CHAPTER: 5

SOURCE OF RENEWABLE ENERGY: AQUATIC PHOTOAUTOTROPHS

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Abstract

The present chapter is focusing on the topic of energy crisis of world because of decreasing the storage fossils fuel and mounting of worldwide climate change. The photoautotrophs are the good substitute of this problem. To make the environment pollution free and production of renewable energy source microalgae are emerging as good sources of biofuels. Microalgae are unicellular or multicellular filamentous, microscopic photoautotrophic organisms. The various macro algae are also a good source of raw biomass material for biofuels production. Algae harvest the solar energy with the help of light harvesting molecules and convert this energy in the form of chemical energy by oxidizing the water molecules and reducing the atmospheric CO_2 in the form of organic molecules. The biomass obtained from microalgae is utilized as the source of raw material for generation of various types of biofuels. The various strains of algae have good growth rate and high contents of biomolecules such as lipids, carbohydrates and other many more value-added foodstuffs. The biodiesel, bio-methane, bioethanol and biohydrogen are the bio-fuels that may be obtained beyond the years. Aquatic plants have been considered as a good renewable energy source. Production of bio-fuel from aquatic flora and fauna is budding energy source now a day. In spite of this, renewable energy sources biofuels to be very gainful and environmentally eco-friendly. It derived from biomass of organisms (either plant or animals). Today bio-fuel is measured to be a good source of renewable energy contrast fuel obtained from fossil for example petroleum, natural gas and coal. So here, this present chapter disclosing some major floating aquatic flora that plays a significant role in the biofuel generation and also in brief describing the harvesting, industrial processing to extract

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biofuel of many categories. The utilization of microalgae in the production of biofuel is the major topic of this chapter.

Keywords: Aquatic Flora; Biofuel; Renewable energy source, Microalgae, Bio-Methane, Bio-Hydrogen, Bio-Diesel

Introduction

Fossil fuel consumption is on the rise, and burning those fuels is contributing significantly to climate change. Countries all around the world have started to put energy policies into practice that incorporate the creation of renewable energy sources. Bio-fuels are preferred among the available renewable energy sources (such as biomass, solar, wind, geothermal, and tidal energy) because they are the only energy source that can be used directly to make fuels for transportation. The bio-fuels are the alternative way of petroleum and fossil fuels of energy source with cost effective and environment eco-friendly [4]**.** Microalgae are accounted to carry out about 40% of the sum worldwide photosynthesis (Falkowsky 1980). Microalgae in particular are a viable feedstock for the manufacture of biofuels [12]. Microalgae and larger plants both carry out photosynthesis to make lipids, carbohydrates, and proteins, among other vital physiological functions [50]. Unicellular photosynthetic microorganisms known as microalgae are employed as sources of dietary supplements, pigments, and bio-fuels and many more value-added products because they are more widely available and produce more oil than other terrestrial plants. Since the 1970s, research on microalgae as a feedstock for biodiesel production has been conducted [25, 63]. Microalgae can be produced on non-arable areas and in non-potable water and grow more quickly than plants do. Furthermore, since microalgae can extract nutrients from wastewater, they are helpful for treating organic industrial effluent [29]. Microalgae can be thought of as a "golden key" with the ability to solve numerous environmental issues because of these benefits and their high carbon dioxide absorption capacity. Indeed, microalgae will emerge as the "supernova" of biofuels, according to Jonathan Trent of NASA's nanotechnology division [28]. In theory, microalgae can be used to create a variety of biofuels, including biodiesel from algal oil, bio-ethanol produced in the dark through anaerobic fermentation, bio-hydrogen produced through photobiology, and bio-methane (also known as biogas), which is created through the anaerobic digestion of algal biomass [3, 8]. Despite the enormous potential of microalgae for the production of proper amounts of bio-fuel, problems like bottlenecks in microalgal growing make them economically unviable and unsuitable for industrial use [63]. Such renewable energy sources can only be produced in a small amount on a commercial scale [19]. Energy is the chief deliberation for the 2030 agenda for sustainable development. Biofuel is a consistent, sustainable, and reasonably priced energy source. Some of the major environmental problems include the rising amount of carbon dioxide in the atmosphere—36.2 billion tonnes—the rising demand for fossil fuels and the diminishing supply of those fuels. Fossil fuels are currently quite affordable; however this situation may change in the next few years, making it vital to find alternative renewable fossil fuel sources that might be carbon neutral. Currently, only 10% of gasoline is produced using renewable resources, with the majority (80–90%) coming from non-renewable sources [39]. In this chapter microalgal biomass has been viewed as a prospective feedstock because of its noticeable growth rate and high concentrations of lipids, carbohydrates, and other value-added compounds. Therefore, it may be utilized for the creation of a variety of renewable, clean, and green fuels, including the generation of bio-methane through anaerobic digestion, biodiesel through transesterification, bio-ethanol through fermentation, and bio-hydrogen created through photobiology [54]. They are sustainable and clean sources and are easily convertible to renewable energy.

Aquatic photoautotrophs used to produce biofuels

Duckweed Fern *(Azolla* sp.)

Azolla is a macrophyte with a high growth rate. Occurs in aquatic habitat (wetland and lakes). Azolla belong to family Salviniaceae. Their faster growth rate is supported by an endophytic nitrogen fixing blue green algae (cyanobacteria). For this reason *Azolla* is used as a biofertilizer for sustainable agriculture [11]. *Azolla* is used in the production of biofuel because of their main constituents of lipids, ash, cyanobacteria components and polysaccharides contents (starch, cellulose, and hemicellulose) [6]. This composition of azolla species makes it suitable raw material for biofuel production. Some species of *Azolla* are listed here *Azolla rubra, Azolla nilotica, Azolla pinnata, Azolla circinate, Azolla Mexicana, Azolla microphylla, Azolla caroliniana, Azolla japonica, Azolla filiculoides.*

Azolla are used in the production of fatty acid methyl esters which can be directly used in diesel engines without further upgrading just after the transesterification process. The process of transesterification is carried out at a specific temperature range which lies between 47 and 60 °C [48]. Yeast isolation techniques are also used in producing ethanol from *Azolla*. In this process H_2SO_4 is used for the hydrolysis of *Azolla* biomass. The hydrolysate is used for ethanol making by the process using fermentation technique. Pyrolysis is a very useful way to produce bio-oil from *Azolla* because by using this process oil can be recovered with a very low emission of sulfur dioxide (SO_2) and nitric oxide (NO_2). Slow pyrolysis is the successful way of producing bio-oil from *Azolla* biomass [10].

Water Hyacinth (*Eichhornia crassipes*)

Water hyacinth (*Eichhornia crassipes*) most problematic weeds a hydrophyte, free-floating, persistent aquatic plant. Their native place is South America. It is a more deleterious weed due to its speedy growth and multiply rate in pond, irrigation and water surfaces [21]. The contents of moisture, unstable matter, fixed carbon, and ash in water hyacinth are 9.95, 56.30, 17.40, and 16.35 (wt %), respectively. The percentage of cellulose and hemicellulose that are higher than the amount of lignin strongly favors the production of biodiesel [30]. Enzymatic hydrolysis methods are used for producing biofuel from water hyacinth. Transesterification is a less common method for producing ethanol because of its high moisture content. So appropriate drying is necessary [1].

Water fern (*Salvinia molesta*)

Salvinia molesta (known as water fern) is the best ever growing hydrophyte. Compared to water hyacinth (doubling time of 7–12 days), the growth rate of water fern (doubles within 3–10 days) is double [2]. It is a damaging aquatic plant covering the entire aquatic body within short time periods. Their higher rate of multiplication and content of lipid used as raw material for the extraction of biodiesel. The transesterification method is used for the extraction of fuel production. The content of unsaturated fatty acids is found in good quantity analyzed by the gas chromatography mass spectroscopy (GCMS). According to fatty acid methyl ester (FAME) analysis, the percentage weight of saturated and polyunsaturated monounsaturated fatty acids are 28.08%, 15.59%, and 56.32% correspondingly. In spite of transesterification anaerobic digestion (fermentation) for production of bio-ethanol, hydrothermal liquefaction (HTL) and pyrolysis methods are also used. Paddy straw is co-digested with water fern for the production of biogas [56]. The yields of biofuels production are less in *salvinia* compared to both *Eichhornia sp.* and water lettuce. The give in rate of ethanol from 12.21 L of fermented solution of *Salvinia* is 62 mL and 2 gL^{-1} were reported by Mubarak et al. and Kaur et al. respectively [33,46].

Water lettuce (*Pistia stratiotes*)

This is an important aquatic genus occurring in the tropical and subtropical surroundings area. It belongs to the family Araceae [35]. By reducing the level of oxygen concentration as well as flow of water, hindering the light penetration to reach up to the submerged organisms creating dangerous effects on water bodies, plants and animals [14]. In spite of these above dangerous effects it has several beneficial roles for the water body because of having phytoremediation properties. It is also used for biofuel (biogas, hydrogen, ethanol, FAME) and production of medicine [5, 22, 24, 36, 53, 57].

Its productivity rate is 60–110 t ha^{-1} $year^{-1}$. Their biomass is used in the bioenergy sector due to following properties found in it- 1. High productivity rate is 60–110 tha^{-1} $year^{-1}$. 2. It has a soft body, comparatively small size to water hyacinth and others discussed above. 3. It is feasible to harvest with low amounts of labor charge. First-rate thermal qualities (moisture 5%; ash 22.7%; volatile substances 48%; and carbon content 24.3%. The sugar filling is usually in the range of 19.2–27 (gLm^{-2}). Biomethanation can be easily performed due to having a good percentage of unstable matter. The high percentage of carbon content makes it suitable for digestion in the absence of oxygen and enzymatic biodegradation. High ash content helps in slagging and fouling [9]. It is noted that due to good concentration of sugar content the production potential of bioethanol is more compared to water hyacinth [22, 24, 43].

Duckweed

Duckweed (smallest aquatic flowering plant) showing more or less distribution all over the world. It is a good source of the raw material for biofuel production. It is used in sewage treatment plants because of their good capacity to pick up mineral nutrients from contaminated water bodies [7]. The five genera (*Lemna, Landoltia, Spirodela, Wolffia*, and *Wolfiella*) with 40 species of Duckweed are placed in the family Lemnaceae. Duckweed under appropriate conditions may double within two days [61]. The composition of carbohydrate (reducing sugar) -16.5%, protein- 34.5%, lignin -7.5%, and ash -14.7% in its dry weight make it good raw material for bio-energy production. The fermentation and digestion of duckweed biomass under anaerobic condition are two appropriate methods. Three species of duckweed (*Landoltia punctata, Spirodela, and Lemna*) are going to be used for bio-energy production. Out of three *Landoltia punctata* has been used more frequently because it shows utmost potentiality. In nutrient poor environment carbohydrate content increase from 18% to 29.8% after eight day of cultivation which is nearer to double than the initial amounts reported by Xu et al.(2011) it means to get good quality of carbon content (carbohydrate) post-harvest procedure are imply. The temperature 35 °C, and a preliminary pH of 5.5 are good for biohydrogen making which was found to be 75.3 mLg^{-1} in acid-treated duckweed [62]. Hydrogen generated in the fermentation processes is collected before the dilution of hydrogen with other gasses present there. Pectinase enzymes are used to improve the quality of bio-ethanol production which enhance the monosaccharide (glucose) production about 142% more compared to untreated duckweed. The fermentation efficiency also increases up to 90.04%. It was possible to make a 142% increment in glucose yielding compared with untreated duckweed, and fermentation efficiency was 90.04%. Little literature is available for the production methods of good quality biofuel from duckweed. Making butanol from duckweed biomass was carried out experimentally. It is an appropriate bio-fuel for internal combustion engines (ICE) reported because of their high energy density of lower vapor pressure. Up to $12.33 gL^{-1}$, $6.17 gL^{-1}$, $0.83 gL^{-1}$ good quality biofuel is obtained are butanol, acetone and ethanol respectively [55].

Microalgae

Microalgae, commonly known as blue green algae due to their unique blue pigment phycocyanin, are extremely diversified, solitary or multicellular, microscopic photosynthetic organisms made up of eukaryotic photoautotrophs and prokaryotic cyanobacteria. They can be found in both freshwater and marine environments, are a vital link in the food chain of the aquatic environment, develop quickly, and operate as a CO_2 sequestering agent in a variety of conditions. A microalga consumes roughly 1.7–2 kg CO_2 and yields 1 kilogram of biomass [13]. It supplies vital nutrients for aquatic species, such as proteins, carbohydrates, and lipids like omega-3 fatty acids. Waste water can be used to grow microalgae since they can make use of the nutrients present there and aid to lower the pollution load. The ideal temperature range for microalgae development is typically 20 to 30 ˚C. Although they require a lot of water to grow—up to 11–13 million L/ha/year—they do so. Depending on the species, microalgae can range in size from micrometers (m) to millimeters (mm).

The complex collection of microorganisms known as cyanobacteria can be found in a wide range of ecological setting, from aquatic to terrestrial and ultra-oligotrophic to hypertrophic. Additionally, they can be found in harsh environments like hot springs and hyper saline lakes. They are common because of their capacity for photosynthesis and nitrogen fixation, as well as their wider environmental adaptability. With over 2000 species and 150 genera, cyanobacteria have a very diverse range of cell structures, from simple colonial to sophisticated filamentous forms with or without branching. These cyanobacteria are important organisms because they increase the amount of carbon in many ecological niches and fix nitrogen, which supports the economics of carbon and nitrogen [43]. On the basis of footstock used biofuels resulting from microalgae are called third-generation biofuels. It is also known as advanced biofuels [31]. The prime sources include reasonable resources that don't influence the food chain and are possible, readily obtainable and flexible to environmental parameters [58].

Algae biorefinery and generation of bio-fuels with biomass of microalgae

Currently, only 10% of gasoline is produced using renewable resources, with the majority (80–90%) coming from non-renewable sources. The amount of biofuel produced globally in 2015 was 130.7 billion liters, with ethanol accounting for 75.2% of that, biodiesel for 23%, and hydrotreated vegetable oil for 3.7% (HVO). Algal biomass is used to produce the bio-products from the algae biorefinery. Algae are autotrophic microbes that can produce their own food through photosynthesis in the presence of water, carbon dioxide, and sunlight [32]. To minimize carbon dioxide emissions from power plants, oil extraction and biodiesel manufacturing sector, algae or cyanobacteria biomass are utilized (may be grown in a photo-bioreactor). In some algae species oil content can be up to 80% of their dry weight. Due to its noticeable growth rate and high concentrations of lipids, carbohydrates, and other value-added products, microalgal biomass has been regarded as a viable feedstock. As a result, it can be used to produce a variety of renewable, clean, and green fuels, including bio-methane produced through anaerobic digestion, biodiesel produced through trans-esterification, bio-ethanol produced through fermentation, and bio-hydrogen produced through photobiology [17]. See figure A.

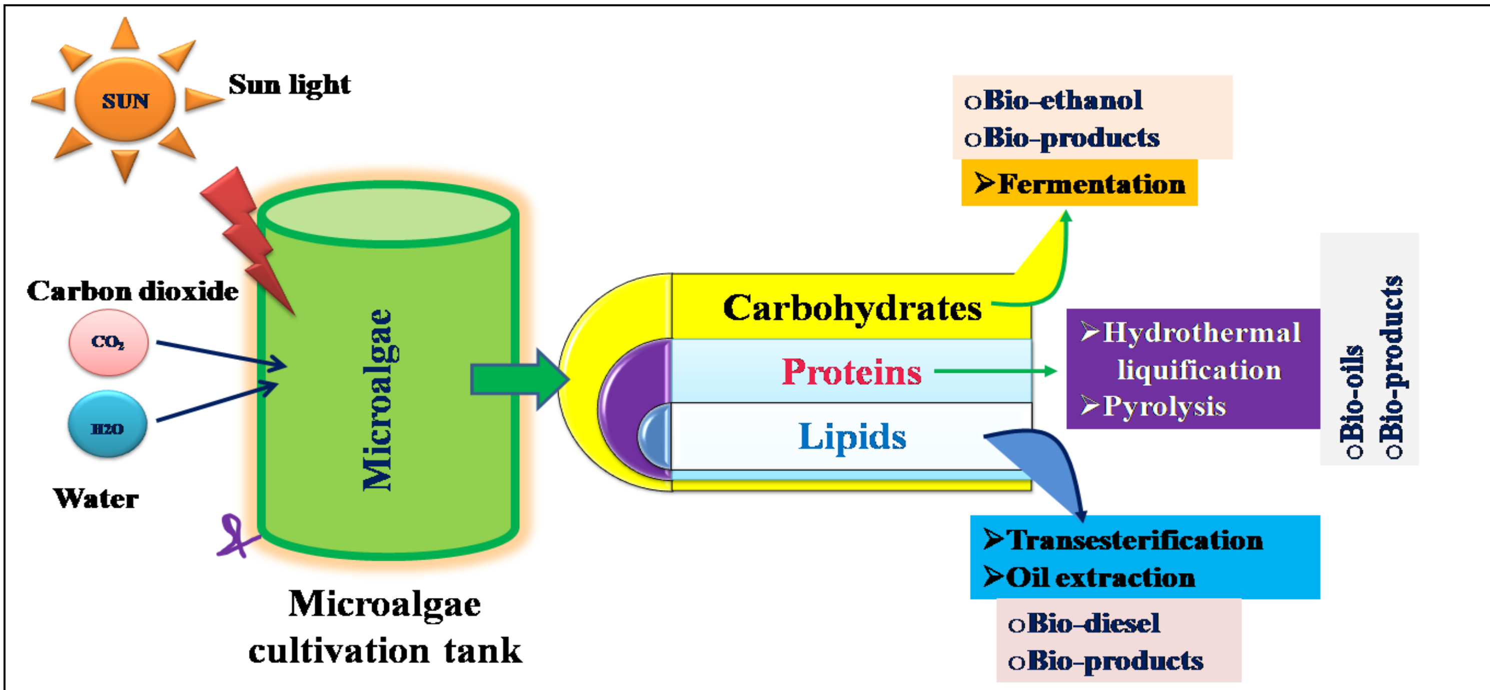

Figure A. Schematic presentation of bio-fuel production from microalgae

Some bio-fuel obtained from microalgae biomass

Bio-ethanol

The majority of bio-fuels are now made through bioconversion from the starch of edible grains, agricultural feedstock, or sugar crops. Cereals and legumes, which are genuinely produced for human consumption, are used as the first-generation feedstock for the manufacturing of bio-ethanol. The substitution of these food crops for the production of bio-fuel increased food costs and depleted agricultural land. The utilization of second-generation feed stocks, such as lignocellulosic materials like agro- or forest wastes and woody biomass, however, partially resolves these issues. Compared to first-generation feedstocks, these feedstocks have certain advantages because they require little land and don't compete with food [45]. As a third-generation biofuel feedstock, microalgae offer an option to first- and second-generation feedstocks because of their high productivity, noticeable growth rate, and ease of year-round culture [15]. Gasoline and ethanol are complementary bio-fuels because they have comparable physical and chemical characteristics. Bio-ethanol has a 21.2 MJ/dm^3 energy content, compared to 31.3 MJ/dm^3 for gasoline. A unit of carbohydrates contains 467 kJ/mol of energy. The energy density of PAR, which has a wavelength range between 400 and 700 nm, is 217 kJ/mol. Eight photons of PAR are needed to create one unit of carbohydrate, hence the efficiency of energy storage in the form of glucose is 11.6% [36].

However, in most cases, microbial biomass undergoes a moderate pretreatment called hydrolysis to liberate the carbohydrate (glucose), which is a preferred carbon source for fermentation in *Saccharomyces cerevisiae* or *Zymomonas mobilis*. Distinct hydrolysis fermentation, in which reactions take place in two separate reactors, and simultaneous saccharification fermentation, in which hydrolysis and fermentation take place simultaneously, are two ways to carry out the fermentation process [26]. Several green microalgae such as *Chlorella*, *Scenedesmus*, and *Chlamydomonas* algae are effective for producing bio-ethanol. 11.66 g/L of ethanol was generated using the fermentation process with 1% diluted acid hydrolysis, yielding an 87.6% theoretical yield in 12 hours, according to Ho et al. (2013) [27,47].

Biodiesel

Microalgae are a well-known option to utilize solar energy efficiently and have a significant potential for producing biomass with no need for additional land. They seem to be the only biodiesel source with the capability of totally replacing fossil diesel. Algae must have a lipid concentration of 20–80% by weight of dry biomass in order to produce biofuel, while oil levels of 20–50% are relatively typical [39]. The most essential process in the commercial synthesis of biodiesel is transesterification, in which triglycerides react with methanol in the presence of a catalyst to create methyl esters of fatty acids and glycerol. At atmospheric pressure and temperatures of around 60 ˚C (140 ˚F) are suitable for this procedure. Triglycerides are first transformed into diglycerides, then into monoglycerides, and eventually into glycerol during the transesterification process. Acids, alkalis, and lipase enzymes all play a role in the transesterification process' catalysis, with the alkali-catalyzed reaction moving forward around 4000 times more quickly than the acid-catalyzed reaction. Normally, the output of biodiesel from transesterification of algal oil is around 80% of the volume of the utilized algal oil [16].

Bio-hydrogen

A possible source of clean, renewable energy is H_2. Since water is the primary byproduct of combustion, hydrogen is viewed as a clean, non-polluting fuel. In addition to being used as fuel, hydrogen (H_2) is also utilized to produce ammonia, clean oil refineries of contaminants, make methanol, and in power rockets. Direct biophotolysis, which includes breaking water molecules into (proton), (electron), and (oxygen) in eukaryotic microalgae and in the presence of sunshine, and then recombining them using hydrogenase enzymes to form H_2, is one method for producing hydrogen. The high photon conversion efficiency of microalgae is >80% in *Scenedesmus* sp., *Platymonas* sp., and *Chlorella fusca* are among the microalgae that produce hydrogen [49]. *Scenedesmus* sp., *Chlorococcum* sp, *Platymonas* sp, and *Chlorella* sp. are among the microalgae that produce hydrogen. The hydrogen yield from *Chlamydomonas reinhardtii*, which has received greater attention for bio-hydrogen generation, was 5.94 mmol/g Chl/h [48]. Another study found that cyanobacteria cells deprived of nitrogen produced the most hydrogen (30 mL H_2/lit/h) (Tiwari and Pandey 2012) [59].

By means of indirect biophotolysis, the BGA produces H_2, during this procedure, proteins in the light-harvesting complex (LHC) guide photons to the photosystem II, which uses the radiant energy from photosynthesis to split water molecules. After oxidation, the Fe-S protein in ferredoxin on the reducing side of photosystem I receives the electrons that were extracted from water molecules photo-oxidation event. In order to create one H_2 molecule, two protons and the hydrogenase enzyme, which is located in the stroma of the microalgal chloroplast, need electrons from reduced ferredoxin. Numerous cyanobacteria, including *Nostoc muscorum*, *Gloeocapsa* sp., *Anabaena azollae* and *Arthrospira platensis*, have been observed to create substantial amounts of hydrogen gas.

The process of fixing nitrogen into NH_3 results in the generation of hydrogen. For the fixation of 1 mol of nitrogen molecules, which produces 1 mol of hydrogen gas (H_2), a total of 16 ATPs are needed. Blue green algae (BGA) may produce H_2 using both nitrogenases (unidirectional) and hydrogenases (bidirectional) depending on the species, whereas eukaryotic microalgae only employ hydrogenases. Uptake hydrogenase, which is in charge of the "Knallgas" reaction, and reversible or bidirectional hydrogenases, which may either make or take up H_2, are two different forms of hydrogenase enzymes that catalyze the process in various cyanobacteria. Microbes commonly use Ni-Fe and Fe-Fe hydrogenases, which use metal as a cofactor [40]. Hydrogen evolution in blue green algae might take place spatially in specialized heterocyst cells or temporally during light and dark phases. Due to the Fe-Fe hydrogenase, an iron-containing protein with a molecular mass of 48 kilo Dalton that is 100 times more than that of other hydrogenases, microalgae have high hydrogen generation efficiency (Happe and Naber 1993) [23]. According to the best predictions, green algae might produce up to 10 mol (20 g) H_2/m^2 every day (Melis and Happe 2001) [41].

Bio-methane

When there is an abundance of nitrogen and phosphorus in fresh water body, a condition known as eutrophication occurs. A variety of microalgae, including *Scenedesmus* sp., *Spirulina* sp., *Euglena* sp., *Chlorella* sp, *Melosira* sp., and *Oscillatoria* sp., have been employed as a feedstock for anaerobic digestion by several researchers to generation of bio-methane [52]. In an Anaerobic digestion, a biochemical process where anaerobic microbes break down organic waste, might be used to make bio-methane from this microalgal biomass. Numerous processes are involved in the procedure, including hydrolysis, fermentation, acetogenesis, and methanogenesis [60]. Complex cell components including lipids, polysaccharides, proteins, and nucleic acids are broken down into its simpler monomeric form such as fatty acids, monosaccharides, amino acids, purines, and pyrimidines during the hydrolysis process. These materials then proceed through fermentation to generate acetate, hydrogen, carbon dioxide, methanol, formate, propionate, butyrate, and other compounds. Acetoclastic (acetate consumer) and hydrogen utilizing (Hydrogen and carbon dioxide consumers) methanogen subsequently produces methane in the last phases. Harun and colleagues claim that, on a dry weight basis, microalgae produce more energy in the form of bio-methane (14.04 MJ/kg) than biodiesel (6.6 MJ/kg) and bio-ethanol (1.79 MJ/kg). Because they contain components that may be broken down by anaerobic digestion, such as carbohydrates, proteins, and lipids. Microalgae are an ideal substrate for the synthesis of bio-methane. Methane production is influenced by microalgae species, pretreatment of microalgal biomass, the presence or absence of inhibitors of methanogenesis, pH, carbon(C)/nitrogen(N) ratio, and temperature (T). It is challenging to extract carbohydrates from microalgae because they are a component of the microfibrillar polysaccharides contained in a matrix of polysaccharides and proteoglycans. To release such fermentable sugars, pretreatment is necessary [20]. By using heat pretreatment, Mendeza et al.

(2015) demonstrated that *Chlorella vulgaris* produced 50% more methane. Numerous studies have investigated microalgae in-depth as a source of methane and found that they may produce between 143 and 400 L-CH_4 (kg/VS) of methane [42, 51].

Conclusion and Future Prospectus

Microalgae are a crucial component of the feedstock used to produce sustainable, harmless, and biodegradable bio-fuels. Today, the manufacturing of bio-fuel is steadily rising. Future energy output from microalgae might potentially replace fossil fuels. Still, there is a lot of research in the field of making biofuels that is required. Future research must locate a promising micro-algal strain with unique characteristics. For the production of algal biomass, the study must also identify effective growing and harvesting methods. The effectiveness of the biodiesel sector depends on the success of oil extraction as well as the standard practices used throughout manufacturing and conversion. The generation of biofuels using enzymatic, biochemical, and trans-esterification technologies is safer and more environmentally friendly than using traditional techniques. The first-generation biofuels have several advantages but suffer from many limitations. Whereas the third-generation biofuels are environmentally eco-friendly because of their easily availability, feasibility and flexibility than second generation biofuels.

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