglected, and no credit for co-products applied

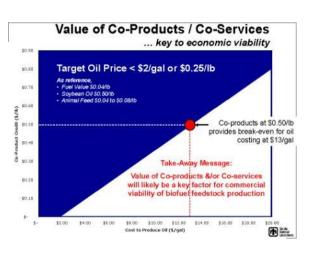
Caveat

Estimates based on limited data so should only be used with caution

Reactor Type	Oil Cost (\$/gal)		
Open Pond	9 - 17		
PBR (\$10/m ²) ³	20 - 38		

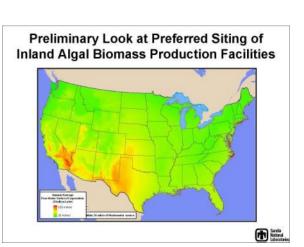
Costs are (1) equipment costs - purchased off-the-shell from vendors, and (2) balance of plant, or everything else
 installation, infrastructure, utilities, controls, et al.
 2 hard Design and Economics For Chemical Engineers by <u>Nac S Peters, Slave D Timmerhaus, Bonald E. Viest</u>, 2002).
 3 Aggressive cost estimate based on thin plastic material PBR approaches that may be scaleable.

The point is here that co-products and co-service value needs to be captured and it is a huge factor for the overall economics.



So this is the challenge, getting from where we are today, approximately, somewhere in the 10-100 dollar/gallon range for oil, getting it down to something that is cost effective; and you got to factor in the value of other co-products and services, but you got a list of things that need work. And some of that has been detailed already.

This is taking a look, if were going to try and site where you would grow inland algae in the United States. What I was showing there was solar resource, temperature ranges; this is looking at municipal waste water treatment; this is looking at fossil fuel emitters; this is looking at sunshine more than 2800 hours of sunshine annually; this is looking at temperatures greater than 55 F annually; days without freeze greater than 200. So you start getting a localization of where you might want to... this is municipal water supply again, fossil fuel emitters in that region, and this is fossil



fuel emitters within twenty miles of the municipal water. So this is beginning to say, ok if you were going to build inland facilities today within the continental United States; you might be looking at this region, based on all those overlay of factors.

Let me go back. This is a quick look at, this is notional, ok, we're assuming...lets scale up to twenty billion gallons of algal oil production per year, 50 billion, a 100 billion. To put that into perspective someone was using cubic meters of oil, that drives me nuts because I cant equate that, but twenty billion gallons per year is about; that would basically satisfy about half of our diesel production, excuse me half of our transportation diesel use, in the United States, 50 would handle all of it. A 100 billion starts to get into the range of all of our fuel; it is short by a factor of two maybe, but it is starting to get into the range, we



use about 140 billion gallons of gasoline and an about 40 some odd billion gallons of

* For the contiguous 48 States

Cost/gal

Past / Current 10 - 100 \$/gal

Algal Oil

10 y

· Algal strain selection / improvem

CO₂ source/infrastructure/cost
 Biomass/oil productivity & reliability

Oil feedstock yield, properties
 Installed system capital costs
 Production system O&M costs

Production systems (Ponds? PBRs?)

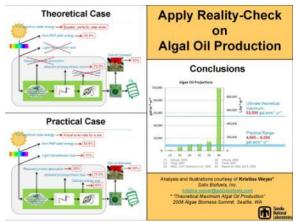
· Harvesting & dewatering processes

> Retional National

Oil extraction & separation process

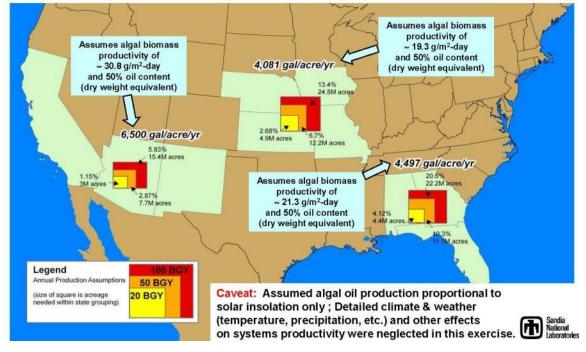
transportation diesel, 60 billion gallons of total diesel, that includes not just transport but fixed facilities.

So again we start with the sunshine, we assume this is what is driving the whole process, we apply here the reality-check on the algal oil production that one of Brian Wilson colleague's did last year. And she estimated that a realistic band for--and it is an optimistic band by the way-- for productivity, is somewhere in the 5,000 to 6,500 gallons per acre per year range. And you can quipple with these numbers. It could be half of that today, probably is half of that today if not less. It could be greater if you really were lucky and did a lot of optimization. This is something that maybe



realizable based on some decent analysis. So we said okay lets use those numbers and lets assume that we take the high solar resource region of the Southwest in the United States.

Consider Three "Notional" Scale-Up Scenarios for Initial Look at Algal Biofuel Production Resource Requirements and Implications



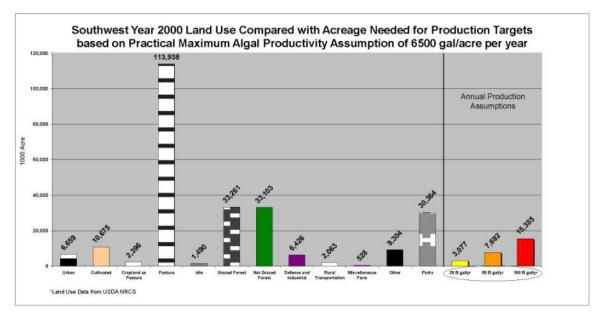
What would be the footprint of land required to achieve those three scenarios of 20 billion, 50 billion, and 100 billion. This is the result you see. We also looked at the central region, the four

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

state region there, and we looked at the far South East region. We made no assumption about the loss of productivity due to temperature effects. This is strictly based on sunlight so it is a very optimistic thing. Realistically if you go to the Midwest you are going to see temperature effects and seasonal variations that will cause those productivities to probably be less. It just gets colder. But anyway given that, we have three million acres as a footprint to achieve 20 billion gallons pr. year, in the Southwest and then you see the range going up, by the way these are calibrated that the areas represented by these rectangles are more or less the area that would be required relative to the size of the United States. So the productivities that are shown in the boxes are what was assumed. Fifty percent oil content on dry weight equivalent bases.

So what does this mean in terms of land that is actually available in the region?

Southwest Region Scenario Land Footprint Consequences Compared with Land Usage*



* Total Combined Land by Category in CA, AZ, and NM



This is looking at the Southwest again and you see the pillars in the far right in those same colors. And this is comparing that land requirement to what is the actual land availability and use in the three states in the Southwest region. And you see we have a lot of pasture land, and we have a good deal of other ag land that is not high, real high value agriculture but could be useful for this.

So the point is—well, there is probably plenty of land. There is not necessarily a show stopper there. This is for the Midwest same kind, different profile, but same kind of conclusion. This is for the Southeast, here it is a little tighter if you start getting to the really high productivities because the green bar is for forest, forest coverage.

Now let us take a look at CO2 sources, we assume now that we want to do CO2 enhancement of the growth. And we assume for this analysis that it is about a two to one factor, in other words two kilograms of CO2 for every kilogram of dry weight equivalent of biomass which is maybe a little high, it is somewhere between one and two. We chose two.

This is a breakdown of what those CO2 emitter sources are.

And this is now looking at what the CO2 requirements are for those productivity scenarios. In the southwest compared with what are the CO2 emitter sources available in that three state region. Now do you see a problem here? Ok, there is a logistical disconnect between what is available and what is needed, likewise for the central state region; like wise for the southeast region. Now caveat, we did not take into account taking the residue of the biomass after you have taken the oil out; doing something with that like digestion, burning that and then using that CO2 to enhance

this. This is really a zero order analysis but it is indicative of what needs to be looked at. I would suggest this sort of thing needs to be looked at for the offshore.

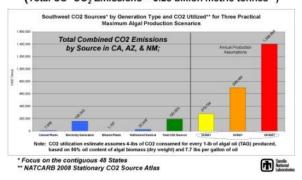


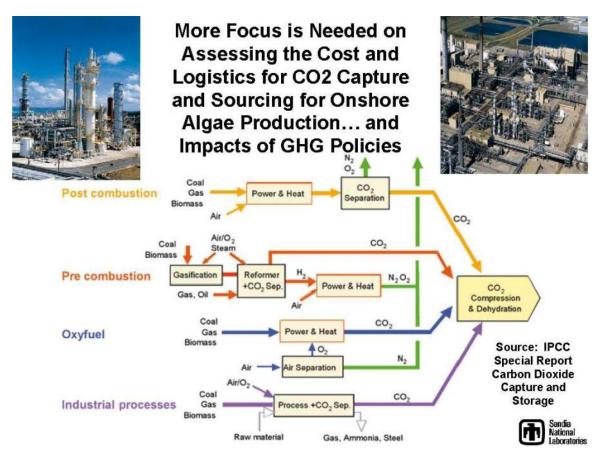


Identified Stationary CO₂ Sources from NATCARB 2008 Stationary CO₂ Source Atlas

Total	3,276.1	4,796	
Refineries/Chemical	196.9	173	
Petroleum and Natural Gas Processing	90.2	475	
Other	3.6	53	
Industrial	141.9	665	
Fertilizer	7.0	13	
Ethanol Plants	41.3	163	
Electricity Generation	2,702.5	3,002	
Cement Plants	86.3	112	
Ag Processing	6.3	140	
CATEGORY	CO ₂ EMISSIONS Million Metric Ton/Year	Number of Sources	

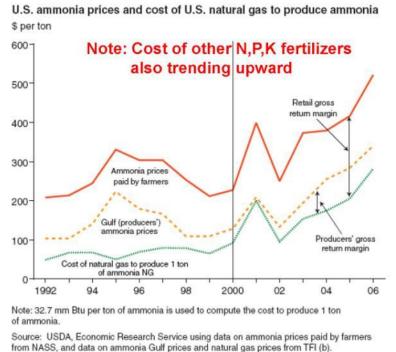
Southwest Region Scenario CO₂ Usage Consequences vs. CO₂ Source Constraints (*Total US* CO₂ Emissions ~* 3.28 billion metric tonnes**)





This is getting to the point that there has been to much hand waving about the sourcing of CO2 for these things. It is not a trivial exercise. Ami made the point that it works well based on using flue gas for what they have done in Israel. Now think in terms of actually scaling this up on a massive scale where you are intruding on the operation of a power plant. It may or may not be easy to retrofit this in a massive way, and if you can not grow immediately adjacent to the power plant you are going to have to move the CO2 to where you can do it. That infrastructure is not going to be cheap, and so that needs to be looked at. In the United States there is a lot of talk about potential cap and trade or carbon tax which could actually... and a sequestration program where this kind of infrastructure could actually get built. Algae production could piggy back off of that if it happens. But that is a huge political and economic issue that is going to play out over a long period of time I think.

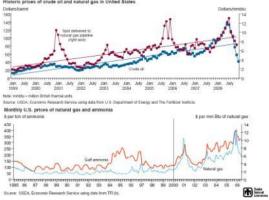
Scale-Up of Algae Biomass Production will Require Other Nutrients (N, P, K) That Are Subject to Increasing Costs Linked to Energy Prices and to Imported Fertilizer Supplies





Okay, fertilizer has been mentioned. I wanted to point it out again, that growing algae in a major scale-up way will cause an issue with fertilizer. If you cant use, and this is an argument for reusing, for capturing waste nutrients in waste water, the cost for nitrogen fertilizer has been trending up and it is the same with the others as well.

Potassium and Phosphate, and this is just showing how ammonia, which is based on natural gas for production has been trending up over the years. And it follows also the price of oil. So that is a factor that also needs to be included in the thinking in terms of major scale-up to the volumes we want. e.g., Ammonia Costs Track with Gas/Oil... Trending Upward !

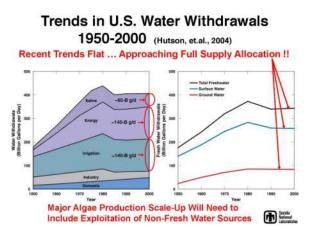


Getting back to the scenarios, lets look at water. This is horizontal pan evaporation for the united states. So this is kind of a parameter that we can use to estimate what kind of water loss one might have for open systems.

This is the result for the southwest region. If you assume open systems with pan evaporation loss as the factor, factored in over those acreages that were required to grow it. You see that the water that is going to be lost through evaporation is on the three bars on the right hand side, the water use in those three states in the southwest region is shown on the left hand side. The obvious thing is that there is a lot of water here. And especially for the really high productivity scenario you are approaching the same amount of water that is used for irrigation throughout those three states. And if you know anything about the western US

and the southwest in particular water is for fighting and whiskey is for drinking. So you are not going to be able to do this with fresh water. This is for the central state region I might add that a lot of their water comes from pumping ground water; the "Aquilolla" aquifer, which is a big problem in terms of sustainability. And then in the southeast. So water is going to be an issue here one caveat is that the pan evaporation is probably the worst case. It probably is going to be less than that. And if it is salty water that you are dealing with, like brackish ground water, it is going to be less, because the evaporative loss there will be less.

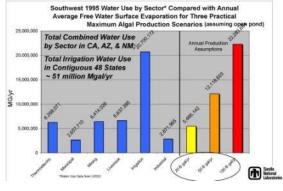
But this is just making the point; hammering it in again that regarding fresh water supplies. This is looking at surface and ground water we are already getting maxed out on our allocation in the US so we can not be looking at using fresh water for this.





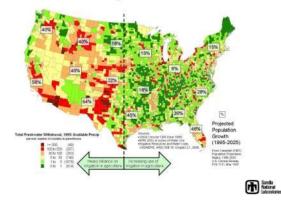
Factor In Evaporative Water Loss

Southwest Region Scenario Water Loss Consequences vs. 1995 Water Use by Sector



This is just showing that it is a stress factor. Everything that is red is right now unsustainable use of water in the US. And you will notice it is not just a Western problem it is also East, Southeast, around the great lakes, it is places you would not expect. And the over pumping of ground water is represented here.

That is just pointing out that we need to be looking at things like this, the ability to co-locate and using waste waters from various sources. That is also a reuse of nutrients which is going to be important. Water challenges are nationwide





This is produced water from oil and gas wells, coal bed, methane that is another potential source. And this is looking at that all overlaying with the brackish ground water aquifers that could be a source. The problem is we do not have good characterization of those. Distribution of Non-Fresh Produced Water, Saline Aquifers, and CO₂ Emitter Sources



Summarizing, we can use a lot less land potentially if we can realize this in an affordable way. Water loss from inland production is going to be an issue depending on where it is. We need to look at the past to mitigate this water loss in particular. And onshore coastal and offshore production are ways to do that. If we can work with marine water that would be great. CO2 sourcing and distribution is going to be a big deal for inland. I think the jury is out on how to do that and what the CO2 issue is going to be for the offshore and the availability of nutrients besides

Summary Observations

- Land footprint required for national scale-up of significant algae biofuel production looks manageable.
- Water loss from inland open algae production systems is likely to be an issue for massive scale-up
- · Need paths & approaches to mitigate water loss
- Closed systems and location of open systems in less arid environments
 Onshore coastal & Offshore production options using ocean water
- CO₂ sourcing and distribution for algae is a key issue
- Availability and cost of other nutrients (N,P,K) is an issue
 Need to exploit capture, recovery & reuse of nutrients from wastewater, etc.
- Salt & thermal management are inland systems issues
 Need to identify and exploit geographically-distributed opportunities for synergistic co-location of algae biofuels production with water treatment, power generation, and other
- co-product industries and markets
 Offshore options worthy of further pursuit to assess opportunities and constraints compared to onshore

CO2 is there. Salt management and thermal management are issues I did not dwell on but they will be a big factor on inland systems. Let's see ...what else. So to bring this to the offshore situation we need to be doing offshore if nothing else then to basically mitigate some of the problems we see with onshore.

Some acknowledgement of contributors.

That's it, thank you.



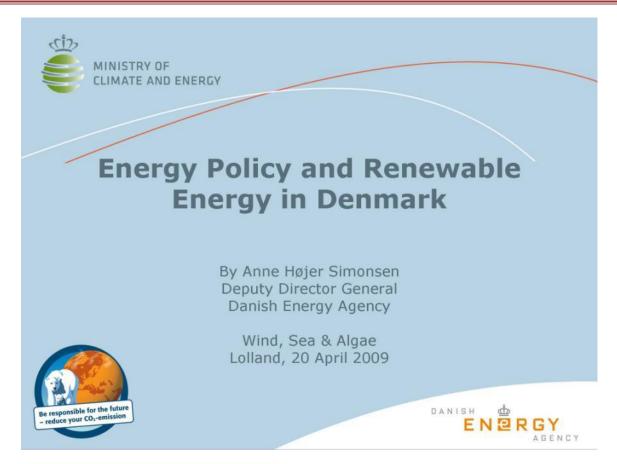
+ numerous others in U.S. government, academia & industry

Thank you ! Questions ?

Anternal Sectoral

Jonathan: Thanks Ron. I am sorry, but we don't have time for questions. There will be plenty of time for discussion. This whole afternoon is going to be an interactive session. Please go get your lunch and bring it back here, we are going to have a lecture by Anne Højer Simonsen about the green revolution in Denmark!

ENERGY POLICY AND RENEWABLE ENERGY IN DENMARK, ANNE HØJER SIMONSEN



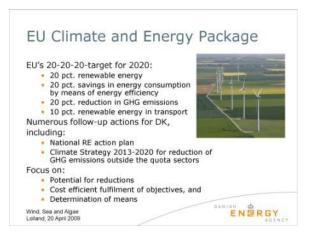
Anders Riel Muller: Anne Højer Simonsen comes from the Danish Energy Agency under The Danish Ministry of Climate and Energy and has over 14 years experience in this field. Welcome!

Anne Højer Simonsen: I'll try to give you a helicopter perspective on Danish energy policy to point out where new energy technologies and also algae fit into the picture. I'm from the ministry for climate and energy and we have one focus in December 2009 in Copenhagen, to reach a new global agreement on climate. This means that everybody in our organization knows what he or she works for this year. They work for a secure, efficient, and environmental-friendly energy policy. And no matter which one of the 250 Danish Energy Agency employees you wake up in the middle of the night, they know that answer. Algae, in that perspective, is perhaps a new way of having a cost-efficient and secure source of energy that could be produced in DK or in the EU, and this why your work is very interesting.

The climate summit: we want a binding global climate agreement on reducing carbon dioxide emissions. The Danish government has committed itself very much to this target, to include as many countries as possible, also to include adaptation to climate change. Adaptation is necessary, because some countries are big emitters and some countries suffer due to emissions. And to have everybody as part of a big agreement, you need to have something in it for everybody. It goes without saying that Denmark is a country that needs to cut carbon dioxide emissions and transfer means to countries that need to adjust to climate change.

The first step up this ladder is an EU agreement on energy and climate. This is necessary because we are among the main reasons for this global problem, but also we have front runners. We have the best economies to retrofit into a more climate friendly society, and therefore we have to take the front run here. And this means that the Danish government works very, very, specifically to have an EU climate and energy package. It was decided in December 2008, and all countries within the EU 17 has committed themselves to a 20% renewable energy, 20% energy savings as compared to what would be





the development otherwise, and 20% reduction in carbon dioxide emissions in 2020. So it's twenty, twenty, twenty in twenty, it's very easy to remember. Denmark, as a front-runner in renewable energy, has committed itself to thirty, 30% renewable energy in 2020. We will reach 20% already in 2011.

Another feature of this EU agreement is that if the world succeeds and we have a global climate agreement, the EU will take a 30% cut of carbon dioxide emissions, and that means that we promise the rest of the world, if you will play with us in this field, we will commit ourselves even more than we have done unilaterally. There are also a lot of follow-up actions for Denmark and others in the years to come. We all have to make a national renewable energy action plan that we have to submit to the commission to show that in 2020 we'll be able to reach the target. We will make a climate strategy for reduction outside the quota sectors, which will include reduction of carbon dioxide in transportation, in farming, and in buildings. These are the three main emitting sectors that are not included in the carbon dioxide quota emissions trading system today. So we are right now trying to make strategies for these sectors, and it's not easy because it's a lot of diffused sources for emissions and it's much more difficult to target than, for example, power plants and so on.

So the headline in EU is promoting renewable energy, a new emission trading scheme, reductions in carbon dioxide emissions outside the quota trading scheme, and a new possibility of also carbon capture and storage, that we have to look into.

EU Climate and Energy Package

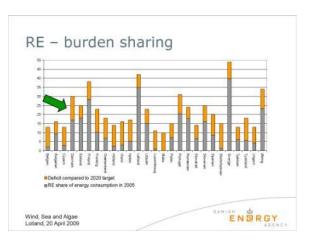
- Promoting renewable energy sources
- Amendments to EU emission trading system directive
- Reduction in GHG emissions outside the quota sectors
- Carbon capture and storage



This is supported by the strategic energy review in the EU, which has more focus on the security of supply. In the EU we are very much dependent on imported oil and imported natural gas, and this is not a situation that is very good for anybody. So besides climate and energy focus and liberalization also of energy markets, there's focus on security of supplies and in Denmark we are right now trying to sketch also a national strategy for security of supply; it goes without saying that it is coordinated closely with climate policies, too.

The renewable energy burden sharing is shown. Denmark has committed itself to 30% by 2020. It's a very large amount and will require a large increase. We are not the top runners; the top runners are countries with natural hydro-power resources or vast biomass resources. May I note, Denmark has none of these resources—no hydro-power and only limited biomass production capacity on land, which is where your activities come into the picture.



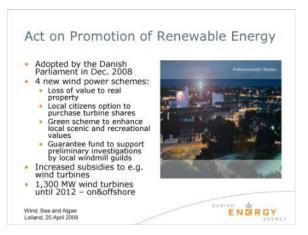


In Denmark, to facilitate the EU agreement in December 2008, in February 2008 the government made an agreement with all but one of the parties in the parliament on a Danish energy policy. This was necessary to agree on what to work for in the EU context; and what we agreed on were renewable targets, but also very importantly, energy saving measures; and I believe that we are the first country in the world, not to decide constant energy consumption but to actually decide to cut energy consumption, as compared to today. This is unique and it's very ambitious. So, it will be difficult for us to



actually make it. The 2011 energy efficiency goal is very difficult to make for us, but we hope the best. The long term vision of the Danish government is that we shall become totally independent of fossil fuels. Is that possible? Well, we are looking into that in different sectors, and we have a climate commission in Denmark that by the end of 2010 will report their roadmap that will sketch how we could reach this. It's secret what they are doing right now. So, I don't know the answer but we believe within the Danish Energy Agency that it IS possible; it's just a matter of the year. Whether it's 2075 or the 21st century, we don't know yet. Another goal is to reduce the use of fossil fuels by at least 15% by the year 2025. This is not very ambitious when you compare to some of the other targets that we have, but, as we all know, fossil fuels in transportation is a specific issue, so this is one important target when we consider what to do about transportation; we need to get something but fossil fuels into transportations.

One of the things politicians agreed upon, on our focus of renewable energy, was the renewable energy act; it's mostly about wind energy on shore. It introduced four new instruments namely that you can be compensated as a neighbor to a wind turbine, if you suffer a loss of property value; That local citizens must be offered a share in wind turbines; That there was established a green scheme where the municipalities, that is the local authorities, can apply for means to make compensating activities, for example, new nature values or recreational values can be kept when you



put up turbines. Last but not least, a guarantee fund, so that local citizens, can try to make their own wind turbine projects, and the preliminary investigations into that - there's a guarantee if it will not end up with a wind turbine project that they don't lose their own money. So it's a way to try to enhance local commitment to onshore wind turbines.

But still, we need more than onshore wind turbines to fulfill these ambitious targets.

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

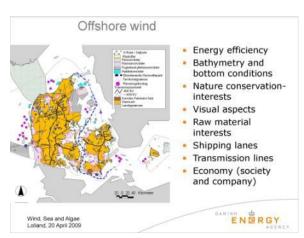
...and in Denmark we went off shore a lot of years ago, actually eighteen years ago we started with *Vindeby*, that's close to where you are now and I believe, that you're going to see it in one of the coming days. And today we are one of the largest offshore wind turbine countries in the world with 424 megawatts up and running. I think the UK has just beaten us this winter, but DK is still a world leader in offshore wind capacity pr. capita. We have in the energy policy agreement improved the feed in tariffs for local projects, and the basis for this was a strategic and environmental resource assessment of



all offshore wind resources in Denmark. I'll come back to that in a minute. But what we see now is that in the coming years we will have another park next to this one of 200 megawatts by this summer. Next summer we will have another one close to where we are now, another 200 megawatts, and right now we are planning for a new park on 400 megawatts. And it is possible, when that is up and running in 2012, that it will be the largest in the world. We know that other countries have larger projects, but they're in trouble and we are known for one thing in Denmark, and that is that we're on schedule with our offshore wind turbines, once we decided it.

So I very much hope that we will make a world record here. We will open a tendering process this week and we have made a whole new procedure where we take out even more risk than earlier in this process by securing that the environmental assessment is already made and known when you have to make your final tender, which means that the insecurities will be very limited as compared to other places in the world. So if everything goes well, in 2012, we will have 1300 MW offshore, perhaps the largest amount in the world.

When we plan offshore wind in Denmark, we look of course on energy efficiency, but we also look on a lot of other conditions, impact on nature, visual aspects, raw material interests, shipping lanes, transmission lines and economies in grids. And this is the result of the resource mapping of offshore wind in Denmark. All those pink dots represent 4600 MW of possible offshore wind localizations where there are no severe problems in placing them. It is all right in considering which grid costs it will be offshore and onshore. It will not destroy any major natural interests. It will not prohibit important fisheries and



so on. So these areas are places where it is good to place wind turbines. And as you see Anholt Windturbine Park for 2012 is here and it was located in this investigation. [13:28] We have an offshore wind turbine committee in Denmark that we chair in the Danish Energy agency and it consists of all relevant authorities and companies so we simply bring everybody together, make a lot of maps of Denmark showing the different interest, put them on top of each other and then map the accessible wind resources so we can sort of "reserve" the best spots

A picture of Horns Reef II; this is the accommodation module for Horns Reef II. It will be the first offshore wind turbine park in the world with accommodation and that is because it is very expensive when the turbines are not up and running when you have a very big park of 200 MW, they are far from the coast and it is difficult and expensive to sail people out there all the time. This accommodation platform will be just next to the turbines and it will have a helicopter platform on top of it so you can bring people in and out and then they can sail from there to the wind turbines close by. We think this will be a

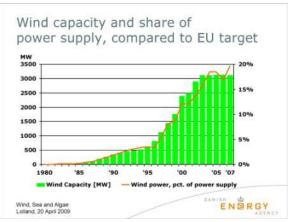


competitive concept as we see more offshore wind farms in Europe.

Rødsand II is just south of the island we are on now. The construction phase is up and running and the picture is from there.

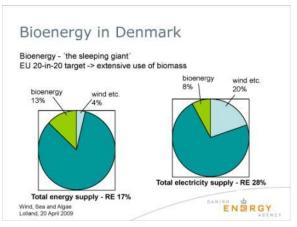
When we look into wind power capacity, you can see here that the share of this is almost 20% of power supply. This means that out of each 5 kWh hour you take out of the plug, ONE is made by wind in Denmark on average. That is a world record.

I will come back to what biomass accounts for, but this is a very important contribution. There has been single hours in the Danish electrical system, where wind power has produced more electric power than we could use ourselves and therefore we are depending very much on strong electrical



interconnectors to our neighboring countries to weigh this out, but it has not been a problem until now; we have never seen a black-out in Denmark due to wind power, even though for example during hurricanes we see a massive and instant drop in production as wind turbines hit their maximum production speed - then they stop and there is no production. We have never seen a black-out from that and that is a very important message, because 25 years ago it was not believed yet that we could incorporate more than 5% wind energy and that has shown not to be right; we are running 20% wind in the electricity system on average today!

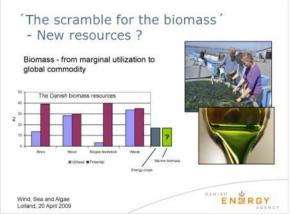
Let's talk biomass and bioenergy, which is why you are here. Out of the total Danish energy supply, bioenergy supplies 13%; out of electricity the supply is 8%. The difference is mainly because of the combined heat and power plants. The main contribution from biomass in DK is straw and woodchips and it is fired in combined heat and power plants in DK; we fire it as we do with coal, in large combined heat and power plants, with flexifuels as we call it. They can shift fuels from natural gas to oil, to coal and to bioenergy. Not all of them, but some of them, not in all instances it is not from



day to day, but within a reasonable amount of time. And that is a very important contribution to security and supply; we have various sources of resources.

We foresee that biomass will need to take a larger share, not only out of total energy supply, but also out of total electricity supply. We will have more bioenergy even though we have a large growth also in wind.

And we also see this as a challenge; because there is a scramble for biomass! We need the new resources, but also they use land, that can be used for other purposes. We saw a food crisis due to rising prices, perhaps because of crops being used for biofuels in the US; we don't know, but that might have contributed. We need to make biomass in a different way and also in ways where we don't necessarily use farming ground for it. [18.12] This picture is from DK, from *Mors*, where they have sea salad/ macro algae that they are growing. That is the largest project in DK today; you will see another



one while you are here in Lolland. This is VERY promising, because this is not something you need anywhere else, no one will miss it and you won't use farming land for it, so if you can drive down energy consumption producing it and the price of it, it is very interesting, not least because it can become fluid and it may some day go into our cars and that is what we need. So this has a very good perspective if developed even more. And what we see today in DK is that when we look at straw—the light one is what we have utilized of our resources and the dark one is we have left of potential. There is still more potential in straw in DK, but wood we have almost exhausted, we cannot expect more energy from there. Biogas has a very large potential, but it's a lot of diffuse sources with different farmers and it is not in all cases that it is actually relevant economically to use the resource for energy; it's too expensive. Also we use our waste in DK for energy; we incinerate it, but also we have opened up for the possibility to burn it in a large combined heat and power plants; off course not daily waste, but for example waste from building industry and so on and we are looking very much forward to what that can be developed into, because as of today we say that about 60% of the energy in waste is renewable. There are still plastic and things in it that can't make it totally renewable, but more than half is renewable, so it is a very important energy source too. But algae, the marine biomass, can make a whole new resource for DK and that is very exciting.

Research & development of bioenergy and Combustion of biomass for large-scale CHP (combined heat and power plant): We say that this mission is complete today. We fire large amounts today, off course you can develop, but that is known technology, there is not too much growth in that.

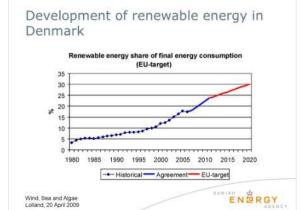
Gasification of biomass: We know the technology in small scale; perhaps we should use it in larger scale. We are looking into can we put the biogas into the natural gas grid. Can we do that? Is it too expensive? Is it relevant from an energy point of

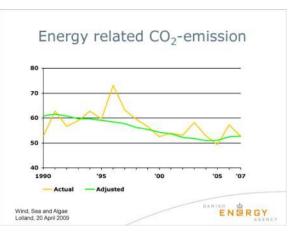


view? The production of liquid biofuels is off course important and we have two very interesting projects in DK. May I note here that it is interesting that we have two projects! Some would say, well choose ONE, but we don't like to do that, we don't like to pick the winner; it's never been shown to be a good idea. We want to have competing projects with the same or like technologies and it will be the same way with algae, we need the friendly competition that is the best way to get a result. If you pick the winner, and you pick the wrong one, you have lost all your money. The overall trend in DK is to develop more advanced and flexible conversion technologies and a broader fuel band. We have more dedicated programs on energy, research and development; one is for large scale demonstration of 2nd generation biofuels, but we also have other programs that supports energy technologies to development and demonstration and right now we are trying to work for a program that takes the technologies the last part of the way to the market, -----the "valley of death" development stage of a new technology has a lot of names ----and we hope that we can get some money for that purpose too. But there is focus on this area in DK and we have great expectations and this is one of the new technologies, one of the new energy sources that might be very interesting and which might contribute to this development.

This is the share of renewable energy in Denmark and as you see it has been rising steadily and slowly and today we are here. This is the expected development due to the energy agreement and when these initiatives are implemented, there is still some way to go to reach the EU-target, which on Denmark's behalf is 30% renewable energy in 2020. So we will need to have new energy policy discussions in 2010-2011 and there is no doubt, that this will also open up for more new energy sources than the most well-known biomass and wind energy, when we are talking about potential renewables.

In the overall perspective we see that the energy related CO2-emissions has fallen in Denmark, both in actual terms and in adjusted terms. It is adjusted for the weather, but also for import and export. We have heavy cooperation with the Swedish and Norwegian hydropower systems. In Denmark when it is very cold and windy we produce a lot of electricity on wind turbines and in the combined heat and power plants; Norway and Sweden have hydropower and when it is not very "wet" years they need our power, so it is a kind of storage for the more fluctuating Danish power production. This



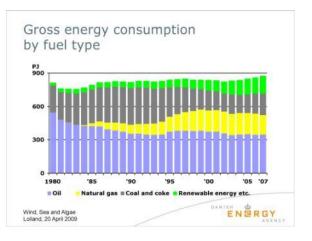


exchange is regulated by the market - i.e. price signals - connected through the Nordic spot market for electricity.

Can we make the change to a totally fossil fuel free Denmark and can we have 30% renewable energy already in 2020?

Off course we can!

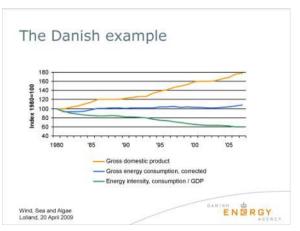
This is a picture showing the Danish energy supply from 1980 until today. In 1973—during the first oil crisis—Denmark was 99% dependent on imported oil for energy purposes, that was transportation, household heating, power supply: Large power plants were running on oil!



Today oil is still important, but not imported, which I will come back to in a minute; but it is almost only in the transportation sector and to some household heating that we use it today, so it is a whole different picture, and it has meant a fundamental change, as you see here: in the short run we substituted oil away and put in coal and then we substituted our own natural gas from the Danish part of the North Sea into the energy system instead of the imported coal and also enhanced the renewable energy, so what you see today is a much more differentiated picture and may I add, that Denmark is today EU's only net-exporter of energy and that is not only because of our own gas and oil in the North Sea, which we decided to exploit during the first oil crisis, but also because of all the power we produce, not least renewable power.

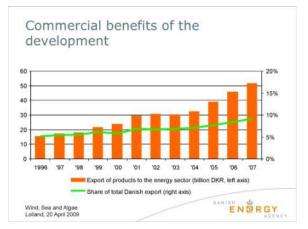
This has led to this example that the Danish climate negotiators travel around the world with!

In the middle you have gross energy consumption. It has been almost constant; it has been rising a little bit in the last couple of years, but almost constant for more than 25 years. At the same time our economic growth has been impressive. This is the only country in the world that can show this development and this means that we need to import far less than we would have had to if we had not had an energy efficient economy. This means better security of supply, this means much lower CO2 emission than there would have been if our energy consumption had been growing like our economy.



So no matter whether you discuss security of supply, climate friendliness or competitiveness, this is a very wise way to go. Energy savings, combined heat and power, clever use of fuels, focus on domestic resources for example wind energy and biomass is the way to reach this balance.

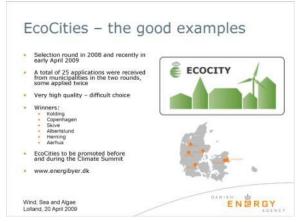
We do not only export energy, we also export energy technologies and we have seen a steady increase in energy technology export and it is off course wind and biomass technologies, but also other technologies. It is a large share of total Danish exports and it is a very important contributor to our balance of payments; the amount is around 50 billion Danish *Kroner*; it is the same amount approximately that we export energy goods for. So all in all, energy and related technology is an important factor in Danish economy also as an export commodity.



We must not only to look into some sectors to enhance energy efficiency and the use of renewable energy. When we had a new minister one and a half year ago, she said we need to do something for the municipalities, they are important players and use much energy [28.23] and have influence on industry and consumers and the public, so we need to make them frontrunners in this climate and energy savings run.

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

So we took an initiative we call EcoCities (in Danish: Energibyer) and two weeks ago we named the last three of them. These are six out of 98 municipalities in Denmark that are special frontrunners in this field. We will not name more of them, but we will make a comprehensive material that will tell what they have done, what is their experience and why is it wise to do it? They have not wasted any money in doing these things, they have gained in their local economies and we make comprehensive documentation that will be accessible on the homepage www.ecocity.dk; it will show the concrete



examples: what did they do in this school building? What did they do to plan traffic better? Concrete examples and we present it in Danish and English and hope that everybody can see that this is not something we do just to be green or to be smart; we do it because it is the most efficient approach and the most wise thing to do, it is simply cheaper to do things this way. So we hope we can make an example with the Danish cities and when the whole world comes to Copenhagen in December, they will be inspired too; after all there are more cities in the world than there are countries.

Last but not least we have a lot of different campaigns in our ministry and some of them are accessible from here; you will have the presentation after this conference and you should be able to click these links and get to them. I have promised to be accessible for questions now and in the break, so I think I will just finish here and open for that!



QUESTIONS:

Mike Seibert: your energy use has not gone up over the last 20 years, which is remarkable, but you've had 80% economic growth; what has happened to the population in Denmark over that same period?

Anne Højer Simonsen: the population has been almost constant. It hasn't risen very much. I don't know the exact number but it is approximately the same.

Dick Seymour: Because of your extensive use of wind power, I'm curious if you have considered compressed air storage as a means of load leveling?

Anne Højer Simonsen: We are very interested in any technology that can store wind energy directly or indirectly. So yes, we are looking in to everything, but we expect it will be a considerable amount of time before it is relevant in large scale, so right now the main way to handle excessive wind power is to have strong inter-connectors to our neighboring countries.

Bjørn Utgård: It is easy to look at the charts and relative performance of climate and say that "yes, we are doing what we need to do." My main concern is that we are not even close to what we need to do. If we look at the IPCC is saying and there is a lot of quoting of the IPCC, for instance the G8 is saying that the IPCC says they are asking for a 50% reduction scenario by 2050....this is not enough. The current concentration of CO2 in the atmosphere is 387 ppm. Now you might have heard of James Hansen from the Goddard Institute saying that we should be returning down to 350 ppm. Now this is the physical basis, but at the same time we have the political basis and I think that is the link between everything you were talking about:how do we make sure we reach an agreement in Copenhagen in November and December. What we are doing in the Bellona Foundation is looking at various technologies and trying to put them together in a new way. One of the things which Denmark can actually be a pioneer in is just because of your coal firing that was mentioned Vattenfall, Europe's second largest power producer is a Swedish one, but big in DK. They are going to coal fire one of their power plants in Northern Jutland with 30% biomass in 2013. Now this requires 450,000 tons of biomass. Why don't we import that biomass for example from India? You can create a lot of jobs, no body mentioned this in the presentations this morning, but we have a lot of people needing jobs so you give poor people a job by producing algae that you can then import to Denmark... Denmark can not only go carbon neutral, but carbon negative, because this power plant that is going to have 30% [biomass] firing in 2013 and can be higher as years go by will actually have a net negative emissions of half a million tons of CO2 per year. Because that biomass absorbs CO2 from the atmosphere ... so there are a lot of interesting things that Denmark can lead the way on and with this I want to lift our scope of looking at solutions.... so we don't look at algae equals oil, but it equals a climate and energy opportunity. Not only in the technical sense, but also in the political sense, because we need to show poor countries that they can actually gain something from an ambitious climate policy. What are we going to buy from the poorest countries in the world? Are we going to buy their food? No we don't think it's secure. You look at the poorest countries in Africa and what do they have that we want to [buy], a global energy commodity where algae are the main component is a possibility. So, this is not a question I guess, but it fits well into all the topics that you mentioned.

Anne Højer Simonsen: A brief answer to that is that in Denmark if we walk the talk, we do the most we can to have a global climate agreement and that's why a Danish agreement and ambitious targets in the EU package on climate and energy I crucial --to walk the talk and I think it will broaden the whole span of energy possibilities in the world if all countries enter this approach. Whether we are ever going to import energy from India, I doubt it, I doubt the energy balance of that, but still we are very aware that all countries need to be seen in this context and that some countries need to gain from this in a more direct way than the rest.

Peter Lindblad: You have reached very impressive numbers for renewable electricity from wind and you also outlined and previous speakers have outlined the problem in transportation, so what do you foresee in Denmark in the transportation sector, how to get renewables into the transportation sector?

Anne Højer Simonsen: Already now, today, we are looking into more possibilities. We have a smallscale hydrogen project, we have a large electric car project, and we have also biofuels. So we are not picking the winner, but trying different approaches, because they have each their own qualities. For example electric cars, the batteries for the electric cars represent a huge potential for decentralized storage of electricity, which is interesting when we produce as much wind power as we do today. So we look into a variety of solutions and I think we'll need all, there will not be one answer here.

ALGAL BIOMASS PRODUCTION IN NEW MEXICO FOCUS ON LIPID MANAGEMENT, PETER LAMMERS

Algal Biomass Production in New Mexico Focus on Lipid Management

Offshore Algae for Biofuels and Beyond Lolland, Denmark April 20-22, 2009







Prof. Peter Lammers Center of Excellence for Hazardous Materials Management, New Mexico State University

Jonathan Trent: I want to introduce Peter Lammers from New Mexico State. I invited him because he is working on the DOE roadmap for algae research for the Department of Energy (DOE). The DOE did a road-mapping exercise last December [2008] for which DOE gathered people from all over the world, although mostly from all over the United States. The goal was a kind of resurrection of the aquatic species program, updated, streamlined, and more relevant. I think that is what we are going to hear from you, right Peter?

Peter Lammers: Actually, No. Al and I talked this over and Al Darzins is going to tell you a little bit about the roadmap activity. I want to tell you a little bit-- well, I want to talk philosophically here for two or three minutes and then actually show some data. I think that where we are right now is embryonic stage of biofuels and we have to recognize that just like in developmental biology, there are things that are done in an embryo stage that aren't done when things are mature. There is a whole series of things we essentially everybody has to do right now, because a lot of the funding now is from private sectors, very few people are talking about it.

But in feeling our way between what is possible scientifically and what might make money from an economic perspective, we have to sort of explore a lot of different pathways. We have to identify where the product streams are going to be, knowing that some of those product streams are going to disappear--in some of them, in relatively short amounts of time--but can be used as a bootstrap target for gaining funding and starting to tackle the large-scale issues we have to deal with and starting to develop algae as a biomass source. So we have to figure out intermediate scales and targets that go with those intermediate scales, and then we have to think about how do we go to really, really large scales so that we get the oil companies, for example, to get actually interested in this. So I think we also have to think... Peter Lindblad's talk was a great futuristic idea how it is we build toward novel fleets of transportation vehicles and so on, but how do we get there from where we are now?

Well, we have to target fleets that exist right now; we have to target the refining industry and their capabilities right now. And then we have to provide feedstocks that they can deal with. So if you go to a refinery and you talk to those folks and you say, you ask them, "What's the complexity of crude oil?" You find out that crude oil has between 50 and 100,000 different compounds in it and the oil companies make their money by knowing that the crude oil from Venezuela and the crude oil from Siberia are different. They send some fractions to one refinery and they make one product and then a different fraction goes to another refinery that specializes in making a different product and so we think about what a large scale algal oil extract might be. It's relativity simple, compared to crude oil. What is different about it is that it has oxygen, nitrogen, and phosphorus, and sulfur in there but those are not insurmountable problems. And if you think about a bootstrap target, what market is out there that we might be able to achieve a significant fraction of? It seems to me and I think a lot of other people that aviation fuel might be that target. You are not going to fly jets on batteries. You need a high-density fuel. And we are going to make a high-density fuel out of lipids and you can do that with technology that exists today. So that would seem to me to be a path that a lot of different groups, a lot of different companies around the world are all targeting.

If we think again about a developmental perspective, it is not that different than the U.S. in the middle of the 1800's. Lots of little pockets of oil, lots of little folks who were drilling and starting to refine; and then the big Rockefellers of the world started to conglomerate those things, but essentially, we need production everywhere and in every different climate. We need to figure out ways of doing it in a distributed way. What are the common issues that we all have to deal with? Let's focus in on those. What I am going to try and do is navigate a path somewhere between Qiang Hu's talk and Ami Ben Amotz' talk... and I'm a biochemist so I am going to talk about how it is that you combine cultivation with management of algal lipids.... So that's where we're going.

And I need to share with you that the funding for this project comes from the state of New Mexico and we are collaborating with folks at Sandia National Lab but most importantly, folks at the Center of Excellence for Hazardous Materials Management in Carlsbad, New Mexico, where we are actually operating a couple of raceways and expanding it.

In the middle here, is one of the raceways over there in New Mexico State University's Agriculture Science Experimental Station near Artesia [New Mexico].

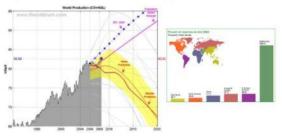
So what's different today from when we killed the aquatic species program? There are three basic things that make me believe that we are in this research game to stay, this time around. There is a general recognition that oil production either has peaked or will quickly, population growth and global warming recognition. Those things are all pretty much different than they were a few years ago.

Why are we here? Why is 2009 different than 1984?

- Oil production has or will soon peak
- Population growth continues to create new demand for energy
- Recognition of the CO2 Global warming connection

Petroleum has been an optimum transportation fuel source, but, bad news: the supply is

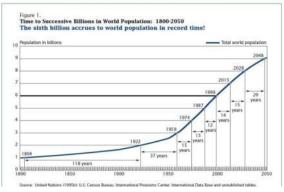
- limited
- non-uniformly distributed
- production may have already peaked



This slide just documents a range of projections about oil production and reminds us that oil is highly non-uniformly distributed around the world and that creates problems.

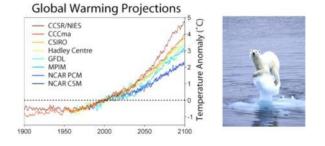
Just a reminder that it took us only twelve years to accumulate the last billion people. Those— decline in oil production and increase in demand driven by population—those trends are not going away. We are going to have fluctuations in price, we certainly saw them in 2008 but the long term projections are pretty clear.

More Bad News



...and of course now there is this general recognition that we have to do something here.

And Yet More Bad News: burning petroleum releases greenhouse gas: CO2



So what are our technological goals here? We need to obviously control the chemistry inside highly efficient photosynthetic organisms and at least initially we need to target existing fleets. And we know that jet A and JP8 can be made with technology that exists today. One of the economic issues that I think we need to deal with is that as you start to scale up production, if you are counting on co-products to make your business plan work, the scaling-up is going to drive down the price of your co-products and you have to be thinking about how you phase that.

Technology Goals:

•Control the chemistry inside photosynthetic cells in order to produce desirable chemical feedstocks •compatible with existing fleets (cars, trucks, ships, aircraft)

Market Options:

Jet-A, JP8 (smaller, markets, e.g. defense, air transport) Diesel, (large market, with correspondingly larger capital investment required co-products, (quickly saturated?)

fossil fuel power plant CO2 capture and re-use (dependent on regulatory authority)

Enabling Technology Development: harness biochemical regulatory pathways, genetic improvements (selections and GMOs), bioreactor design, harvesting, extraction, conversion – Algal Agronomy

And there's also a lot of uncertainty around what is going to happen in the regulatory context in terms of will we be paid over the long term for carbon capture or carbon-neutral fuels, and how is that going to work? And so the enabling technology is what we need to work with and on the biochemical level, regulation at both the enzyme level, the transcription level and through genetic engineering are all targets that we need to put in play. I think we have to be really wise about drinking the Kool-Aid about genetic engineering being always a negative thing. Let's try to get some studies that put that into a real world context. Those studies that I've read anyway indicate that putting a trans-gene into an organism, by definition, weakens it and puts it in a situation where you have to control the environment to select for it. I think we need to stand up and say let's not be afraid of that technology. Let's study what the risks are and let's have a dialogue about what an acceptable risk might be and get on with transforming our energy sources.

So integrating bioreactors with metabolism--that's what we want to talk about and focus on today.

I would like to point out, to build on a concept that Bryan Willson talked about in terms of good design; you want to separate growth from chemistry. Right now, I am now going to show you some data that is how nature does it. We need to think about that in terms of how we design our bioreactors. If we think about a batch mode where we essentially harvest everything and restart, maybe that will be the most efficient way. But maybe you can design a bioreactor so that you have separated in time and space a mode where the cells are growing absolutely as fast as you can make them grow and then move them into a different bioreactor where they're changing

Overview State of New Mexico Algae Project – Energy Innovation Fund to CEHMM

- Technical Challenges
- Cultivation and algal oil content management
- Predator, pathogen, competitor control
- Integrated bioreactor testing and modeling

New Mexico-State University



their internal chemistry to make it more like what you want it to be at harvest. We need to put those alternative ideas out in the field and test them through several growing seasons and see which one wins out. So that's a plan of what it is that we are trying to do.

There already have been some nice papers talking about the theory of serially-linked turbidostats. The references are up on the slide here.

Raceway Agronomy

Balance the needs for rapid growth and high oil content knowing growth rate and oil content are inversely proportional

- A. Option 1 batch culture/ harvest and manage nutrient limitation to trigger increase oil content
- B. Continuous culture/harvest and manage nitrogen levels to find sub-optimal maxima for both growth rate and oil content
- Continuous culture/harvest using hydraulically linked serial turbidostat culturing (Rasch and Christiansen, 2003 Aquaculture Engineering 27, 249-264; Bensen et al. 2007 Aquaculture Engineering 36, 198-211)
- D. Need for accurate, rapid and small scale methods for determining oil content and metabolic status of cells

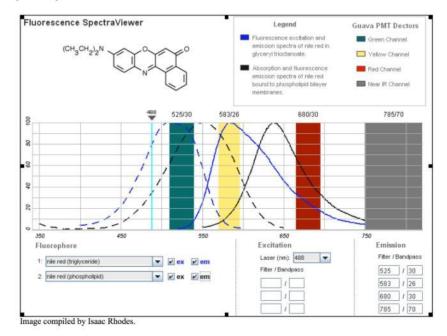
So if you are going to follow algal lipids you need a good assay; that's what biochemists do. So we consulted the literature and some people said Nile Red does not work with *Nannochloropsis*, which is the strain we are working with. And another report said it did. So we decided that we would go in and re-do everything and optimize things if it turned out that we could do it. So the principle behind Nile Red is that there is an excitation maximum, and

Nile Red Validation Study

Does NR work with *Nannochloropsis?* A review of the literature suggests both yes and no.

How good is the correlation between NR fluorescence and total lipid content?

there is a difference in excitation of fluorescence, depending on whether Nile Red has moved into a neutral lipid or a polar lipid.

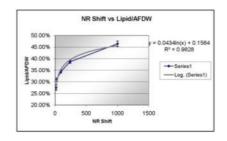


Excitation and Emission Data for Nile Red in Triacylglycerol and Phospholipids.

This slide represents excitation maximum in phospholipids. Then the emission wave lengths are also shifted, so if you-I won't go through this in too much detail for those of you who aren't scientists-- but the notion is that Nile Red gives you an enrichment for the presence of triacylglycerol over phospholipid.

And to make a long story short, we looked at whether or not you add the Nile Red to the fresh cells or cells that have gone through a freeze-thaw. It was more convenient for us to work with cells that have gone through a freeze-thaw. It turns out it is a saturatable, concentration-dependent phenomenon. You pick times where you are saturating the cells and the emitted fluorescence is constant and you implement that on a flow-cytometry and you actually figure out a way to get algal cells at different

Plot of Gravimetric Total Lipid versus Nile Red Fluorescence



lipid concentrations and I'll show you how I did that, in a couple of slides. But then you use a gravimetric assay, where you actually extract the oil and weigh it and then you plot that against the fluorescence and you get this nice exponential function where the lipid content normalizes at Ash-Free-Dry-Weight (AFDW) vs. the Nile Red shift. The shift is just the fluorescence minus their subtracted back

ground; it gives you a nice function. You can plot it on a log scale and get a nice straight line. So now that we have an assay, let's take a look at what it takes to control the lipid.

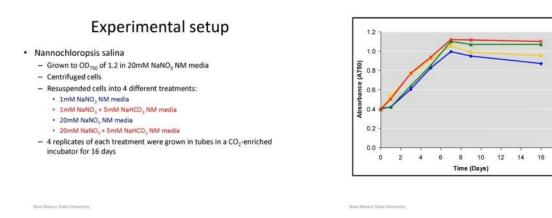
Again we looked at the literature and we found a nice experiment done by Cooksey at Montana State, back in the 90's where he actually looked at the ratio of carbon and nitrogen. So the notion here is that the absolute value of nitrogen is less important than the ratio of nitrogen to carbon in terms of metabolic regulation. The experiment was essentially designed on the one Cooksey did and we are just looking to see whether *Nannochloropsis* would respond to that and we had four different treatments—two levels of nitrate and plus or minus bicarbonate. So *Nannochloropsis* is Eustigmatophyceae and it does

Nitrate and Bicarbonate Alter Lipid Content in Nannochloropsis





not take up CO2 directly; it's specialized to take up bicarbonate.



Some interesting differences between *Nannochloropsis* and true green algae: here, we did four replicates, we followed growth; we did Nile Red on a time-course basis and if this is a growth curve now we're just looking at the absorbance at 750 to follow growth, and we have a color coding here on our different treatments. So here's our low nitrogen, and low nitrogen with bicarbonate, and then the two higher nitrogen treatments are up here. Note that around seven days into our system we are leveling off; we are hitting stationary phase. Now stationary phase is not death for microorganisms. It's a period in which there is a whole program change in gene expression and the cells are starting to yellow in this stage, they become chlorotic and they are essentially getting themselves ready for a long winter's nap. These organisms evolved in the ocean and they circulate vertically and they spend lots of periods of time where they are just waiting for an up-welling event where they can be close to the surface so they can bloom.

1 mM NaNO3

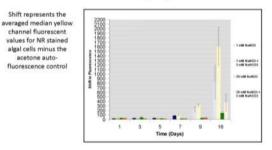
- 20 mM NaNOS

18

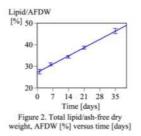
We need to harness what goes on in this phase for the following reason: This is the Nile Red data-again, color-coated treatments here. This is right where the growth stopped; note that's where we start to see this increase in Nile Red fluorescence, indicating that we're starting to make lipid. And note that when we have the highest ratio of carbon to nitrogen is where we get the most dramatic response in terms of increasing the lipid. So, based on that data, we then scaled it up, and this is actually how we did the validation. We had to take a leap of faith that the Nile Red was going to work; figure out what conditions give you high lipid and then we took a 20-liter carboy, grew it up to end of log-phase growth, we harvested it and resuspended it in fresh media containing the 1 mM nitrate and 5 mM bicarbonate and then we followed it for thirtyfive days. This time we did the gravimetric truthing of the total lipid content, using a classical Bligh and Dyer procedure and that would get a linear increase in total lipid, to 0.5% per day.

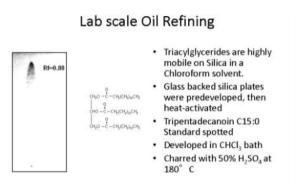
Now the question that you should be asking yourself now is, "What is happening to the lipids as it goes up?" We need to think this is a total lipid; we are getting all different kinds of different things in there. So, as a biochemist you want to say--well, "Now let's drill down into these details and figure out what's changing?" as we start to accumulate, in stationary phase now, different lipids. So we need to do some lab-scale refining and you can use thin layer chromatography and standards to figure out where your triacylglycerol is.

Nile Red/Lipid



Linear rate of increase in total algal oil is observed when low nitrate plus 5 mM bicarbonate is added to a dense culture of algae

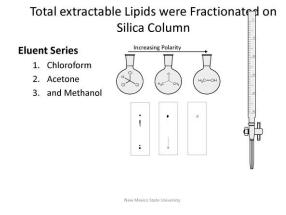




You can then do preparative scale work where you are putting your Bligh and Dyer extract onto a silica gel and then you elute with three different solvents of increasing polarity. This is a TLC plate of what you get from each of those different eluents. This chloroform fraction is going to contain the

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

triacylglycerol fraction and it's going to contain the carotenoids. This acetone fraction is going to have more polar lipids and chlorophylls and things like that are going to come out in this fraction.



So, based on that then, you can follow the composition of the Bligh and Dyer extract as a function of time while you're accumulating your lipids. At day 14, where the carotenoids and the triacylglycerols are around 18%, note that between day 14 and 21, this more than doubles, at the expense of the material in the acetone fraction and in the methanol fraction. So the carotenoids--this is reflecting the chlorosis that's happening right here. The more interesting thing here, is between day 21 and day 35, the triacylglycerol fraction is actually going down. So we have this growth phase, and

Preliminary Data: Column Fractions

N = 1: from time course treatment 1 mM nitrate, 5 mM bicarbonate treatment

Day	CHCI3	Acetone	Methanol
14	3.1	6.8	7.3
	18.0%	39.5%	42.4%
21	28.8	17.6	8.9
	52.1%	31.8%	16.1%
35	20.7	19.8	10.9
	40.3%	38.5%	21.2%

then we have this remodeling, and then we have something else that is going on in this phase, which has probably gone beyond where we want to go when we harvest them.

So we need to be thinking about how do we design large-scale cultivation systems that tune you onto this sweet spot. Then we need to think about how we put that into a refinery and start to make fuel.

So we have been growing *Nannochloropsis* outdoors in Carlsbad with the Center of Excellence for Hazardous Materials Management. We have a really good team over there of engineers and microbiologists that have been tracking the composition of the ponds, and a team of aquatic biologists at NMSU that have been following what is in there. We have enough material that we can do composition from field-grown material; this is the

Polar Lipid FA Composition Field – grown *Nannochloropsis*

Fatty Acid as Methyl Ester	Percent composition	
C14:0	6%	
C16:0	28%	
C16:1	26%	
C18:1	8%	
C20:5	32%	

fatty acid composition of the polar lipid fraction from field-grown material.

Note that we have quite a bit of the C-20's in the polar fraction. If we look at the non-polar fraction, the C-20 goes way down and we have much more of this material here, so this is again fodder for the people in the refineries because these are materials for which we know how to convert them directly into aviation fuel.

So, if you scan through the literature, you will also see reference to salt stress as being a lipid trigger, and so here's an experiment just to give you an idea of the range of things that you can do that mimics the serially-linked turbidostat approach. And here what we're doing is running in the laboratory: [we] built some little mini raceways and lit them, and so we have 2X seawater in this raceway, and ocean strength seawater in this one. We are essentially running, these are about 18 L capacity each, and every other day we take 6 liters from here and put it down in here, and we take 6 liters from here and we Neutral Lipid FA Composition Field – grown *Nannochloropsis*

Fatty Acid as Methyl Ester	Percent composition	
C16:0	35%	
C16:1	30%	
C18:0	6%	
C18:1	21%	
C20:5	8%	

Two-Stage Raceway Experiment: Semi-continuous, linked, serial raceway

Raceway 1: algae grown at 3.5% NaCl

Raceway 2: algae grown with 7% NaCl

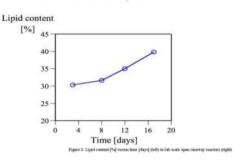
Semi-continuous dilution from 1X to 2X NaCl raceway, maintaining differential [NaCl], constant pH, Temp, nutrients added to Raceway 1 only.



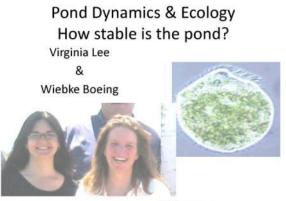
harvest. And when we started out at day 1, both of them were at the same cell density. Then you do this semi-continuous harvesting, maintaining 2X seawater here, and ocean strength seawater here and you follow what happens to lipid over time.

And what you see is this: we're sampling now the Nile Red fluorescence in the 2X ocean strength reactor, and you see that we go from a starting point of about 30% of total lipid via a Bligh and Dyer gravimetric assay and we're building up toward 40%. So this demonstrates that you actually can use the notion of separated bioreactors, one designed for growth and one designed for stress and chemistry.

Total oil content (lipids/fatty acids) increases in response to increased NaCl in a two-reactor, linked turbidostat system



So what about the stability of outdoor ponds? Wiebke Boeing and Virginia Lee have been working on this, taking the samples grown outdoors in southeast New Mexico, and following what else is in there. We know that there's quite a list of goblins that can come in there and wreak some havoc and so we used a couple of different methods for counting both a hemocytometer and a microscope, as well as flow cytometry.

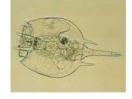


New Mexico State University

New Mexico State University

Predators / Pathogens

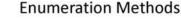
Ciliates, Rotifers Heterotrophic flagellates Chytrid fungi Bacteria, Viruses Other algae: green, yellow, diatoms, cyanobacteria





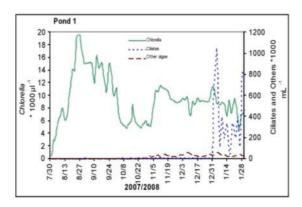
New Mexico State University





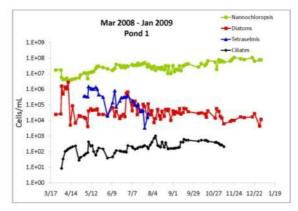
- Counting (hematocytometer) – No pretreatments required, but laborious with limited sensitivity
- Flow Cytometer
- Pretreatments required (filtration)
 - Size limitations (ciliates and some diatoms too large)
- Large counting range gives excellent sensitivity and statistics

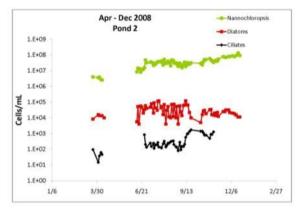
I will show you three data slides here; this was from 2007 and 2008, where we were growing *Chlorella* out there. The scales, this is times 1000 per microliter and this one is times 1000 per milliliter, so for the ciliates and the other algae, these numbers are three orders of magnitude less than these numbers. *Chlorella* did really well; we had this little spike in ciliates here toward the end of the year in the wintertime, but it went down again.



This is for the last growing season; we had two different ponds growing, trying to establish this in the spring, we had quite a few diatoms, a little incursion here; this is a log scale now. So when summer came along, we had quite a bit of a gap between *Nannochloropsis* and other things. There is a continuous load of grazers that are consuming these bugs that you'll have to deal with.

So in the second pond, we started a little bit later; this is an indication that failures do happen. We had some mechanicals and we had to re-start this thing, and when we re-started it in June, it was very, very happy.





Question from the audience: Is this is the high salt pond?

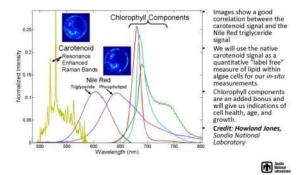
Peter Lammers: These are at 1X: 3.5%.

Question from the audience: Have you tried 2X?

Peter Lammers: So we haven't tried 2X [seawater] outdoors yet; that was an indoor experiment. But believe it or not, you see indoors some of the same things as you do outdoors, I don't know where they come from...

These are based on microscopic counts for the most part because we wanted to see what was in there. We can go back and double check with flow cytometry to automate this. You absolutely need automation, you've got to have automation. If there was a take-home lesson from Ami's talk, although all this is fine, if you're going to do this in New Mexico, you have to do it the same way as they did it in Israel-- you have to automate everything. Otherwise it's going to kill you, price-wise.

Hyperspectral Imaging of Confocal Microscope Data Results for Chylamydomonas Reinhardtii



Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

So where do we go from here? A collaboration with some really smart folks at Sandia National Laboratory that are going to allow us to look at the chemical composition in more detailed ways than we have yet. They do this thing called hyperspectral imaging, where you can de-convolute an envelope of fluorescence as a function of wavelength into its individual component parts. You also can add Nile Red to it and separate out the triglyceride peak and the phospholipid peak. Some really cool stuff that you can do: This is the carotenoid peak and the structures in here are



resonance enhanced Raman bands, so Raman gives you vibrational data. And from that vibrational data you can start to figure out [from this kind of data,] the fatty acid composition of any voxel inside a confocal image. Let me back up: confocal microscopy, most of you know, gives you a three-dimensional image of a single cell, and when you couple this sort of hyperspectral imaging to a confocal microscope, then a voxel is a three-dimensional pixel; you can essentially look at a cell and see whether or not these carotenoid peaks are in the same voxel as the Nile Reds, and it turns out that they are, demonstrating that the carotenoids can be used as an internal flourophor to assay for your lipid content. If you collaborate with folks at Sandia (I think there's only one of these things in the world), it's going to allow us then to take a look at these lipid triggers and find out, on a cell partition basis, where these things are happening on what kind of a time course. And then we use that data again, go back, collaborate with the engineers, figure out how best to separate in time and space the two different basic needs that we have to meet--biomass and chemistry.

[SLIDE]

So there's a picture of the overlap of the carotenoid signal with the Nile Red signal, which I should have put up here sooner.

[SLIDE]

I would like to stop there and take any additional questions you might have. First, I want to acknowledge the group at CAM; Doug Lynn here is the Executive Director; Lou Olgard is the project manager; Leslie Kirkus is an NMSU graduate that is doing a lot of the day-to-day pond maintenance over at NMSU. Several of us are working on this from different angles, from engineering to economics to biochemistry to biology, and then the group at Sandia that is doing the imaging.

Thank you very much.

QUESTIONS:

Jonathan Trent: Thank you Peter. We have time for questions. Yes?

Ihab Farag: In your growth curves, did you notice any lysis if you continue measuring absorbance beyond your 15 days that were on the chart?

Peter Lammers: Well, there was a slight decrease in absorbance, but that does not indicate there was a lot of lysis going on. If you look at the cells microscopically, they're chlorotic but they're not lysing. They are not dying; they are just remolding themselves and going into stationary phase, very different than death.

Jonathan Trent: So the data you showed for the carotenoids versus the Nile Red, that correlation, when you did the analysis of the lipid, was it the Nile Reds looking at both types of lipids or? Does it work for correlating with the neutral lipid?

Peter Lammers: Was it this one?

Jonathan Trent: Yes. You have triglycerides versus phospholipids. That's right; good, thanks.

Zbigniew Kolber: The relationship between the carotenoids and the lipids. The physiological state that is inducive to lipid production that is manifested by the presence of carotenoids, or is there just a proportional relationship between making both of them together?

Peter Lammers: So if you add an inhibitor of fatty acid biosynthesis and then induce for a carotenoid isoprenoid pathway, it shuts off the enhancement of the isoprenoid pathway because the products of that pathway partition into lipid droplets. So in the absence of that partitioning capability, there's feedback inhibition on carotenoid biosynthesis. So those two things are coordinately regulated but it appears as though the lipid synthesis is more important because if you knock it out, then you knock down the carotenoids but they're co-localized under the same sorts of conditions.

Jonathan Trent: Qiang Hu, did you have another question about this?

Qiang Hu: You have made some comments about the relationship between carotenoid biosynthesis and fatty acids. So these two pathways are physically coupled and all the functions are coupled, so basically, if you block one pathway you will block the other, so vice-versa and so there is a ratio between how much pigment in a carotenoid made and versus how many fatty acids, so this ratio is around 1 to 5, so [for] every carotenoid made, at the same time the cell makes five fatty acids. Basically that's the rule.

Jonathan Trent: Thanks for that clarification... George has a question up here, and then we are going to go on, unless there are other burning questions.

George Oyler: It was very nice seeing hyperspectral data but now that Qiang was speaking, his work has indicated that the triglycerides are within the chloroplast, which is a little different from other eukaryotic cell localization of the lipid bodies. For instance, in higher plants, they are derived from the ER, and with this imaging, you can image where the chlorophyll is and where the triglycerides are

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and by imaging, verify his bio-chemical separation work, where the lipid is, which would be very interesting; and you probably already have the data available.

Peter Lammers: Yes, this is for chlamy. We'll be doing it for nano and, as you know, they are in very different organisms and different taxonomic differentiation. There is a lot of potential for what we can do with this work.

Jonathan Trent: There's one more question.

Unknown: The graphs that you showed of lipids with time. What would be the optimal time to harvest? It seems the longer you wait, that lipid would increase but you went all the way to 50% over 25 days. Did you determine what would be the optimal time for harvesting?

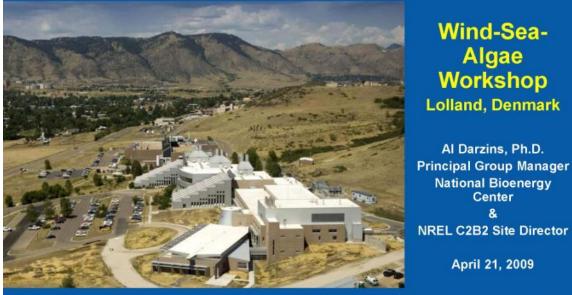
Peter Lammers: The optimal time for harvesting would be the minimal amount of time to get the maximal amount of the quality product that you want. It would appear under those laboratory conditions, it would be 21 days. Now you have to [go out and] figure out what's it going to be in the field in April; what's it going to be in the field in May; what's it going to be in the field in June, and so on. There is lots of work to be done. Essentially this is what everybody is doing everywhere; I'm just talking about it.

Jonathan Trent: Thanks, Peter.

OFFSHORE ALGAE CULTIVATION FOR BIOFUELS PRODUCTION, AL DARZINS



Offshore Algae Cultivation for Biofuels Production: Parallels to Past and Current Based Efforts



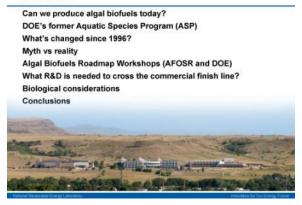
NREL is a national laboratory of the U.S. Department of Energy Office of Energy Efficiency and Renewable Energy operated by the Alliance for Sustainable Energy, LLC

Jonathan Trent: Al Darzins is going to talk about the DOE algal biofuels technology road map. He is from the National Renewable Energy Laboratory (NREL). Al actually invited me to an algal biofuels workshop sponsored by the Air Force Office of Scientific Research (AFOSR) in Arlington, Virginia in which I did a thought experiment and brought up the idea of doing offshore algae. So it was only poetic justice that I invited him to this meeting, So, welcome Al!

Al Darzins: Thank you very much everyone. I would like to thank Jonathan for giving me the opportunity to speak here today. What I am going to be doing today is giving you a United States federal government prospective of where we currently are with the development of algal biofuels. So, this first slide contains a photo of the National Renewable Energy Laboratory in Golden, Colorado.

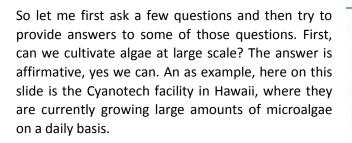
The next slide contains my outline for today's talk. First, I'm going to ask the \$64,000 dollar question, "Can we produce algal biofuels today?" Next, I will talk about the DOE's former Aquatic Species Program and about some of the lessons learned during that successful program which ended in 1996. Next, I will talk about what has changed since that program ended in 1996. We have heard a little bit about what has changed. Next, I will address the myth versus reality of algal biofuels, because frankly there is a lot of myth out there with regards to algal biofuels development. Next, I will talk a little bit

Outline



about the algal biofuels road map both from the Air Force and DOE perspective. More specifically, I will address what R&D will be needed to cross the algal biofuels commercial finish line. Lastly, I will talk about some of the biological considerations of algal biofuels. While engineering is a huge component in the development and commercialization of algal biofuels, biology is an equally essential component.

Let me begin with a little information about the National Renewable Energy Laboratory (NREL). On this map we are represented by the red star in Denver Colorado, located at the foothills of the Rocky Mountains. NREL is really unique amongst all the other national laboratories. There are a number of national laboratories around the country. Some are defense orientated, some are funded by the DOE's Office of Science, some are funded through the Office of Nuclear Energy while still others are focused on fossil energy. However, NREL is the only national lab that is focused on renewable energy and energy efficiency.



Wind, Sea Algae Workshop, Lolland, Denmark, April, 📼





We can cultivate algae at large scale...

1 460 1 10

My second question, is can we harvest algae? Again, I must answer in the affirmative. We can harvest algae using flocculation and centrifuges. We talked about these technologies yesterday as a means to get an algal paste.

We can harvest algae



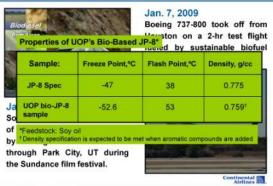
My third question, is can we today extract oil from algae? Absolutely. There are a number of ways this can be accomplished. In the laboratory we can use organic solvents to effectively extract the oil from algal biomass. Organisms like *Botryococcus* can secrete hydrocarbons which can aid in the collection of oil.

We can extract oil from algae....



Lastly, we can also convert these microalgal oils to transportation fuels. For example, Solazyme has been promoting its process of making oil from algae grown heterotrophically and using the recovered oils to produce biodiesel. Many of you also probably heard about a Continental airlines test flight in Houston, that used biofuel derived from microalgae and Jatropha. So we can make biofuels successfully from microalgae.

We can convert algal oil to fuels....



UOP has reported that it can actually make a very decent bio-based jet fuel using in this case soy bean oil, but you could just as easily use algal-derived oil here as well. And the specs are pretty close to what they are seeing with the standard JP-8.

So the \$64,000 dollar question is, "Can we produce algal oil cost effectively and sustainability today?"

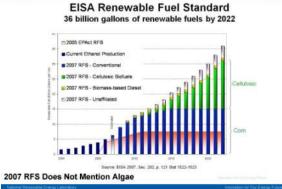
I will let my comic strip colleagues answer that question (Calvin and Hobbes laughing uncontrollably).

The answer, is no we can't, not today at least. We are still many, many years away from cost effective and sustainable algal biofuels production.



In 2007, the United States passed a very aggressive renewable fuel standard (RFS). This new RFS mandated that by the year 2022 the US will be using about 36 billion, that's with a 'B', billion gallons of biofuels. Corn ethanol production is currently expected to top out at about 15 billion gallons per year, which is called the blend wall because the US currently uses about 140-150 billion gallons of gasoline. Fifteen billion gallons of corn ethanol are sufficient to blend all of the US gasoline at 10% percent. The remainder of the 36 billion gallons is going to be made up of cellulosic ethanol, other

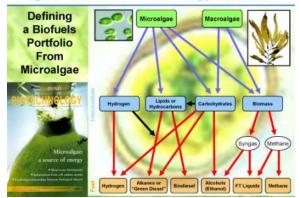
Advanced Biofuels in 2007 EISA



advanced biofuels as well as a category known as unaffiliated biofuels. It's potentially in this category where we think algal biofuels can actually have a huge contribution.

As a lot of you know algae have the ability to produce a wide array of different chemical intermediates that can be converted into biofuels. Peter just talked about algal biohydrogen production. We have been talking about lipids, but algae also have the capability of making large quantities of carbohydrates. Lastly, one could use the biomass itself to produce methane through anaerobic digestion or syngas and bio-oil through various thermochemical conversion processes such as gasification and pyrolysis. This is applicable both to microalgae and to macroalgae. So the nice thing

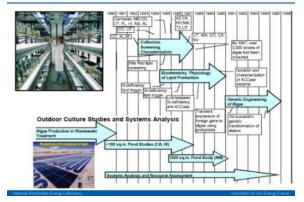
Algae: Numerous Bioenergy Routes



about the algae is that they are very flexible in terms of the intermediates that can potentially be made and converted into biofuels.

From 1979 to about 1996, the DOE sponsored the Aquatic Species Program (ASP), which was run by NREL. On this timeline you can see that from the early part of the Aquatic Species Program they were really focused on collecting organisms and characterizing them for growth and oil production. researchers used really The brute force methodologies and it took them a long time to collect about 3,000 microalgal strains. During the middle of the ASP they really started concentrating their efforts on the biochemistry and the physiology of lipid production --some of the things that Peter just talked about in terms of inducing the lipids. One

DOE's Aquatic Species Program

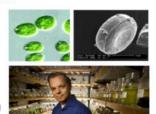


major finding of the ASP was that nitrogen depletion in green algae and silica depletion in diatoms was found to trigger oil production in these organisms.

In the latter years of the ASP the researchers focused on developing genetic engineering tools for microalgae. They, for example, reported one of the first successful genetic transformations of a diatom and then went on to attempt to genetically engineer a diatom to produce more oil. Researchers were able to over-express the Acetyl CoA Carboxylase (ACCase) gene, the first committed step in fatty acid biosynthesis, in the diatom *Cyclotella cryptica*. However, they found this genetically engineered

Microalgae Collection and Screening: Lessons Learned

- Many microalgae can accumulate neutral lipids
- Diatoms and green algae most promising
- No perfect strain for all climates, water types
- Choosing the right starting strain is critical



Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

variant did not produce any more oil than the parental control. While the demonstration of being able to engineer microalgae was a huge milestone, it was nevertheless a simple attempt at trying to use genetic engineering. Well, we now know that in the absence of some fundamental knowledge of lipid biosynthetic pathways in microalgae it may be more difficult than just cloning and expressing a single gene.

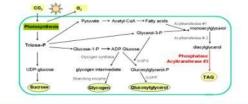
In addition to the ASP bench-scale studies there were also open race way pond growth studies in California, Hawaii and, of course, the Roswell, New Mexico study that took place over a number of years. During the growth studies in Roswell they were getting peak area productivities of 50 grams per square meter per day. That level of productivity wasn't sustainable over the entire year. On an average the researchers were able to obtain about 10-15 grams per square meter per day.

The ASP ended in 1996 largely because of federal budget cuts. However, there were a number of important lessons that we learned from the Aquatic Species Program during its almost 20 year run. First, many different microalgal species have the ability to accumulate neutral lipids with diatoms and green algae representing some of the most promising producers. No single algae strain will be perfect for all climates and weather. That's why we usually start with the best strain in the local environment. Again, as my colleague and friend, Jerry Brand, who is the culture curator down at the University of Texas algal culture collection points out that.... "Choosing the right starting strain will absolutely critical to any algal biofuels commercialization effort." In addition to the lipid biochemistry and genetic engineering lessons learned during the ASP we know that lipid induction through nutrient deprivation really affects biomass productivity in a negative way because frankly the organisms stop growing. To date, we have not identified an obvious lipid trigger.

We've really only—I think—begun to scratch the surface with regards to our understanding of microalgal lipid pathways and their regulation. So we need to devise and utilize all sorts of genetic strategies in order to alter some of these pathways with the purpose of improving lipid production.

Physiology, Biochemistry, and Genetic Engineering: Lessons Learned

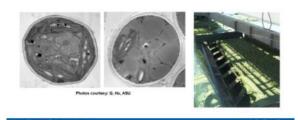
- Lipid induction with nutrient stress doesn't help productivity
- Key enzymes increase upon induction, but no obvious "lipid trigger"
- We have only begun to scratch the surface
 - · Understand lipid pathways & regulation; devise genetic strategies



In terms of downstream process engineering it is thought that microalgal cell flocculation is probably going to be one of the most promising routes for harvesting and dewatering. We know that we can do solvent extraction at the lab scale, but the real question is will we be able to do this on a commercial scale? So if flocculation is to be the method choice for harvesting in large scale algal cultivation facilities we will really need to start focusing our research efforts on the cell wall ultra structure. As algae grow its very likely that the cell wall ultra structure changes and its these changes

Process Engineering: Lessons Learned

- Flocculation most promising route for harvesting & dewatering?
- Solvent extraction of oil is feasible; but not economical
- Development of extraction methods will need a better understanding of cell wall ultra-structure and composition



that are going to give us some critical hints about how to better harvest and then extract oils from these organisms.

The ASP final close-out report was published in 1998. It contained a good summary of the major findings of the program and highlighted some major recommendations for future research. For me as a microbiologist it was rather satisfying to see that the ASP report first recommended "put less emphasis on outdoor field demonstrations and more on basic biology". What we really need to do is to ratchet up the engineering along with the biology. Given the fact, that we don't know a lot about the biology, what changes you effect in engineering can seriously effect the biology, and vice versa.

ASP Close-Out Report: Future Directions

- Less emphasis on field demos; more on basic/applied biology
- Take advantage of plant
- biotechnology
- Start with what works in the field
- Maximize photosynthetic efficiency
- Set realistic expectations for the technology
- Look for near term technology deployment such as waste water treatment



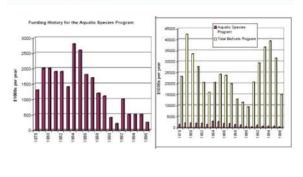
A second important recommendation was to "take advantage of plant biotechnology", There has been a virtual explosion in biotechnology over the last 10 years that can be brought to bear on improving lipid production by microalgae.

The ASP also recommended to "start with what works in the field". The ASP report also recommended that work should continue to "maximize photosynthetic efficiency", --which, of course, is easier said than done.

We as industry need to "set realistic expectations for the technology" because right now there is so much hype out there in the public. If we continue down this "hype" road and can't deliver on some of the outrageous claims out there, the public will stop taking this developing technology seriously. We have to become a very credible industry by setting very realistic goals and milestones. The ASP report also recommended that we "look for near term, intermediate deployment opportunities such as wastewater treatment.

The funding for the ASP over the almost 20 years was approximately \$25 million. However, as you can see by the graph the funding level started to tail off dramatically after 1990. During the later years of the ASP funding levels on an annual basis was at a half a million dollars or less. I'm sorry, but in my opinion, you cannot continue such an important program on that reduced level of funding. If you take a look at the spending on the ASP and compare it to the total funding for the DOE biofuels program you can see by this graphic that funding for the Aquatic Species Program was very small indeed. Over the

ASP funding



12 or so years that followed the ASP funding levels for algal biofuels research have been quite inadequate both for academia and the national labs. Although just recently funding for algal biofuels research is beginning to improve.

What has changed since the end of the ASP in 1996, you may ask? Pete eluded to some of this earlier; record crude oil prices; increasing worldwide energy demand; environmental concerns over increased CO₂ release; and a substantial increase in interest in algal biofuels by industry. For example, there is growing interest in algal biofuels by oil companies e.g., NREL is currently working with Chevron; Shell is working in Hawaii through a joint venture known as Solana; Conoco Phillips is sponsoring algal biofuels research through the Colorado Center for Biorefining and (C2B2). In addition to oil companies, we have



had significant interest in the development of algal biofuels coming from end users, engine manufacturers, and the airplane manufacturing industry, In fact, Boeing has actually come out and stated that bio could soon start replacing fossil fuel in jet aircraft with 3-5 years. Departments within the US Federal government are also now funding algal biofuels research like the Air Force Office of Scientific Research (AFOSR) which focuses in on basic science and the Department of Defense's DARPA program which has funded two large algal biofuels projects: one led by General Atomics and the other by SAIC. In addition, many of the US DOE national labs are jumping into this research area again. NREL actually restarted its algal biofuels research program about two and a half years ago.

Public perception of algal biofuels is actually quite amazing. For some reason people resonate with the concept of developing transportation fuels from algae. You can try to explain cellulosic ethanol to them and they kind of scratch their head, and ask "is that corn ethanol"? Well, no it's slightly different. But when you tell them about algal biofuels, very simply put they get it really quickly and they go "yeah, that's really cool". My wife is actually an ambassador for algal biofuels. When she's asked: what does your husband do?" She replies: "he works on renewable energy and is working on

Algal Biofuels In the News......

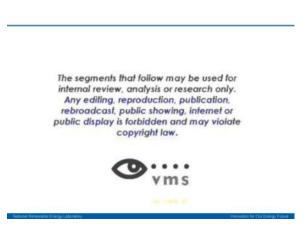


producing oil from algae." And people respond, "Yeah, I get that." So it is actually really capturing the public's attention. The next slide shows you just a sampling of where algal biofuels has been in the news such as National Public Radio (NPR) and various other publications.

But now what I would like to do –hopefully if you'd indulge me is to show a news video that came out last December on NBC's Nightly News. I am hoping it will play.... there we go...

video: Now when can something we normally consider a nuisance actually be a real source of energy in the future? When you sit down and make a list of issues we will be dealing with over these next few years there is; the economy, there is our security, and there is energy, pretty much at the top of that list.

And because the search is on for any source other than oil, that brings us to our closing story here tonight what can safely be called an alternative fuel. Our report from NBC's Kerry Sanders in Miami.



Kerry Sanders: Thirty years ago when American drivers were outraged as gas prices spiked to fifty five cents a gallon, government scientists wondered if somehow algae could be used as fuel.

Bill Grieco: This is an algae paste.

Kerry Sanders: It was one of those tiny federal research programs--mostly ignored--until gas hit three dollars a gallon.

Bryan Willson: There is no doubt that we can get oil from algae. The challenge is scaling it up to very high productivity at very low cost.

Kerry Sanders: Nationwide at more than 70 private labs backed by 100s of millions in venture capital and oil companies like Chevron and Shell; scientists are now reporting successes, squeezing enough biodiesel from slimy algae to make this a viable enterprise.

Al Darzins: Algal oil has the capability of really replacing or displacing quite a bit of the petroleum diesel that we produce here in the United States. Which is to the tune of 66 billion gallons.

Kerry Sanders: Researchers discovered when this primitive organism is exposed to extreme sunlight or cold water it creates stress which causes the algae to produce oil.

Fred Tennant: The great thing about algae is that mother-nature made it to grow very, very fast. So instead of harvesting once a year or twice a year we harvest every couple of days.

Kerry Sanders: While it grows faster than corn used for ethanol, getting algae enough to eat has been a problem. Every pound of algae consumes more than two pounds of carbon dioxide. One solution: grow it where there is an abundance of CO_2 : Power plants. In one small scale test in Arizona the power plant pumped its CO_2 into water where the algae then absorbed it.

Bill Grieco: We are able to convert, a renewable product like algae into fuel and at the same time consume CO_2 . It is a perfect story.

Algae to oil, literally the greenest of fuels.

Kerry Sanders NBC news. Thelzmeer, Florida.

So I thought that news piece was a fairly balanced story on algae. It really did not promise a lot, it just simply stated that the potential for algae as a feedstock for biofuels is really there. As I said earlier there are really a lot of unsubstantiated claims out there right now. It is these sorts of things that we as an industry have to constantly fight. The other thing we have to contend with is the growing drum beat of those people who are denouncing algae. We are going to have to counter those deleterious comments with credible, and I do mean credible numbers with regards to cost, sustainability as well as appropriate use of water, land and nutrients.



As I mentioned earlier, there is a great deal of federal interest now being generated with the potential of algae fuels. The Energy Independence and Security Act (EISA) passed in the US by President Bush in December of 2007 had specific language centered on algal biofuels. Section 228 of the Act explicitly stated it required the Energy Secretary of the United States, at that time Sam Bodman (currently its Dr. Steven Chu), to present to Congress a report on the feasibility of microalgae as a fuel feedstock. Wow, that was big; that was absolutely huge for algal biofuels to be recognized in that

Congressional Algae Report

2007 Energy Independence and Security Act (EISA)

- Increase availability of renewable energy that decreases GHG emissions
 Increases Renewable Fuel
- Standard (RFS) to 36 B gallons by 2022.
- (Section 228) Requires Energy Secretary to present to Congress a report on the feasibility of <u>microalgae</u> as a feedstock for biofuels production





manner. NREL actually helped in writing this report. As of today it hasn't made its way to congress yet, but the hope is it will make its way to congress very soon.

Let me now talk about some previous algal biofuels road mapping activities. Jonathan, I believe you mentioned this activity at the outset of my presentation. NREL was instrumental in helping the Air Force Office of Scientific Research hold a joint AFOSR-NREL algal biojet workshop in Arlington, Virginia in February of 2008.

Unfortunately, I do not have enough time to go through everything covered in the workshop but I wanted to highlight some of the biological considerations that were discussed during the roadmap. First, we are lacking comprehensive, publicly available strain databases regarding biofuels applications. We have strain collections located throughout the world but those only have limited information in their databases regarding specific information on growth rates, tolerance to salinity and temperature ranges, lipid content/composition, and general physiology.



Strain Research I

Publically available strain database and resource center
growth rates, temp. ranges, lipid content/composition, general physiology **Jolation of novel strains**issues: use of culture collection strains vs diversity in environment
consensus exists for the need to isolate novel (biofuels/feedstock producing) strains from a variety of unique habitats
needs of the community are diverse; max. genetic diversity, models strains, and outdoor applications **Model organism(s)**issue: one (or few) vs many models systems
consensus: in addition to C. *reinhardtii*, multiple model organisms should be pursued
selection criteria: lipid content/profiles; rapid growth productivity; available genetics; robust in mass culture

Next, is the issue of isolating novel algal strains from novel habitats. The issues with some culture collection strains that have they been kept in culture collection for years and years and probably no

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

longer resemble the phenotype that what was initially deposited nor is there a concerted effort to go out and look for algal diversity in the environment. I will show you a little bit of that.

At the workshop, we arrived at a consensus that we need to bolster our isolation of novel organisms because that is where we will accumulate some of our best sources of genetic diversity to do metabolic engineering improvements of oil production. Another issue centered on model organisms; do we use one or a few? The consensus was we need to look at many organisms with *Chlamydomonas reinhardtii* being one of them, but other organisms need to be pursued as potential model organisms.

Yet another issue that was discussed was the selection criteria for a model organism? It should include such parameters such as lipid content, rapid growth and productivity and available genetics.

We also discussed the need to speed up the sequencing of algal genomes. As most of you know there has only been a handful that have been sequenced to date. We know there is going to be more in the future. In order to make sense of those genomes we are going to need to train scientists that are able to annotate those genomes and do comparative analysis between known genomes. We need to start developing this capability on a large scale right now. We don't currently have a good understanding lipid metabolism and carbon partitioning pathways in algae, so to some extent





understand we are going to be running blind if we are going to be talking about commercialization this technology over the next number of years.

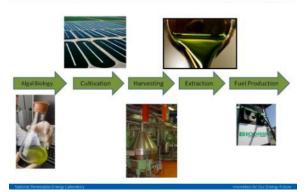
The AFOSR, as part of its algal bioenergy program, has decided to invest some of its funds in a systems biology approach to oil production and will be starting an integrated program designed to look at the *transcriptomics, proteomics,* and *metabolomics* of algal lipid production.



I mentioned that DOE actually sponsored a road mapping effort in December of 2008. NREL and Sandia National labs helped plan and execute the workshop for DOE. The goal of this workshop was to identify key technology hurdles, and I have listed some of those key hurdles already. Basically, we had about 200 stakeholders attend the workshop mostly from the United States, but a few stakeholders from outside the US also attended. These stakeholders represented universities, national labs, companies, algae companies, end users, and utilities just to mention a few.

The input that was received as part of the DOE workshop is being used to draft a comprehensive national algal biofuels road map for the United States. The completed road map would then be used to recommend research strategies to address those key technology barriers. Additional information regarding the DOE workshop can be obtained at the website provided here.





Day 1 Breakout Sessions

December 9 Breakouts	1	
Algal Biology (including strain selection) – Organize time & focus by obvious categories (marine vs. fresh; closed system vs open ponds; predation vs symbiotic)	Al Darzins (NREL)	Qiang Hu (ASU)
Cultivation – applied biology, scale up, including virology (and predation)	Pete Lammers (NMSU)	Eric Jarvis (NREL)
Harvest/dewatering	Tony Martino (SNL)	Ron Putt, Auburn University
Extraction/fractionation	Nick Nagle (NREL)	Mike Cooney, U Hawaii
Conversion to fuels	Blake Simmons (SNL)	Steve Przesmitzki (NREL)
Co-products	Michael Huesemann (PNNL)	Molly Hames (DOE)
Systems integration	Len Malczynski (SNL)	John Hogan (NASA Ames)
CO ₂ sourcing & Siting; land, water resources	Ron Pate (SNL)	Anelia Milbrandt (NREL)
Regulatory/Policy	Phil Pienkos (NREL)	Grant Heffelfinger (SNL)

Day 2 Breakout Sessions

December 10 Breakouts		
Fossil fuel producers, refiners, catalyst suppliers	Mike Pacheco (NREL)	Chris Shaddix (SNL)
Algal biofuel companies	Grant Heffelfinger (SNL)	J. Brainard (NREL)
Biodiesel/bioethanol renewable fuel producers and appropriate user groups (e.g. auto industry)	Mike Cleary (NREL)	Chuck Mueller (SNL)
Co-product and competing product companies (ag-bio, food/pharma/nutraceutical, chemicals, bio- based materials)	Maria Ghirardi (NREL)	Leslie Pezzullo (DOE
Input Groups/Sourcing (utilities, wastewater, CO ₂ sources)	Tryg Lundquist (Cal Poly)	Steve Gorin (NREL)
Policy, Regulatory, and Standards	Steve Mayfield (Scripps Inst.)	Tom Gross (IF LLC)
Financial interests and risk analysts	Joel Serface (Entrepreneur in Residence NREL)	Tom Brennen (Entrepreneur in Residence at SNL)
Possible public-private partnerships and interests	Bill Buchan (NASA)	Joyce Yang (DOE)

The DOE workshop contained numerous break-out sessions devoted to several key barrier areas. For example, Qiang Hu and I were part of the algal biology session while Pete Lammers was part of the algal cultivation break-out session. Other breakouts included sessions on harvesting/dewatering, oil extraction, conversion to fuels, co-product generation (we know these are going to be important), systems integration, siting, resources management and regulation and policy.

This is what the road map outline looks like right now. It's still in the drafting process but it will include an executive summary, introduction, and sections on standards, regulation, policy and partnerships; Ron Pate talked about the systems and techno-economic analysis yesterday.

Roadmap Outline

- Executive Summary
- Introduction
- · Leadership & Collaboration to Achieve the Vision
- · Standards, Regulation and Policy
- Partnerships
- Systems & Technoeconomic Analysis of Algal Biofuels Prospects
- Science, Engineering and Scale-Up Strategy
 Feedstock
 - Processing and Conversion
 - Closing the Fuel Cycle Starting with the End in Mind
- Summary and Conclusions

What I would like to do is to focus on the science. engineering and scale-up strategies. Even more specifically address feedstock issues. Here are some of the areas that are under the algal biology section which includes some of the topics we have already talked about such as strain isolation and screening, cell biology and physiology, the development of an algal genetic toolbox. Chlamydomonas has a pretty good developed genetic system but most all the other organisms do not. Lastly, a systems biology approach will be necessary to find out where the bottle necks are in these various biosynthetic pathways.

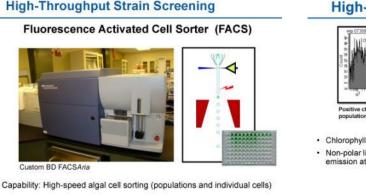
We talked about algal biology and screening, novel habitats and ecosystems using high-throughput screening methods, I'll talk about that in just a minute. We also discussed developing standards for procedures, reporting and facilitating comparative analyses and data sharing as well as improved biological resources such as the public algal culture collections like UTEX and CCMP. The ultimate goal is to develop is a public database infrastructure that everyone has access to with regards to important parameters on important strains.

Roadmap Outline

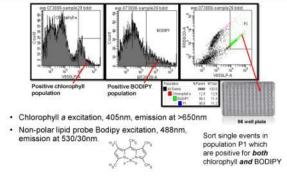
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Feedstock: Algal Biology

- Strain isolation and screening
 - Novel habitats and ecosystems
 - Creation of high-throughput screening methods to identify promising strain characteristics
 - Development of standards for procedures and reporting to facilitate comparative analyses and data sharing
 - Improved resources for public culture collections (e.g. UTEX and CCMP)
 - Public database infrastructure on strains and their characteristics including regional environmental variability on cultivars



High-Speed FACS Sorting



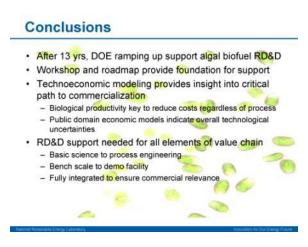
NREL after re-establishing its algae program is now using rapid, high-throughput methods to identify novel algal strains from unique aquatic environments. In this endeavor we are using a fluorescence

Wind, Sea Algae Workshop, Lolland, Denmark, April, 2009

activated cell sorting (FASC). Here is a photo of NREL's FACS instrument. Basically, what the instrument does is to interrogate small aqueous droplets containing algal cells with a laser beam of light. If the droplets containing the cells have the right parameters they are either collected in a tube or in a 96-well plate containing growth media. Droplets that do not meet the right parameters are shuttled off into waste. If all 96 wells grow, you have 96 unialgal cultures. So in a very rapid fashion you have unique algal cultures at your disposal for screening purposes.

We have done this to sort out those organisms that have high lipid content. We first sort populations based on the presence of chlorophyll. Chlorophyll positive populations are then sorted for their ability to be stained with a fluorescent dye called Bodipy which is a new neutral lipid dye very analogous to Nile red. Micro droplets that are both chlorophyll-positive and Bodipy-positive are deposited into a 96 well growth plates. Wells positive for growth are first examined using light microscopy. In this image you can see a diatom and here is a green algae. Algal cultures are then stained with Bodipy and examined using confocal fluorescence microscopy which reveals oil droplets which stain green. Here is a confocal fluorescence micrograph movie showing a diatom stained with Bodipy that is absolutely chalk full of these oil droplets. It is absolutely amazing how much oil some of these algal strains can produce. This particular organism is fairly large and is on the order of about 50-60 microns in size. So it is a very large organism compared to something like a *Nannochloropsis* species which you know is very small.

In conclusion... After 13 years, the US Department of Energy, is beginning to ramp up its support of algal biofuels research. We are hopeful that the algal biofuels technology road map derived from the workshop will provide a foundation for that support over the next couple of years. Techno-economic modeling and life cycle assessment is going to provide us with the necessary insights as we move towards the critical path to commercialization. I can't stress enough how important techno-economic analysis of algal biofuels processes will be in the future.



R&D support will be needed for all elements at the algal biofuels value chain. I am not talking just about the biology. I am talking about the biology and the various downstream processes such as harvesting, extraction and fuel conversion.

Despite the potential of algal biofuels there are still many challenges associated with commercializing this technology. Anybody who is in this and thinking this is not a risky game is, I think delusional; they are fooling themselves because the regulatory landscape is confusing and contradictory. We don't know what regulation policy is going to say about algal biofuels plus the relevant regulations and policies that are out there are not really crafted with algal biofuels in mind, so we are going to have to help them along as they craft some of those policies.

While there is still a lot of uncertainty the near term potential for algal biofuels here is for water remediation, job creation, and education. So based on what I have told you about, I think the potential for algae biofuels is absolutely immense. What we need to do is keep a level head with regard to that potential and not over sell the technology. Because that is the worst thing we can do with this technology; that is, to over promise but under deliver. With that I would like to thank you for your attention and I invite any questions.

Conclusions (continued)





QUESTIONS:

Jonathan Trent: Thank you Al!

Wow the Department of Energy is underway! I should say the Aquatic Species program was quite under-funded. The project I am doing is funded by Google--Google.com--not NASA. But lets have some questions and lunch, I hope, is waiting for us outside. We can come back and ask more questions later, but Robert?

Robert Baertsch: Al I just want to share that, one of things we did for the human genome project which I think is really great is to have an international consortium where we have meetings to present results. We set goals to what we want to achieve in the next few years in terms of annotating the human genome and developing new assays. Then we would all put the data in one place, in this case the encode genome browser was done in Santa Cruz. And I think we could do a similar encode project for the algae group. I think they are using it now for fly and other model organisms.

Al Darzins: But I think you still need to train people to do the annotation. We just don't have the resources to do this. We are also talking about sequencing probably hundreds of algal genomes just for comparative purposes. We really do need the education to bring these people on board. For example, anyone out there who is trying to hire algal phycologists knows that it is hard to find such expertise. So in order to bring people into this field we need to provide the appropriate training.

Jonathan Trent: I have a question for you Al. In the road map, is there going to be any discussion at all about offshore algae cultivation?

Al Darzins: That's a really good question based on what we are hearing here in Lolland. I am going to have to review the current roadmap input to make sure that the off-shore option has been captured. Oh, it is in there? Then OK, it is in there.

Jonathan Trent: So we are on the map.

Al Darzins: We are on the map.

Jacob Davis: In one of your slides you said oil extraction is not economical... I guess I have a couple of questions about that. What is that compared to? Do you have any comparison on a per ton basis with other oil seeds? How much is it not economical by? And where is the data to support this?

Al Darzins: Yeah, I don't have the data in hand but the folks I have talked to say that at the scale where we are going to need to do this, we are literally looking to be able to produce 10s of billions of gallons of oil to displace some of that petroleum diesel usage... right now the United States I think produce about 3 billion gallons of soy bean oil to purify that oil the industry uses expellers to remove the oil or they use solvent extraction to some extent. It may be very difficult from a cost perspective to use solvent extraction on that scale as well as to try to recycle all that solvent to being green. I don't think this is going to fly... I'll get you the data though. I just don't think it is going to be the way to go.

Jonathan Trent: I think everyone is hungry and is holding their questions back.. Oh, Travis... Better be careful. It better be a good question, everyone is hungry...

Travis Liggett: This is not a question. I will make it quick. My ideas for the geometry of the bags....If you would like to look at them. Please take one.

SEAWEED AQUACULTURE IN OPEN COASTAL WATERS, BELA BUCK

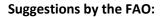


Jonathan Trent: When I first thought of OMEGA as a part of an integrated windfarm system, I did what everyone does these days, I did an Internet search on "windfarms and aquaculture," and that was my introduction to Bela Buck—our next speaker. Bela's inspiration is to use the windfarm infrastructure to support macro-aquaculture, which includes seaweed and mussels and a variety of other possibilities. I don't know if he wants to use any of the other windturbine features for pumping water or lighting or anything else, but I'll let him tell us about his plans and hopefully he'll inspire us to think about how this applies to OMEGA...

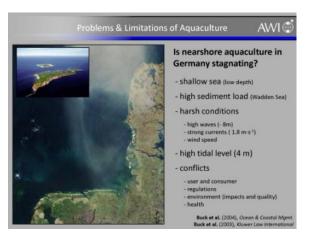
Bela Buck: Thank you for inviting me to this workshop; it's a great pleasure for me to be here. I will not talk about microalgae, but will present some of our experiences when we moved aquaculture or macro candidates offshore. We don't have any experience with microalgae, except in the lab; we focused on moving offshore with macro algae candidates.

Problems & Limitations of Aquaculture

Actually, there's a reason why we do not have that much aquaculture in Germany. It is because the *Wadden Sea* is very shallow and we have a very high sedimentation load that has some shadowing effects. In addition to that, there are harsh environmental conditions: we have high waves, strong currents, wind speeds, a tidal range of about four meters, and the biggest problem we have is this one (refers to slide). Maybe all of you who have not worked offshore have no idea about user conflicts, but they do exist and that's the biggest problem: if you don't have any acceptance with other users, you can forget all your ideas.



So then we decided to move offshore; if there is no good site in coastal areas available, moving offshore is an option; such a move that was also suggested by the FAO in 2002, especially for fish and mollusks. Neither seaweed nor microalgae is mentioned by the FAO, but this was seven years ago and there are a lot of scientists who now run projects with seaweed offshore. Actually, what is offshore? For example, this cage is offshore; the same as this one, but during the conference in 2005 in Limerick, James Ryan defined offshore for aquaculture, saying it should at least be an exposed condition. There's no need to have it one mile or 10 miles, 5 kilometers or 15 kilometers off the coast; it should just be in an exposed condition. This is an exposed condition; it's actually our farm but you cannot see it because it is submerged. I want to go into a little bit more detail about our biological investigations and will follow with some techniques. I will finally suggest how to combine these technical designs with wind farms, as well as some management and site-selection criteria.



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AWI

Suggestions by the FAO:

A STRATEGY FOR THE SUSTAINABLE DEVELOPMENT OF EUROPEAN AQUACULTURE, COM(2002) 511 final, 61 pp.

<u>Competition for space</u>. Many complaints against aquaculture development reflect competition for space, the recent growth of aquaculture, particularly on the coastline where there is already a high concentration of activities, put it in the place of the newcomer disrupting the long-established *statu* quo between existing users. Land and water for aquaculture will be more and more expensive in future. Aquaculture establishments may be forced to move offshore, but this is a possibility for some species only.

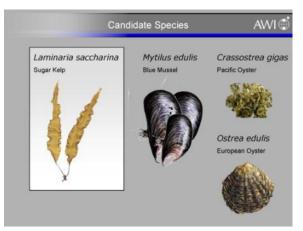
Here offshore technology needs to further develop.

Develop closed water recirculating systems, offshore fish cage technology, mollusc offshore rafts and long-lines.



Candidate Species

There are a number of species we tested off shore but today I only want to go into detail about *Laminaria saccharina*,. I will focus on that *L. saccharina*, although that species is currently being reclassified. For those of you who have experience in seaweed taxonomy, there is *Laminaria* and there is a new genus called *Saccharina*. , However, I still use the old name, for the "sugar kelp," *Laminaria saccharina*. Why do we use this seaweed? In Germany, we have a name for it; it's called a *Tausendsassa* and I think in English, it's called a "Jack of All Trades," something like that, because there



are so many possibilities for which you can use Laminaria. For example, we use it to absorb heavy metals and, two days ago, Jonathan, you asked me how it works, so I put together all the [publications] literature, which I found, so you can actually have a overview. I forgot one additional potential utilisation: the Biogas operators are looking for new crops. They usually use wheat and maize and, actually, the north of Germany is now only used for bio-gas agriculture and not for human consumption. All biogas plants currently suffer a bottleneck since there is not enough space for all the maize and all the wheat required for biogas production. As a consequence, the idea came up to culture seaweed as an additional product for biogas production.

Reviews on Laminaria Cultivation

The other reason that Laminaria is an ideal candidate for aquaculture is that a lot of research is done already. The first review article was published in 1949 by Parke and there was another one by Kain in 1979. There's now a new one-- this one came out last year—by Bartsch et al. 2008 with a special chapter concerning the potential utilization of laminarian plants. These scientific experiences are necessary when moving offshore.

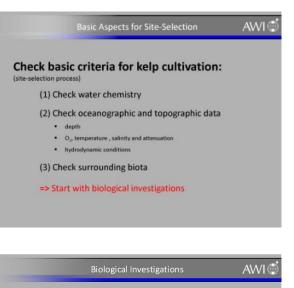


Check basic criteria for kelp cultivation

When you want to move offshore, you have to do some site selection investigations in advance; you have to check the water chemistry of course; you have to check your oceanographic and topographic data and you have to check the surrounding biota. If there are no laminarian plants but you have hard substrate, there should be a reason for that. In some areas, you could have predators (e.g. sea urchins) so it may not be a good site for culturing Laminaria. You start your biological investigations, which also include various cases on carrying capacity.

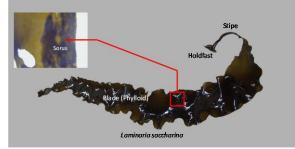
This is an image of the Laminaria saccharina; it's a blade about 2 meters long with a stipe and a holdfast. I do not want to go into detail about the reproduction cycle but you do need sorus (a cluster of structures producing spores), and first of all, a lab. As soon as you have the sorus and you have your spores, the spores will by geotaxis settle on these ropes. When they are about 1 millimeter in length, you have to transfer the ropes with the young plants off shore. This takes place at an early stage of the life cycle. The reason for this early transfer is that the holdfast adapt to strong conditions (waves and currents). If you bring the plants to the grow out site later, the holdfast will be dislodged.

There is a rope around each drum and the drums rotate, thus allowing a minimum current at an early stage and the holdfasts can adapt already in the first month after settling. Within this time they reach this size here, (about 1 or 2 millimeters), you can transfer them to sea.



Biological Investigations:

(1) Understand biology and carry out the reproduction cycle

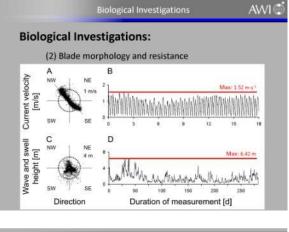


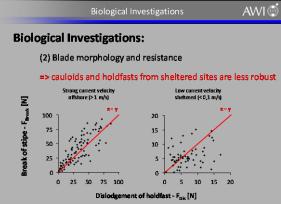


Biological Investigations: (2) Blade morphology and resistance

You never know how the conditions are offshore and you never know if you are going to lose your culture installation. As a farmer, you want to be sure that you will have your farm tomorrow after leaving the farm today.

There are two possibilities: one is that the holdfast will be dislodged, and the other is that the stipe will break. So we tested both parameters: this is a sheltered condition and this is an offshore condition. These are the long seaweeds, and these are the small ones but the breakage of the stipe and the dislodgement of the holdfast are very similar and the same is true for sheltered conditions. But you'll see that 15 Newton is the force you need already to dislodge the holdfast; offshore it's 75 to 100 Newton for the same size of species, about 1.5 meters in length.





If you want to test whether the seaweed will be in the right position and you don't want to lose them offshore, you will need to have some information about the drag on your seaweed. When you want to calculate drag, you need the drag coefficient of the cultured candidate.. Drag-coefficients exist for nearly everything in water and wind but not for seaweed.

So we had to do some towing experiments. We did those in a towing tank in Hamburg to find out the drag coefficient. We towed different sizes of Laminaria, from sheltered and offshore conditions, and also bunches of Laminaria, and then we included the data that we measured at our offshore site. This is the current velocity; 3 hours after slack tide (slack water high tide). There was a current velocity of approx. 1.5 meters per second, which is very strong. It's' a bi-directional current due to the tidal current regime. There are also waves of about 6.5 meters in height. The site is 17 nautical miles off the estuary of the river *Weser* and, of course, here in the south, there's the shore, so most of the high waves come from north to south.

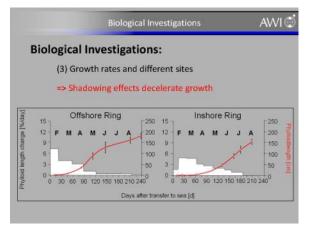
What we found was that offshore seaweed adapted at an early stage to their harsh environment. Because of being transferred to the sea, they do not have the high drag force, as do seaweed from sheltered conditions. These are different sizes--this is a long Laminaria, this is a small one, the same here. But, as you can see, in current velocities of about 2 meters per second we only have a drag force of about 5 Newtons.

So if you take these data and include them in this model for the worst- case scenario, which is about 6.4 meters wave height, 2 meters per second velocity and a wave period of 6 seconds, you will have 35 Newtons. But what we measured (before we come back to the beginning) was that if you want to break the stipe or dislodge it, you will need up to 72 or 77 Newtons. In this way, you can be sure that if these algae can learn in the beginning to adapt to offshore conditions, you will not lose them.

The reason for this higher resistance is that offshore seaweed has a streamlined morphology; it's very flat. And this is the seaweed you may know from sheltered conditions; it's undulated and not that long. And this is one of our *Laminaria* we grow on-shore in rotating tanks with bubbles, so we have this corkscrew morphology.

Biological Investigations: (3) Growth rates and different sites

Of course, everything that is offshore has better O_2 conditions-- still enough nutrients because the North Sea or the German Bight tends to eutrophication anyway. If you have enough nutrients in the water, you will have a very good growth rate and your seaweed will have a length of about 2 meters after 7 months. You cannot grow them longer; you have to harvest them, because after that time, you will have a lot of fouling organisms. If you have fouling organisms, the surface of your seaweed will enlarge; then you can lose it to what is often described as "tip loss." Because of this fluttering in the water, you

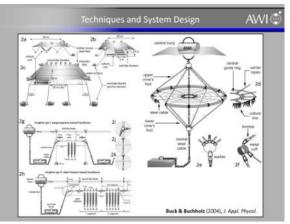


will lose the tip of the seaweed and that is always a loss of biomass; furthermore, the seaweed is not growing any more at that time. It has no more length change because it has entered the reproductive cycle.

How did we culture Laminaria offshore? There are a lot of techniques used worldwide.. You have long line construction, ladder construction or grid construction-- everything that looks very nice. You have buoys floating on the water, this line and the other line; and then, on another day, when you come back, there's nothing anymore. You find them all washed up on the beach or entangled in a ball-like structure somewhere in the ocean.

Techniques and System design

So that's the lesson I learned, because I'm a biologist and when I started doing these things, I wanted to do everything alone: [I thought I knew] how to use shackles and swivels and concrete blocks but yes, that was the lesson I learned. If you want to move offshore as a biologist, you should include engineers, at the early stage, (laughter) of course. Sometimes biologists forget that, because they think they know everything. There are other constructions, which we use for long lines for mussel cultivation. Mussel cultivation is very easy because you can submerge the whole system at about 5 or even 10 Meters.



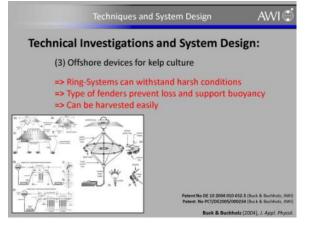
Richard Langan from New Hampshire submerges his mussel long line 10 Meters below the surface so he does not have wave action anymore; but you can't do that with seaweed, because you don't have enough light. For that reason, we developed and designed another construction-- this ring construction-- so that all the forces acting on this buoy by wind or wave or swells are going all along this wire onto the concrete block. And from a bird's eye perspective, the whole thing can move in a circle around the one-point mooring within which you have this spider-web-like construction. Here you have all the ropes, where your seedlings are with the Laminaria. One ring with a diameter of 5 meters can have a biomass wet weight of 800 to 1,000 kilograms.

Comment from the audience: You can produce power with this?

Bela Buck: Maybe; that is another question, another vision...

Technical Investigations and System design (3) offshore devices...

The ring system was the first technique that we found which was able to withstand harsh conditions. Next we tried different kinds of buoyancy, one buoy, different buoys...elongated buoys, ball-like buoys...so you should invest in a lot of techniques. It depends on the size of the ring and on the species; this is good enough for *Laminaria saccharina* and *Laminaria digitata* if you change the species, it could be a different system. This is not the end, I think; the next step would be, how can we construct a modular system to connect different rings or even a pentagon-like structure.



Wind, Sea, Algae Workshop, Lolland, Denmark, April 2009

Technical Investigations and System design (3) offshore devices for kelp culture

This is the ring after harvest; it's an old design with different buoys (it's not the one with the one buoy on top) and this is a photograph from a byssus plaque of a mussel. But I put it in because I have no photo of a holdfast available right now. You should also look into details regarding the ropes and the hard substrate, because PE and PP, the nano-surface, is very different and the holdfast will not hold on every surface. It's not only a question of having something cheap, like *cocos* or sisal; you should have an idea about which species will hold, where and how.



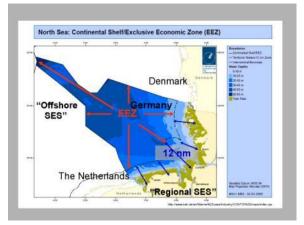
Jonathan Trent: Do you understand the basis for the holdfast or is it just intrusion into cracks and crevices?

Bela Buck: It's going around the rope; but what happens if there's no connection to the surface of the rope, and then you cut a rope here and there?. You can take the rope all out of the holdfast, so it's like a tunnel of little roots. You should have a rope, which is well connected to the holdfast of the seaweed.

North Sea: Continental Shelf

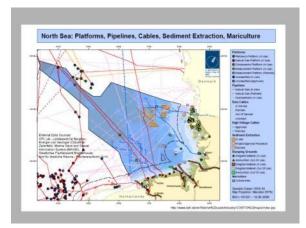
Now something about management and site selection; this is the North Sea coast of Germany.

This is the coastal sea with the most integrated coastal zone. Managers say that it's a social-ecological system (SES)--a regional social-ecological system-- and this is the offshore SES or the exclusive economic zone (EEZ).



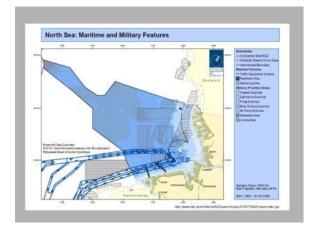
North Sea: ...

Here on this map, you'll find all the platforms, pipelines, cables, mariculture, and sediment extraction.



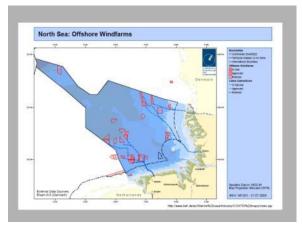
North Sea: ...

Here are the maritime and military features.

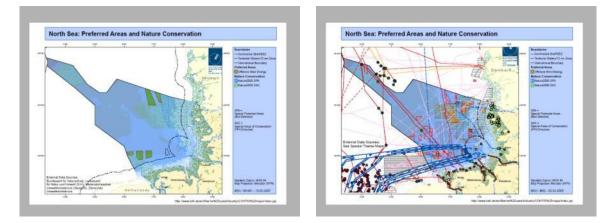


North Sea: ...

These are the planned windfarms, some of them, this one, these, and this one are under construction. They're a little bit further away from the coast than those that you see in Denmark; from here to there, from Borkum Island to here, is for instance about 45 kilometers!.



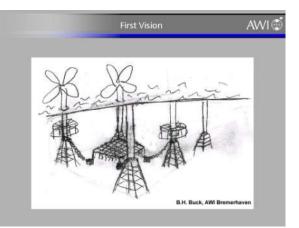
North Sea: ...



Here you have all these nature conservation areas, important bird habitats, and Natura-2000 habitats and if you include them all in one map, there's no more space left. That's why I told you in the beginning that stakeholder conflict is the crucial element in moving offshore. We don't talk about waves and currents. That is something, you WILL find a technique; maybe it will be very expensive but you will find a technique! But with stakeholders, especially fishermen, it's another kind of human being. (laughter) That's my experience; they are very friendly, but they are totally different. Actually, what you said yesterday about the fishermen, that they like to go in the farm, yes, they do. Actually, when I wanted to have this test site, I organized a fisherman meeting, because they had to agree, and somehow it appeared during this meeting that on this little, little test site, about 4 hectares every flatfish fisherman and shrimp fisherman somehow had to go to and to fish on this very spot. Perhaps it was a secret upwelling site, I have no idea ?! There's another group, the private boat-owners with their sailing boats. When they go offshore and they are very far away from the coast that is a little bit like Texas. They take everything they find. If there's a buoy, maybe with a very expensive instrument hanging on the buoy, they cut it because it's a buoy offshore, and whose buoy is it? There 's no name on it, so they take it and you lose your instrument, of course—with all your data.

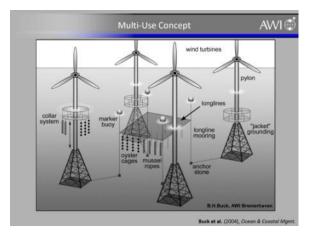
First Vision

Nine years ago, in the early 2000's, we had the first idea about how to resolve this problem: make it like a co-management situation. Why not have various people use one space? Actually, this is a drawing on a beer map in a pub at 2 AM, but somehow because of it, I got the position at the AWI.



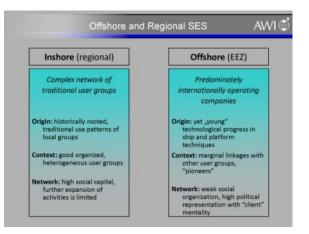
Multi-Use Concept

This is a new design. In between, you have a mussel farm. I don't have any ring system or oyster cages here but I'll show that later..



Offshore and Regional SES

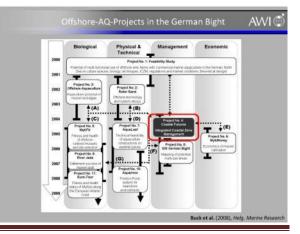
On shore and offshore are very different. It's not only a question of hydro dynamics; everybody knows that offshore is a high-energy environment and that you should know something about waves and currents. But the wind farm planners or operators have a lot of economic and political power. They have a strong political will as backup; they're subsidized, they're operating more internationally. But they don't have any social capital. The fisherman, however, are historically rooted; they have a network and have been here for hundreds of years. How then to bring them together? That was one of the biggest problems we had. Don't be afraid,



one of the biggest problems we had. Don't be afraid, I will not go into detail with all of these projects, but I will consider here only what was very important.

Offshore-AQ-Projects in the German Bight

This project,, coastal futures, was an integrated coastal zone management project. Bringing all the stake holders to one table was the most difficult problem that we had, but it worked, and since then the fisherman have actually helped us in doing aquaculture offshore First they hated us, because we had somehow something to do with the wind farmers, and wind farmers and fishermen don't like each other because the wind farmers take the fishing grounds.



Involved Parties

Here you have some of these users; we had all these authorities: mariculturalists, wind farmers, other companies, research institutes ... in our projects.

Involved	Parties	AWI 🌐
- Authorities:		ave i
- 6 Water and Shipping Agencie	s	
- 2 Federal Water Ways Director	rates	NO THE
- Federal Fisheries Agency		and the second second
- Federal Maritime and Hydrogr	aphic Agency	
- Food Quality Testing Laborato	ries	
- 12 Mariculturists		
- approx. 30 Fishermen		All a statements
- 4 Offshore Wind Farm Planers & C	Operators	TA L
(local player, int. player)		Att at
- 9 Research Institutes		THE
- 10 Companies (Engineers, Econon	nists,	
Food Production)		
- Senate of the City State of Bremen	n an ann an the second	
Krause et al. (2003). Rights & Duties		Buck et al. (2004 Ocean & Coastal Man

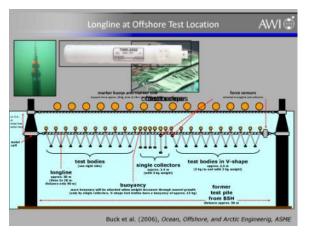
System Design for Grounding Structures

The idea came up to not only use the same space, but also to use the same grounding construction. That is a way to get rid of very expensive mooring systems, so how can we combine a seaweed cultivation design, or mussel cultivation, or whatever, with the grounding construction?



Longline at Offshore Test Location

We had a project to measure all these loads. This is a test body that has the same size, the same volume and the same weight as a fully grown market-size mussel collector. We also had some of these force sensors, and the whole longline was connected to two piles. The force sensors are connected here and within the system, and we also measured, of course, the current velocity, the wave height etc, to find out how high the loads are from long line on the running construction. This mussel long line was 60 meters long. What were the depths? That side was about 20 meters and the long line was submerged about 5 meters.



Long line at Offshore Test Location

Here you have the long line still on the surface. This is one of the piles at high tide and we submerged the whole system down to 5 meters by using these big rings..



Diving is only possible at slack water (about 20-25 min.). Work on zodiacs is possible for 1.5 hours.

Research and Seasickness

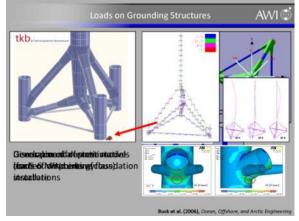
Actually, the problem is that it's not only a matter of the applied technique but also a question of the people who should do this work. This is our research vessel. Although it's not a normal condition, this is something that you have to confront frequently and then there's no chance to do any work. You're fighting seasickness. Some people say, we have so many unemployed fishermen that could do this work. But that will never happen. Most fishermen will not do aquaculture—this is also one of my lessons I learned --maybe some will, but the majority will not do aquaculture. They are fishermen and



have been fisherman for 5 or 6 generations thus it is a major part of their social identity..

Loads and Grounding Structures

This is one of the grounding constructions of a windmill off the coast -- 50 kilometers North of Borkum, an island off the west coast of Germany. It's a different construction from those you know, because in Denmark you use a mono-pile construction. This is a tripod; to see how big this tripod is would be to compare the size of this nice French car to this grounding construction. Here you have the water surface. We each started calculating different materials, different models, and which material, which thickness, is where? How do we find



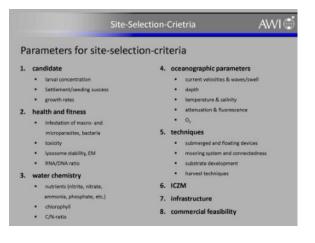
a place where we can connect a long line? In the beginning, I thought okay, just weld a shackle and I'll put my long line on it; done! But that will never happen. Also, we tried to find alternative connection points for this shackle; should the shackle be here or there, or here? Also, you have to include your vibration by rotating of the blades and also by the currents; you have to include that in your calculations. We found out that on this very short, 60 meters long line, you already have a force of 20 tons. So if this long line is 700 meters long, because the actual size between the turbines is one kilometer, if it's 700 meters long and you have 70 parallel long lines, you will never ever connect this to a wind turbine. It's different from seaweed because seaweed does not have the same force as mussels; it's another surface. But with mussels, it will never work, so you have to invest in your mooring systems.

Marine AquaKulturFlachen in der Deutschen AWZ

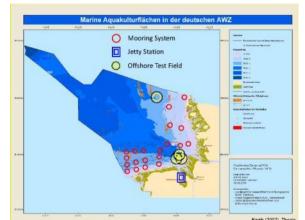
Again this is the German economic exclusive zone and here you have some site collection experiments around planned wind farms. We had some of these mooring systems to calculate growth rates, to take water samples, to measure the growth of mussels, etc, and we had these test sites. We had these long line systems and ring systems and also one jetty station, which is a bridge to deliver coal; that is about 1.2 kilometers long, so you are very much in the center of the Jade bay, where you have a very high current velocity.

Parameters for site selection criteria

For the site selection criteria process, there are a lot of things you have to take into account. It's not something you should do in only two weeks. All of these things--lava concentration is of course only for mussels-- but for sediment or seeding success, how many *Laminaria* do you have per centimeter of rope? This is what you need to calculate to break even: the sediment or seeding success and the market price. We enlarged our site selection criteria program so that we are starting south of Portugal and going all along the coast to north of Denmark, mainly focusing on shellfish, but we will now also include seaweed.







Three last slides



Since I am coming to the end of my talk, I must say that I did not do all of this alone; we have a lot of partners, which you'll find here. And we also have other partners, like ships, most of the ships, like this one-- it's an army vessel--also, this one, this one and this one. The other ships helped us to find all the things that we lost, like buoys, long lines, and all that kind of stuff.



QUESTIONS

Jonathan Trent: We have some time for questions. Actually, I have a question.

Bela Buck: Barry Costa-Pearce from Rhode Island asked me to make an announcement about a symposium "Renewable Energy and Offshore Aquaculture" which will be held in November in Newport, Rhode Island. It's within the Sea grant college programs; the Baird symposium. Maybe we could put that on Google later.

Jonathan Trent: When you looked into the turbine extension as a basis for anchoring, you said that you can't possibly hook between the one-kilometer distance. But could you use it as a center point and then just put buoys in, half the number of buoys that you need?

Bela Buck: It depends on the candidates; seaweed is not possible, because the seaweed has to be at least one meter below the surface. Next, between wind farm operators and aquaculture, there's a big gap. First, there's the wind farm, and later comes the interest of the aquaculture operator So, all service boats for maintenance or whatever should enter or should be able to access the wind turbine, and you cannot do that if you have your farm. And they will never go around maybe 100 meters to enter a waterway to come to the turbine, if something happens. It's a question of money, so if the wind turbine is not working, they go directly to it and they don't want any farm in-between. The idea was to have different plots within the wind farm. That will work, provided that the wind turbines have free access at all times.

Jonathan Trent: Yes, I think Dick Seymour brought that up in a design that we were talking about, and this is a very important point. Some of this is so much social psychology and branding. I once had some experience with the Navy and when I went to talk to the Navy guys, if I had asked them to do research for me, they probably would have said "No." But I asked them to envision themselves as being like astronauts, except that they were going to this place underwater. I mean, this is just a matter of branding; I think you have to work on that-- how you can get fishermen to work for you. But you can't use the word "aquaculture." You have to use some other concept.

Bela Buck: This is an early stage; we don't have any commercial operation yet, because we have no wind farm.

Jonathan Trent: They have . .

Bela Buck: Yes, actually there is a wind farm in Wales, North Hoyle; we will install a long line, a mussel long line there, maybe next year.

Jonathan Trent: Well, there may be some possibilities here, too, I guess.

Bjørn Utgård: In Norway, we have a lot of fish farms. And the fish farmers are very different from fishermen, I think, because along the coast they are the industrial tycoons. They are the guys that are the richest guys along the coast. The problem is that in many of the enclosed areas, there's going to be too much fertilization from the fish farms, so you need to deal with that somehow. Clearly a macroalgae or any installation that would remove some of that fertilization could actually increase the ability of the sea area to take fish farming, while, at the same time you'd be given an extra .

Bela Buck: You want to move fish farms offshore?

Bjørn Utgård: Well, there's a, different technology that some people are working on in Norway but are actually putting the fish farm offshore. I'm thinking, did you talk, did you look into cooperation with the fish farmers instead and [establish] synergies because, like you said, we haven't found a fish farm, we haven't found a wind farm yet, but it might be that a different connection can actually be more feasible from a stake- holder perspective.

Bela Buck: Yes, that has already been done. A lot of projects worldwide deal with seaweed and fish farms. One of the scientists doing this is Thierry Chopin in the *Bay of Fundy*. He is connecting Laminaria saccharina to a salmon farm, so this is already occurring on practice. It's not new; we can do the same thing but I am focusing on something different. It's a good idea if you have no wind farm but if you want your seaweed farm, you should connect with something different, like a fish farm.

Jonathan Trent: Have you tried to grow other brown algae, like *Macrocystis*? Does it grow in cold water here?

Bela Buck: We don't have Macrocystis, no, it's an invasive spcies.

Jonathan Trent: Ah, you're not allowed to introduce a new species,...

Bela Buck: You should know.

Jonathan Trent: Yeah, of course, I'm sorry.

Bela Buck: There's no doubt; you will never be able to; it's a nature conservation area. So if you really want to have problems, do it.

Bela Buck: In my current experience, offshore fish farms will never work in Germany. There will be no acceptance. We are very close to one of the key nature conservation areas of the world. And if the fish farm is maybe, let's say, 20 Kilometers off the coast and no feeding barges or no feeding containers are available which are able to feed this farm, for let's say two weeks, in case you have a storm condition, you cannot say OK, I won't go today, I'll go tomorrow. They're fish, they are hungry, they will die; that's your money. And there's no chance; you cannot submerge it to 70 Meters, which is done like in ocean spar technology cages and other cages, because it's only 20 meters deep in the *Wadden* sea area.

Jonathan Trent: Are the surface conditions and the waves large enough to actually scour the bottom? Do they have an influence all the way down to the 20 Meters?

Bela Buck: Maybe we should ask you, I think..

Brian LaPointe: Did you monitor the seed end ratio, the plants, through the growing?

Bela Buck: The sea end ratio?

Brian LaPointe: Yes, the carbon-nitrogen ratio.

Bela Buck: In the water

Brian LaPointe: In the algae?

Bela Buck: No, in the water.

Brian LaPointe: In the water?

Bela Buck: Yes, for the mussel cultivation.

Brian LaPointe: Oh, okay; were the plants' nitrogen limited to the Laminaria?

Bela Buck: I don't think so; actually, I never did that but they looked very healthy. And they tasted the same as in shore seaweed. Maybe I should do that. Why do you ask?

Brian LaPointe: Ken Mahan [sp?] did work years ago, and published a Science paper back in the early 1970's, showing *Laminaria* in St. Margaret's Bay in Nova Scotia, showing a very seasonal pattern of storage of nitrogen in the winter and then becoming depleted in the late spring, summer months. For this depletion, you could enhance the growth and yield by fertilization, during the late part.

Bela Buck: Then we come back to the nature conservation story. We cannot put anything into the water. Our institute fertilizes the Antarctic; it's a different story and I don't like it but they do it for experiments with iron, but there's no fertilization of the North Sea.

Garcia Reina Guillermo: You don't fertilize your farm. Have you drafted the number, which is the yield per linear meter of your long lines?

Bela Buck: Yes. You're talking about seaweed, not mussels.

Garcia Reina Guillermo: Yes.

Bela Buck: Okay; this ring will have a rope of 18 meters and in this 18 meters, after 7 months you will have a biomass, of between 700 and 1,000 or 800 and 1,000 kilograms wet weight, which is equivalent to 10- 20% dry weight. So it depends on what you want to do.

Garcia Reina Guillermo: You have 80 meters after 7 months, from 700 to 1 ton fresh weight.

Bela Buck: Yes.

Garcia Reina Guillermo: Okay, thank you.

Jacob Davis: Do you know what the load capacity utilization rates are for these offshore wind farms, meaning, is there access load capacity to actually attach the OMEGA systems to these wind farms?

Bela Buck: I think you cannot connect to a turbine but they have other artificial platforms to store tools, or maybe people can live there for some days, in case of emergency, if they are repairing something on these platforms. But you cannot connect to a wind turbine.

Jonathan Trent: Thank you then; I think that's all the questions for now.



Cultivation of the Seaweeds and Its Usage for Reducing Eutrophication

Guang-Ce WANG

Institute of Oceanology, Chinese Academy of Sciences

Jonathan Trent: The next speaker is Guang-Ce Wang from the Chinese Academy of Sciences. Our colleague on the Science advisory team Qiang Hu, invited him, so would you please say a few words about Guang-Ce Wang?

Qiang Hu: Professor Wang is the director of Qingdao Institute of Oceanology Algal Research Laboratory. He is also the president of Chinese Phycological Society, so he has spent many years working on seaweed cultivation as well as microalgae biotechnology. Welcome.

Guang-Ce Wang: Thank you Jonathan and Dr. Hu Qiang. I am very pleased to be here to introduce you to seaweed cultivation in China. I will say something about why we do seaweed cultivation in China and finally, in the third part, I will make some suggestions and recommendations about OMEGA.

Part I Cultivation of Seaweeds in China

First I would like to say something about the cultivation of seaweed in China and introduce you briefly to the history of seaweed cultivation in China. As a matter of fact, the Chinese have a very long history of seaweed cultivation.

The first evidence of algae cultivation goes back to the Song Dynasty [960-1279], with a type of seaweed we call glueweed (*Gloiopeltis furcata*). This cultivation is known to have begun over a thousand years ago—a very long history. The second type of seaweed known to be widely cultivated, we call purple laver, and now *Porphyra haitanensis*. This is a red alga that has been cultured for more than 200 years. These are the two historical species of algae cultured in China and the first two species known to be cultured.

The first place these two species were cultured is in the Taiwan Strait, which is located in the southern part of China. In ancient times Porphyra haitanensis and Gloiopeltis furcata were cultured using a very simple method, which is called the rock cleaning method. It involves simply knowing what time of the year the seeds of the seaweeds are abundant in some areas of the coastal regions, and cleaning the rocks in the intertidal just before the seeds are released. That is to say that the ancient seaweed farmers scraped off all the other organisms, especially the barnacles and all other seaweeds, making the rock surface clean and accessible to the intended seaweed, which can grow on the clean rock surface and grow there well. It's a very simple method, but it's very effective and can yield a large harvest. This was the method used hundreds of years ago by the ancient Chinese people.

In 1949 the scientific cultivation of seaweed was established, beginning with the cultivation of two specie: *Laminaria japonica* and *Undaria pinnatifide*. Bela [Buck] just told us about *Laminaria saccharina*,



Both by the rock-cleaning method.

2. Scientific Cultivation Systems for Seaweeds

The modern cultivation system was established with the cultivation of two types of economic seaweeds, *Lamilaria japonica* and *Undaria pinnatifide*. Both were introduced into China in 20 century, and *Lamilaria japonica* was from Japan and *Undaria pinnatifide* from Korea.

but in China, we culture *Laminaria japonica* and *Undaria pinnatifide*, although neither are local to China. *Laminaria japonica* was introduced from Japan and *Undaria* was introduced from Korea.

When we talk about modern cultivation of seaweed in China, we should mention professor C.K. Tseng, who is a very famous phycologist in China. Professor Tseng established marine algae research and many application in China after he returned to China from the USA in 1946.

To understand how the scientific cultivation of seaweed was established, we should consider the conditions in the coastal regions in China. Professor Tseng and his colleagues gave us some of the critical scientific issues that must be studied for the successful cultivation of seaweeds:

First they focused on the seeds, because the different species have different seeds. We say "seeds", but as a matter of fact, we really mean spores. Different species have different spores and different species form spores at different times and under different conditions. So we should study how the seeds are formed and where it is formed. Since the 1950s, Professor Tseng led scientists to do some research concerning this.

Second we should study the substrate because the seaweed spores attach to the substrate to grow and develop. So we must understand which substrate the spores of seaweed like and which substrate the spores of seaweed dislike. Different species have different characteristics and requirements.

Third we should study the light factors because light factors are very important; the seaweed is a photosynthetic organism and different species have different light requirements. For example, when we study the *Laminaria*, we should know the water clarity and light requirement for the *Laminaria* and from this we can decide which depths are appropriate. If the seaweed needs high light we should keep the seaweed at the surface of the water. The red algae are less adapted to high light and we According to the conditions of coastal regions in China, some scientific bases involved in the successful cultivation of seaweeds should be studied by the research group of Prof. Tseng and other research groups in the early of 1950s. The scientific bases include as follows.

1. The seeds

The fertile fronds of *Laminaria* was put in a seawater tank where some hemp or palm

fibers existed, and the spores from fertile fronds were attached to the fibers, which would be floating in the sea.

2. The substrate

There are two types of substrates, natural ones and artificial ones. The natural ones were stones and rocks, et al, which was used in early days. In China, the substrates used before 1960 were hemp or palm ropes.

3. The light factor

The light factors include light intensity, regulation of light intensity in the field, the relationship between the light and individual development of seaweeds.

- 4. The temperature factor
- 5. The inorganic nutrient factor include need for fertilizer application in *Laminaria* cultivation, fertilizer application by porous container method, fertilizer application by spraying, fertilizer application in *Laminaria* cultivation.





C.K. Tseng (1909-2005) should put them at greater depths, depending on the water clarity. Therefore, the light factor is very important for understanding how to cultivate seaweed.

Fourth, the temperature is also very important. China it's a very big country and the coastal line is very long. From the south part to the north part, different places have different temperatures. For example in the island of Hai Lan we have no winter; it is always summer. All the year is hot, but in the northern part of China temperatures are very cold. So we need to study the temperature and its effects on seaweeds.

Fifth we should study organics and nutrient effects. In the 1950's, Chinese coastal regions had very clean seawater because it was deficient in nutrients. To culture seaweed required adding fertilizer to the seawater. Now, it's different and much of the Chinese coast is having eutrophication, which means it is unnecessary to fertilize seaweeds.

Sixth is the weed factor. The need to study how different seaweeds compete with the type of seaweed of interest. We need to know how to avoid the weed species, so we should also study this deeply.

Seventh, is the disease issue. There are two types of diseases. Those that can be considered physiology disease and those that are based on pathogens. Sometimes seaweed cultures are severely infected by diseases and this must be understood to be controlled.

Finally, if in one place we just culture one species of seaweed, the ecological and economic advantages will gradually disappear. This means we should breed new strains. For example, for the *Laminaria* and *Porphyra* we should be selecting for higher quality strains.

All of these questions are resolved when the modern cultivation of seaweed is established.

6. The weed factor

- In Laminaria cultivation, the weed algae include Ectocarpus, Enteromorpha, Licmophora, etc.
- (2) In Porphyra cultivation, the weed algae include some green algae (Monostroma, Enteromorpha and Urospora) and some diatoms such as Licmophora, ect.

7. Disease

include physiological disease and pathogenic diseas

8. Breeding of new strains

- (1) genetic studies of Laminaria and Porphyra
- (2) breeding of new strains of Laminaria japonica with distinct morphological characteristics.
- (3) breeding of new strains of Laminaria japonica with high productivity and high iodine content

Cultivation of *Lamilaria japonica* and *Undaria pinnatifide*

Now, I would like to say something about *Laminaria japonica* and *Undaria pinnatifide* because these are the first two species cultured in China and they have been actively cultured since 1949 and even today these two species have among the largest production. I will introduce something about *Laminaria*, because the Chinese culture style is different from what we saw for Germany.

I will begin with the life history of *Laminaria* because both *Laminaria* and *Undaria* have similar life histories, so a review of the life-history of *Laminaria* covers the history of *Undaria*.

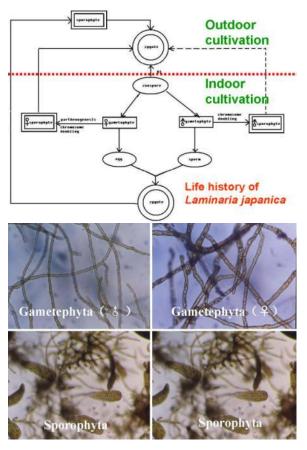
With regard to cultivation you can divide the process into two stages, the first stage is indoor cultivation and the second is outdoor cultivation.

With regard to the life-history of these seaweeds, we have to divide it between the gametophytes (male and a female stages) and the sporophytes (asexual stages).

As a practical matter, the modern cultivation system includes five parts. The first is spore collection and indoor cultivation of the sporeling. The second is sporeling transplantation. The third is setting up cultivation raft in the field. The fourth is cultivation management in the field. The fifth and last is harvesting.

In some ways, the most important stages are the first stage (the spore collection and indoor cultivation of the sporeling) and the second stage (the setting up of cultivation raft in preparation for transfer to the field because this is indoor cultivation). The third, fourth, and fifth stages involve outdoor cultivation.

For indoor cultivation of sporeling, I should mention Professor Tseng again, because in 1955 he devised some interesting sporeling methods. In these methods, *Laminarian* zoospores are collected in the early summer and both the young gametophytes and the juvenile sporelings are first cultivated in enriched



● 中国科学院 备译研究研

The modern cultivation system include 5 parts:

- I) Spore collection and indoor cultivation of sporeling
- II) Sporeling transplantation
- III) Setting up cultivation raft in the field
- IV) Cultivation management in the field
- V) Harvesting.

seawater in the greenhouse where the temperature is about 10°C. These cultures are maintained for about 2 or 3 months. When the sea temperature; that means the outside seawater temperature is about 20°C, the young sporelings are moved to the field and cultured. In China in the summer the

temperature of the seawater is very high, so if you export the sporelings to the field under inappropriate conditions, the cultivation will fail.

The indoor cultivation occurs in a greenhouse, also known as a breeding house, as shown in the picture. The water processing involves pumping seawater into the inside pond to cool to about 10°C. The pond has substrate and fertilizer and then fronds of Laminaria with spores are introduced. The fronds release spores, which attach to the substrate.

The substrate in the **pond** is a screen that provides a favorable structure for the spores of Laminaria to attach.

In the green house or breeding house...

SPORELING TECHNIQUES --- indoor cultivation of sporeling

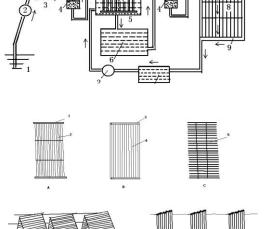
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Tseng et al. (1955) devised the summer sporeling method, in which the Laminaria zoospores are collected in early summer. The young gametophytes and juvenile sporelings are first cultivated in enriched seawater in a greenhouse maintained at about 10 °C and moved to the sea when seawater temperature drops to about 20 °C in the autumn.

Seedling house

Page 180







The culture of sporelings develop for about 2 or 3 months

Then we move it to the field



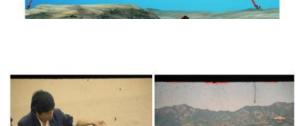
Young sprelings

derwater floating raft

Floating raft

This is a modern culture system, but the design of the device goes back to the 1950's because it is very effective. A worker at our institute designed it. These are very thin ropes and the two ends are fixed to the bottom of the sea. There is a floating ball that makes the string stay at the surface of the sea. There is a small stone that keeps the string vertical. The young sporophytes are attached to the string.

I'm showing you old pictures because in China the cultivation of *Laminaria* is currently replaced by other organisms, other seaweeds.



The Cultivation of Porphyra

I have introduced the cultivation of *Laminaria* and I will now introduce something about *Porphyra*. *Porphyra* is the second largest seaweed industry in China.

If we speak about the cultivation of *Porphyra*, we should mention something about the history of *Porphyra* because in the higher plants or other seaweeds, the life history is relatively simple. The life-

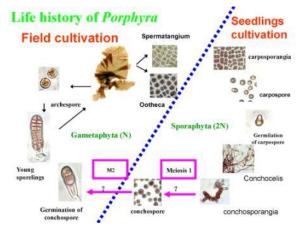
history of *Porphyra* is very complicated and if we speak about the life-history of *Porphyra*, we should mention the very famous phycologist: Kathleen Drew.

Kathleen Drew (1949): Porphyra spores do not germinate to become leafy Porphyra, but gave rise to the shell-boring microscopic plant, the conchocelis

Kurogi (1953a, b) in Japan and Tseng & Chang (1954) in China : the *Conchocelis* grows on molluscan shells and its spores, the conchospores, develop to become the leafy *Porphyra*.

Porphyra yezoensis: hermaphrodite Porphyra haitanensis: dioecism

In the ancient times, the Chinese people that cultured *Porphyra* didn't know where the spores formed. They just put Porphyra into ponds. While the fronds of *Porphyra* release some spores, these spores cannot develop into mature individuals. In 1949 Kathleen Drew discovered the carpospores, which are released from the frond of *Porphyra* and develop into a very small plant called the *conchocelis*. This *conchocelis* develops very slowly in liquid medium and forms an useless structure, but if the *conchocelis* finds a mollusk shell, it enters into the shells and develops very quickly into a conchosporangia.



Professor Tseng and a Japanese phycologist named Kurogi independently found that the role of the *conchocelis* was to form the conchosporangia, which releases conchospores. It is the conchospore that forms the useful fronds of the mature *Porphyra*. This is the life-history of some *Porphyra* species, while others have another complexity—they release another kind of spore called an archespore and the archespore can also form into a mature individual.

Based on the life-history of *Porphyra,* we also divide the cultivation process into the two stages of indoor and outdoor cultivation as for *Laminaria*.



Wind, Sea, Algae Workshop, Lolland, Denmark, April 2009

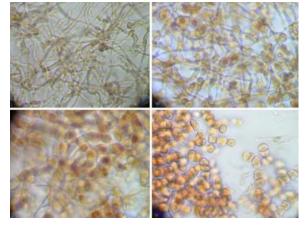
Indoor cultivation takes place in the breeding house as shown in Figure x. By the way, this breeding house is on the beach near Shanghai in the central part of China where there are also windmills.

There are ponds in the breeding house with their bottoms covered with shells. The *conchocelis* released into the pond enter into the shells and noticeably change their appearance as they progress from their early stage to their late stage of growth. In the late stage, which takes about two months, the *conchosporangia* is formed.

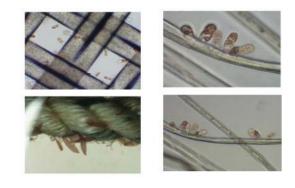


Breeding Stage

Under the microscope, both the *conchocelis* and the *conchosporangia* are visible. The *conchosporangia* produce conchospores and archespores. At the time these spores are released, strings are suspended in the pond and the spores will attach to these string within 3 or 4 hours.



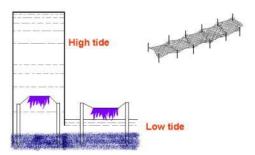




The strings are then taken to an outside pond for further development. At the appropriate time, the strings with attached spores are moved to the field to develop into individual plants.

Porphyra has a high economic value in China and its cultivation in the field is very popular and extensive. There are three different field cultivation methods used. The first, called the fixed pillar method, involves attaching the strings of *Porphyra* to poles or pillars driven into the bottom in shallow coastal waters so that at high tide the strings are submerged and at low tide the strings are exposed to the air.

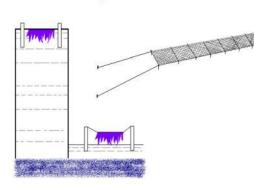






In the second method, called the semimethod, the strings are attached to a poles or pillars extending downward, fixed to the bottom. In this method, at the frame floats on the surface and at the pillars are standing on the bottom, the strings of *Porphyra* to the air.

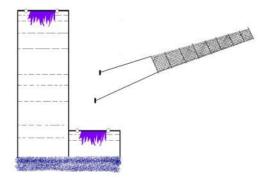
Semi-floating method



floating frame with but not high tide low tide exposing



In the third method, called the fully floating method, the frame of *Porphyra* strings is floating at high and low tides, which means it will not experience desiccation because it is in the water at all times. Fully floating method



Methods one and two take advantage of the fact that *Porphyra* can be exposed to the air for several (3-4) hours and survives desiccation, while other seaweeds attached to the string do not tolerate this desiccation and die. Using these methods, produces quite pure *Porphyra*—not contaminated by other seaweeds. The floating methods (two and three) have the advantage of improved irradiance for higher productivity. Method three however, which does not have a desiccation period, does not produce as pure *Porphyra* cultures unless the seawater is clean of other contaminating seaweeds.



We cultivate two species of *Porphyra* in China. One cultivated in the northern parts of China, *Porphyra yezoensis*, is similar to the species cultivated in Japan and Korea. The other cultivated in the southern part of China, *Porphyra cantanis*, is a local species. This Figure downloaded from Google Earth indicates the enormous scale *Porphyra* is cultivation in the coastal regions around China.



Jonathan Trent: Are these floating systems or are they anchored? Floating?

Guang-Ce Wang: Floating.



原藻加工



Harvesting



紫菜食品加工



紫菜产品交易

Finally, there is harvesting, processing, and of course the business trade of *Porphyra*. As you may expect for this large-scale effort, the harvesting and processing systems are elaborate as shown in Figures x,y. Also, the *Porphyra* trade market is very advanced in China.

Cultivation of Gracilaria

Most of the agar in China comes from *G. lemaneiformis*, while *G. tenuistipitata* var. liui is used as a good feed for growing marine animals.

Another seaweed that is cultivated extensively in China is *Gracilaria*. *Gracilaria lemaneiformis* is cultured for the production of a very high quality agar. *Gracilaria tenuistipitata* produces a low quality of agar, but it is used extensively in aquaculture as food for marine mollusks, such as abalone.

Unlike *Porphyra* and *Laminaria*, which form spores that develop relatively quickly, *Gracilaria* spores take 4 or 5 months to develop into mature individuals. Therefore, *Gracilaria* propagation does not use spores, but uses re-growth. In this method *Gracilaria* branches are cut into very small pieces, attached to strings, and put in seawater. Both species *G. lemaneiformis* and *G. tenuistipitata* var. liui, grow very well in saltwater ponds with little management to be successful. The scale of *Gracilaria* cultivation in China is also very large.

Both *Laminaria* and *Porphyra* utilize their spores to germinate into sporelings, but *Gracilaria* is very difficult to use spore to grow into mature individuals. Spore germination of *Gracilaria* would gives rise to disc-like thalli and requires several months to give growth of erect branches. The idea of employing spores in mariculture of *Gracilaria* was therefore abandoned, and the cut branches for propagation were used .

G. tenuistipitata var. liui







The production of marine aquaculture in China and in particular the production of seaweed, has increased significantly with time.

Jonathan Trent: Is that tons per year?

Guang-Ce Wang: Yes, tons per year, dried tons.

Jonathan Trent: That's a lot!

Unlike western countries, where seaweed cultivation is a small industry, seaweed cultivation in China is very popular and is now a very large and diversified industry. This includes seaweed cultivation, and the production food, feed, fertilizer, medicine, and chemicals. In China, the seaweed industry involves several hundred thousand people, if we include production and trade. It is interesting to consider how the seaweed industry got so big.

Theproduction of marine aquaculture in China

year	Total production of marine aquaculture	Production of seaweeds	Production of Laminaria	Production o Porphyra
1985	71.2	27.0	25.4	
1988			27.0	
1990	162.0	27.5	24.0	0.8
1992	242.0	55.0	49.0	1.6
1993	308.7	68.1		
1994	345.6	73.0		
1995	412.3	73.8		
1996	763.9	91.3		
1997	791.0	96.1		
1998	860.0	104.1		
1999	974.2	119.4	89.4	4.1
2000	1061.2	122.2	83.0	4.8

There are now large-scale industries in seaweed cultivation, including phycocolloids, chemicals and medicines and food, feed and fertilizer, involving several hundred thousand people in China being engaged in the production and trade of algae and their products.

The Purpose of Largescale Cultivation of Seaweeds?

You may know that in the 1950's, the Chinese cultured seaweed primarily for food because at that time there was a severe food shortage. There is a long tradition of using Gracilaria and Gloiopelti for producing colloidal substances and a host of seaweeds such as Sargassum for medicine. Recently, there has been much interest in using seaweeds for bio-energy and for bioremediation. This last point about using seaweed for bioremediation, I think is very important, because it may be a way to address the problems of eutrophication in China. There are coastal regions that are severely impacted by eutrophication and the Chinese Government is encouraging seaweed farmers to cultivate seaweed to reduce eutrophication. I think this is very important for China to be proactive in this way.

In China, aquaculture of finfish, mollusks, and shrimp is very popular and the scale and production is very large. Caged finfish aquaculture, for example, covers large areas in the north and south, with cages floating on the surface in coastal regions, that can be seen on Google Earth.

Usage of Algae in China

- 1. For food : *Porphyra*, *Laminaria* and *Nostoc*
- 2. For colloidal substances: Gracilaria, Gloiopeltis
- 4. For the medicine: Sargassum
- 5. For the bio-energy
- 6. For reducing of eutrophication

PART II

Seaweeds Used as Alleviation of Marine Eutrophycation

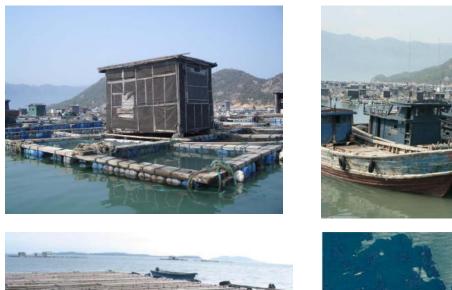
..... Integrated Cultivation of Finfish and Seaweeds

It is possible to use controlled cultivation of seaweed to reduce eutrophication. We speculate that it

should be possible to attach seaweed spores to aquacultured animals in a way that would be beneficial to both. The algae will produce oxygen for the animals and the animals will produce nutrients for the algae.

Integrated aquaculture: the future!







10.3.2004

The large-scale aquaculture of marine animals, produces a lot of waste materials. This includes a lot of solid waste from uneaten food and feces, as well as dissolved metabolic wastes, including carbon dioxide, ammonia, and phosphorus. This huge load of wastes from aquaculture causes extensive eutrophication in coastal waters and has the potential of causing ecological disasters. You may recall the "green tide" that occurred in the waters around Qingdao (my city), and impacted the 2008 sailing competition in the Olympic games. It took many people to clear that seaweed and we need to be able to understand and control these kinds of events.

Finfish Aquaculture Waste Production

- Solid wastes
 - Uneaten Food
 - Feces
- Dissolved Metabolic Wastes
 - CO2
 - NH₄ – PO₄



You may not realize that in Chinese the character for animal means "moving organisms" and the character for plants is "fixed organisms," which means if we realize this scheme to attach algae to animals, we will have to change our characters.

Kirsten Olrik: This idea to attach seaweed to animals is not yet realized, is it?

Guang-Ce Wang: No, it is not yet realized—it's just my dream.

Part II suggestions

Integrative Cultivation of seaweeds and fuel microalgae

In this final part of my presentation, I would like to make some suggestions about OMEGA. As a matter of fact, I found the presentations yesterday afternoon very stimulating, because I think the OMEGA system is very good—the idea is very nice, There are of course many factors to consider. For example: How to make the OMEGA system stable in currents and surface waves? Yesterday morning Professor Seymour gave a very good report about the ocean currents and waves, which can obviously become so strong as to damage any OMEGA system. The proposals for microalgae cultivation system offshore is great and there are so many advantages, however, I am concerned for two points:

(1) will the cultivation system pollute the seawater?

if yes, I would say the system will cause the eutrophication of seawater because both the supplement of nutrients into the system and the harvest of microalgal biomass has possibility to discharge some nutrient media to seawater.

So the question is how to reduce the current and waves and their effects?

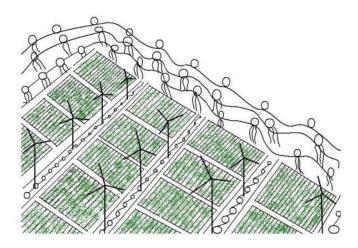
(2) is the cultivation system which will lie on the surface of seawater stable?

Therefore, I propose the cultivation of seaweeds, which is very popular in China, should be included in the the system, because the seaweed can allivate or prevent the eutrophication possibly resulted, and further the seaweeds cultivated can obstruct the strong sea current to some extent. That is to say, the microalgae cultivation system can be surrounded by the raft cultivation of seaweeds.



I would like to suggest that the large-scale culturing of seaweed around an OMEGA system has the potential of reducing the surface waves and stabilizing the system. This surrounding growth of seaweed could also help contain the impact of any nutrients that could leak out from the OMEGA system and provide a safeguard against OMEGA contributing to local eutrophication. **[Likewise, OMEGAs with algae can be built in conjunction with the large aquaculturing systems in China to control eutrophication].** If the OMEGA is built in the vicinity of a wind-turbine farm, the structures could include a seaweed component to include the benefits and products of the seaweed as indicated in my drawing

Thank you very much.



Q & A SESSION

Bela Buck: In the first half of your talk you presented a technique when you cultured *Laminaria* vertically. Do you have any growth limitations for those seaweeds, which are growing in the deeper water, because of maybe shadowing effects?

Guang-Ce Wang: We are not in the deep water, we are just in the surface of the ocean because you know in China the seawater is not very transparent, because it's very dirty. We culture the seaweed in the surface of the seawater, not at depth or on the bottom.

Bela Buck: Yeah, but the image you presented showed ropes with the *Laminaria* hanging vertically. That's why I asked you. They were not spread out horizontally, just in case, if you're doing it vertically I have another comment.

Guang-Ce Wang: Ok

Bela Buck: Place the ropes at an angle. That way you prevent entanglement, and you can increase your biomass by 2X.

Guang-Ce Wang: Uh, yeah.

Jonathan Trent: I have a question about the cultivation of algae for the cleaning up of eutrophication. Was that the *Gracilaria* you were using to clean up the eutrophied areas?

Guang-Ce Wang: Yeah.

Jonathan Trent: The question is whatever algae you use for clean up eutrophication, do they also take up heavy metals and does that create a problem for using the algae as a food source?

Guang-Ce Wang: I think it's no problem because in China we are using *Gracilaria* for dealing with eutrophication, because *Gracilaria* grows very fast. I think it's no problem because *Gracilaria* absorbs some heavy metal and the other things...

Jonathan Trent: How do you get rid of the heavy metals from the *Gracilaria*? I assume you don't use the *Gracilaria* for food if you are using it for remediating eutrophication?

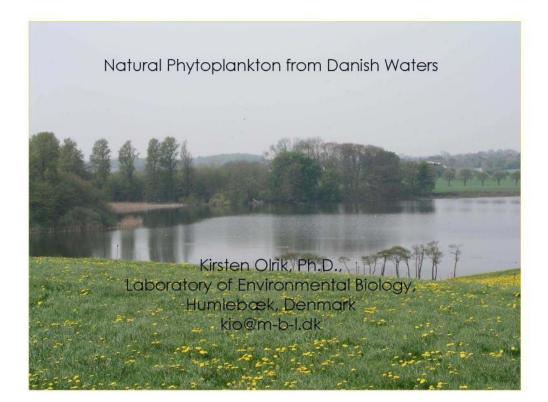
Guang-Ce Wang: We use *Gracilaria* to produce agar and to feed marine animals, but it is not used as a food directly in China.

Garcia Reina Guillermo: Can you give us a figure on the yield of the *Laminaria* that you produce in China per hectare per year, do you have any idea on the yield per year?

Guang-Ce Wang: Yeah. I can give you some information about it in later

Jonathan Trent: Thanks you very much...

NATURAL PHYTOPLANKTON FROM DANISH WATERS, KIRSTEN OLRIK



Kirsten Olrik: Good afternoon everybody. I will take this opportunity to tell you about natural phytoplankton in Denmark - or microalgae, as you call them. (We normally use that term for small algae in culture only).

I have tried to get hold of some phytoplankton data from *Fehmern Belt*, where the experiment with the OMEGAs was thought to take place. Unfortunately, such data do not exist, but from the environmental center in Nykøbing Falster, instead I received three years' cycles of phytoplankton biomass on species level from Hjelm Bight, which fairly close to Fehmern Belt. The important phytoplankton maxima in this water are peaks of diatoms, dinoflagellates, and cyanobacteria. The biomass of three groups varies quite a lot both during their seasonal cycle and

autohoptas Dictorm 19 O t ^a		Hjelm Bugt 1998-2000 Mean depth 22 m Not far from Fernem Belt
Miconophic Lorger dino Flogel ales 193 C F I	Richard Control of Con	Departure of
Cyano- bacteria µg C+1		
Michophic Namo- Rogetales pg C/I	10 10 10 10 10 10 10 10 10 10 10 10 10 1	

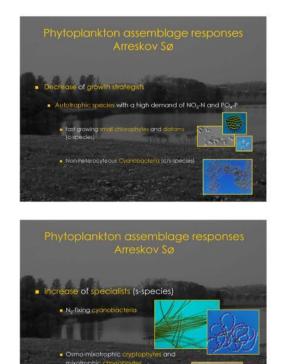
between the years. The biomass peaks were repeatedly high, but one of the years, there was an extraordinary high maximum of the N_2 -fixing cyanobacteria *Nodularia spumigena* (11 g carbon per liter water), which is one of the highest values ever measured in Danish waters. These results tell us that there is a strong seasonality as well in the growth conditions in the Baltic Sea areas around Denmark,

which will regulate growth of microalgae in plastic bags placed in Fehmern Belt as well. Sometimes The Baltic Sea and its surroundings are haunted by enormous masses of *Nodularia* from *The Baltic Sea Proper* where they act in strongly stratified water in the photic zone in the upper 10 meters of a deep water column. They sink down and catch phosphorus, and when they are oversaturated with phosphorous, they float up into the surface water and catch light. These algae turned up in 2006 in enormous amounts, where they also hit Zealand, floated up to Copenhagen and its harbor, and further north. It was unaesthetic, foully smelling, toxic, and associated with sea cholera bacteria and other bacteria. When cyanobacteria strand in the coastal zone in such masses, they are not in active growth, they have been forced up into a narrow upper layer, where they sooner or later get photo-inhibited and finally die. These decaying masses of cyanobacteria in brackish waters keep floating around for some time.

<u>Question from Audience: where is this area situated?</u> Hjelm Bight is the closest area to the *Fehmern Belt*, where we could get some phytoplankton series from the environmental centre of Nykøbing Falster. It is situated on the eastern side of Falster, the island you cross when you go from Copenhagen to Lolland, but it is more or less the same water as in Fehmern Belt.

I will also show you long term series of phytoplankton from two eutrophic lakes. In earlier years, both of these lakes received urban sewage that was cut off around 1990. The data presented here are means from March through October, which was the growth season before the global warming started.

Arreskov Sø is a typical shallow, alkaline, eutrophic East Danish lake, like the ones in Lolland. After the urban sewage was cut off in the early 1990es, concentrations of nitrate and ammonia in the lake water declined, whereas that of phosphorous is about the same, due to remains from the earlier pollution. Over the last 10-12 years, there has been a rise in the mean temperature in the lake from around 13 °C to above 14 °C, and the growth season has been prolonged to almost the whole year. Over the summer, the temperature rise enhances the microbial oxygen consumption in the bottom water and the sediment, and anoxia allows phosphorus to release from the lake sediment. The oxygen is also taken from nitrate, the nitrogen circle runs quicker because of the temperature rise¹, and nitrogen is transformed into N₂. Consequently, the lake water during summer in Arreskov Sø contains high amounts of phosphorous and is severely nitrogen depleted.



¹ Weyhenmeyer; G.A. *et al.*2007Nitrate-depleted conditions on the increase in shallow northern European lakes. – Limnol. Oceanogr. 52/4: 1346-1353.

The total phytoplankton biomass of autotrophic species (green algae and the non N_2 -fixing cyanobacteria *Microcystis*) decreased over the 1990es with the urban sewage cut off to Arreskov Sø. The chlorophytes have not returned, but 5-10 years ago, cyanobacteria turned up again in extremely high biomasses of approximately 60 milligram ww per liter. These cyanobacteria are species with other characteristics than the previous ones in the lake, primarily N_2 -fixing species of *Anabaena* and *Aphanizomenon*. They are short-lived,

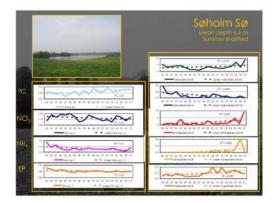


and when they die, they leave organic nutrients in the water that are utilized by non N_2 -fixing cyanobacteria and small mixotrophic flagellates².

Paradoxically, the warming of Arreskov Sø, that induced the N₂-fixing cyanobacteria in the phytoplankton have turned the shallow lake into a sink for CO₂ and conserve it as organic matter. In this stage, the lake is useful in the combat for a better climate, and it can go on for some years, until the whole water column one day turns anoxic. From a climatic point of view it is so far a success, but from a nature conserving point of view, it is a disaster.

Søholm Sø is also an alkaline, eutrophic lake with a phosphorus deposit from earlier pollution with sewage. It is deeper than Arreskov Sø, and stratifies during summer, with a warmer upper layer (epilimnion), separated by a thermo- and oxicline from a cooler bottom layer (hypolimnion). The temperature rise in the epilimnion over the last 10 years is evident. Like in Arreskov Sø, nitrate and ammonia have decreased in the epilimnion after the sewage cut off, and for some years, severe nitrogen



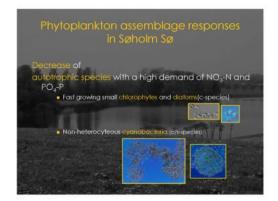


² Olrik, K. *et al.* 1989-2008. Annual reports on phyto- and zooplankton from Arreskov Sø and Søholm Sø. –The Laboratory of Environmental Biology Aps for the County of Funen/ Odense Miljøcenter.

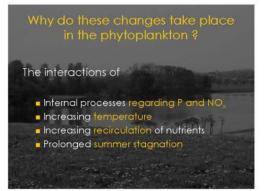
depletion has taken place there every summer. In the cooler hypolimnion, the nitrogen circle runs slower, and some ammonia is preserved. But every summer, anoxia rises from the bottom up in the hypolimnion, and phosphorus is released from the sediment.

After the sewage pollution was cut off from Søholm Sø in the early 1990es, autotrophic phytoplankton species (fast growing small chlorophytes and diatoms, and non N₂-fixing cyanobacteria) with a high demand of NO₃-N and PO₄-P decreased significantly. But during the later years with global warming, the summer phytoplankton biomass has increased tremendously. The strong vertical separation between sunlight in the upper water and nutrients in the bottom water has intensified so much during the summer period that N₂-fixing cyanobacteria no longer can compete.

Under these conditions, species of the mixotrophic dinoflagellate *Ceratium* are strong competitors. These species are fast swimmers and perform diurnal vertical replacements between the upper layer with sunlight and the deeper thermocline, where they catch phosphorous and graze upon microorganisms. Over the later years, they have ruled the summer phytoplankton in Søholm Sø completely.







In the unstratified, fully mixed Arreskov Sø with a phosphorous waste deposit in the sediment, the temperature rise in the summer period induces anoxia at the bottom and release of phosphorous from the lake sediment to the water phase. Denitrification is enhanced, and severe nitrate depletion takes place in the water every summer. This situation is utilized by N₂-fixing cyanobacteria to build up huge biomasses. These species are more or less toxic. They are succeeded by colony forming non N₂-fixing cyanobacteria (also mostly toxic), and small



mixotrophic flagellates that utilize the organic matter released by the N₂-fixing cyanobacteria.

In the deeper temperature- and oxygen stratified Søholm Sø with a phosphorus waste deposit in the sediment, the temperature rise induces prolonged periods of thermal- and oxygen stratification during summer and an increased internal release of phosphorus from the lake sediment to the hypolimnion. The impact on denitrification in the cooler hypolimnion of Søholm Sø is less than in the epilimnion, and in the shallow Arreskov Sø. In the temperature stratified Søholm Sø, the response on phytoplankton in summer is an increase of the strong swimmers among the mixotrophic dinoflagellates, especially *Ceratium* species who are unique competitors under a strong separation between sunlight in upper layers and nutrients in deeper layers of the water.

We intend to continue our work on improvement of the nutrient enriched lakes by altering their nutrient balance, and simultaneously collect relevant and potentially valuable biomass for feedstock of specialized bio-refineries.

Jonthan Trent: Do you know if they have interesting oils in them? Are they peculiar types of oil?

Kirsten Olrik: Cyanobacteria contain a very simple lipid composition with typical 'chloroplast' lipids predominating³. The dominant sterols found in dinoflagellates are dinosterol, 4,24-dimethylchlestanol, amphisterol, and cholesterol⁴

Jonathan Trent: Do they have large amounts?

Kirsten Olrik: Yes, I think so.

Jonathan Trent: Thank you very much.

³ The major glycolipids are MGDG and DGDG but others have been found (Harwood, L.J. et al. in Rogers, L.J- 6 J.R. Gallon (eds.) 1988. Biochemistry of the Algae and Cyanobacteria. - Proc. Phytochem. Soc. Europe 28. Oxford Science Publications.

⁴ N. Withers in F.J.R. Taylor (ed.) 1987: The Biology of Dinoflagellates. Bot. Monogr. Vol 21. Blackwell Scientific Publications.

I assume everyone has heard of "dead zones," so I will keep my description short and focus on ideas for their remediation. In August 2008, it was reported in the journal *SCIENCE*_that there are about 400 dead zones around the world (Figure1). These are places around the world where algae blooms are leading to the formation of an hypoxic condition, i.e., places in the water with low oxygen, due to decomposition of the algae. The red dots in Figure 1 indicate areas of hypoxia; the Baltic Sea is clearly an area with significant dead zones, as is the Gulf of Mexico. The algae blooms that lead to dead zones in the Gulf of Mexico and off the coast of China can be seen in satellite photographs (Figure 2).

Dead Zones 2008

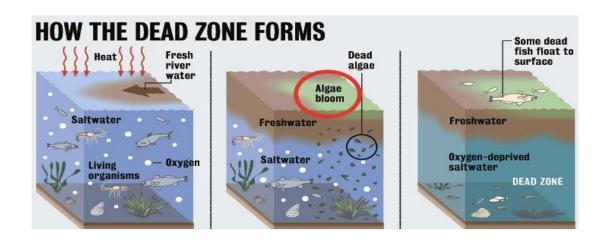


Science vol. 321: 15 Aug 2008



Figure 2

Dead zone formation is due to nutrient enrichment in coastal regions from municipal wastewater and farm runoff, stratification of the water column, and suffocation of the organisms in the deeper waters (Figure 3). The nutrient-rich freshwater enters coastal regions primarily does two things: 1. It stratifies the water column because of the density difference between freshwater and saltwater; and 2. It produces algae blooms at the surface. The stratification from freshwater floating on seawater maintains a stable water column and the nutrients from fertilizer in the surface layer cause the local algae to bloom. In the normal progression of an algae bloom with time the algae aggregate and sink to the bottom. The sinking algae decompose and the oxygen in the water is used up by the respiration of the bacteria involved in the process of decomposing the sinking algae. The combination of the stable water column, which prevents oxygen from the atmosphere from mixing into the deep water from waves and the high-levels of respiration from algae decomposition cause the deeper water to become low in oxygen or hypoxic. This hypoxia suffocates many of the fish and invertebrates that live in these regions; hence the name "dead zones."



Fertilizer runoff

Stratified water column

Suffocation

Figure 3

Dead Zones and OMEGA: It was in the process of considering ways to remediate dead zones that I thought of "Offshore Membrane Enclosures for Growing Algae" (OMEGA). The idea for OMEGA emerged by analogy in contemplating the process by which people use algae to scavenge CO2 from the flue gas of power plants, which they do by bubbling the gas through cultures of algae. I reasoned that nutrients could be removed from polluted rivers by passing their water through cultures of algae enclosed in nutrient-permeable plastic bags. Using the system in rivers required freshwater algae, and by combining the idea of freshwater in containers with semi-permeable membranes, and water flowing into the ocean, I realized that by floating the OMEGA downstream into the ocean, the salt gradient between the freshwater inside the OMEGA and the saltwater outside could be used to dewater the algae by forward osmosis. The basic idea for remediating dead zones is to use the algae contained in the OMEGA bags to "filter" the nutrients out of the polluted river water, the same way

Wind, Sea, Algae Workshop, Lolland, Denmark, April 2009

that algae filter the CO2 out of flue gas. In the case of OMEGA, we have the advantage of being able to facilitate the harvesting of the algae by floating the OMEGAs downstream into the ocean and use osmosis to remove most of the water.

The OMEGA is an enclosed photobioreactor submerged in the water, which has a number of advantages over land-based bioreactors. I focused on the nutrients in the surrounding river water as a source of nutrients for the enclosed algae culture, but in addition to providing nutrients, the river also provides physical support, temperature control, and energy for mixing. Unlike bioreactors on land, which enclose water in air, the OMEGA encloses water in water, which means the surrounding water is a support and the bioreactor itself can be made of less robust materials. On land, the closed bioreactor must be cooled or heated to maintain internal temperatures appropriate for algae growth, but the OMEGA submerged in water, uses the heat capacity and the temperature stability of the surrounding water to maintain temperature—OMEGA floats in a water bath. Finally, if the OMEGA is constructed from flexible plastic, the movements of the surrounding water provide mechanical energy for mixing the enclosed culture.

The way an OMEGA system works for dead-zone-remediation would involve anchoring a string of OMEGAs in a polluted river, so the enclosed algae scavenge nutrients from the surrounding river water as it flows by. When the algae are fully-grown, the OMEGA is floated or towed downstream to the ocean for dewatering by osmosis and harvesting. The harvesting can be done on a barge or on offshore platforms. The partially dewatered algae are transferred from the OMEGA to holding tanks on the barge or platform and the dewatering continues by a combination of filtration and wave-powered centrifugation. The concentrated algae slurry would be further processed on land by electrokinetic dewatering and extraction procedures for products. For continued dead zone remediation, the empty OMEGA would be returned upstream by truck or train to be refilled, redeployed, and to repeat the cycle.

OMEGA in **Denmark** would use nutrient-rich freshwater in rivers or fjords. The saltwater dewatering. In some fjords or in the Baltic, this may require that the OMEGA be

submerged to depths below the halocline, in order to find a high enough salinity for osmosis.



The OMEGA system removes nutrients from the river to grow algae inside the OMEGA instead of leaving them in the river or sea. The OMEGA algae therefore use nutrients that would otherwise contribute to the blooming of wild algae, which, in turn, contribute to dead-zone formation.

In one version of a river-OMEGA system, the OMEGAs extend out into a river with connections that allow them to separate from each other, as ships pass. In this configuration, the OMEGAs would use the flow of the river to work their way downstream, by flipping end to end.

If OMEGAs are installed in fixed locations in the river, there is ample evidence that they can be towed without difficulty. This action is demonstrated, for example, by the simplicity with which Jack LaLane pulled 70 boats across the San



Francisco Bay on his 70th birthday, with his feet and hands tied.



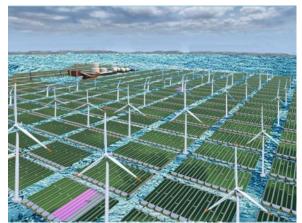
Tow the OMEGAs

to sea for dewatering

Nutrient scavenging or nutrient sinks: If OMEGAs are to be effective nutrient scavengers in rivers, we must consider questions of scale. How big should they be? How much algae biomass is required? What efficiency of nutrient uptake would be required to effectively "filter" nutrients from contaminated river water? What would be required to impact algal blooms in coastal regions down river? What would be required to impact dead zone formation?

Wind, Sea, Algae Workshop, Lolland, Denmark, April 2009

In addition to OMEGA river deployments for scavenging nutrients from river waters, before these nutrients reach coastal waters, OMEGAs could also be deployed in coastal waters to soak up nutrients in their vicinity. Here, too, the questions of scale are relevant; in addition, there are questions of infrastructure and logistics for deploying, maintaining, and harvesting OMEGAs in coastal regions. To address the question of infrastructure, we are considering the possibility of associating OMEGAs with wind farms. Could the wind farm structures be used as attachment points for

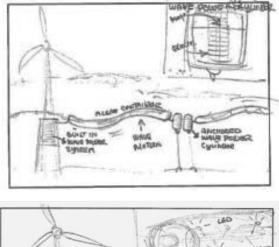


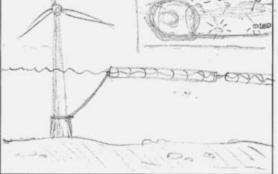
anchoring OMEGAs? Could the wind energy be used to pump water, provide lighting, dewater the algae, move OMEGAs underwater, and/or impact cultivation temperatures?

In an artist's concept of an wind/algae farm, we begin to consider what advantages there are in colocalizing wind and algae farms offshore and what issues need to be addressed.

For example, in the drawings, the attachments of the OMEGAs to wind turbine stanchions are illustrated and there are suggests of how mixing and electricity can be generated by using wind or wave

energy. Mixing the algae can be done by using electric pumps, air spargers, mechanical devices, or waves. The electricity generated by wind or waves can also be used for inexpensive lighting, such as LED, which can be used to control the photoperiod of the algae.





Our goals in this session are to discuss OMEGA remediation of dead zones in rivers or in coastal regions. Will these systems be free-floating or anchored? Will they be independent or anchored in arrays or associated with wind farms?

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In our discussions, let's assume that we are going to write a report to the United Nations on dead zone remediation and offshore algae farms. Indeed, we represent many countries from many parts of the world, with many different backgrounds and interests. We are going to report tomorrow, not to the UN assembly, but to Danish government officials and the press, so it is not unreasonable for us to take this exercise seriously. If possible, at each of the tables, we would like to have at least one expert in each of the four areas: biology, engineering, environment, and economics. You are allowed to go to other tables for consultation and if you feel frustrated or uninspired at the table you chose, you are free to get up and go to another table. Each table will meet for one hour and then we will have one person from each table report to the plenary session.

OUTLINE:

- 1. Discussion
- 2. Uffe Toudal Pedersen
- 3. Stig Vestergaard
- 4. Panel Session
- 5. Final Remarks

Jonathan Trent: We want to try to organize what we will do this afternoon with the people from the Ministry and from the consulates. I want to summarize what we discussed during the meeting at the break. First, we talked about what we are not going to do, which is that we are not going to be prescriptive and try to tell the people of Lolland how to "solve" their problems, which we don't adequately understand. What I think we should do is to try to convey our feelings of solidarity and support for what Lolland and Denmark are doing to promote environmental awareness and develop green and sustainable technologies and lifestyles. I think we should also present ourselves as the international team that we are, and show people that, in the global scientific community, there is a unified sense of urgency that something should be done to put human society onto a trajectory toward sustainable living.

We have come to Lolland to advocate for the development of algae cultivation. As a part of the world's portfolio of sustainable practices, which can impact some of the more pressing global problems, such as fuel, food, fodder, and wastewater treatment. We need to acknowledge Lolland's generous invitation, wonderful hospitality, and, most importantly, the support we have gotten from the leaders in Lolland, who have expressed their interest in algae-based technologies. For the latter point, we should mention the possibilities of utilizing Lolland's *"experimentarium,"* as Lene called it. We should ask ourselves, if we, as an international group of scientists and engineers, could focus on Lolland as a place to do research, collectively and interactively? This is an idea we should seriously consider. Do we want to try to use Lolland as a place to do demonstration projects because of the willingness, openness and commitment to doing this kind of research by the people here Lolland or are there too many disadvantages because of the location and the winters and whatever?

The other thing we need to decide this morning is how we should constitute the panel for this afternoon, how many people and what topics should be represented. I supposed we should have as broad a range of backgrounds as possible. The subgroup decided that we should include a Chinese, and Qiang Hu, you were elected; we wanted to include our Israeli, so Ami Ben-Amotz, you were elected; and we wanted to have one representative from India. We have one Dane, and we thought that should be Lene Lange; we wanted to have another American that is a big part of the algae story— and we thought Al Darzin should do it. Beyond that, does anyone have any other recommendations?

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Any volunteers? Do we need another Scandinavian? Peter Lindblad, you are part of the science advisory team; would you be wiling to sit on the panel? By the way, you can come up later and make recommendations for other representatives to me, if for some reason you don't want to voice your opinion publicly.

Finally, we talked about topic areas-- what we should present and how we should present it on the panel. In my opinion, the most important thing we need to convey is our level of enthusiasm, as we saw in the presentations this morning and in the afternoon sessions yesterday. I suspect the enthusiasm part will come through, but we do need some topic areas. Are there any recommendations?

Lene Lange: I think that enthusiasm can be conveyed by the wealth of ideas. It doesn't have to be by a person looking happy, or a person looking enthusiastic, because in most people's minds, biomass is for bio-energy, and that is bio-fuel, or diesel, or ethanol. That's the one-track mind you're **talking** to. So we must have as one of the main messages that algae is part of the solution--not just algae as energy, but maybe more importantly as fertilizer, animal feed, and nutraceuticals or other chemicals that are now derived from fossil oil. The point is people need to recognize that oh wow, many things come from algae. That may be the most productive, most important thing to convey—there is enthusiasm for this wealth of possibilities from algae cultivation. Then the next part is to not commit ourselves to just have one model. There are microalgae AND macroalgae and both can be cultivated offshore and onshore. It's not just about energy, it's about a wealth of sustainable possibilities...

Jonathan Trent: I'd like to show PowerPoint material, but it makes sense only if we can compile a collection of images from what we saw during the whole meeting. Could someone put that together over lunch?

Jens Holm-Nielsen : For the PowerPoint, I have the idea that we could do 4 or 5 of these PowerPoints, a few of your own slides and then the drawing (pointing to back) that really highlights and zooms in on Lolland and gives a lot of ideas, and then a few more slides.

Jonathan Trent: I'm also thinking about the morning presentations we had. There were some great PowerPoints. There were some good pictures of the ocean, and some good material on algae, and algae cultivation... What do you think?

Bjørn Utgård: I think that we need to ask ourselves, what's the goal here? Is your goal to get some good press or is the goal to impress people that are here, because if you're talking to journalists, what you need to do is convey the main messages and you need to give them take-away sound bites. We don't want a PowerPoint that narrows the scope of the debate and preparing a good PowerPoint is challenging. Giving a mediocre PowerPoint is not a good idea.

Jonathan Trent: Yes, it's important to do a good PowerPoint. It's a good question to consider what we want to convey to the press? I've had a couple of interviews since I've been here and the first question from the press is always: "Why Lolland?" I suppose there's a perception in Denmark that Lolland is kind of a provincial part of Denmark. We need to be able to answer this question...

Bjørn Utgård: The main reason we are in Lolland is that the challenge to the environmental problems is getting solutions implemented. The way you do that is, you take business, you take research, and you take politics together, and you make the platform on which all this can be implemented. Lolland, at the moment, is the hot spot in the world for making these solutions become reality. That's why we're here; we're not here because we were invited; we came here, we wanted to come here, because Lolland is the hot spot. Denmark is the hot spot this year and if we can't make it HERE, we can't make it!

Jonathan Trent: Lagree, Lagree! (Clapping) . . . Please make that statement. Lagree completely with what you've said. The fact that they invited us here is already indicative of what they are talking about.

Person in back???: . . . The reason that we are in Lolland is that they have the force to connect us.

Jonathan Trent: That's right, and they have the will that we be here, right.

Kirsten Olrik: To me, we have to focus on the fact that Lolland is open to new solutions.

Jonathan Trent: Yes.

Kirsten Olrik: And maybe we can provide some ideas and some solutions.

Jonathan Trent: And mostly because . .

Kirsten Olrik: not everybody is open to solutions.

Jonathan Trent: Exactly, that's what I meant when I said they invited us; that Lolland is open to explore new things with regard to clean energy... Phil, do you have a comment?

Phil McGillivary: My comment is, I think that for each one of the panelists, you should have a slide; one slide from China that shows what they are doing in China; show one slide when you introduce them; one PowerPoint slide, a picture of what's happening in Israel. It's easy to take the pictures from these presentations, and for each person where there is a slide that would be applicable, I think there were enough. Show what each panelist is doing in his/her country. That's what we call "telescoping." Telescope in--you bring it from the world community to Lolland and then you telescope it backs out. Herb Wenham used to tell me, "Telescope it in, telescope it out. and keep it very simple."

Jonathan Trent: Right, OK. I like that idea,...

Lene Lange: I would like to support that very strongly, because when I have the interviews, my first question has not been, "Why Lolland.?" It has been, "How far out in the future is it?" We never heard a journalist say, "We never heard about algae before; will it take ten years, decades and decades?" So if you actually show from different parts of the world that there is already something going on, then you already have pictures where there's an incredible story that shows that this is not just something like pie in the sky.

Jonathan Trent: Right, that's always been the second question. "Why algae?" and "Why here?" are the two questions.

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Phil McGillivary: Show the picture when you introduce the person.

Jonathan Trent: Yeah, that's a great idea; thank you Phil.

Phil McGillivary: It's all in a name.

Jonathan Trent: Now, we'll have to put that together; I think Kim and I can put that together. Leo, do you have something to contribute?

Leo Christensen: Thank you, there's one thing I would really like. I'm a practical guy; I am not an academic. I really like the idea that the knowledge, the ideas, whatever we have collected here today is put in a system, so we can be sure that under the COP15 in December, whatever we have learned here, is also presented there by people who are involved in this process;. We have the foreign ministers here, we have the universities here, we have politicians and contact to them, and we are very, very close to COP15. We know that we will have something like three hundred television stations coming down here over the summer.

Jonathan Trent: What do you mean "presented at COP15?" What does that mean?

Leo Christensen: I mean that we must have this message about the algae moved into COP15. There will be a lot of exhibitions going on at COP15; there's a lot of politics going on up to the COP15. And we have a once-in-a-lifetime chance to present what we have learned here in the COP15.

Lene Lange: We want to do exactly that, but to be even more specific, we want this to be captured and to be a starting point to go on from here. We are recommending that we make Lolland our experimentarium for new technologies for algae, and that we want this international group to be the international network for that objective, and we want to convince the Danish minister, and so on, to support this kind of activity. We're recommending that there actually be a demonstration at some scale, because these ideas cannot be proven just in labs; we need to get out there and try things for real. And then the last point is aboutCOP15, we want to have algae as one of the take-home messages from there. We want to announce that we will meet and have this kind of summer school and we have teaching and student projects going on here and so on. So the concept for how to do that should be ready in a small concept note on an ad, and in the paper, for the COP15, and that will bring activities to Lolland and it will bring forward the whole area. This is the kind of thinking. You have to be very concrete, we need to have buzzwords and one-liners... We need to make sure we have some administration and I think that BASS could be the consultancy that could actually be a small secretariat. We have to have things already going on from now; otherwise, we will all go home and all of us are so busy, that we need someone to keep this going, someone to keep the momentum going.

Jonathan Trent: I like that idea and, Bjørn, you have something to add?

Bjørn Utgård: There's a question on this sheet that we got, and it says: "What should we do for a follow-up"? And I think that's exactly what Lene Lange is addressing here. I would like to propose an international competition for the concept, so that all these ideas can really get going, so that they have a deadline for making all these ideas come to real concepts, and so that you're going to have competitive concepts. That way, it's not about "My bag is better than your bag;" "Well, prove it."

Jonathan Trent: I like that idea, Bjørn; in fact it segues perfectly into an idea that Anders Muller-Hansen has just presented as a very pragmatic way to go forward, and let me put it up so that we can project it. Can you get that on the camera? Let's see, we might have to remove your head, Mike. (laughter) OK; , is that readable? I'll go through it so you can understand it. What Anders was telling me at the break is, I think, a powerful way to go forward, and that is to develop as a team, and I've got to tell you, it's remarkable that there are nearly 80 people in this room who are functioning like a much, much smaller group. That, to me, is exciting and indicative of what can be done when we really get focused.

But Anders had the idea of having a series of projects, and it could 5 or 6, maybe up to 10, that are specific projects related to algae and we would have specific milestones, and he put COP15 as one of the milestones at which we might want to aim for these different projects, that we could develop even remotely. We can develop them intellectually over the Internet. They could, indeed, also be the basis for us writing grant proposals together, through a unifying manager who will be keeping things going on this axis. So these are the (I can't read it very well) these are the persons in the group and there will be a manager, probably Bass, that would somehow organize this and the projects would have milestones and these would be "Go", "No- go," decision points that we would develop. These different projects would develop with a number of people and they would be interactive, so they would be interlaced. Do you want to go on and make some comments about this?

Anders Hansen: All the people who want to be involved later on are here in a group, and first something about what we do, e.g., I have some knowledge; I can be a part of this project at the moment here; we say where we want to be in each project. That way we know what the status is, so the knowledge from one project can go from one person to the next one, and so on. We have the projects here, and the persons involved.. And that way we make a matrix, so all that we know gets into all the projects.

Jonathan Trent: Yes, and the idea would be that it isn't going to be limited to the people in this room; it includes people that are within the matrix of our knowledge groups. Is there a comment back there?

Raman Saravanane: Getting back to the discussion, I want to add a few points. Basically, the technical recommendations of the Lolland idea can be treated as a role model. I underline the word "role model," which needs to be extended, especially for the developed countries, where we have the estuaries and the Dead Zones as a protected area which would work well, with a lot of NGO's possible, as an elaborate network, and that would add a lot of input [and I would say] from the NGO's community as well. So that's my point, because we've got a lot of NGOs, because many wars have been going on. Especially as a carbon credit, because they are being paid, we get the project that the NGOs are being paid for and therefore I thought, "Why can't such a system be induced?" So that will make it a viable transfer on the ground.

Jonathan Trent: Yes, I like that idea; it's a good idea... Are there any other issues that we need to discuss as a group right now, because I think that what we might want to do, is take some time and figure out what this PowerPoint is supposed to be like... Robert, did you have something?

Robert Baertsch: I've been talking to three or four people here about setting up an algae consortium to do biological research along the lines of the encode project that we did with the human genome. We

annotated one percent of the human genome with a large consortium of 20 universities. I think we could really benefit from having a consortium of algae researchers that go to DOE or NSF and ask for a large fund to annotate the genome, with the ...

Jonathan Trent: We have it.

Robert Baertsch: Well, this is the consortium. We need to start to write a white paper right away and actually do this.

Jonathan Trent: OK, but that's what we were proposing up here, which is that we're now going to define projects, and one of the projects could very well be what you just described. And then, if there's enough buy-in from the other people to make it into a coherent group, then, it's a project and we'll go with it... Jens wanted to have a couple of minutes to talk about something, right?

Jens Holm-Nielsen: Yeah, but it's along the same lines. I've had a concern since yesterday and also there's a common frustration among some of the Europeans: how not to make algae developments a new bubble of hot air. Definitely, we all have to think about that..

Jonathan Trent: Can you elaborate on that, "...a new bubble of hot air?"

Jens Holm-Nielsen: I have to be honest; it's from my heart, and I mean it, of course. Your presentation from NREL yesterday that biofuel will have the solution for jet fuels and for transportation, and solve all the problems-- we have to take the situation a little bit more calmly and easily. It means making a pragmatic...

Al Darzins: Excuse me, but I don't think I communicated that. . . I tried to make it a little bit more balanced that just that. It's part of a solution. I presented a fairly even...

Jens Holm-Nielsen : Yes, you are correct, but the video turned me off a little bit., Never mind; it's just to make the next step a pragmatic approach for how to make a new algae movement. Basic research, implementation research, realistic algae resource studies, because these algae studies until now-- I don't believe in them. We have to have some more resource studies on the table, and then, of course, international networking and we must define how to make common algae platforms and common understanding. We have to be really pragmatic, step-by-step by step, to reach the new level-- a paradigm change in algae research. It was just from my heart to dare to open up this discussion a little bit.

Jonathan Trent: Yes, OK. I think that's true; I think we need to be working towards making ourselves credible as a group, and I think that's probably the primary message we want to convey, to this team of outsiders, including the press, which is that is that algae is really a credible research area, with a lot to offer, both in terms of biofuels and all these other ancillary and important products that we've been talking about for the last couple of days. OK... Are there any other issues? Maybe it's not as productive to continue in this complete sort of quorum, plenary discussion and maybe there would be a better use of time if we were to break up into small groups, and we need some time to figure out how to do the PowerPoint.

Lene Lange: Just a very quick point and that is, that all the time you're preparing for communicating with journalists and politicians and opinion and decision makers, make sure that you are aware of some pitfalls that you should take care not to forget... There have been a few people saying, "Oh, if there is not enough nutrient in that, we'll just make sure that they release some more, or "They don't have to clean it so well, and then we'll have more nutrient." That's not an option. It's not an option in Denmark. The problem is that well get, for instance ...we'll get some more pig farms here; then, we'll have wonderful wastewater and we can make all the algae. We get a lot of serious pollution from the rain carrying down the nitrogen nutrient because of all the pig farms. We are destroying the biodiversity on land based on that; it's not just in the nutrient and the water. So don't come up with that answer or we will be associated in this group with suggesting more pig farms or more nutrients in our wastewater... I mean that's a "No-no" in the Danish mind; I mean, really, we will be on the wrong side of the fence.

Jonathan Trent: I agree; in other words, we have to be careful of what we say. That's true.

We have an hour's time, so I would propose that we break and you're welcome to hang out and network.

Jonathan Trent: We're going to start the afternoon with the last part of our incredible couple of days that we've been having in all of our discussions. We have the permanent Secretary of Energy here, who's going to talk to us about some of the issues with regard to Denmark. I'm not going to attempt to introduce him; rather I'm going to let Lene Lange introduce him, because she knows him much better than I do. I know him from a couple of visits that he almost made to NASA, but didn't quite get to NASA and Lene Lange is going to talk to him. Then we are going to hear about Lolland, from Stig Vestergaard, the Mayor of Lolland, , who hosted us the last couple of days, and after that, we are going to have the discussion that we talked about this morning..

Lene Lange: This is a great pleasure for me and I'll be as informal as the whole workshop has been, which I think has been shown, Jonathan—to be the way to be: to be to the point, and keep the level of energy and enthusiasm high. So, yes, with enthusiasm, I also now introduce the permanent Secretary of the Ministry of Science and Technology in Denmark., Uffe Toudal Pedersen, who has actually been with the Ministry for 4 years. A lot of things have happened in that time; we have lots of different strategic research coming up much more strongly, and a lot of interaction between the industries and academia. We have even been trying to work between different ministries, which is a huge challenge in Denmark that we have to overcome. So, we are really looking forward to hearing what you have to say to us, but I promised to say that you were not an expert in algae and that you are not an expert in bio-energy either. But this session will also be for non-experts,, so Uffe, we are looking very much forward to hearing you. The floor is yours.

UFFE TOUDAL PEDERSEN:

Thank you very much for the kind invitation, and the almost overwhelming introduction. I have to say that changes in ministry were under way, even before I came, ust to put matters in the right order, so

to speak. But first of all, I have to say that it's a great pleasure and a great honor for me to be invited to open this concluding part of the workshop, although it's a bit complicated to arrive at the end of a seminar, because you missed so many things in-between. So I might say something that perhaps is a bit out of order but nevertheless, it should be seen in that light.

I would like to say a few words about the government's position in relation to climate, energy, and the environment. First of all, and perhaps a bit more formally on behalf of the government, I should very much like to express strong recognition to the municipality of Lolland and to Stig Vestergaard, to the scientific team and in particular, to Jonathan Trent, for having organized this workshop, together with the Baltic Sea Solutions. At the same time, we would also like to express a deep respect for the fact that it has been possible for so many people from across the world to come to Denmark and to come to Lolland today. World-class researchers and many companies have been here during these few days. I know that many of you have traveled long distances to be here, and we see this as a clear signal, of course, of the relevance and the importance of the seminar and also, of course, hopefully, of the things we have to offer here in Denmark.

Basically, one could say and perhaps it's a tedious fact, which this workshop springs from man-made climate changes-- a deep, worrying challenge that we, across governments, countries, regions, must overcome as a unity. We are morally, politically, and economically obliged to do our utmost to create sustainable development. We do see research and forward-looking investments as some of the keys to finding solutions in this area, solutions that are so necessary I know that since Monday, you have been through a number of presentations, challenging presentations, and that you have been through a number of discussions. I hope that during these debates and different encounters, you have had the ability to create very good connections across the world. I have to say that we, from the outside world, are really looking forward to hearing the conclusions of the seminar, to be presented shortly by the scientific team, and I do hope that we will get a lot of information that we can bring back.

Globalization is a reality and the world is more complex than ever--that is common knowledge and has been said many times, but still it's true. There are no easy solutions to the climate/ energy problems that face us; it is therefore essential to understand that there is not only one solution to our challenges. In parallel, we must focus on and invest in many different initiatives, including initiatives within wind energy, biofuels, solar cells <u>depositing</u> (?) of CO_2 etc.

But it's not done with that; we must also understand that there is not one model that fits all. Models or solutions should be judged according to the context in which they are supposed to operate.. What I am trying to say is, simply, that we must be open in our approach to the different solutions put forward and thought of. Wind power is obvious in some parts of the world; it's obvious here. In other parts of the world, it's not obvious at all. Production of first generation biofuels, bio-ethanol—in Brazil, for instance--, may be a good idea in Brazil; it's disputed, but it may be a good idea in Brazil, but under different circumstances, it is perhaps not a good idea at all. We all realize that the urgent need for multiple solutions implies that we do not burden agricultural land and fresh water—that area, in my view, is one about which we could all agree.. But still, I would urge that we be open in our approach to the solutions that are put forward to us. Sometimes when we have debates about these issues, one tends to be located in separate trenches and that's not what we should aim at.

The ambition to create a sustainable society has been very high on the political agenda in Denmark for many years and we are persistently working to maintain and develop Denmark as an international hub for sustainability. In a long-term perspective, the Danish government has said that it would like to see a Denmark free from fossil fuel, free from coal, oil, and gas. Of course, it's an ambition that can only be realized in a very long-term perspective. And it's an ambition that can only be realized through research and innovation.

Back in 2006, the government decided, with a broad majority in Parliament—and I have to say that this broad agreement has been consistent since—that by 2010, 1% of our GDP should be allocated to publicly funded research in general. We will meet this target in 2010 and unfortunately, one could say that the financial crisis has made this task easier but that was not thought of when it was decided in 2006; I have to say that. Of course, we have heavy political discussions about how our research money should be allocated. I guess that's the case everywhere, and also in Denmark. We do allocate a substantial amount of our research money in the area that we are discussing here today. In order to allocate our research money in the strategic area, as we call it in Denmark, we have had, together with researchers and businesses, a very open process where we have identified a number of priority areas. We call this *"Basic catalog research, 2015."* This summer, we intend to launch a green research strategy that will take as a starting point this catalog. .

In this catalog, we have identified, together with the whole environment, six themes as core areas for green research and innovation:

- 1. Energy systems of the future.
- 2. Future climate and climate adaptation.
- 3. Competitive environmental technologies.
- 4. Bio-resources, food, and other biological products.
- 5. Intelligent solutions for society.
- 6. Sustainable transport and infrastructure.

The overall ambition of the green research strategy is to embed climate and energy as a distinct and highly prioritized seam within the overall Danish research policy.

I think it's fair to say that both in terms of focus, and in terms of timing, the conference here in *Maribo* is taking place at the right time, at least from a Danish point of view, because right now in my job, we are preparing the draft body for 2010; furthermore, the next round of political negotiations regarding the distribution of research money will take place after the summer break. During that round of negotiations, we are not going to allocate specific money to individual research projects; rather, we are going to distribute the money according to, among other things, the areas that I just mentioned. After that, I do hope that research applications will follow, among other sources, from a seminar like this--

applications that will be handled by our scientific research councils, according to the way that it is done. But I do hope sincerely that out of this will flow, perhaps not this year, perhaps it's too early, but in the years to come, applications, projects, ideas and initiatives that could be eligible for funding for our research system.

From an outsider's point of view, the perspective of this conference sounds almost too fantastic to be realistic or even true-- that we might some day be able to replace our dwindling fossil fuel resources by algae conservation and biofuels. It's really a vision that I can tell you (as we put it in Danish) will "sell tickets" in Denmark. But of course, there is always a certain "but'" to visions, one of the very strong pre-conditions that I see is that production and testing and experiments must be organized in such a way that they are not harmful to our environment.

We are not responsible, in our Ministry, for environmental issues, but there is no doubt that the environmental aspects of algae production will be an issue that will attract much attention and I think that the environmental impacts of this concept will have to be analyzed very carefully, simply because activities, perhaps even offshore, can have or might have, or should not have, very dramatic impacts on the open sea. I understand now that this is not the only solution, but it could be one of the solutions. I say that, and what I say here should not be heard or seen as a fundamental reservation about the vision, because we do find the vision to be enormously interesting. It's only to stress it as a point of attention and a point of focus that one should not underestimate.

Lolland is the site of the first and largest offshore windmill farm, as I'm sure you have heard several times during the last few days. With the establishment of an offshore research or an on-shore research and development algae facility, Lolland will certainly complete the picture of a region that has put sustainability high on its own agenda. And thereby Lolland has demonstrated that, for a change, it is fair to say that here a region has been able to think globally and act locally. Sometimes this phrase is a bit worn out, but I think in this particular case, it's fair to use it or reuse it. Everywhere in the world, everywhere in Europe, and in particular, in Denmark, 2009 will be the year of climate change, of environment, where climate change, where environmental issues and energy will be very high on the political agenda and in Denmark of course, because Denmark is hosting the United Nations COP 15, to take place in November this year. The government, is only part of that effort. The government as such is struggling hard these days, weeks, and months with many other actors in Denmark and, in particular, across the world, to prepare a summit that will hopefully provide changeable solutions, or at least ideas for solutions.

I have the personal view that, when it comes to the real world, it is the sum or rather the accumulation of many ideas, many initiatives that provide, so to speak, the fundamental changes, and I do see that the conference you've had over the last few days, the exchange of ideas, the innovation, the things that will follow from this conference, are one of these building blocks that we do have to see as part of the present solutions to our future problems, or the future solutions to our present problems, I think it should be phrased in that way. As I said before, I do hope that out of this-- and I will be listening very carefully to the summing up in a few minutes-- I do hope that out of this, will follow new ideas that we can bring back into our political circle, both in Denmark and more globally.

Having said that, I just once more would like to thank you for having chosen to spend your time in Denmark and in Lolland. I am sure that Stig Vestergaard will say more about that in a few minutes,

but it's really an honor and a privilege for the country to host you. I wish you all possible success with the follow-up to this workshop and I do wish you all a safe return to your home when it comes to that. So thank you for the attention and have a nice week.

Jonathan Trent: Thank you very much, Uffe...

Next, we're going to hear from Stig Vestergaard. We heard him speak at the gala dinner, thanking us for being here, and we thanked him as well for having us here and now we look forward to hearing your next input for our workshop.

STIG VESTERGAARD

Dear friends, I hope you have enjoyed your stay here in Lolland. I will be very glad to meet you and to talk with you. Lolland will develop energy and create growth, based on its own natural resources. Lolland wants to develop and attract business, education, and research, based on available natural resources. (It was a difficult word. (laughter) The Lolland town council, which was established in 2006, agreed with these visions.. Since then, Lolland has been working towards a sustainable future. Focusing on energy and the environment, using its own natural resources and potential, Lolland already has an impressive list of initiatives involving innovative technologies for sustainable energy solutions.

We believe we are contributing to making a difference in tackling the climate challenges that the globe is facing. There are several reasons that these initiatives are taking place on Lolland. Lolland County has provided the physical framework for establishing full-scale testing and demonstration facilities. This is a great advantage for researchers who want to test their lab results on a full scale. As you saw yesterday in *Onsevig*, we will soon be able to take steps toward full-scale experiments with algae in the algae innovation projects.

We have an important position and a special status in Denmark. Growth forum Zealand and region Zealand have appointed Lolland, not only as a climate and energy county, but also as a showcase county for climate solutions. Denmark's Climate and Energy Minister, Connie Hedegaard has stated that Lolland has succeeded in attracting new technologies and is the magnet for sustainable initiatives within renewable energy. It is an achievement we are proud of, but also a commitment. Lolland County has a political will and courage to implement large investments. We are a rural county, which has understood how to think in new and innovative ways, by connecting international networks and using the county's natural resources to the advantage of the residents and as an example for other regions. And Lolland County has the competence and know-how.

The latest years of investment and full-scale demonstration of innovative and sustainable technologies have given Lolland a good head start in the sectors of alternative energy and environment--something which is of great value, now that everybody has suddenly realized that something must be done in order to adapt to future climate change. I look forward to the outcome of this workshop. I

believe the impressive preparation of this unique workshop, headed by Jonathan Trent will be the start of something that will grow and develop and I would encourage all of you to continue sharing the experiences you have harvested during this conference.

In a little while this workshop will be over. I hope you will not say goodbye, but rather "på gensyn," which is Danish for "See you again" on Lolland. I would like to give a special thanks to Jonathan Trent and to Baltic Sea Solutions for this arrangement.

Thank you for your attention. I hope that we will have the opportunity to meet again. Please know that you are always welcome here. And thank you.

I hope you understood most of this ! (smiling)

PANEL SESSION:

Jonathan Trent: Because we have had such a vivacious and intense discussion the last couple of days at our workshop, and because we now have people from the press and from the Ministry and from the consulates here, we thought it would make sense for us to have a panel, which is a representative subsection of our group and our discussions. I will give an overview, with some commentary by the panel. Then we'll open it up to questions and discussion.

Let me begin with a couple of comments that are captured by some of the inspirational quotations we have hanging on the walls. The first quote from former US Vice President Al Gore, in 2007 he said: "we face a genuine planetary emergency. We cannot just talk about it; we have to act on it and we have to solve it, and we have to solve it urgently.

The next quote is from one of the founders of OPEC Sheikh Ahmed Zaki Yamani The oil Minister in Saudi Arabia for 25 years and the former OPEC Secretary General, when asked in 2006: "What will happen when we run out of oil?" He answered, "The Stone Age did not come to an end because we had a lack of stones..." Indeed, the oil age will not come to an end because we have a lack of oil. The oil age will only come to an end when we have a good alternative to fossil fuels. This is the implicit motivation of all our conversations during the last two days. Some of us have spent year and even careers looking into the possibility that algae could be one of the alternatives to fossil fuel.

This brings us to our last quote from Harry Truman: "There is no limit to what you can accomplish, if you don't care who gets the credit." While this may be contrary to our academic upbringing, particularly in the US, it's going to be essential in the rapid development of new technologies for cultivating algae, such as OMEGA.

Algae is and has been our primary source of oil for some time. What you may not realize is that most (perhaps 90%) of the oil that we are now pulling out of the deep earth in the form of fossil fuel is believed to have been deposited by algae that lived in the shallow seas that covered most of the earth

over 200 million years ago. Descendents of those algae still exist today and they continue to produce oil, which we could, if we knew how to cultivate those algae appropriately, tap as a sustainable supply of carbon-neutral oil.

We've heard from our Israeli colleague, Ami Ben-Amotz, that it's possible to grow large amounts of algae on land, but that there are problems and expenses associated with that process, which make it non-competitive with fossil fuels. One of these problems is freshwater. Even if we cultivate algae in salt water, there are problems with evaporation in open ponds and with the use of freshwater for cooling in closed bioreactors.

Freshwater is a potential crisis that rivals or exceeds our fossil fuel crisis and we obviously don't want our algal biofuels plans to exacerbate the water crisis. There is also a food crisis on the horizon and we don't want the production of algae biofuels to compete for water, land or other resources that can be used for agriculture and food production. In other words, we want to be able to produce algal biofuels without using agricultural resources and in a place that is out of the way. The answer that we have been considering for the last two days is to grow algae on the municipal wastewater that we are currently dumping into the ocean and to grow them in the ocean in OMEGAs (Offshore Membrane Enclosures for Growing Algae).

In addition to providing biofuels and other algal products, the OMEGA system is designed to improve the situation in coastal areas where municipal waste is currently causing blooms of wild algae that are contributing to the formation of hypoxic or dead zones. The OMEGA is a controlled and contained algal bloom that enables us to bring the algae back on land to be use for biofuels and fertilizer, and etc., and prevents the wild algae from blooming and causing problems in our coastal zones. In other words, OMEGA transforms what is currently considered a waste-stream into a resource for growing algae, which captures the significant amount of nutrients we are currently losing in the ocean, and allows the ocean to resume its more natural cycles of algal blooms.

OMEGA obviously seems like a wonderful plan, but as my engineering friends tell me: "the devil is in the details." The details have been the topic of our discussions--and we had some very intense discussions in the last couple of days. The all seem to focused on the four major problem areas I outlined of Biology, Engineering, Environment, and Economics. Some of the questions we addressed were:

[Biology]: What kinds of algae would we grow in OMEGA? Would we rather grow seaweed than microalgae? What are the advantages and disadvantages of each? What do they need to grow and are the conditions available here in the vicinity of Lolland and elsewhere in the world?

[Engineering]: How can we do it? What are the engineering constraints? What are the materials that can be used? How will the be secured? How do we engineer the logistics of filling, growing, harvesting are the materials that we would need to use?

[Environment]: In considering the environment, we also consider policies, permits, and politics. We obviously need to ask what is the impact of floating large OMEGAs on the surface of the ocean? How will the shading impact the underlying water column? What are the consequences for marine mammals and birds? How will it impact water movement, larval dispersion, and fish populations?

What are the consequences of leakage? What will happen to the intertidal if OMEGAs wash ashore? With regard to policies, permits, and politics, how should we be rethinking what we as environmentalists have fought so hard to establish? What should the permitting process look like in light what looks like an imminent crisis for the oceans from acidification and stratification? How do we, as scientists and engineers, advise and inform the politicians so they can act rationally and quickly?

[Economics]: This includes issues about whether the implementation of OMEGA could be economical as well as whether we can raise the necessary resources to develop the needed technology. Considering how important these issues are, we have spent a relatively short time focused on them. A thorough economic analysis would have to include biofuels, fertilizer, fodder, and all the other potential products that can be made from algae, as well as the value of the services provided by OMEGA for wastewater treatment. The costs of OMEGA in terms of design, materials, construction, deployment, maintenance, utilization, harvesting, processing, and all the implications this could have on local economies, boat traffic, fisheries, and etc. is the topic for a separate workshop and one that will be needed if the basic concept of OMEGA proves to be viable.

We all recognize that we have little time to be proactive in dealing with the problems that are on the horizon. I fear that when these problems are upon us, our responses will be reactive, as they have been in the past, and the environment will be the first casualty in our desperate efforts to cobble-together solutions. In fact, I think many of the global problems we are confronting are a consequences of this kind of shortsighted reactive behavior and a denial of our longer-range responsibilities. Many of the policies we now have in place, designed to "protect" the ocean, fail to take into account the mass extinctions that are predicted by the end of this century.

One of the beauties of the OMEGA system is its attempt to utilize what is now considered "waste;" the nutrients in wastewater, the CO2 in the exhaust gases, and to deal responsibly with all aspects of the system. At the beginning of the conference, I said that I am not speaking on behalf of NASA, but this parsimonious utilization and recycling of materials that is fundamental to OMEGA has its roots in NASA life-support systems. In NASA terms, this is called closing loops--that is, in life-support for astronauts in space, everything must be found for survival. Not just food and water, but even the air the astronauts breathe and everything is expensive to carry into space, which means that everything is optimized and carefully considered and everything must be considered and reconsidered for its value or potential value, its direct use, and its alternative uses. Applied to OMEGA, we are collectively considering the optimization of resources on our Spaceship Earth.

There's an old saying that states, "Genius generates new ideas and labor alone finishes them." I think we've certainly had some ingenious ideas in the last couple of days. I hope that there are those among us sufficiently inspired to want to put the labor into following up on at least some of these ideas.

I am convinced that the prospects for successful OMEGA demonstration projects are within the people represented in this room and the potential for a full-scale implementation of an OMEGA system is within the network of our combined networks. Whether or not any scale of OMEGA is realized

anywhere in the world, will depend on our energies—individual and collective to bring this concept into focus for our colleagues and for the world.

I was struck by what Uffe told us, with respect to the six themes that he described as the focus for science and technology development in his ministry:

- 1. Energy systems of the future.
- 2. Future climate and climate adaptation.
- 3. Competitive environmental technologies.
- 4. Bio-resources, food, and other biological products.
- 5. Intelligent solutions for society.
- 6. Sustainable transport and infrastructure.

Algae research and development seems to be relevant to all of these six areas in one-way or another. Clearly, algae is an energy system that impacts climate and climate adaptations. It has the potential to be a competitive environmental technology and it is undoubtedly a "bio-resource." The intelligence of using algae for solving a variety of our social problems with fuel and wastewater treatment, and the use of a biofuel from algae for transportation touches his last theme. Indeed, algae technology really does fit into all of the Ministry's future themes and I think this will become even clearer to the audience after we have discussed as a group what we have been doing for the last two days.

I would like to ask my colleagues to come forward for the panel. We're going to review some of our ideas and show you some of the ideas we have for the future.

You should understand the format of the workshop we had for the last two days. In the mornings, we had informative lectures because we brought together people from many different disciplines. This includes ocean engineers, phycologists (people who study algae), biotechnologists, environmentalists, ecologists, university professors, researchers, business people, and even rocket scientists. Because of all these different backgrounds, we had tutorial lectures in the mornings. We will show you some of the information from those lectures and some of the ideas that emerged to give you an overview of what we accomplished.

I will go through some of the images shown to give you an impression. I would like to acknowledge that the reason we came to Lolland is because Lolland demonstrated to us this very important idea: that we need to think, or swim. And that is to say that they are certainly open to the possibilities of research and the kinds of research that we want to do. As Stig Vestergaard indicated, we are welcome here, and finding that kind of openness, provided us with a forum for doing the research and experimentation that are necessary for progress. That attitude of openness is really something that is very important to us.

One of the issues we discussed that is a local and a global problem is the problem of so called "dead zones." These are places around the world where there's now so much fertilizer being dumped into the ocean, running down rivers and streams, and getting into the ground water, that it ends up forming

places where the local algae bloom because of the fertilizer. When they die as a natural part of their lifecycle, they sink through the water and decompose as they sink. This decomposition, uses up the oxygen in the water column and the organisms that live there that require oxygen are suffocated—hence the name dead zones. The number of dead zones around the world has been increasing every year for the last few decades and their numbers have become quite alarming; particularly in places like the Gulf of Mexico and the Baltic Sea. In some places, this phenomenon is only seasonal, limited to the summer months. We had some interesting discussions about how algae, which are the root-cause of dead zones can be a basis for preventing the formation of dead zones if they are contained in OMEGAs. Algae are a perfectly reasonable and sustainable way for treating municipal wastewater and have been used historically in many parts of the world. We discussed how we could use this traditional approach of algae wastewater. We talked about how we could use the algae or in this case algae of our choice to clean up wastewater and in so doing produce biofuels and food and fertilizer.

This kind of "industrial ecology" in which the waste product from one industry is a resource for another, is interesting, but our plan is to push this kind of *ecology* even further. Based on a meeting I had with Stig Vestergaard about a year and a half ago, we discussed the possibility of combining wastewater treatment by algae growth with offshore windfarms. In this case the windfarms represent an existing infrastructure in the Baltic around which an OMEGA algae farm is constructed. It's not just infrastructure, the windfarm represents a valuable source of energy for pumping wastewater and compressed air and providing electricity to provide light for the algae to control their growth rates. The algae farm in turn, adds value to the expensive infrastructure used to hold the turbines aloft in the Baltic. There was a lot of discussion about this, and whether the wind-turbine stanchions are strong enough to support the algae farm a if the algae farm would be in the way for fixing the turbines, etc.

In keeping with a good scientific meeting, there were arguments on both sides and lively discussion about how or if to proceed. Indeed, there are scientists in our group that have developed algae farms over the years and who know what is really involved in this long and difficult process.

Pre-eminent among our successful algae farmers is our colleague from Israel, Ami Ben-Amotz, who is the chief scientist for Seambiotics and NBT. Ami, would you please say a few words about who you are and how the system you created in Israel is working so successfully.

Ami Ben-Amotz: I have a different comment. First, I've been working on algae for a long time, since 1964, so it's almost 45 years. I remember when my advisor convinced me to work on algae, as he said, for a few reasons: First, algae have no age, so, usually by definition, we say "She." I have lived with algae more than with my wife. She is here and as algae are young all the time, they don't change. When you talk about micro-algae, you don't see any change in the algae and you don't develop any sentiment for them, so that's one of the best reasons to work with algae for a long time. The other reason is our plant in Eilat. Yesterday, I talked with the mayor and I think there is a very critical issue of which I want to remind you regarding onshore or offshore and that issue is related to tax: city tax. Originally, when we started the pond, it was aquaculture, so the mayor said, "Zero tax," and for many years we've been growing algae. Now, about a year ago, the city sent a revision to the tax and said, "You aren't growing algae; you're producing algae."

producing algae is almost 15M dollars in city taxes. Originally, the mayor said "You grow and you don't have to pay tax." Now, twenty years later, you see the plant,; then the new mayor, the 4th mayor already, said, "I'm sorry; I don't remember what the other mayor said and now you have to pay industry tax, 15M dollars."

We went to court, to the lower level court, and we won. The court decided that we actually grow algae. The city appealed to the next, to the middle court, and we won again. And now it will go to the high court in Israel. So, are we growing algae or are we producing algae? Why I say all that, is because one of the conclusions we came to was that instead of working onshore, we have to work offshore. No tax, free land; even the government cannot say anything and the land will be available for hotels and other resort areas. So if you're looking for several years of experience with algae, I can tell you that it's not only biology, as you can see by the question with the city, which is very important, and there are other matters. Actually, I think working with algae is fun; if you don't feel that it's fun, then don't work with algae. For anyone working with algae, I really think it's very nice, and being in Lolland, seeing the facilities that you have--flue gas, land, sea and know-how, I think that the people here can grow algae and I wish you a lot of success and luck. And if you have some difficulty, you are welcome to come to us and get some of our experience and some of our know-how. Thank you very much to the Minister and the Mayor.

Jonathan Trent: We also heard from a colleague from a culture that's been growing microalgae, seaweeds for over a thousand years. We heard pretty impressive ideas from professor Wang about how the Chinese grow seaweeds and have been involved in this type of aquaculture for many centuries. They use simple, labor-intensive, but effective methods that produce huge amounts of biomass. We had a picture of what the Chinese seaweed aquaculture areas look. Qiang Hu also told us about his extensive research experience about how and under what conditions microalgae produce oil. He also told us about the effects of light on algae growth. Qiang Hu, did you want to say a few words?

Qiang Hu: Later!

Jonathan Trent: In that case, we'll hear about seaweed cultivation from our Germany colleague, Bela Buck. Bela told us about the possibility of combining seaweed culture with different kinds of aquaculture, e.g., oyster cages and mussel ropes; with a seaweed called *Laminaria*. These ideas have been developed in the North Sea and Bela showed us some striking pictures of actual structures that he designed and deployed and were recovered with a lush growth of *Laminaria*. Bela, you also told us about some problems in the ocean; do you want to say a few words?

Bela Buck: We have been doing this kind of aquaculture for the past nine years. I started with this subject for my Ph.D. thesis. In the whole institute, I was the guy with the visions--with the visions that were impossible, of course. Today, it's very different. There are other countries doing the same thing; the only difference today is that all the other countries have windfarms, and Germany has no wind farms yet. We try to use other constructions offshore to combine with aquaculture and I can say that today there are various candidates that you can use to grow algae of other things offshore. It's still somewhat of a problem of biology. I put some slides in my talk for those of you who have not worked offshore, so that you know what is waiting for you when you go offshore. It's difficult, but I

will never say "no," because I started with visions and I'm still open for visions. I am an experimentalist and I think we just have to test one of our ideas on a pilot scale.

Jonathan Trent: We are with you Bela... We heard from Ami Ben-Amotz about an onshore algae farm in Israel and there are also existing algal cultivation systems that are now being used in Hawaii on the Kona coast, and yet another system on the coast of Hawaii, and you see the different algae strains, represented by different colors. These systems were discussed by our colleague, Al Darzin, from the National Renewable Energy Lab, who told us about some of the practical issues associated with cultivating algae and compared for-profit companies growing algae for high-value products like carotenoids or astaxanthin, which sell algae/kg for thousands of dollars, in contrast to the would-be companies that would produce algae for biofuels, which would have to sell algae/kg for \$0.10 or \$0.50. Al also told us about the DOE roadmap for the future of algae research and development in the US. Al, would you please say a few words about NREL, Doe and beyond?

Al Darzins: First of all, I would like to thank the Minister and the Mayor for having us here. This has been a wonderful opportunity. I work at the National Renewable Energy Laboratory in Golden, Colorado, and it is the only national laboratory in the Department of Energy in the United States that is solely devoted to energy research. NREL re-established its work in algae a few years ago, after working almost twenty years in the area. In the U.S., we know that there are probably going to be multiple solutions to our energy challenges; there is going to be wind and solar; there is going to be bio-energy. Within the bio-energy community, we also know there is not going to be a single winner; there are going to be multiple solutions that will include things like ethanol and biobutanol and algae, so that's why there has been such an immense interest over the last number of years in the development of algal biofuels. The world has several challenges, but within the world, there are several regional areas that I think could really be starting to be used as test beds to test some of these bio-energy solutions. We think that Lolland and the Baltic Region can be set up as a very unique test bed to evaluate and implement interesting bio-energy solutions that involve algae, both microalgae and macroalgae, for the production of not only bio-energy but feed, high-value products and fertilizer as well.

Jonathan Trent: There's a lot of discussion about how we're going to move ourselves around in the future—maybe hybrid or electric vehicles on land, but what about long-distance travel that is current done in airplanes? The possibility of having an energy density that is adequate for batteries that are adequate for flying airplanes remains a very distant dream. This need for aviation fuel for the foreseeable future may be the niche for algae, as Al presented. This brings us back once again to the question of how are we ever going to produce enough algae to meet even only our global aviation needs?

Among the intense discussions we had about algae cultivation was the possibility of cultivation offshore as opposed to on-shore. How can we use Lolland as a test bed, as a kind of experimentarium to move from onshore sites into the Baltic and ultimately out into the sea?

The successful evolution of this technology will obviously involve a great deal of ocean engineering and Feargal Brennan from Cranfield University provided some valuable insights into how this process may develop. Feargal, would you please comment on this?

Fergal Brennan from Cranfield University in the UK. The reason I'm sitting up here is to represent the considerable engineering expertise we had here over the last three days. We've had engineers from France, from the UK, from Denmark, from the Baltic States. In addition, to looking at economic and environmental, as well as the biological aspects, it's essential that the engineers were invited and that we would try and guide the discussions to pragmatic paths through the thicket of our arguments. Don't forget, Northern Europeans have been the world leaders, for I would say thousands of years, in shipping and offshore structures. We've exported our technology from Denmark, from Norway and the UK, to Brazil, throughout South America and in fact, all over the planet. So we do have the wherewithal to help the biologists to be able to produce cost effective and reliable engineering solutions.

I would say, inspired by the Danish lead on offshore wind, the UK is in deep water, literally (laughter) as far as its 2020 targets and it needs to produce 12 gigawatts of offshore wind to meet its 15% by the year 2020 target. That means you have on average 2 megawatts turbines; that equates to about 6000 turbines. That's not going to happen in ten years and nine months. This has to come out of a billion pound fund from the Energies Technologies Institute in the UK to try to look at diverse solutions. We've been successful in the first round of this funding cycle and designed a windturbine called the NOVA (The Novel Vertical Axis) or offshore vertical axis machine. I say to people that when we start to go into deeper waters (the Danes know this very well) we need new designs to deal with different pressures for functionality and reliability. Trains don't look like boats and there are good reasons why deep-water offshore wind turbines will not look like onshore wind turbines, particularly when the get very large. What we are doing with our colleagues in Europe is working to transfer know-how from the offshore oil and gas industry to the emerging and embryonic renewables industries. I'm afraid they will have to go through many of the same pitfalls that we did, but at least we in the oil and gas industries can see the learning curves that are ahead of them.

Jonathan Trent: The engineering expertise gave us confidence that offshore algae cultivation systems are possible, because indeed almost anything is possible for the engineers if there is "enough money" involved. So what's enough? How much should we (the world) be spending on replacing our fossil fuels, which are known to be so problematic? This is a 150-year-old technology that is currently earning on the order of \$2,000,000,000,000/yr. How do we work out the economics for this? What are the oceans and the atmosphere worth? What's civilization worth? What is the human population worth? What is biodiversity worth? How do we get our thinking to this scale as we confront the transition to sustainability?

Our colleague Peter Lindblad gave one of the most stimulating presentations we heard about a transition to a hydrogen-based alternative. Peter would you please tell us a little about the worldview that you have from your vantage point in Sweden using some of the oldest organisms on the planet to come to our rescue?

Peter Lindblad: Thank you very much, Jonathan. I'm Peter Lindblad from Sweden; I work at the university but also at the Swedish Energy Agency and at Nordic Energy Research I represent a cluster of scientists doing R&D on future applications for biofuel. I think, from a Nordic perspective, that we are all very aware of algal blooms. We have algal blooms in the Baltic and we are trying to address the question of can we utilize the organisms that we find in the algal blooms, specifically the cyanobacteria, to produce bio-fuels? We have a system today in which we have other cyanobacteria in the R&D systems. We have systems in which we can produce different kinds of biofuels that can be utilized but that are still on an R&D schedule. What we also see happening is that in parallel the same kinds of organisms are being used and developed for products on a commercial schedule and for me this is very encouraging. We see an example here: it's a greenhouse whose owner was traditional, selling green plants. He is now making more money by growing microalgae--the same ones we have in the algal blooms. He has made an algal photobioreactor, a cheap one, and he is selling the products. So we see today a development: yes, it is possible to grow the same kind of organisms for a product. It's not bio-fuel, but we are convinced that the biofuels are next... We already have today the possibility of developing cheap bioreactors and harvesting for example. We would be happy to discuss that further.

Jonathan Trent: It is clear we are on the cutting edge. At the interface between what is new and exists now and what will soon exist. We had a lot of discussion about how we could do things so they could scale to include algae for biofuels. I kept trying to lead the discussion back to the idea of using OMEGAs, large plastic enclosures that would float on the sea surface with algae growing on wastewater inside. The group however, was not only thinking out of the box, they were thinking out of the bag.

The lectures we had from our colleague from New Zealand, Yusuf Chisti, not only covered the range of existing algae cultivation methods, but presented us with some pretty wild ideas about how we could cultivate algae in architectural structures in the cities of the future. Yusuf, would you tell us about some of your insights?

Yusuf Chisti: I'm from Massey University in New Zealand. Currently it is possible to commercially produce microalgae biomass for relatively expensive products, using open raceway systems that you saw earlier, as well as various kinds of photo-bioreactors. So there are engineering solutions to produce microalgae; what is not currently possible is to produce it at such a low cost that it's competitive with petroleum diesel, to make biodiesel from this kind of source, but that's not to say that this cannot happen in the next twenty years or so. That's a kind of time frame that I'm putting out there for my own projection; I don't want you to be quoting me here twenty years later about this.

Yes, the technology is there to do it, but it's not there to do it economically, yet. I think it can be done and a lot of people here are working on the biology of these algal systems, as well as production technology, on how to harvest them, how to extract the oil from them, to make it all a commercially viable venture. I believe other than algae, there is no other biological system that can provide you amount of biofuel that you actually require for sustainable use currently. You can't do it from palm oil; you cannot do it from soybean; you cannot do it from corn, nor from any other crops. Algae, I believe, are the only option in the long run. **Jonathan Trent:** We talked about algae; we talked about the importance of algae, and I think there's a growing concern and a growing commitment that algae really are, especially the microalgae, critical for biofuels. We also discussed the combination of microalgae, macroalgae, and fish farming. I think it's important to realize that we were discussing all this in relation to an integrated approach.-- a "system of systems," as it would be described at NASA. The engineer's approach to putting things together into an ecosystem within a system to produce a system of systems. At this point we should hear from someone who puts people together to make systems that work. Leo Christensen, our local Lollander who has been helping to motivate the workshop with his enthusiasm for science and engineering projects in the context of Lolland. It was Leo's vision and perseverance that got me to Lolland and out to see the offshore windfarm. It is Leo's aggressive vision for the future of Lolland that is pushing Denmark into the lead in clean technology. The American 19th century philosopher Ralph Waldo Emerson once wrote that "all great discoveries begin with enthusiasm." I think we need to hear some of Leo's enthusiasm at this point.

Leo Christensen: Thank you. I would like you to remember that we call the way we are working in the EU, "the triple helix." The first time I explained it to the Mayor, he thought I was joking but actually, think about how the universities are knowledge drivers. Having us in the public system building the frames, look, remember it took 2,000 documents just to make this small dike you saw here. If the private companies or the universities did this work, actually it would never be started. Use the public as a frame shaper and then have the private companies come and do the job; and, as I said out there, remember that we have to earn money. As soon as we can earn money on those systems, people start believing it and we can keep going by building on what we learn to get grants and get wiser and wiser about how to make the system work. Thank you.

Jonathan Trent: In discussing what we were considering as to how we would interact with the community of Lolland, our colleague from Norway Bjørn Utgård, has some really important points to make about why Lolland is the right place for us to initiate our new green visions. Would you please say a few words?

Bjørn Utgård: I represent the Bellona Foundation; it's a Norwegian NGO that was founded in 1986. For the first few years, we worked on getting attention for the problems and then we realized that people were tired of hearing about the problems. They wanted to see the solutions. They wanted to be optimistic about the future. So, we have been working on solutions for a long time.

The latest discoveries we made in facing the future, which is what I think we are doing here, were algae. We have to acknowledge that there is a climate crisis, that there is an environmental crisis; there is a resource crisis, and there is an economic and a social crisis-- all at the same time. Crisis is a bit apathy- inducing, but what we see in looking at all these problems is that you put them together in new systems, and if you start thinking, not only about the technological solutions, but also about the societal systems that surround them, the technologies that you can actually come up with then reflect some really amazing results.

What I see here in Lolland is a happy integration of Industrial Interest in knowledge, in terms of research and education, and the most important element-- and I think that this is the most crucial thing to get in place--is the political leadership, the brave political leadership that can, as Leo said, make the platform on which these solutions can be tested.

We find algal biomass to be a crucial element able to handle all of these problems. We need a lot of new energy to fuel 3 billion new people for the next 40 years. At the same time, we need to produce a lot of food. Clearly, we need a large amount of biomass. We did a study last year that showed that biomass is at the core of the climate problem. We need to supply more freshwater; globally, we need to provide a billion new workers with jobs, over the next ten years.. Most importantly, we need to make sure that we can get those brave political decisions through. If, in December in Copenhagen, we can succeed in inducing an optimism for the future, if Lolland can be the evidence to those political leaders who are going for those new technologies and putting them all together and merging political processes with industrial processes, with high tech or with cutting edge knowledge, if Lolland can be the example that proves that you can actually create a place which is very good to live in, I think we should now look towards Copenhagen [COP15].

We should be very practical about how we communicate the opportunities and the knowledge that we have obtained here. As a Foundation, we are planning a lot of activities in Copenhagen and I'm very interested in hearing any ideas that we can show to the world in December that can convince them that this is actually going to work. Also, what I realized here, is that there is a desperate need for a great deal more funding for research, so that researchers don't have to rely on single companies that will then want to own the knowledge that these researchers produce. That's what we are going to work for in Brussels. We are now going to take a seat on the bio-fuels platform in the European Union, and that's what we are going to really push forward. Thank you.

Jonathan Trent: As I mentioned before, we spent a lot of time, in the afternoons designing projects that we might do. This was partly done as an exercise, although it was very realistic, in that we would pick topic areas that were substantial and real. One of the ideas that came forward was presented by, was chaired by, a whole group of people working on it with great enthusiasm and Aaron Baum was the person who chaired that session. I thought it would be instructive for you to get a feeling of the kinds of projects we were discussing that might in fact be implemented. That is not to indicate that this is something that we would necessarily do, but we want you to know that it has been studied and was really the efforts of about a two-hour discussion--about an hour-long discussion and about an hour of follow-up discussion. You may notice on the walls around the room all the different types of sketches, which reflect the interaction that was generated, just from these intense meetings, which were really meant to just begin to develop an intellectual relationship among the people in the room. Aaron, do you want to say a few words about the proposed systems for Lolland's dikes?

Aaron Baum: I think this is a great example of the kind of creativity that you can get when you bring together people from all different fields, who are both global and local. We were really inspired when we were brought out to the dikes and we saw the existing infrastructure there and the storage of the agricultural run-off, which is so damaging to the ocean when it's released with all the extra nutrients. So a NASA scientist got talking to a Lolland agronomist and then biologists and chemists, and ecological specialists and economic specialists, got together. And we came up with this concept that is so simple and effective, that we think that it will actually pay for itself and, in addition, pay for the dikes that are so necessary here and, of course, increasingly around the world. This is how it works: The agricultural run-off water accumulates in a fetch ditch that's behind the dike here. This is the ocean, and that water runs out to this small pond here, that's being drawn on a siphon, and the critical part of the system is a membrane, that's a technology that's been worked on at NASA, that will actually pull the water out of

the algae, which is the critical thing, because you've got all these nutrients in here but they're mixed in with water and there's no economical way to bring them back to the fields. Therefore, the membrane that takes the algae that are growing in the water and that have absorbed all these nutrients, and separates them from the water and then, you have an algal paste that you can return to the fields. We were able to calculate that we ought to be able to pay for the whole system with the value of the fertilizer that we're pulling out of the water; it's that simple. And furthermore, it's so low-tech that we ought to be able to take this system all over the world and eliminate dead zones everywhere. We're very excited about this concept and we're already starting to put together a proposal.

Jonathan: Do you want to say a bit about what your background is?

Aaron Baum: Oh sure, yes. I'm from AlgaeLab.org; we develop open source technology internationally.

Jonathan Trent: Qiang Hu do you have some comments you would like to make about the algae research that you've been doing, before we go on?

Qiang Hu: My name is Qiang Hu, I'm a phycologist or algologist working on algae, I was at Arizona State University in the U.S. and I'm Chinese. I don't know which country I'm supposed to represent. I should just present as myself (laughter) There are so many Americans in this room, that I better represent China.

Jonathan Trent: Because you've done research in this area for so long and because you've worked in China and Japan and Israel, as well as now in the United States for a long time, you, in and of yourself, are an international component of the workshop.

Qiang Hu: OK; we can talk a little bit about that. I spent more than 20 years working with algae and, the more I study, the more I feel that I understand very little and so that's a problem. The point is that today there are many hypes about algae-based biofuel or bioremediation or about how to use algae to make tons of biomass, as a feedstock or a human food supplement. That can be true to a certain extent. For instance, in China last year, right before the opening of the Olympic Games in Qingdao, we have one professor in that institute, Professor Wang. A couple of weeks, right before the Olympic games, in that bay area, all of a sudden there are microalgae and seaweed growing in a million tons of biomass, just within a few days. So algae do have the potential to grow fast.

Right now we understand very little about what's the growth mechanism? How do you induce the algae to grow in that explosive way? Before we really understand the mechanism; what's the reason for it and can we understand this well enough to control it and control the algae? I'm afraid to really understand this is going to take some time and therefore it's going to take a long time before we can grow algae biomass in sufficient quantities to replace our fossil fuels. If you look at conventional techniques for energy production like coal-fired generation or oil or natural gas, it has taken over a hundred years or at least many decades to reach the level of today's sophisticated energy generating systems. Algae have great potential, but at this moment, society puts very little money into this kind of research and it will take some time before producing algae based biofuel or bioremediation becomes common practice. So please, politicians and communities, you must be patient with regard to this kind of technological innovation.

Lene Lange: I can be patient, even though it's not close to my nature. The most important part from science, I would say, is that we need cross-disciplinary thinking. In Denmark, we have also had biologists in one silo and engineers in another, and we need these to come together. We also need a new attitude towards actually sharing knowledge, because of this sense of urgency. We need to share knowledge and in that sharing of knowledge we will be building know- how and technologies. And with all the comments here, I think that is what we have been demonstrating. As for the rest of it, I can wait, but I want to emphasize the importance of this cross-disciplinary thinking and doing facilitated by combining industry and academia, is my main point. I want to add however, that we need public money to be really innovative, which is why I was so happy to hear y**our** statement that you welcomed such creative thinking [pointing to Uffe Toudal Pedersen]. I think we cannot rely on just Lolland to pay for everything in this collaboration. I will reserve the rest of my comments for closing remarks. Thank you.

Jonathan Trent: In fact, Lene Lange just introduced the last quote, which comes from President Harry S. Truman, and is somewhat symbolic of our meeting. He said that there is no limit to what you can accomplish, if you don't care who takes the credit. That is one of the themes of our workshop. Thank you very much..

Questions:

Uffe Toudal Pedersen: I would like to ask a question and I hope it's not hopelessly stupid. I wonder if it's possible to distill from the last two days of debates and presentations a number of research challenges that we need to tackle first. Is it possible to identify some bottlenecks in our knowledge that we should address first...?

Jonathan Trent: You mean with regard to algal research?

Uffe Toudal Pedersen: Yes. Is there a general consensus about what we should address first?

Jonathan Trent: You mean what is the hierarchy of challenges in algal research and how would we addressing these challenges?.

Uffe Toudal Pedersen: Yes. Is there a general consensus about what we should address first?

Jonathan Trent: I'm afraid there will be nothing resembling a consensus even among the friendly group here in this room. I don't think it's a problem that there will be no consensus, because it will be like the old proverb about the blind men arguing about the nature of the elephant. The blind man holding the trunk has one clear idea, the one holding the leg has another idea, the one holding the tail has another, the one touching the stomach has yet another, and as the proverb goes they are arguing about the nature of the elephant because each of them is convinced of their own data and refuses to conceded that the others could be right because they do not want to try to take on their blind colleagues perspective. Well, I cannot speak for everyone here, but for me the wonderful thing about the last couple of days has been the opportunity to see the algae "elephant" from a lot of new perspectives. We spent some of our time arguing, but there was something of a consensus about the importance of including an offshore component to algae cultivation—microalgae and seaweeds. With regard to the challenges, we divided them into the four broad categories (listed in no particular order of priority): 1) biology, 2) engineering, 3) environment, and 4) economics. There are formidable problems in each of these categories, but I think it's fair to say that our consensus as a group is that none of these problems in fundamentally impossible to overcome, e.g., in biology we would have to genetically engineer a super-alga or in engineering we would have to defy the laws of thermodynamics or the environmental impact would be unacceptably devastating and even for economics we are not orders of magnitude away from what is feasible. To quote Sherlock Holms: *"when you have eliminated the impossible, whatever remains, however improbable, must be the truth?"*

Indeed, this is what our workshop was ultimately about, to consider the feasibility and desirability of offshore algae cultivation as an option in general and as a possibility in Lolland.

Does somebody else want to tackle Uffe's question about consensus of ideas and hierarchy of challenges? Robert, you want to say something about this?

Robert Baertsch: I have actually been going around informally and talking to different algae researchers and we've come up with a document that identifies some of the crucial research questions [in biology] that we need to answer to make bio-fuels a reality and I would be happy to share that document with you. It relates to algal genomes.

My name is Robert Baertsch and I'm working with Jonathan now, but I spent the last five years working on the human genome project with David Haussler at UC Santa Cruz. Haussler's group assembled the human genome in Santa Cruz in a graduate student's garage. It was a competition between our public effort to assemble the information, which was an open effort, and Craig Venter's company, which was a commercial effort. I'm happy to report that the public project beat Venter's private company and we published the human genome before him, which meant it stayed in the public domain. Since then, a huge consortium of labs all over the world has been developing tools to annotate the human genome data. The plan now is to take these tools and transfer them to the algae community. There's a wealth of people around the room that are interested in doing algae genome research and we've identified a list of basic projects that we think are important to enhance growth and lipid production in algae.

Jonathan Trent: This is a good focused plan in the category of biology, although it may take many years to reach the goal of genetic control of "enhanced growth and lipid production." We could debate, if intervention at the genetic level is more effective than traditional horticultural selective methods, but we have already acknowledged that we have agreed to keep our minds open with respect the many features of this algae elephant.

Are there other ideas in other challenging categories?

I should say that as a group we did not agree that offshore microalgae cultivation was a better project than macroalgae (seaweed) cultivation; and certainly there was no consensus about this for the Baltic area. We discussed at length the advantages and disadvantages of being offshore and it's hard for me to say specifically that we agreed on what could be a model system. In fact, as a team we agreed that to take an engineering approach would require that we identify specific projects with specific goals and a list of possible locations and then to see if we could map the projects into the locations. In other words, as long as we continued to talk at the 10,000-meter level, it is not going to be possible to make the hard decisions about implementation. In two days, we didn't have time to do more than break ground in our understanding of how to do something as ambitious as OMEGA, but if nothing else, we now have a lot more minds thinking about the topic.

Sherwin Gormly: Although you covered it to some extent, one of the things I'm interested in is looking at better algae strains. My name is Sherwin Gormly and I work with Jonathan at NASA Ames Research Center; I'm an engineer. Basically, I agree that each solution has to be tailor-made for specific applications and where you're applying that solution. Therefore, there are as many solutions as there are people in this room and as many application envelopes in which we're going to be growing that algae...

Rathinam Raja: I am Raja from southern India. I would like to mention just one particular important point. As you mentioned today and we discussed yesterday, there are a lot of dead zones in the world and in India it is estimated that there are two thousand kilometers in the eastern end of the western part of India. We have a lot of dead zones in estuaries and backwaters. Keeping these things in mind, and transferring our attention to low land, I would firmly address the idea that this should be the right time that the lowlands become a place to do a major OMEGA demonstration project. We should have definite concrete ideas in mind for specific locations, but these ideas should be generic enough to be transferrable to other developing countries.

Tomoko Nielsen: I'm originally from Japan, but I now live in Lolland. I have a question. I've been seeing a very successful workshop about algae. I understand that you guys are planning to work together on various project and you will study various aspects of algae cultivation in the future, but how will we be able to follow the progress, to see the updated information? How can we look forward to seeing your results in the future?

Jonathan Trent: There are possibilities for us to continue to communicate how things develop in our group by keeping our Internet site active, but you have to realize that this is the first time many of us have met and it remains to be seen how things develop. In other words, to borrow a phrase from Churchill, what we've accomplished so far "...it is not the end; it's not even the beginning of the end, but it is perhaps, the end of the beginning." Having spent the last couple of days together, you know people you can contact and I should hope we all feel comfortable that we have expanded all our networks. We're also already planning the next workshop so there could be some developments there also.

Aaron Baum: I just want to say that the website is live and looks great. It's really functional and has a lot of capability for people to exchange information and stay in touch. It would be really neat if we could put it up on the board so people could see it.

Travis Liggett: My name is Travis Liggett. I work very closely with Jonathan; I design the existing OMEGA modules. I would like to point out, just very briefly, a trend that we seem to be experiencing-- which is reduction in the half-life of the development cycle for technologies. Just think about how quickly the Internet emerged, and also how much it has changed all of our lives... I invite all of you to consider the possibility that this will be the most difficult thing that we've ever done and that it's going to happen very, very quickly. Just think about technical people having an emotional experience and I'll leave it at that.

Jonathan Trent: There's a website that's been developed by Matt Attwood from Algae Systems called *algaepedia.org.* Would you please tell everyone about this Matt?

Matt Attwood: The idea is to keep the conversation going after this event, in a collaborative kind of open source environment where we can all discuss what we're working on, talk about some of the problems we're trying to solve, deposit it back to the community, and work collaboratively to try and work on what are the best ways we can solve these problems. It's something like a *Facebook* interface for algae. You can have your own membership; you log on, and we'll make accounts for everybody. We'll also have photographs, and we'll send everybody an email that says how to log in. We'll put up all the audio and video for this event up there. You can have your account login. There are forums based on topic areas that Jonathan has defined. There's a news section where everybody can post different ideas, different topics, different challenges, different things like that. It's pretty self-explanatory, and very simple: you create your account, you have your own little picture, there's email and there are ways to communicate inside this website. I hope it's useful to the group and I hope it continues to keep the conversation going.

Jonathan Trent: If there are no other questions or comments, we would like to adjourn this part of the meeting and maybe we'll look forward to seeing you again in Lolland in the not-too-distant future. Thank you Steve; thank you. Uffe. for coming, and thank you all for making what was a fantastic meeting for me. I'm saying my goodbyes and Lene Lange our Danish representative on the Science Advisory committee for the workshop, is going to give the last remarks. Do you want to say something Stig before we adjourn this part of the meeting?

Stig Vestergaard: Once again, thank you for visiting us. I would like to invite you for the next conference in Lolland. In two years, you'll come back to Lolland and see what's happened since you will have been here. I hope you will come back and I hope a lot will have happened. I'll see you again in two years.

CLOSING REMARKS:

Lene Lange: This has been completely inspiring and I would like to mention just a few things in closing. First, when Jonathan asked about the press and there was only one person answering, although we had expected more. I want to say that Jonathan and I, and others have been giving interviews. There will be planned interviews in the next days from the national TV, from the national radio and national newspapers. I'm sorry, Mayor, but some people think that it's too far to come to Lolland,; they are terribly mistaken. So we'll go to Copenhagen and give interviews there, by phone and so on, wherever there is interest and we will of course talk about our meeting in Lolland. Second, to address Uffe's question about the challenges. I would say very briefly that the biology has been described well enough to see that the potential is there, it is there in both the oil and the carbon, as well as in different materials for energy feedstocks and for animal feed. That part is done. What remains to be done is to come up with an economical framework that maps out the a practical way to reach commercially viability. In the map, there must be directions to enough production in a short enough time to satisfy the commercial needs and the system must be sufficiently robust to amortize its own costs and to survive winter storms and anything else. Basically, to make the OMEGA project or something like it possible, it has to make a commercial product or products or services and preferably products and services.

These are details that need to be worked out and they will have to be worked out for each location and circumstance. It's not that one-size fits all. In temperate regions, light is special and in the tropics, it's different and temperatures are an issue. There will be many different conditions that will require many different ways of dealing with them. What we can conclude from the workshop is that we all see algae as part of the solution for the future and not only in Lolland. It will be part of the solution in many parts of the world where they will integrate the system into their environment. Wind will be one source of energy; solar will be a source, other types of biomass will give some energy, and algae will be another important part of the solution. Now [the wagon is rolling.] we are off to a good start.

Finally, I want to stress one more point here--one that is very important--and that is a sense of urgency. We are in a dilemma now as scientists: we don't want to promise too much, and we say, "Oh, it takes time,." I've heard, "Oh, twenty years" and so on. We have to be courageous and say that this is a technology we can deliver now and that there are already algae which are commercial in their production schemes. It's not just far-fetched, but at the same time, we have to do it in a solid way.

If we are too patient, if we have to be responsible, and say it takes a long time, then we will not be part of the solution and the world needs all the solutions it can get-- and this is one of them. We need to share this sense of urgency. We must stand together to work on this and see that it actually moves. I don't want to oversell, the idea but I'm worried about when we say, I heard about a second generation. We'll say, "Oh, it will be decades," and so on, which means that it wasn't taken seriously, which means that it didn't get the investment, which means that this part of the world moved too slowly. US, Brazil, China move faster; we in the EU move too slowly.

As for the lab scale, we have a lot of scientists who work on the lab scale and that's not sufficient. Along with all of the engineering, labs and environmental interaction and robustness are needed. We need to get out there. That's why we also need the test beds, and different types of *experimentaria* out in the real world. That's where we see Lolland as really interesting. We could really do it here and that's how we could really capture the overall result of what this workshop has done. That is to say, OK, here we have very forward thinking leadership, political leadership. We can use that as a good-will invitation, to build on that, and to have more people coming back here and form an International network that gets things done.

Jonathan, I'm impressed that you've been able to invite so many of the leading experts in relevant fields from around the world and with your invitation, they have all come to this small part of the world. That's extremely impressive, but we need to do one more thing that's very important and that is we need to be able to attract young talent. Next time we need to attract the young students with

their supervisors. By having them come and maybe by creating summer schools here through collaboration with universities and by establishing student projects here that is the way we will make things flow. Then there will be more people working and openly sharing their knowledge and building the know-how and the technologies that will be implemented by the next generation.

What we want to do it soon and not wait two years. I think we should have a concept note on the website and in hard copy, ready for Cop15, describing the visions, how we would like to go from here and the type of activities we foresee, all ready to be distributed among the Cop15 members coming to Copenhagen. Since this meeting is going to be in Denmark, it creates one more source of hope that there are ways of overcoming the crisis and making very substantial progress. We should take advantage of the fact that Cop15 is going on here and that we can be ready for it. We have the vision, we have seen where the problems are; we know the need for cross-disciplinary and International collaborations and we need to be sharing this knowledge. But we have a time constraint because we have to act now to be ready for Cop15. It's good to be ready. We could use Bass as the secretariat and keep the momentum going. Otherwise, it will be too slow. With all this kind of planning, we have a very good chance, not just to meet again in two years, but to have accomplished significant things and have made significant progress in that time.

As a final comment, for me, it's been fantastic to be along with all of you here; it's been a great pleasure. Thank you so much.

Peter Lammers: Jonathan, I think you have done a fantastic job as the Master of Ceremonies. We need to acknowledge Jonathan.

Jonathan Trent: Thank you, it was my pleasure. I was very glad to have had this experience with you all. Thank you all very much and I look forward to interacting with you both in cyberspace and in the future, and on some projects in Lolland or wherever.

Thank you all for coming... To be continued...

APPENDIX A - LIST OF PARTICIPANTS

Last Name	First Name	Job Title	Company
Alvi	Jakob Khan	Head of Section	Ministry of Foreign Affairs of Denmark
Atwood	Matthew	Founder	Algae OMEGA
Baertsch	Robert	Researcher	Algae Omega Project
Barlow	John Perry	International Relations	Algae Omega, LLC
Baum	Aaron	Dr.	algaelab.org
Bech	Karin Svane	consultant	Danish Technological Institute
Bech	Brit Kim	Market Consultant	Korea Trade-Investment
Ben-Amotz	Ami	Professor	Seambiotic
Börjesson	Johan	Scientist	Novozymes
Born	Jens	Professor	Flensburg University of Applied Sciences
Breddam	Klaus	head of programme	Risoe DTU
Brennan	Feargal	Head of Group	Cranfield University
Bruhn	Annette	Scientist	National Environmental Research Institute
			Institute for Marine Resources, Alfred Wegerner Instiute for Polar
Buck	Bela	Professor	and Marine Re
CANIARD	Thomas	Scientific Attaché	French Embassy in Denmark
Chisti	Yusuf	Professor	Massey University
Dahl-			
Madsen	Karl Iver	President	Dansk Akvakultur
Darzins	Al	Principal Scientist	National Renewable Energy Laboratory
Davis	Jacob	Finance Director/Interim CFO	Algae Omega, LLC
Edwards	Maeve	Postdoctoral researcher	National University Ireland, Galway
	Waleed		
Elmazny	Nazmy	Director	Vacsera, Innovation Development Unit
Elsvor	Torsten	Journalist	Lolland Falsters Folketidende
Fagerberg	Tony	PhD Student	Lund University
Farag	Ihab	Professor	University of New Hampshire
Frigaard	Niels-Ulrik	Assoc. Prof.	University of Copenhagen

Garcia Reina	Guillermo	Dr	University of Las Palmas
Ghirardi	Maria	Principal Scientist	National Renewable Energy Laboratory
Gormly	Sherwin	Senior Technologist	Education Associates
	Anders		
Grauslund	Handlos	Business Development	Novozymes
Gregersen	Во	Scientific Affairs Specialist	US. Embassy
Guðfinnsson	Hafsteinn	Scientist	Marine Research Institute
Hall	Erik	Regional Environment Officer	US Embassy Copenhagen
Hallgreen	Lars	Investment manager	Forskerparken CAT
Hansen	Gert	Curator, PhD	Scandinavian Culture Collection of Algae & Protozoa
Harpøth	Jytte	Sektorchef	Lolland Kommune
Heijenga	Anthony	Science Officer	GIA Consultancy
Herheim	Jack Olav	Advisor	The Bellona Foundation
hestevik	svein	СТО	One Earth Technology Group AS
Holdt	Susan L.	Scientific Assistent	DTU Aqua
Holm-			
Nielsen	Jens Bo	Ph.D. Centre for Bioenergy	Aalborg University
Hough-			
Maguire	Zoa	Managing Director/Interim CEO	Algae Omega, LLC
Hu	Qiang	Professor	Department of Applied Biological Sciences, Arizona State University
Jacobsen	Jacob	PhD student	University of Copenhagen
Johannesen	Flemming	Project assistant cand.com	Ministry of Foreign Affairs of Denmark
Johansson	Jan	Advisor, Energy and Environment	Baltic Sea Solutions
Junker	Helle	Senior Engineer	Dong Energy
Kaas	Hanne	Business Developer/Senior Manager	DHI
Kim	Stacy	Research Professor	Moss Landing Marine Labs
Klinger	Markus	Scientist	Danisco
Knudsen	Søren	Editor-in-chief	Lolland Falsters Folketidende
Knudsen	Thomas	Kommunaldirektør	Lolland Kommune
Kolber	Zigniew	Research Engineer	Monterey Aquarium Research Research Institute
Krøyer	Jesper	Trade Officer	Korea Trade-Investment
Kudela	Raphael M	Associate Professor	University of California Santa Cruz
Lammers	Peter	Professor	New Mexico State University

Lange	Lene	Professor	Aalborg University
Lapointe	Brian E	Research Professor	Harbor Branch Oceanographic Institute
Larsen	Birgit Hartvig	Direktør	Grønt Center
Lassen	Susanne	Freelance journalist	Bass
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