CHAPTER 3

MICROBIAL PROCESSING OF NATURAL RUBBER WASTE

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ABSTRACT

This paper points on the importance of research for competent microbial isolates for the development of microbial process for the recycling and treatment of waste rubber materials. Microbial flora are found in many latex rubber wastewater effluent representing an untapped resource, which could harbor important functions in bioremediation. In the ASEAN region, a sizeable sink of spent raw materials are extruded in the output of rubber processing. Reports in Malaysia showed that approximately 20 tons of rubber and 410 thousand litres of effluent per day are produced by the rubber factory. This study provides background and suggested methods for studying microbial flora for conversion of rubber waste stream.

SIGNIFICANCE

This research study is geared towards the isolation, as well as the characterization of competent microbial isolates for the development of microbial process for the recycling and treatment of waste rubber materials. The primary sources of isolates are soil at locations in rubber producing plants, latex processing residues or leftovers various tissues from rubber plants or amendments via enrichment procedures of collected polluted local soil, effluent, and environmental samples from various sites with latex

waste residues. Since rubber waste biodegradation involves gaseous by-products, which are difficult to characterize, the Denaturing Gradient Gel Electrophoresis and quantitative RT PCR will be used to detect genes active during the microbial processing. Process treatment with microbial isolated biomass will be conducted in bench-scale application to obtain recycling with the recovery of isoprene and co-products. Bench-scale treatment of wastewater from primary processing of rubber will likewise be considered in order to reduce their environmental impacts.

The use of bacterial strains such as the purple nonsulfur photosynthetic bacteria (PNSB), *Rhodopsuedomonas* is a favorable technology which is ecologically and environmentally promising since better than 90% of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) in treated rubber wastewater effluent could be reduced by these organisms. The treatment of latex centrifugation effluent with free cells of *Bacillus* sp. SBS25 showed 68% reduction in COD while treatment method in an activated sludge reactor using the consortia and the SBS25 isolate resulted in 92.5% reduction (Cheriana and Jayachandrana 2010). Microbial flora are also found in many latex rubber wastewater effluent representing an untapped resource, which could harbor important functions in bioremediation.

A recent paper reported the use of quantitative real-time PCR for assessing water quality in landfills in correlation with BOD/ COD associated microbial gene expression (Han and Kim 2009).

In the ASEAN region, a sizeable sink of spent raw materials are extruded in the output of rubber processing. Latex waste residues accounted to about 12% of the total daily effluent of a Vietnamese natural rubber factory. In local terms, this translates to about 30,000 kg/ day assuming a minimal 10%. Reports in Malaysia showed that approximately 20 tons of rubber and 410 thousand litres of effluent per day are produced by the rubber factory.

THE EMERGENCE OF THE BIOECONOMY

The twenty-first century has been characterized by the emergence of new challenges faced by globalization amidst the need for new socioeconomic and resource scarcity caught by rapid urbanization and population surge, environmental protection and regulation, an expanding global class hungry for automobiles and modern technology, and more volatile finances that face the global market. The term Bioeconomy was the product of these global mosaic of challenges. What Golden & Handfield (2014) had put it, Bioeconomy is global industrial transition of sustainably utilizing renewable aquatic and terrestrial resources in energy, intermediate, and final products for economic, environmental, social, and national security benefits. The White House (2012), declared “bioeconomy is one based on the use of research and innovation in the biological sciences to create economic activity and public benefit.” Quoted in the Organization for Economic Co-operation and Development (OECD 2009): “From a

broad economic perspective, the bioeconomy refers to the set of economic activities relating to the invention, development, production and use of biological products and processes. If it continues on course, the bioeconomy could make major socioeconomic contributions in OECD and non-OECD countries. These benefits are expected to improve health outcomes, boost the productivity of agriculture and industrial processes, and enhance environmental sustainability."

TECHNICAL JUSTIFICATION

The assessment of aquatic ecology has relied most commonly on the macro invertebrates as indicators. However, bacteria and other microorganisms may also be informative of the condition of aquatic ecosystems (Wakelin et al. 2008) and could thus be considered as environmental bioindicators. These are common biota in sediments and biofilms, in the water column but also are also ubiquitously present at high abundance in aquatic systems. However, much research on microbial bioindicators in aquatic systems has been limited to heterotrophic bacteria in relation to the decomposition of dissolved organic matter (Geldreich, E. E. 1976) and as a measure of sewage pollution (Miescier, J. J., and V. J. Cabelli. 1982). Technical understanding of basic processes and their relation with environment is clearly sought after since the microbial interaction is based on the function of genes and this will contribute to our understanding of microbial bioindicators, which has been quite limited as stated.

Molecular characterization including the sequencing of specific bands in the DGGE will then constitute a major effort and has to be included in the screening objective and in objective #5. This is perceived in an apriori premise that the information of genes and their sequences is a basic information and is inclusive in the screening objective. This is inherent in studying the distribution as well as the dynamic structure of microbial communities with the functional niche (as to where in the environment they could function), which will help us achieve environmental efficiency.

Environmental protection is grounded in to basic principles: Principle 4, Rio Declaration on Environment and Development and the International Declaration on Cleaner Protection.

Thus, there is a pressing need to study and implement wastewater treatment in the country. Based on a recent study (Water and Environment Partnership in Asia WEPA 2012) among non-pollution sources, agricultural runoff is the major source at 74% in terms of BOD. According to a recent report (ADB, Urbanization in Asia, 2011) Most Asian cities do not have effective wastewater treatment systems. In the Philippines, for example, only 10% of wastewater is treated while in Indonesia the figure is 14%, in Viet Nam, 4%, and in India, 9%. As published in the WEPA report 2012 the country experienced increasing trends for BOD levels as observed in many priority rivers (525

bodies of water) some of which exceed 300% in BOD levels in 2010 (compared to 2003 levels). Based on the data of a commercial website, surface water is the major source (at 73%) of potable water in the urban population.

Pollution is constantly eroding our water resources and could have adversely affect the health of the population in the long run; such as the prevalence of antibiotic resistance genes (ARGs). Even subtoxic levels of zinc, which is used in the rubber latex processing could cause induced antibiotic resistance. Stoll et al. 2012 reported the wide distribution of ARGs for sulfonamide, trimethoprim, macroline, β-lactams and chloramphenicol in the aquatic ecosystems, which serve as the reservoir of ARGs genes and could potentially be transferred from commensal microorganisms to human pathogens; furthermore the ARGs have been found to be resistant to UV irradiation.

OBJECTIVES

The study aims to develop microbial process for the recycling and treatment of wastes from the primary processing activities of rubber.

Specifically, the project shall have the following objectives:

1. Isolate microbial strains for the degradation of wastes from rubber primary processing
2. Screen microbial strains for waste degradation properties and translucent halo formation in agar-latex media plates
3. Screen microbial strains for extra-cellular protease activity and clearing zone formation in gelatin/ or casein
4. Characterize microbial isolates and assess the potential application in rubber waste recycling and treatment
5. Characterize microbial processing using Denaturing Gradient Gel Electrophoresis and PCR

6. Conduct bench-scale rubber waste processing/ wastewater treatment using microbial isolates

LITERATURE

Technical with Economic Justification

A report assumed that in Vietnam about 80 percent of the rubber plants use open lagoons for wastewater treatment (Resource Assessment Report for Livestock and Agro-Industrial Wastes – Vietnam 2010). In the South East region, treatments are based on conventional biological processes, for example: pond system, oxidation ditch, anaerobic digestion and activated sludge. However, based on current literature (Nguyen & Loung 2012), these processes have not sufficiently met the quality requirement for the effluent of natural rubber processing industry in Vietnam (QCVN 01:2008/BTNMT). Moreover, there has been no implementation of a full combination of the biological, physical and chemical processes. Technical treatment processes in rubber factories in this region are shown in Table 2.

Table 2. The average capacity of influent and the technical processes for wastewater treatment system of some rubber processing factories in South East region, Vietnam

No.	Factory	Company	Average volume capacity of influent (m^3/day) *		Technical process for wastewater treatment of the processing of concentrated latex rubber **
			Concentrated latex wastewater	Other kinds of wastewater (miscellaneous latex, SVR3L, SVR 10)	
1	Loc Hiep	Loc Ninh One Member Co., Ltd	450	550	Decantation - UASB – aeration tank – settling and filter
2	Quan Loi	Binh Long Rubber Company	500	-	Decantation – oxidation ditch – settling and filter
3	Tan Lap	Dong Phu Rubber Joint Stock Company	300	-	Decantation – oxidation ditch – settling and filter
4	Tan Bien	Tan Bien One Member Co., Ltd	300	700	Decantation – oxidation ditch – settling and filter
5	Ven Ven	Tay Ninh Rubber Joint Stock Company	550	950	Decantation – flotation – oxidation ditch – settling and filter
6	Bo La	Phuoc Hoa Rubber Joint Stock Company	400	-	Decantation – flotation – UASB – aeration tank – settling and filter
7	Xuan Lap	Dong Nai Rubber Coporation	700	1,000	Decantation – oxidation ditch – settling and filter

* The average data were calculated during November, 2011
** Source: Conference of the summarization of mechatronics, processing and environment of Vietnam Rubber Group, 2009

The technology of membrane bioreactor (MBR) can overcome the disadvantages of biological methods. According to the study (Nik et al 2010), the process could be continuously operated for more than one month without the chemical cleaning of membranes. The removal efficiency of COD was 96.99% at the initial concentration of 3,500 mg/L COD, of BOD was 96.78%, total-N 65.17% and N-NH3 61.35%.

In Malaysia, aerobic and anaerobic treatments are the most common biological method used for treating rubber wastewater with high efficiency, low in capital costs but required land space. In some rubber factories where land area is limited, aeration systems are used as an alternative of settling ponds. The best novel methods also are shown in Table 4 (Mohammadi et al. 2010).

Table 4. Various rubber wastewater treatment systems and their efficiency

Treatment	Description	Initial COD (mg/L)	Initial BOD (mg/L)	Initial TKN (mg/L)	Initial sulphide (mg/L)	COD removal efficiency (%)	BOD removal efficiency (%)	TKN removal efficiency (%)	Sulphate removal efficiency (%)	SS removal efficiency (%)	Reference
Conventional/Current technologies											
Anaerobic filter.	Packed with aquarium media with dimension of 30 cm *100 cm; OLR = 11.8 $gCODL^{-1}$ day^{-1} and HRT = 10 days.	18219	12750	-	-	92	-	-	-	-	Anotai et al. (2007)
Up-flow anaerobic sludge blanket (UASB).	Steel cylinder shape with dimension of 600 m^3 *250 m^3, consists of a waste water distributor, a lid for scraping sludge, dry rubber content and a gas-solids separator.	6100	-	315	-	80	-	80	90	-	Taechapatarakul (2008)
Biological method incorporated with sulphate reduction system (purple non-sulphur photosynthetic bacteria).	Optimum growth in latex rubber sheet wastewater with 0.50% ammonium sulphate and 1 mg/l nicotinic acid in a pure culture and or a mixed culture.	7328	4967	-	-	90	90	-	92 - 96	-	Kantachote et al. (2005)

Several types of enclosed anaerobic digesters were evaluated; however this resulted in frequent clogging of biomass in the packed bed system. This led to the development of anaerobic sludge blanket reactor (UASB), which can control the fault smells emission from the oxidation and stabilization ponds. However difficulties remain in developing the granular sludge blanket and maintaining its stability. However, this system was used in many industries without the legal procedures that this could be appropriate for the treatment of natural rubber wastewater.

An essential difference between anaerobic and aerobic wastewater treatment systems is that the loading rates of anaerobic reactors generally are not limited by the supply of any single reagent, like oxygen in aerobic systems. During the last three decades several high rate anaerobic reactors configurations have been developed.

A study (Jawjit and Lliengcharernsit 2010) indicated that the application of the two-stage upflow anaerobic sludge blanket UASB to concentrated latex processing wastewater is feasible. Nevertheless, combination with other treatment systems (e.g., oxidation pond, aerated lagoon) is necessary to meet Thailand's industrial effluent standards (in the case of COD). Using the Hydraulic Retention Time (HRT) at 24 h and 48 h (optimal HRT for the acid tank and the UASB tank, respectively) resulted in a reduction of 82% for chemical oxygen demand (COD).

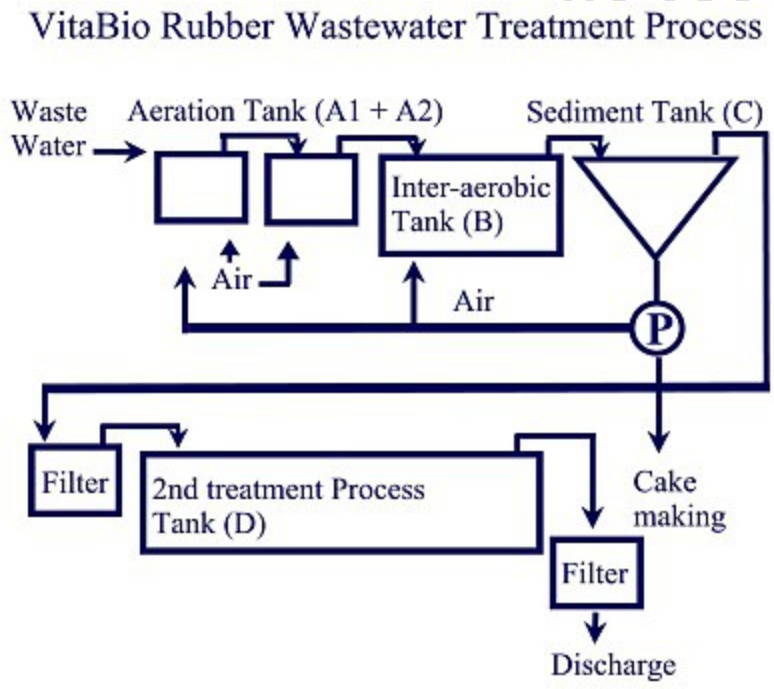
In a recent report (Tanikawa et al. 2012), treated natural-rubber latex wastewater containing a high concentration of sulfate using a combined system consisting of a two stage reactors of up-flow anaerobic sludge blanket (UASB) and a down-flow hanging sponge (DHS) reactor as a post treatment for a period of 10 months and maintained an 11.1 days of the hydraulic retention time however recycling was needed to improve the COD reduction beyond 70.2%. In terms of power consumption, the two stage system

was 93% less than that of the conventional lagoon system, reduced the amount of excess sludge discharged by 90%, and reduced the GHG emissions from the aerated lagoons by 95% by recovering methane.

In another report (Phoolphundh et al. 2013) using a two-stage upflow anaerobic sludge blanket reactor results showed a relatively high-rate treatment for latex-processing wastewater having a hydraulic retention time of about 2.5 days and the removal efficiencies of the system were 51.6 % (COD) and 75.9% (sulfate). Monitoring the microbial diversity with DGGE revealed that there was more sulfate-reducing bacteria in both reactors than *archea* bacteria.

In a recent publication, Nurul Zaizuliana R. A. et al. (2013) used an effective microbial technology in Anaerobic Sequencing Batch Reactor (ASBR) system to treat rubber processing wastewater. Pollutants concentrations were reduced to 60% of COD and 62% of BOD5 reductions.

The VitaBIO Treatment Process, features a phasic Inter-aerobic Tank (B), which could periodically function under (micro)anaerobic and aerobic conditions depending on the amount of oxygen pumped into the system.



The major steps of Rubber Wastewater Treatment Process of VitaBio:

1. Aeration Tank Operation (A1 +A2)
This stage oxidizes and reduces the concentration of chemical preservatives to

become biologically degradable. The pump-aerated wastewater initially is treated in the Tank A1, and the flow-over water is continuously treated in the Tank A2. Some of the formalin materials are oxidized, and some of the formalin materials are absorbed by the sludge in the aeration tank.

2. Inter-Aerobic Operation (B)
This stage consumes the major portions of organic chemicals in the wastewater. In this inter-aerobic tank (or also called intermittence-aeration tank), the amount of oxygen supply is controlled. Because under the low dissolved oxygen condition, when aeration operation is closed, the remained oxygen in the water will be consumed in a short period of time, therefore the tank which was original in aerobic operation can turn into anaerobic operation within short time frame. Through the periodical aerobic and anaerobic operation, the microorganisms "digest" the chemicals and/or release them while the decomposition speed can be greatly improved. Thus this operation procedure can have much better effective performance than single aerobic or single anaerobic operation procedure, and it also shows better efficiency.

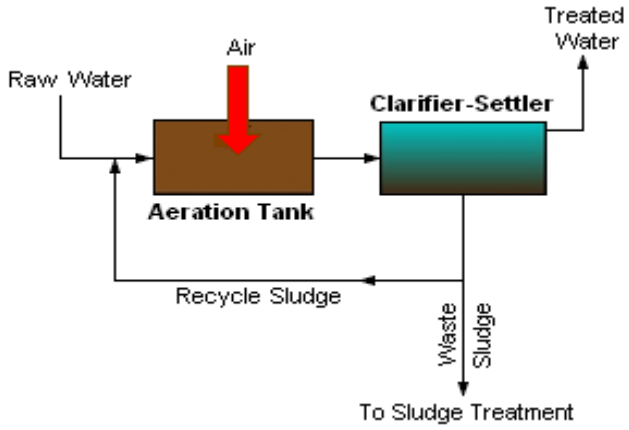
3. Sediment Tank Operation
This stage collects solid materials through sedimentation process and provide activated sludge to inter-aerobic tank and aeration tank. These sludge is recycled back into the inter-aerobic tank for further process and the extra sludge also can pump into the aeration tank for the oxidization process.

4. 2nd Treatment Operation
This stage performs inter-aerobic operation in a longer resident time. The wastewater from sediment tank passes through the screen filter and enters into the 2nd treatment operation. By controlling the amount of oxygen supply, the tank can be in the mode of aerobic and anaerobic operation sequentially. After this treatment, the water passes through a screen filter for discharge.

For high COD content, the aeration in inter-aerobic tank operates 40 to 50 min., and shut off 10 to 20 min. For low COD content, the aeration of inter-aerobic tank operates 10 to 20 min., and shut off 40 to 50 min. Dissolved Oxygen maintenance range is between 0.3 ~ 2.5 mg/l, the preferred range is between 0.3 ~ 0.7 mg/l. The total capacity of activated sludge is about 5% ~ 10% of the size of inter-aerobic tank, in order to maintain the biological cycling period between 24 to 48 hours. The amount of activated sludge in the activated sludge tank is about 30% ~ 40% range.

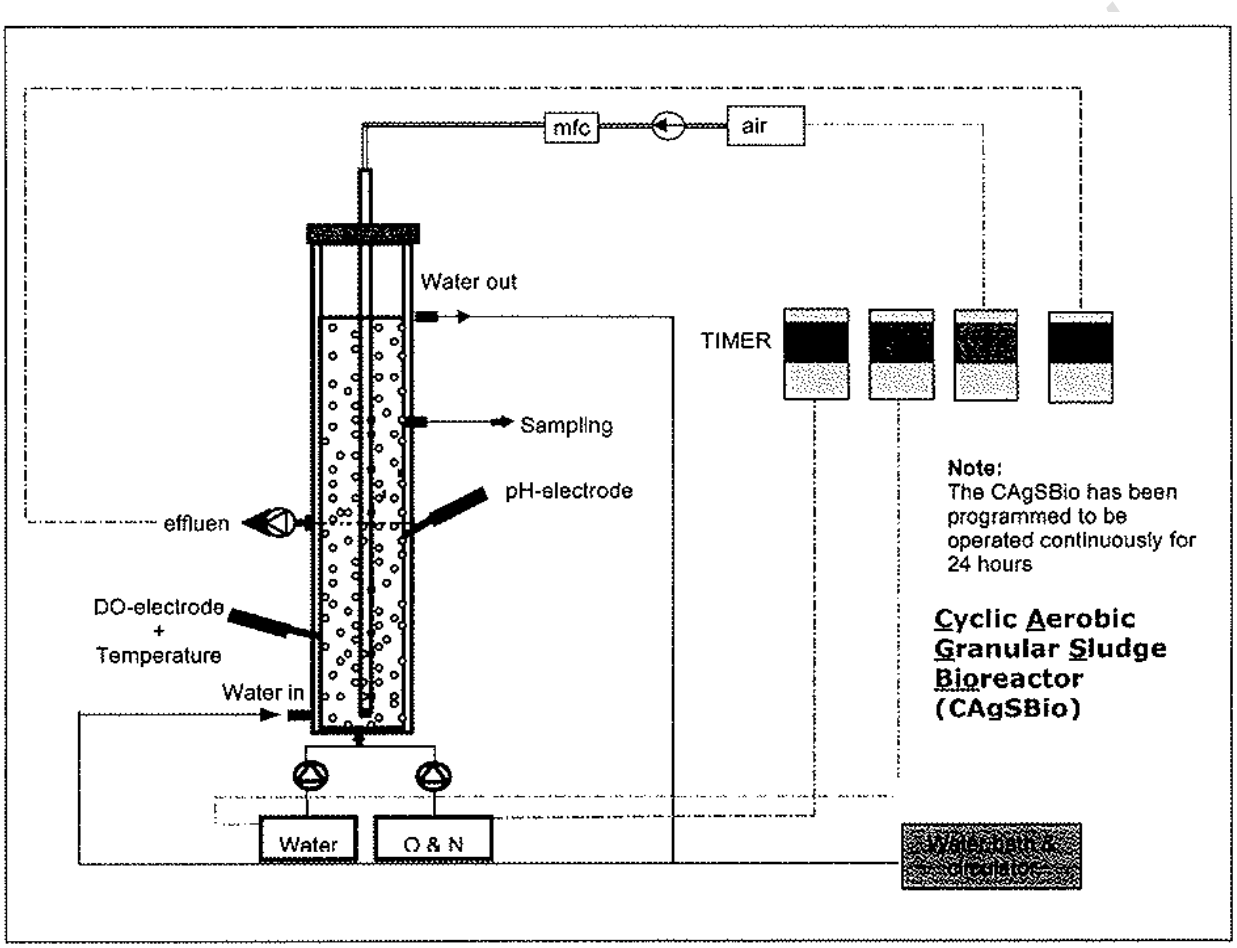
The activated sludge is a process in waste water treatment in which air or oxygen is forced into waste water liquor to develop a biological flock which reduces the organic content of the waste water.

This process is used by the Bridgestone Sumatra Rubber Estate (BSRE) at Medan, Indonesia.



The activated sludge technology requires large footprint (big settling tank) due to the relatively slow settling characteristics of sludge flocs. In contrast, the Aerobic Granular Sludge (AGS) technology offers a possibility to design a compact system based on simultaneous organic and nutrient removal and because of the good settling characteristics of the AGS, the use of a big settling tank is not necessary. Thus, the AGS installation can be cheaper and more compact. This has been studied to improve sludge settling and behaviour in activated sludge systems. The main advantage is that aerobic granular sludge (AGS) can settle very fast in a reactor or clarifier because AGS is compact and has strong structure. It also has good settleability and a high capacity for biomass retention. This has been used by Rosman et al. (2012), for rubber

wastewater treatment using sequential batch reactor with a cycle time of 3 hr using a single column reactor. However the system requires regulated temperature and pH (27±1°C and pH 7.0±1). In this system similar physical properties could be developed in single reactors however, different nutrient elimination performances and microbial communities are affected by temperature.



3L CAgSBio Cyclic Aerobic Granular Sludge Bioreactor on 24-hr continuous operation. (Nor-Anuar 2008 Institute of Environment and Water Resource Management (IPASA), Universiti Teknologi Malaysia).

A new technology, swim-bed combined with aerobic granular sludge (Zhang et al. 2007) was developed for wastewater treatment on the basis of the biofilm process and activated sludge process, and results demonstrated notable performance of high-efficiency treatment capability and sludge reduction. The hydraulic retention time (HRT) was only at 3.2 h. The results showed that COD removal and nitrification efficiency were high at the volumetric loading rates (VLRs) equal and less than 1.0 kg COD/m³/d, corresponding to 0.13 kg N/m³/d. COD removal and nitrification efficiency were above

90% and 73%, respectively using an acryl-fibre biomass carrier (biofringe) for the treatment of latex wastewater (Le, Nguyen et al. 2012).

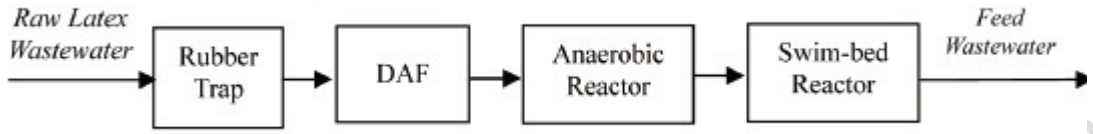


Diagram of pretreatment of feed wastewater effluent with a swim-bed reactor.

Aerobic granulation constitutes a novel technology, which was recently developed (Beun et al. 1999; Tay et al. 2001). This process is a microbial cell self-immobilization forming microbial aggregates that have a strong and compact structure, consisting mainly of aerobic and facultative bacteria and is distinct from anaerobic granular methanogenic sludge. The settling velocity and density of aerobic granules are much higher than those of conventional bioflocs, while the granules have a large surface area and high porosity and have been demonstrated for treatment of a wide variety of wastewaters. Stable aerobic granules could be cultivated in substrate with high levels of ammonium salts that could stably exist for 216 days in continuous-flow reactors with or without submerged membrane (Juang YC; Adav SS et al. 2010). Single strain dominant (*Candida tropicalis*) aerobic granules have been observed in studies of phenol degradation showing that primary microbial strains could enhance degradation of pollutants (Adav SS; Chen MY et al. 2007). Stable aerobic granular sludge could be developed in an SBR (Sequencing Batch Reactor) for palm oil mill effluent. Following granulation, good accumulation of biomass in the reactor and good settling characteristics were observed. The granules showed results of 91.1%, 97.6% elimination rates for COD and ammonia respectively (Abdullah et al. 2011).

Using a strain isolate, *Bacillus* sp. SBS25 with native consortia flora (Cherian, and Jayachandran 2010) in an activated sludge system had reduced the pollutants to 92.5% of COD. The sole strain isolated contributed about 68% reduction. A set up of aerobic biofilm reactor in H_2S removal were assessed by Chaiprapat S et al. 2011. It was found that H_2S removal efficiency increased with increasing air mix ratio and retention time (RT) with the average removals of 94.7% at 160 RT under a 1:4 biogas-to-air ratio with the acidic biofiltration conditions. This technology research could turn to the a development of efficient and low-cost metal absorbents for cadmium, cupper, zinc (Liu et al. 2002) which are often associated with wastewater from natural rubber latex.

In Sri Lanka based on 10-year study (Vithanage 2003), mechanical aerated lagoon system was more cost effective wastewater treatment process for natural rubber

industry where the land is available. However, the initial capital cost of the activated sludge process at Parakaduwa Factory was lower with aeration tank and a sand bed.

According to cost estimates (Lehmonen 2012, Thesis), the use of membranes would be more expensive than oxidation or adsorption treatment, but the estimates are not comparable to each other, because some of the calculations only include the operational costs and some other also include the investment costs and calculations are based on different size wastewater volumes. Thus, based on these estimated costs, it is not possible to say which of these technologies are the most economical. When advanced oxidation processes are viewed, it can be said that the use of H2O2 increases the treatment costs. In addition, cost efficiency of different technologies in addition to the costs treatment efficiency must also be taken into consideration.

The configuration of a working batch reactor for treatment of rubber latex wastewater will be designed based on the use of cultivated isolates as monocultures or in combination with the normal flora:

i) built in an activated sludge setup

ii) built in a granular sludge setup

iii) built with a combination of a biofilm/or biomembrane setup

The wastewater footprint for natural rubber processing is considerably large. For the production of one ton of rubber about 150 cubic meter of wastewater is produced compared to 10-20 cubic meters in the processing of an equivalent amount of fruits. The effluent liquor discharged directly from the natural rubber process has very high concentration of Biological Oxygen Demand (BOD of 5,000 – 6,000 mg/l) and Chemical Oxygen Demand (COD of 9,500 – 12,000 mg/l) for the centrifuge process, which also contains significant amount of ammonia nitrogen.

Based on the studies in the upstream intermediate natural rubber production in the ASEAN region, a sizeable sink of spent raw materials are extruded in the output of rubber processing. Latex waste residues accounted to about 12% of the total daily effluent of a Vietnamese natural rubber factory.

In Malaysia, rubber wastewater contains considerable amounts of skim, latex serum, uncoagulated latex and washings from the various processing stages. Approximately 20 tons of rubber and 410 thousand litres of effluent per day are produced by the rubber factory. However, reports in several studies showed daily discharge of about 80 million litres of untreated rubber effluent to nearby streams and rivers. The high concentration of nitrogen (including ammonia-nitrogen), sulphate, and heavy metals such as zinc, copper, and cadmium, pose a threat to the environment.

Microbial treatment of latex wastewater is currently limited by low viability due to high organic load and high ammonia concentration severely affects the anaerobic process. Odor and gaseous pollution are mitigated using various solid scrubbers but are also a major environmental issue since this could affect water palatability. Gaseous emission from the residual latex could be considered as a renewable source of added raw materials, which could be enhanced or processed by synergistic microorganisms.

Anaerobic digestion is an attractive waste treatment practice in which both pollution control and energy recovery can be achieved. However, the inhibitors commonly present in anaerobic digesters such ammonia, sulfide, light metal ions, heavy metals, and organics tend to be concentrated along the latex wastewater streams. The use of Coconut shell fibre in anaerobic and aerobic processes has encountered difficulties such as clogging, which necessitated a filtration step before treatment. With the development of anaerobic sludge blanket reactor (UASB), high organic loading could be achieved in the digester. The main difficulty with the UASB lies in developing the granular sludge blanket and maintaining its stability.

The use of bacterial strains in rubber wastewater treatment is considered as an ecologically and environmentally favourable technology. *Rhodopseudomonas* DK6 isolated by Kantachote et al. 2005 showed the best potential for effluent treatment since it can grow well under microaerobic-light conditions and a mixed culture. This purple nonsulphur photosynthetic bacteria (PNSB) from rubber sheet wastewater (in Thailand) grows optimum in a mixture of 0.50% ammonium sulphate and 1mg/l nicotinic acid with latex rubber sheet wastewater. A 90% reduction of COD and BOD concentrations have been reported with this PNSB isolate. In other treatment regimes such as in sewage wastewater, selected microbial consortia comprising *Bacillus pumilus, Greviacterium* sp, and *Pseudomonas aeruginosa* could effect percentage degradation of 79% for COD and 85.5% for BOD in 4 h incubation (Dhall P et al. 2012).

The EMMC technology or the entrapped mixed microbial cell technology is a system that entraps the mixed microbial cells into the polymeric carriers; membrane bioreactor (MBR) consisting of membrane sheets/fibers to effectively retain the biomass in the reactor. These two biotechnologies increase the solid retention time (SRT) and are thus able to retain high concentration of biomass in the reactor. A membrane bioreactor consisting of two Kubota flat sheet membranes (pore size 0.4 um), with biomass acclimatization, has been used to treat latex wastewater. The BOD and COD removal efficiencies were 96.78% and 96.99%, respectively. This in technical aspects would suggest that entrapped mixed microbial cell (EMMC) technology could considerably enhance the efficacy of the system using microbial strains. Recent technologies using expanded bed biofilm reactor and sequencing batch biofilm reactor results showed that heavy metal adsorption by these reactors are 50 - 95%.

Several procedures consisting of physical processing, co-digestion with other waste, adaptation of microorganisms to inhibitory substances, and incorporation of methods to remove or counteract toxicants before anaerobic/ aerobic treatment can significantly improve the waste treatment efficiency.

Other components present in natural rubber (NR) latex, such as proteins and phospholipids have been shown to be associated at the rubber particle surface. These phospholipid-protein layers are important in the colloidal stability of the NR latex. The presence of protein degrading activity during the upstream processing of natural rubber will be addressed using samples from various processing steps to be able identify prospective hotspots of microbial biodegradation of latex and thereby improve on the processing recovery.

In the processing of skim latex, trypsin is used to further increase the rubber content (and remove the proteins). The quantity of the enzyme used is however limited to 0.5% since allowable protein content must not exceed 3.1% in the finished product. Sourcing a microbial process for the removal of proteins from latex wastewater (containing latex residues) would present a biotechnological advantage for scavenging latex from wastewater. This procedure could be used in combination with the process of assisted biological coagulation (ABC), in which the microbial growth necessary for the production of acid is accelerated by adding sugar.

THE BIOTECHNOLOGICAL SIGNIFICANCE OF ISOPRENE

Bio-isoprene accounts to as much as 27% of the contents of new tires (Biofuels Digest 4 May 2010), while one liter of petrochemically derived isoprene requires about 7 liters of crude oil. Thus, the raw materials for bio-isoprene from natural products (plant, microbes, or biomass derived) have the potential for reducing (GHG) emissions.

Tire rubber usually consists of 40 to 50 percent rubber (styrene-butadiene rubber, natural rubber, and butyl rubber), 25 to 40 percent carbon black, and 10 to15 percent low-molecular-weight additives. The exact composition depends on the type of tire and the design process of the individual tire manufacturer. ADVAC Elastomers, Inc. reported that it has successfully developed a proprietary product (TIRECYCLETM) which can be blended with virgin rubber and contains up to 87 percent recycled content.

Isoprene (boiling point, 34 ^{0}C) is a gas at low temperatures and bubbles out of the fermentation process in the gas phase thereby ameliorating costly downstream processing. However, bio-isoprene has a better environmental performance than

synthetic rubber, but neither is biodegradable under natural conditions. According to Goodyear, the "BioIsoprene™ product will serve as a renewable and cost-competitive alternative to isoprene since it could provide a hedge against rising crude oil prices".

THE MICROBIAL CHALLENGE

At the turn of the century developing renewable biofuels will be a key factor to meet global demands for energy and synthetic chemistry feedstock without effecting climate change and environmental complications.

Based on a news report of Chemical & Engineering News (Dec 12, 2011), microbial fermentation holds promise for making three renewable rubber intermediates: isoprene, isobutene, and butadiene. The demand for the five- and the four-carbon products will continue to rise in the near future. However these processes have to be cost competitive with the petrochemical pathways. The enzyme isoprene synthase has been identified in plants and through synthetic biology its expression has been optimized in several microorganisms.

Waste microbial degradation involves a number of microbial communities and the by products of rubber, polyisoprene are gaseous in nature and are difficult to characterize.

The basic molecular mechanism by which rubber is degraded is not known. Tsuchi and coworkers were the first researchers to isolate and identify low-molecular-mass oligo(cis-1,4-isoprene) derivatives with aldehyde and keto end groups from rubber-grown cultures of *Xanthomonas* and *Nocardia* species. Several Actinomycetes isolates of the genus *Nocardia* have been shown to degrade trans-polyisoprene. The trans-isomer of polyisoprene [poly (trans 1-4 isoprene)] found in the plant, Gutta-percha, is being used for several technical applications due to its resistance to biological degradation.

According to previous research, natural rubber degrading bacteria mostly belong to the group of Actinomycetes. Recently, certain thermophilic bacteria were also reported to be rubber degrading. Degradation of natural rubber latex by two gram negative bacteria, *Xanthomonas* sps. and *Pseudomonas aeruginosa* were reported in previous works. But there were no reports on gram positive bacteria other than the Actinomycetes. Biodegradation of natural rubber latex is a rare event.

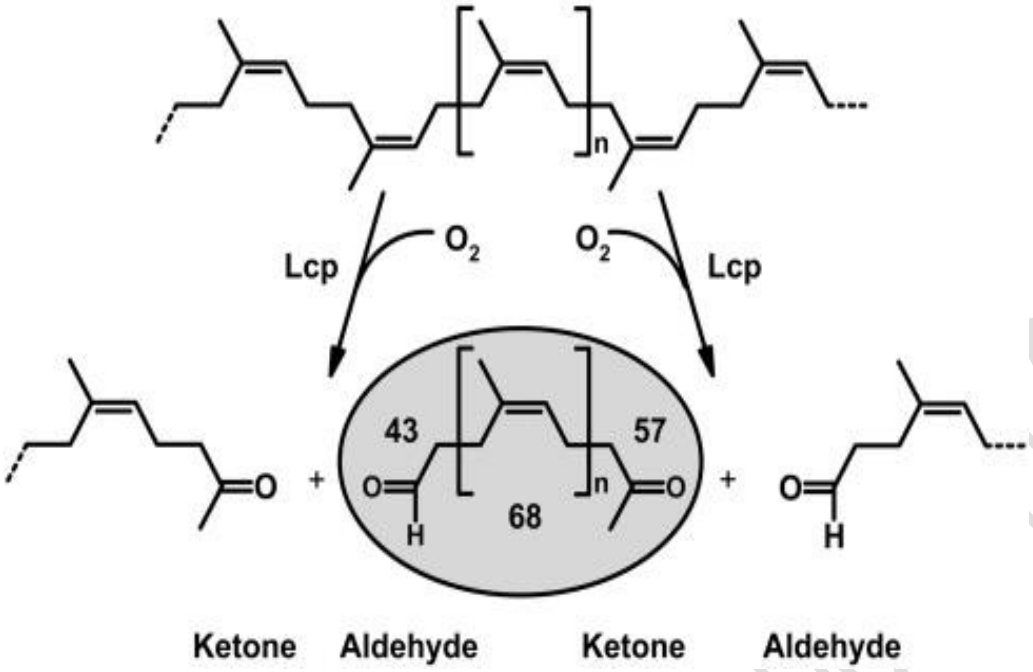
With regard to their decomposition strategies, two different groups of rubber degrading bacteria can be distinguished. While bacteria forming clear zones (translucent halos) on latex containing mineral agar have been repeatedly described, only few representatives of the second, adhesive growing group were so far isolated and

described and were classified into the so-called CMN group (*Corynebacterium, Mycobacterium, Nocardia*). Of these, *Gordonia polyisoprenivorans* Kd2 (DSM 44302T) is the most comprehensively characterized and taxonomically investigated strain. Until now only species belonging to the genera *Gordonia* (formerly known as *Gordona*), *Mycobacterium* and *Nocardia* were identified as non-clear zone forming rubber degrading bacteria that are dependent on direct contact to the substrate. Compared to clear zone forming rubber decomposing actinomycetes, the adhesively growing bacteria represent the more powerful rubber degrading bacteria.

THEORETICAL FRAMEWORK

The scientific basis is the depolymerization of poly(cis 1, 4-isoprene) of the equivalent enzymatic reaction. Tsuchi and co-workers were the first researchers to isolate and identify low-molecular-mass oligo(cis 1, 4-isoprene) derivatives with aldehyde and keto end groups from rubber-grown cultures of *Xanthomonas* and *Nocardia* species. Expression of *lcp* gene in *Streptomyces lividans* TK23 resulted in the accumulation of 12-kDa degradation products containing aldehyde groups, which are metabolized via beta-oxiditive pathway. Xanthomonas polyisoprene enzyme gene has the name Rubber oxygenase A (*roxA*), which revealed two heme binding motifs. In the *Nocardia*, and *Xanthomonas* the dioxygenase endocleavage of the double bond is the initial step.

Ibrahim et al., (2006) proposed a pathway for the cleavage of poly (cis-1,4-isoprene) via the *lcp* gene. Two individual cleaving reactions presumably catalyzed by Lcp result in the formation of a bifunctional isoprenoid species with a keto function and an aldehyde function.



The feasibility of using solid waste and effluent output from the natural rubber factory showing sizeable sources of valuable spent resources for bioconversion is shown in the figure below.

By-product/ Solid Waste/ Effluent (Based on Production Capacity 11,000 tons/yr)
Xuan Lap Natural Rubber Factory, Vietnam.

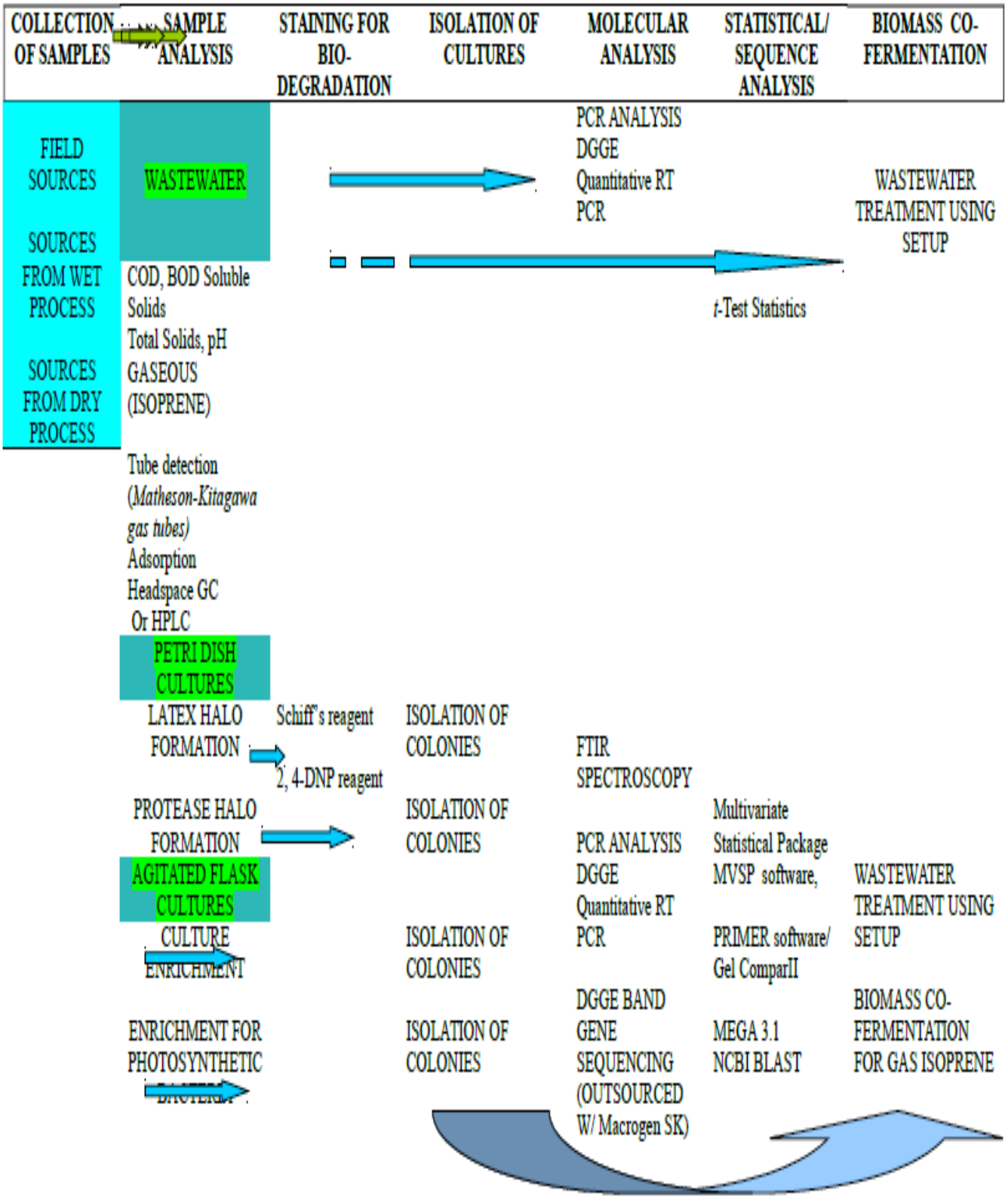
Inputs	**Kg/day**
Field Latex	106,000
Chemicals	476.2
Water	554,000
TOTAL	**660,476.2**

OVERALL CENTRIFUGATION PROCESS

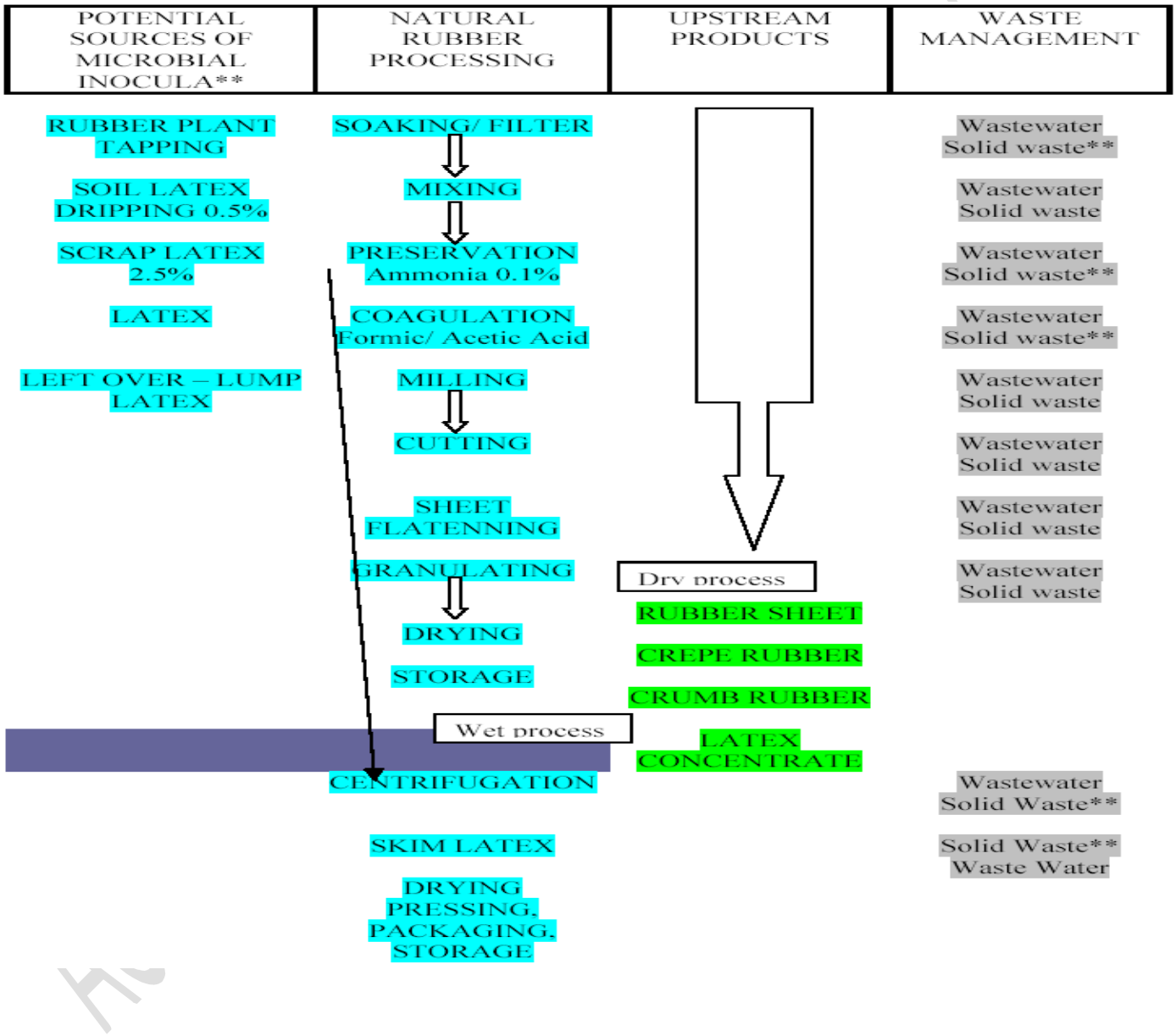
Output	**Kg/day**
Centrifuged latex	35,000
Skim latex	7,740
Gaseous	(Could be considered in the unaccounted 48,000kg/day lost in the output balance)*
Wastewater	570,000 (high Nitrogen, Ammonia)
TOTAL	**612,740**

METHODOLOGY

Experimental Design – (Please see separate next page.)


COLLECTION OF SAMPLES
SAMPLE ANALYSIS
STAINING FOR BIO-DEGRADATION
ISOLATION OF CULTURES
MOLECULAR ANALYSIS
STATISTICAL/ SEQUENCE ANALYSIS
BIOMASS CO-FERMENTATION
FIELD SOURCES
SOURCES FROM WET PROCESS
SOURCES FROM DRY PROCESS
WASTEWATER
PCR ANALYSIS
DGGE
Quantitative RT PCR
WASTEWATER TREATMENT USING SETUP
COD, BOD Soluble Solids
Total Solids, pH
GASEOUS (ISOPRENE)
t-Test Statistics
Tube detection (Matheson-Kitagawa gas tubes)
Adsorption
Headspace GC
Or HPLC
PETRI DISH CULTURES
LATEX HALO FORMATION
Schiff's reagent
2, 4-DNP reagent
ISOLATION OF COLONIES
FTIR SPECTROSCOPY
PROTEASE HALO FORMATION
ISOLATION OF COLONIES
PCR ANALYSIS
DGGE
Quantitative RT PCR
Multivariate Statistical Package
MVSP software,
PRIMER software/ Gel ComparII
AGITATED FLASK CULTURES
CULTURE ENRICHMENT
ISOLATION OF COLONIES
ENRICHMENT FOR PHOTOSYNTHETIC
ISOLATION OF COLONIES
DGGE BAND GENE SEQUENCING (OUTSOURCED W/ Macrogen SK)
MEGA 3.1
NCBI BLAST
WASTEWATER TREATMENT USING SETUP
BIOMASS CO-FERMENTATION FOR GAS ISOPRENE

Sources of Microbial Inocula along the upstream processing of natural rubber are shown in the next flow diagram:



PREPARATION OF MATERIALS

Rubber latex materials. Rubber latex will be prepared from freshly tapped *Hevea brasiliensis*. crude latex contains approximately 35% rubber and 1 to 1.5% proteins. Latex will be purified from soluble proteins by repeated (three times) centrifugation and

washing with 0.002% Tween 80. The top layer (cream) from each centrifugation step is used for the next centrifugation step, while the bottom fractions are discarded. Latex is heat sterilized and stored at 4°C. Analytical grade rubber latex concentrate will also be purchased from local distributors.

Adsorbents for gaseous products isoprene to be used are matrix of Tenax® TA, Activated coconut charcoal sorbent tubes, Supelpak 2SV Matrix (purified Amberlite XAD-2 resins). Some reagents will be obtained commercially packed in tubes, Supelpak 2SV Matrix will packed in stainless steel tubes to be obtained commercially.

Bacteria, media, and culture conditions are to be made in nutrient broth or in a mineral salts medium described by Tsuchii and Takeda with 0.5% glucose or 0.2% purified rubber latex at 30°C. Latex cultures also contained 0.002% Tween 80 and sometimes contained 0.05% yeast extract. Solid media contained 1.5% agar. Latex agar will be prepared by the overlay technique; a bottom layer (~30 ml) of mineral salts agar in a petri dish overlaid with the same agar supplemented with 0.2% purified latex from *H. brasiliensis* (percentage of solid rubber) with or without 0.05% yeast extract, resulting in an opaque overlay. Colonies are screened for translucent clearing zones upon incubation at 30°C within 2 to 4 days, indicating utilization of the latex. Enrichment of photosynthetic bacteria such as *Rhodopsuedomonas* (an example of purple nonsulfur phosynthetic bacteria PNSB) will be done using high lux illumination light source during microbial isolation.

Wastewater pollution indicators tests for COD, BOD, TSS, SS, pH, temperature will be analyzed using standard methods. COD will be measured using the HACH Spectrophotometer; TSS, SS will be analyzed using pre-weighed filters after oven-drying.

Determination of Isoprene Gas from Sample/ Culture Preparation Gas formed in flask-incubated samples from various latex effluent or cultures will be trapped using a temperature-defined (for isoprene adsorption) collecting system in closed temperature vessel with adsorbent tube (packed commercially or laboratory prepared) attached to a vacuum/ aspirator/ pump. A semi quantitative method will use a Matheson-Kitagawa Toxic Gas Tube Detector System with a measuring range of 1 to 16 ppm, which is connected to an air sampling pump. Glass vessels are preferred in our experiments since plastics are permeable to light hydrocarbons and have been shown to absorb them. The gaseous hydrocarbon mixing ratios in the headspace (HS) above the medium are defined as ‘‘control’’. The emissions from samples are defined as positively occurring only when the HS mixing ratio for a given sample is higher than the control HS mixing ratio.

A 200 ml of sample suspension (collected from various latex process points) or sample cultures will be separately transferred from Nalgene incubation bottles into 250 ml Duran glass bottles fitted with a PTFE-septum or a suitable amber bottle filled before analysis. A further identical glass bottle (volume) is left empty as a gas blank and one was filled with the same liquid concentration of blank medium. A head space gas chromatograph/mass spectrometer (HS-GC/MS) instrument will be used for the analysis of VOCs in the headspace of the samples. A volume of 10 mL of headspace sample will be cryogenically concentrated at -70 oC (with a circulation cooler) in a stainless steel microtrap packed with porous silica beads (Unibeads 1S, 80/100 Mesh, Alltech) under a flow-rate of 40 mL min-1. A RTX-VMS capillary column (40 m-long, 0.18 mm ID, 1 mm film thickness) supplied by J & W Scientific (California, USA) or a suitable column will used for the separation of sampled compounds. After sample injection, the column oven will be maintained at 50 oC for 4 min. After the initial isothermal step, the temperature is first increased to 100 oC at 9 oC min-1 and then from 100 to 230 oC (2 min) at a rate of 40 oC min-1. The mass spectrometer detector will be operated in electron impact mode with the following conditions: potential ionization 70 eV; source temperature 230 oC ; and selected ion monitoring (SIM) mode. (The detection limit is in the range of 0.05 to 5 pptv and the uncertainty of 15%.) For each at least 3 replicates will be collected and analysed.

Extra-cellular protease activity is assayed with azocasein (0.1 mg/ml) as substrate in a reaction mixture of 1 ml of Tris-HCl 0.1 M, pH 9, 200 ml of CaCl2 0.2 M and 50 ml of azocasein, and 1 ml of sample supernatant and further incubated at 37 °C for 1 hr. After which, trichloroacetic acid 5% (1.5 ml) was added. The proteolytic activity is defined as the change of one absorbance unit at 440 nm. Isolates are screened for extra-cellular proteolytic activity in culture petri dishes containing gelatin/ or casein.

Detection of aldehyde groups with 2, 4-DNP reagent. 1 mL of 2, 4-DNP reagent will be added to the sample, and the yellow precipitate that developed over 1-2 min at room temperature is noted. Any precipitating yellow colour denoted the presence of aldehyde groups produced during the degradation of the polymers. The composition of the 2, 4-DNP reagent is as follows: 3 g of 2, 4-dinitrophenyl hydrazine dissolved in 15 mL of sulfuric acid plus 70 mL of 95% ethanol plus 20 mL of H2O. Aldehyde group staining of sample preparations will also be compared with the adsorbed gaseous isoprene formation.

DNA extraction from microbial cultures will use the Fast DNA Spin Kit (MPbio.com) and will be performed according to kit protocols. DNA extraction from wastewater samples will be using the PowerWater® Sterivex™ DNA Isolation Kit (Mo-Bio.com) Extracted DNA samples obtained by the two methods will be purified using a PCR purification kit, and DNA concentration is measured using an UV/Vis spectrophotometer, at 260 nm

wavelength. Coefficient at 260 nm (i.e. an A260 of 1 gives the following µg/ml): DNA: 50; RNA: 40; Oligos: 33. The DNA extracted is resuspended with 100 ul of sterile distilled water and stored at -20 C until use.

Primers

The 17-mer canonical forward primer, designated F-968 (Brons and van Elsas 2008) amplified the 14 different bacterial phyla namely, *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Verrucomicrobia*, *Chloroflexi*, *Gemmatimonadetes*, *Chlorobi*, *Bacteroidetes*, *Cyanobacteria*, *Chlamydiae*, *Ferribacter*, *Deinococcus*, and candidate division TM7, the dominant phyla being *Firmicutes* and *Proteobacteria*. The bacterial 16S rRNA specific primer F-968 (5′-AA CGC GAA GAA CCT TAC-3′), to which a 40-mer GC clamp (5′-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA CGG GGG G) is attached at the 5′ end. The reverse primer R1401-1b CGG TGT GTA CAA G**A**C CC**G GGA ACG** is specific at about position 1400.

lcp gene primers are as follows:

forward primer 5'-ATGGAGAATCTCAGCAGGCGA

reverse primer 3'-GGTCAGCCCGGCCTGTTG.

roxA primers are as follows:

(i) P1-R2: 5P-AARTCRTGSCCSCCRTTRTC,

(ii)P1-NR2A:5P-TCRTGSCCSCCRTTRTCRTTRCC,

(iii) P3-F5: 5PTGGGGSCTSCCSAACWSSGCSAACGAYGC,
(iv) P3-NF5: 5P-CCSAACWSSGCSAACGAYGCNGG.

expected PCR product 1.3 kbp

primers for α-methylacyl CoA racemases
5´-GGA TCC AGG GAG GAC GTC CAT GAC AGC AGA TTC GAC AC
3´-TCT AGA TCA GTC GGT CCA GAT GGT G

Primers will likewise be generated and selected from the following Open Reading Frame (ORF) genes specified for the corresponding enzymes involved in the beta-oxidation pathway for rubber latex biodegradation (as described by Hiessl S et al. 2012) using the Open Reading Frame Retrieval java tool.

Acyl-CoA synthetase

GPOL_c26980
GPOL_c49330

Acyl-CoA dehydrogenase
GPOL_c06060
GPOL_c10630
GPOL_c11980
GPOL_c15560
GPOL_c36890
GPOL_c45280
GPOL_c45460

2,4-Dienoyl-CoA reductase
GPOL_c19120

Enoyl-CoA hydratase/isomerase
GPOL_c09320
GPOL_c30630
GPOL_c36880
GPOL_c41700
GPOL_174p01070

3-Hydroxyacyl-CoA dehydrogenase
GPOL_c09390

Thiolase
GPOL_c05990
GPOL_c14950
GPOL_c18410

α-Methylacyl-CoA racemase
GPOL_c36450

Ribosomal Database Collection II Release 9.50 (option Probe Match; http://rdp.cme.msu.edu/probematch/search.jsp) will used to analyze the 16S rRNA gene sequences.

PCR amplification.

PCR mixtures are composed as follows. Seven microliters of 10× PCR buffer 100 nmol $MgCl_2$, 0.5 µl formamide, 0.5 µg T4 gene 32 protein, 10 nmol of each deoxyribonucleoside triphosphate, 10 pmol of each primer, and 3 U of 10 U/µl Ampli*Taq* DNA polymerase, Stoffel fragment (Applera), are combined with st.H_2O to 50 µl in a 0.2-

ml Microfuge tube. After the addition of 5 ng of template DNA, the mixtures are incubated in a PCR system programmed as follows: initial denaturation of double-stranded DNA for 5 min at 94°C; 10 (touchdown) cycles consisting of 1 min at 94°C, 1 min at 60°C, and 2 min at 72°C with a decrease in the annealing temperature of 0.5°C per cycle; 25 cycles consisting of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C; and extension for 30 min at 72°C. All amplification products were purified with the Wizard PCR DNA purification system (Promega, Madison, WI) and analyzed by electrophoresis in 1.0% (wt/vol) agarose gels, followed by ethidium bromide staining (1.2 mg/liter ethidium bromide in 1× Tris-acetate-EDTA).

Nested PCR DGGE

Three-step nested-PCR-DGGE.Two strategies will be used to analyze the bacterial communities. First, the 16S rRNA fragment will amplified using the primer pair F-968(GC)/ R1401-1b. The PCR was performed using a touchdown annealing protocol with decreasing timperature. Second, a three-step nested amplification was performed to obtain different rubber biodegradation primers (*lcp, roxA, mcr*) associated 16S rRNA fragments suitable for DGGE. In the first step, a nearly complete 16S rRNA gene fragment will be amplified using a canonical primer pair F-968 . The product obtained will used as a template for a second amplification with rubber group-specific primers. Finally, to generate products suitable for DGGE, a third round of amplification will be performed with DGGE primers GC clamped using the product of the second round as template.

A total of 300-500 ng of PCR product is loaded into each lane for wastewater community DGGE, while separately, 50 ng of DNA is loaded for pure-culture DGGE. A denaturing gradient of 35–65% denaturants [100% denaturants is a mixture of 3.5 M urea and 32% (v/v) formamide] is used in 6% (w/v) polyacrylamide gels. Electrophoresis was performed in 0.5 × Tris-acetate EDTA buffer at 60 °C and at a constant voltage of 70 V for 16 h using a DCode system (BioRad). The wells are loaded with roughly equal amounts of DNA, and electrophoresis is carried out in 0.5× TAE buffer at 70 V for 16 h at 60°C. The gels were stained for 90 min in 0.5× TAE buffer with SYBR gold (final concentration, 0.5 μg/liter; Invitrogen) or an alternative staining. Gel images will be captured using a Gel Doc (BioRad), and analyzed using quantity one software (BioRad). For this analysis, each DGGE band is assumed as operational taxonomic unit (OTU) or phylotype. Bands will be detected using the band-searching algorithm of the software, which takes care of background subtraction. Gels will be checked visually for ensuring the number of bands. The background is subtracted using a rolling disk set at 20, and band density at positions is converted to intensity per *R*f value between 0 and 1. After normalizing for total intensity across lanes, data were input into the past software package and analyzed using multivariate principal component analysis (M

Statistical Package (MVSP v3.1) software, and PRIMER X software for non-parametric multidimensional scaling (NMDS). Alternatively, the DGGE patterns will be compared by clustering the different lanes by Pearson's product-moment correlation coefficient with GelCompar II software (Applied Maths) by the unweighted-pair group method with arithmetic mean, rolling-disk background subtraction, and no optimization.

Quantification of functional genes

RNA from microbial cultures will be isolated using Qiagen RNeasy Mini kit. Alternatively extracted DNA will be used for qRT PCR. qRT PCR will be used to quantify the abundance of *lpc, roxA, mcr* and selected genes of the beta-oxidation pathway in rubber bioprocessing. All reactions will be conducted on a BioRad real-time PCR instrument in 25-µl reaction mixture volumes. The PCR chemistry was based on QuantiTect Sybr green *Taq* and buffer (Qiagen). The quantification of the *lpc, roxA, mcr* and selected ORFs of the beta-oxidation pathway are based on the given primer sequences provided above. Primers are added to give 0.4 to 0.8 µM in the PCR master mix, and 5 µl of DNA is pipette into each reaction. The themocycle conditions are based on touch-down PCR. In the first 8 cycles, the annealing temperature is decreased from 60°C to 55°C, and then maintained at 55°C for a further 30 cycles. Denaturation is at 94°C for 30 s, primer annealing for 30 s, and extension at 72°C for 45 s.

Outsourcing of sequence analysis, construction of trees, and statistical analyses

To obtain a substantially pure PCR product for DNA sequencing, individual bands from DGGE gels are carefully excised using sterile razor blades, placed in 1.5-ml microcentrifuge tubes containing 40 µl of 1× Tris-HCl buffer, and stored for 48 h at 4°C. Analysis of the sequences will be done with Macrogen, South Korea. Chimera check with Bellerophon was used to check for chimeric sequences (http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi). Bellerophon is a program for detecting chimeric sequences in a multiple-sequence data set by comparative analysis. It is specifically developed to detect 16S rRNA gene chimeras in PCR clone libraries but can be applied to other gene data sets. The partial 16S rRNA gene sequences are compared with sequences in GenBank with nucleotide-nucleotide BLAST (BLAST-N) to obtain the nearest phylogenetic neighbors (www.ncbi.nlm.nih.gov/BLAST/). Sequences showing more than 97% similarity are considered to belong to the same operational taxonomic unit (OTU). Trees are constructed from libraries obtained with each reverse primer by neighbor joining within the program MEGA 3.1 (The Biodesign Institute) and bootstrapped with 500 repetitions. These trees are used to obtain broader groupings—supported by checks with the Ribosomal Database Project (RDP) database—which will served to prepare histograms revealing the compositions of the bacterial communities.

FTIR-ATR spectroscopy Sample aliquot will be subjected to FTIR Spectroscopy. Spectra will be recorded by a Fourier transform infrared (FTIR) spectrometer (Thermo Scientific Instruments) with the attenuated total reflectance (ATR) technique. The angle of incidence is set at 45° by using a ZnSe crystal with 20 active internal reflections. Sixty scans will be co-added with a resolution set at 4 cm^{-1}. Transmittance will be recorded using Thermo Scientific software with baseline analysis. For comparative analysis, spectra are standardized by applying a vector normalization. No further spectral processing will used to ensure band frequency and band shape quality. For spectral control, measurements in the transmission mode had been performed by using ZnSe disks as sample holders.

Bench-scale fermentation will be performed using wastes from primary processing of rubber. Microbial isolates biomass obtained would be co-fermented with the rubber waste using a suitable preliminary flask setup. Test conditions will be designed to process the waste as well as to be able to recover as much the gaseous by product A suitable material interface will be used in the initial set up for entrapment and adsorption of gaseous products and commercially available activated carbon and hybrid products available. A bench-scale bioprocessing setup will also be designed for the treatment of latex waste effluent using microbial biomass. Estimates for floc-granule formation will be based using a test procedure in a beaker reaction to determine the conditions and residual pollution indicators.

General Statistical Analysis Descriptive statistics include the mean and standard deviation or the median and 5th and 95th percentiles, where specified. P-values <0.05 were considered statistically significant. The *t*-Test based on F values will be used to analyze the difference between treated and wastestream COD/ BOD values. A Mann-Whitney-U-test will be used to test differences in peak area per compound between treated and control sample preparation. A non-parametric Spearman's rank correlation coefficient will be assessed between the culture and qPCR measurements to compare quantitative results.

EXPECTED OUTPUT

Microbial strains will be characterized and possible properties for isoprene processing as well as extra-cellular protease activity. A bench-scale microbial bioprocessing setup will be designed for the recycling and treatment of wastes from the primary processing of rubber. Initial studies will include polymer entrapment of volatiles to measure production of isoprene.

TARGET BENEFICIARIES

- Natural rubber latex tappers

- Natural rubber and technically specified rubber users and producers

REFERENCE

Alave, Kristine L. Metro Manila produces a fourth of Philippine garbage Philippine Daily Inquirer. Tuesday, August 16, 2011.

Bode HB, Kerkhoff K, Jendrossek D. 2001 Bacterial degradation of natural and synthetic rubber. Biomacromolecules. 2:295-303.

Braaz R, Fischer P, Jendrossek D. 2004 Novel type of heme-dependent oxygenase catalyzes oxidative cleavage of rubber (poly-cis-1,4-isoprene). Appl Environ Microbiol. 70:7388-95.

Cheriana E, & K. Jayachandrana 2010 Biological treatment of natural rubber latex centrifugation effluent using activated sludge system enriched with Bacillus sp. SBS25. International J of Environmental Studies 67:725-733 DOI: 10.1080/00207233.2010.513588

ctasgis02.psur.utk.edu/.../solid%20waste%20documents/.../... Recycling Marketing Cooperative for Tennessee MATERIAL RECOVERY FACILTY -- Handbook 2003 Dec

Dhall P, Kumar R, Kumar A. 2012 Biodegradation of sewage wastewater using autochthonous bacteria. ScientificWorldJournal. 2012:861903. doi: 10.1100/2012/861903. Epub 2012 Jan 4.

Han JS, Kim CG. 2009 Comparative assessment of gene quantification using real-time PCR and water quality parameters in unsanitary landfill. Water Sci Technol. 2009;59:331-8. doi: 10.2166/wst.2009.855.

Ibrahim EM, Arensk̈otter M, Luftmann H, and Steinbüchel A. Identification of poly(cis-1,4-Isoprene) degradation intermediates during growth of moderately thermophilic actinomycetes on rubber and cloning of a functional lcp homologue from Nocardia farcinica strain E1.. Appl Environ Microbiol. 2006 May;72(5):3375-82.

Imai S, Kazuya Ichikawa, Yuki Muramatsu, Daisuke Kasai, Eiji Masai, and Masao Fukuda 2011 Isolation and characterization of Streptomyces, Actinoplanes, and Methylibium strains that are involved in degradation of natural rubber and synthetic poly(cis-1,4-isoprene) Enzyme and Microbial Technology, Volume 49:526–53.

Jendrossek, D, Tomasi, G, Kroppenstedt, R.M. Bacterial degradation of natural rubber: a privilege of actinomycetes. FEMS Microbiol. Lett. 150, 179–188.

Juan-Peiró, L, Anne Bernhammer, Agustin Pastor, and Miguel de la Guardia. 2012 The Use of Amberlite Adsorbents for Green Chromatography Determination of Volatile Organic Compounds in Air. Journal of Analytical Methods in Chemistry, vol. 2012, Article ID 728143, 6 pages. doi:10.1155/2012/728143

Kantachote D, Torpee S, and Umsakul K 2005. The potential use of anoxygenic phototrophic bacteria for treating latex rubber sheet wastewater. E-J. Biotechnology, 8: 314-323.

Kawahara, S.; Kakubo, T.; Sakdapipanich, J.T.; Isono, Y.; Tanaka, Y. 2000 Characterization of fatty acids linked to natural rubber—role of linked fatty acids on crystallization of the rubber Polymer, 41: 7483-7488.

Linos, A, Steinbüchel, A Microbial degradation of natural and synthetic rubbers by novel bacteria belonging to the genus Gordona. 1998 Kautsch. Gummi Kunstst. 51, 496–499.

Linos, A, Steinbüchel, A, Spröer, C, Kroppenstedt, R.M. Gordonia polyisoprenivorans sp. nov., a rubber degrading actinomycete isolated from automobile tire. 1999 Int. J. Syst. Bacteriol. 49, 1785–1791.

Linos, A, Berekaa, M.M., Reichelt, R, Keller, U, Schmitt, J, Flemming, H.C., Kroppenstedt, R.M., Steinbüchel, A Biodegradation of cis-1,4-polyisoprene rubbers by distinct actinomycetes: Microbial strategies and detailed surface analysis. 2000 Appl. Environ. Microbiol. 66, 1639–1645.

Makiko Enoki, Yoshiharu Doi, Tadahisa Iwata 2003 Oxidative Degradation of trans-1,4-Polyisoprene Cast Films and Single Crystals by Enzyme-Mediator Systems Macromolecular Bioscience 3: 668–674.

Meriam N, Nik Sulaiman, Shaliza Ibrahim, Sarah Lim Abdullah 2010 Membrane Bioreactor for the treatment of natural rubber wastewater. International Journal of Environmental Engineering (IJEE) 2(1/2/3).

Mohammadi M, Hasfalina Che Man, Mohd Ali Hassan and Phang Lai Yee 2010 Treatment of wastewater from rubber industry in Malaysia. African Journal of Biotechnology 9:6233-6243.

Rose K, and Steinbüchel A. Biodegradation of natural rubber and related compounds: recent insights into a hardly understood catabolic capability of microorganisms. Appl Environ Microbiol. 2005 Jun;71(6):2803-12.

Sansatsadeekul J, Sakdapipanich J, Rojruthai P. 2011 Characterization of associated proteins and phospholipids in natural rubber latex. J Biosci Bioeng 111:628-34.

Schloman Jr, W.W.1; McIntyre, D. 2001 Reduction of resin in polyisoprene latices by lipase-catalyzed hydrolysis of triglycerides Industrial Crops and Products 13: 131 – 134.

Steinbüchel A. Production of rubber-like polymers by microorganisms. Curr Opin Microbiol. 2003 Jun;6(3):261-70.

Tsuchii, A, Suzuki, T, Takeda, K Microbial degradation of natural rubber vulcanisates. 1985 Appl. Environ. Microbiol. 50, 965–970.

www2.adb.org/Documents/Reports/Consultant/.../full-version.pdf. Metro Manila Solid Waste Management Project Final Report ...

www.biofuelsdigest.com/.../what-is-bioisoprene-and-why-should-you...What is Bioisoprene and why should you master it? 4 May 2010..

MAKING RUBBER FROM RENEWABLES - Chemical & Engineering ... pubs.acs.org/doi/abs/10.1021/cen-v089n050.p018 12 Dec 2011 – Chemical & Engineering News Archive

www.polymersolutions.com/.../more-companies-making-tire-rubber-...More Companies Making Rubber from sugars 19 Dec 2011

White House, National Bioeconomy Blueprint, (2012) (from http://www.whitehouse.gov/sites/default/files/microsites/ostp/national_bioeconomy_blueprint_april_2012.pdf)

OECD, 2009, The Bioeconomy to 2030, Designing a Policy Agenda, www.oecd.org/publishing/corrigenda

VitaBIO Treatment Process (<http://www.vitabio.com/ves/rwad_rb1.htm>)