Biofuels from Microorganisms

Mariam Amer, AbdelGawad Saad and Nahed K. Ismail

Abstract Biofuel as a renewable energy, can be produced from many resources, but the easiest, safest, and most economic resources used are organisms—natural materials like algae—especially microscopic organisms. Microalgae are characterized by their ability to be grown both naturally and quickly, and represent a source of carotenoids, lipids, and polysaccharides. *Chlamydomonas reinhardtii*, *Dunaliella salina*, and various *Chlorella* species permit the extraction of about 5–7% biodiesel from their cells. Producing bioethanol to a higher concentration of 60% can be obtained using *Chlorococum* sp. The best technique for using microalgae to produce biofuel as biodiesel and bioethanol is a biochemical technique, that is, the photo-fermentation technique used to produce biohydrogen. The biochemical technique uses a process known as pyrolysis in which biomass is heated, in the absence of air, to temperatures above 500 °C for short periods (a few minutes). Also, *C. reinhardtii* can generate high condensation levels of biohydrogen. To produce biohydrogen, a quick fermentation process is required using non-sulfur bacteria, with light as an energy source , to produce organic acids by dark fermentation.

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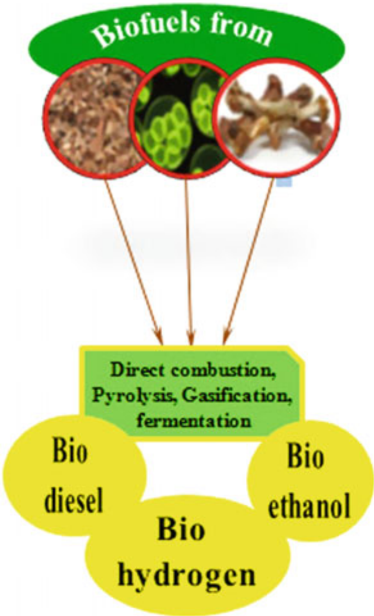
Graphical Abstract



Keywords Biofuel · Biodiesel · Biohydrogen · Bioethanol · Macroalgae Microalgae

1 Introduction

Due to the continuous decline in natural energy sources and the fact that most sources are harmful to the environment, an attempt is being made to search for other sources of energy which are safer, faster, and more productive. The most important of these sources, coming from natural materials, is called biomass. Biomass can be used as the raw material for bioenergy derived from biodiesel, bioethanol, biogas, and biobutane. These are collectively known as biofuels. These biofuels can be produced mainly from the materials within hemicellulose, cellulose, and lignins. Biomass sources include bones, wood, maize, grass, algae, oils, etc.

Fossil fuels damage the environment by producing greenhouse gas emissions leading to an enhanced global warming (Chisti 2007; Medipally et al. 2015). The negative effects of using fossil fuels are: reducing fossil fuel reserves; diminishing available resources leading to increasing CO_2 concentrations in the atmosphere causing a change in climate; and geopolitical strife. Therefore, the greatest challenge is searching for "clean" energy resources (Mata et al. 2010; Medipally et al.

2015; Shuba and Kifle 2018). In addition, the provision of multiple sources of energy protects against human conflicts driven by, for example, the demand for oil.

Biofuels are classified into four generations based on their production technologies: first-generation fuels which are made from vegetable oils, starch, sugar, or animal fats; second-generation fuels which are made from corn, wheat straw, non-food crops, wood, or solid waste; third-generation fuels which are made from algae; and finally fourth-generation fuels made from the conversion of vegetable oils and biodiesel for biogasoline. Giving particular attention to third-generation biofuels, algal biomass can accumulate considerably high amounts of lipids comparing with the biomass of oil plants (Abdelaziz et al. 2013; Voloshin et al. 2016).

Algae grows naturally and quickly and produces oxygen by photosynthesis. In addition, macroalgae does not require land, so there is no competition between algae and plants in terms of space. Therefore, macroalgae biofuels have little effect on farms or food supplies and do not require compound treatment methods as compared with lignocellulose-enriched biomass (Voloshin et al. 2016).

The main difference between algae bioenergetics and plant bioenergetics is the technology used to increase biomass. Plant bioenergetics requires the utilization of valuable resources and provides a relatively low yield in terms of the proportion of the organic feedstock mass to the mass of the biofuel synthesized. On the other hand, plants do not require any additional production methods, besides the standard growth techniques already used in agriculture and the creation of specific growing conditions. Microalgae can grow in conditions which are unsuitable for plant growth, that is, saline soils, wastewater, etc. (Chisti 2007; Wang et al. 2008).

Phytoplankton or microalgae are commonly found in oceans: the most well known being dinoflagellates, diatoms, green algae, and blue–green algae. The most important resource for carotenoids, lipids, and polysaccharides are marine unicellular microalgae, which have been extensively studied in the scope of biofuel production and fodder supplements (Liau et al. 2010). Furthermore, land plants can realize a photoconversion productivity of less than 1% in temperate climates, whereas microalgae can convert 5% of solar energy into chemical energy (Rösch et al. 2012).

2 Energy Conversion Process

The actual conversion of biomass into biofuels comes after its cultivation and preparation processes (Chisti 2007; Nigam and Singh 2011).

There are different techniques for converting biomass to energy. The first uses a chemical technique (hydrolysis and/or transesterification) which provides certain reactions in the presence of a catalyst. The second is a biochemical technique (fermentation and/or hydrolysis) which depends on the nature of the chemical processes which occur in living cells. Direct combustion represents a third technique where heat energy is converted to electrical energy. The last technique is called thermo-chemical (gasification, pyrolysis, and liquefaction) and includes treatments of feedstock, under

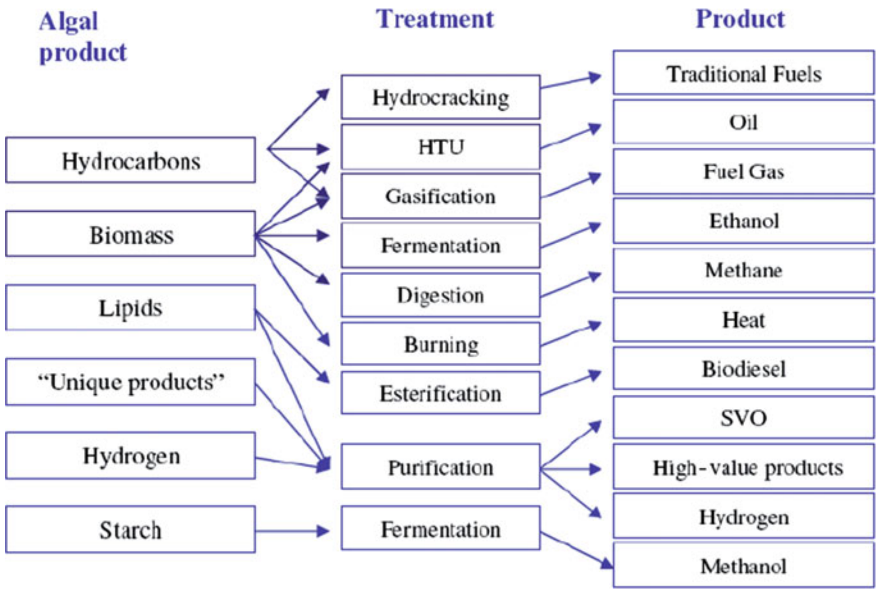


Fig. 1 Different of methods used to produce biofuel from algae (Voloshin et al. 2016). HTU—Hydro thermal upgrading, SVO—Straight vegetable oil

high pressures and temperatures, to obtain compounds at both low O_2 content and molecular weight.

There are different methods used to produce algal biofuels—Fig. 1 illustrates these methods (Voloshin et al. 2016).

3 Biodiesel

One of the methods of multiplying the production capacity is the production of biodiesel. Biodiesel is one alternative fuel which is obtained by a transesterification reaction in the presence of triglyceride oil and monohydric alcohols. Biodiesel is non-toxic, technologically sensible, and biodegradable when it is obtained from renewable resources. It can be obtained from residues of vegetable oil, fish oil, chicken fat, and algal oil (Lang et al. 2002; Spolaore et al. 2006; Sharif et al. 2007) which therefore partly decreases our dependency on oil-based fossil fuels.

Algae (macro and micro) generally has a greater photosynthetic effect than other biomass. Algae is the many sources of bio-diesel and the highest supply from feed-stock for biodiesel. It can produce more than 250 times the oil produced per acre of soybeans. Similarly, algae produces 7 to 31 times more biodiesel than palm oil. It is thought that biodiesel automotive fuel, produced from algae, could be used to replace gasoline. The preference is to use microalgae rather than macroalgae to pro-

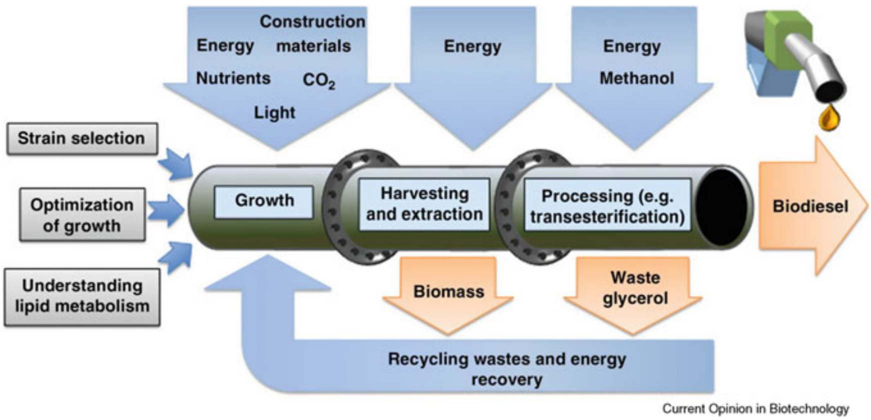


Fig. 2 Processing algal biofuel in a pipeline (Scott et al. 2010)

duce biodiesel. Microalgae are categorized as organisms which are less than 2 mm in diameter and are capable of photosynthesis. Microalgae is characterized by the fact that it is faster and easier to grow and produces greater oil yields than macroalgae. Macroalgae is not usually used to produce biodiesel. However, biodiesel can be produced from macroalgae if it contains a lower lipid content than microalgae. Some researchers concluded that using *Oedogonium* sp. allowed them to obtain higher contents of biodiesel than *Spirogyra* sp. (Hossain and Salleh 2008).

Some polyunsaturated organisms may contain a high amount of fatty acid as docosahexaenoic acid (DHA), which consists of 22 atoms of carbon in 6 dualties which belong to the so-called w-3 group. These organisms can be grown without light on heterotrophically organic substrates. These types of macroalgae contain from 1.3 to 7.8% dw of lipid (Sijtsma and Swaaf 2004).

3.1 Producing Biodiesel from Algae

Figure 2 illustrates the major stages which must be taken into consideration. There are many factors which must be optimized such as material inputs (nutrients and growth energy for homogenous), energy, suitable treatment of spent media, residue products, and residual biomass (Scott et al. 2010).

3.1.1 Algal Strain Selection

One vital consideration is algal strain selection. Algae is a non-flowering plant as an aquatic organism which feeds by photosynthesis. Approximately 300,000 species of algae have been identified from different sources. Some species of green algae such as *Chlamydomonas reinhardtii*, *Dunaliella salina*, and various *Chlorella* species, as well as *Botryococcus braunii* contains more than 60% of its lipid weight. Much of the lipid is secreted from the cell walls (Metzger and Largeau 2005). Other important algae groups include the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* and other heterokonts including *Nannochloropsis* and *Isochrysis* spp.

3.1.2 Production of Fuel Molecules by Growing Algal Biomass

The process of biofuel production by growing algae, raises some concerns: (1) the feasibility of closed or open bioreactors, (2) how nutrients and CO_2 is supplied to the bioreactors, and (3) avoiding contamination from adventitious organisms. For most microalgae, the combination of fuel molecules such as TAGs is at the expenditure of growth, thus, circumstances need to be improved to optimize TAG production (Scott et al. 2010).

3.1.3 Harvesting and Extraction

As shown in Fig. 2, producing biodiesel requires that biomass be harvested and processed. The obstacles to making biodiesel, in terms of selecting the best method to release fat from the cellular wall, are characterized by the low-energy requirements and economic avoidance of the unreasonable use of solvents such as hexane as well as the increased output of liquid carbon. The safest method is to extract oil without contaminating other cell components such as chlorophyll or DNA. Some of the methods use selective enzymes and decomposition of the cell wall (Scott et al. 2010) (Fig. 3).

3.1.4 The Final Process and Use of Its by-Products

The standard industry method is to extract biodiesel from converted substitutes of TAG transesterification using methanol to obtain methyl esters of fatty acids. Some evidence exists that the fatty acid composition of some types of TAG will be greater in unsaturated acids than is permissible in biofuel components. These substances can then be used to produce glycerol-based products (Scott et al. 2010).

There are a number of methods that can be applied to extract oil from algae, like mechanical compressing, hexane solvent extraction, and so on.

Solvent Extraction

Addition solvent from 0.5% to 0.7% to raw materials for all residuals and leaves for improving the oil extraction. The solvent extraction method can obtain materials with low oil contents. The solvents can use to pre-pressed the high-content materials

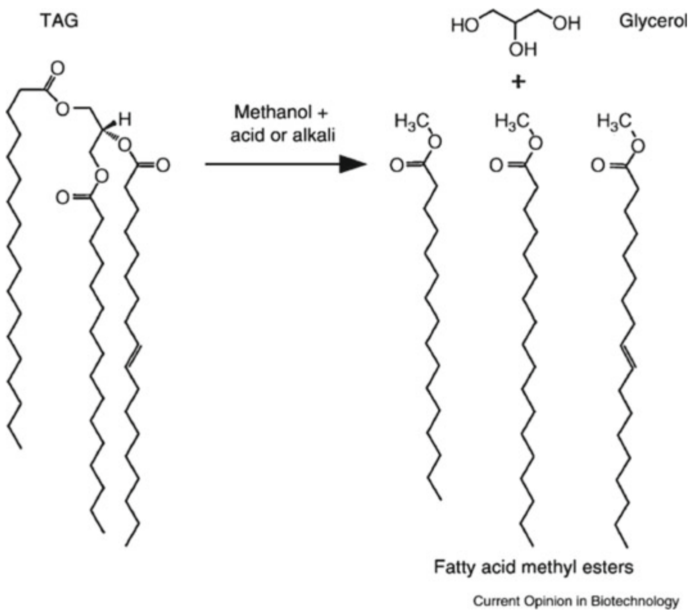


Fig. 3 Esterification of triacylglycerides extracted from algal oil for biodiesel (fatty acid methyl ester) production (Scott et al. 2010)

as oil cakes. This process obtains a high percentage of lipid. Extracting oils or lipids from algae is completed in the following manner. The first step is to obtain algae from a system of open ponds. The algae is then dried in air. Thereafter, the algae is ground. The dried algae samples are then placed in a thimble within a Soxhlet extractor which is placed into a flask filled with extraction solvent and a condenser. Next the solvent is heated until it flows back. The hexane solvent's vapors travel through a distillation arm and flood the chamber housing the thimble. The condenser secures any dissolved vapor in the chamber containing the solid material. The warm solvent floods slowly into the chamber containing the solid material. The desired compound will then dissolve in warm hexane. When the chamber in the Soxhlet extractor is filled with solvent, it is automatically emptied by a siphon arm, with the hexane returning back to the distillation flask (Fig. 4) (Topare et al. 2011).

Oil Extraction Using an Expeller

The expeller method presses the algae mechanically. Algae is acquired from open pond systems and then dried in natural air. When using the expeller method temperatures may exceed 49 °C between the pressed raw material and screw. The screw presses the oil seeds into the cavity of a barrel. Algae is introduced into the expeller on one side of the screw with the oil being output on the other side of the screw. The

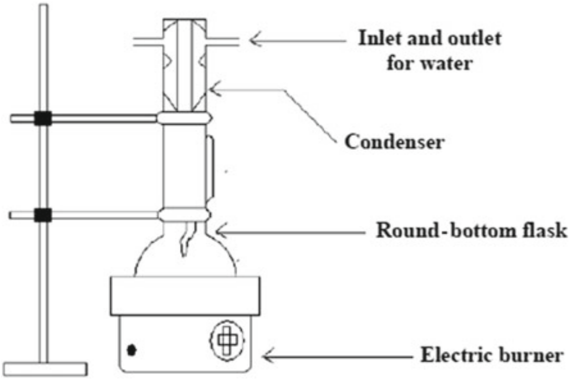


Fig. 4 Solvent method for oil extraction from algae (using a Soxhlet extractor) (Topare et al. 2011)

continuous pressure and friction of the screw drive thereby presses the filamentous algae (Topare et al. 2011).

4 Bioethanol

Bioethanol fuel is an alcohol produced by fermentation, generally using carbohydrate products which are found in sugar and starch crops like corn, sugar cane, and sweet sorghum. Ethanol can be utilized as a fuel for transportation in pure form, normally used with additives to increase the gasoline octane content and improve vehicle emissions (Hossain et al. 2015). During the first generation of bioethanol use, there were concerns about mounting food prices and the use of agricultural fields for the production of bioethanol from feedstock. That problem was countered partially by using lingo-cellulosic materials like crop residues or wastes in second-generation feedstocks. The main advantage of second-generation feedstocks over first-generation feedstocks was the reduced use of food materials and the lesser requirement for land. Nevertheless, purification, production, and several pretreatment requirements have made their production very challenging and uneconomical (John et al. 2011).

Algae used in third-generation biomass used to produce biofuels represents an alternative to first-generation and second-generation biomass because of its high productivity and the ease with which it can be planted (Daroch et al. 2013). The production of ethanol from algae depends on the fermentation of algal polysaccharides, that is, cellulose, starch, and sugar. Under special conditions, the carbohydrate content in microalgae is rich—about 70% (Branyikova et al. 2011). The cell walls of microalgae are split into inner and outer cell wall layers. The cell structure of the outer cell wall can be formed as a trilaminar outer cell wall layer and a thin outer monolayer (Yamada and Sakaguchi 1982). The microalgae outer cell walls contain polysaccharides like agar, alginate, and pectin. Nevertheless, their cell structure can differ from species to species (Yamada and Sakaguchi 1982). Conversely, the inner cell walls of algae are mostly composed of cellulose, hemicellulose, and other substances (Yamada and Sakaguchi 1982). Microalgae is considered a feedstock for producing bioethanol, this is because it has both starch and cellulose in its cell walls (Brennan and Owende 2010). Generally, the polysaccharides and their cellular walls can be fermented to produce ethanol (Hall and Payne 1997).

Their are many ways used to produce ethanol—digestive enzymes (discharging sugars from stored starch), carbohydrate fermentation, distillation, and drying. Ethanol can be used as an alternate to gasoline in gasoline engines by mixing gasoline with ethanol to any percentage. Most current automotive gasoline engines can operate on a bioethanol mix of 15% gasoline or petroleum. Ethanol contains less energy than gasoline. This means it requires much more fuel to produce a similar quantity of energy (Bruhn et al. 2011). Ethanol's advantage is its higher octane ratio, allowing increased engine pressure to increase thermal efficiency. Compared to gasoline, ethanol contains approximately one third of the energy content per unit volume (Hossain et al. 2015).

4.1 Pretreatment of Lingo-Cellulosic Materials

The purpose of pretreatment is to separate or deposit hemicellulose and lignin, decrease cellulose crystallization, and increase material porosity. Pretreatment must fulfill the following requirements: (1) use enzymatic hydrolysis to form sugars; (2) circumvent degradation or carbohydrate loss; (3) avert inhibitory formation of some by-products produced during the hydrolysis and fermentation process; and (4) be economical (Sun and Cheng 2002).

4.2 Bioethanol Production

Extraction of ethanol from biomass is achieved in two stages: carbohydrate hydrolysis to simple sugars (xylose and glucose) and sugar fermentation into alcohol. During the hydrolysis process, the carbohydrate is divided into glucose molecules, where the efficiency of cellulose conversion is based on mechanical and chemical preprocessing (Demirbas 2007). As shown in Fig. 5, the enzymatic hydrolysis process is followed by carbohydrate enzymatic hydrolysis with the assistance of acidic and cellulose enzymes. A high concentration of ethanol can be obtained by the fermentation of C_5 and C_6 sugars and subsequent ethanol distillation (Demirbas et al. 2011).

4.2.1 Ethanol Production from Microalgae

Some species of microalgae are ideal for producing bioethanol using their carbohydrates, which can be extracted, to make fermented sugars. These species of microal-

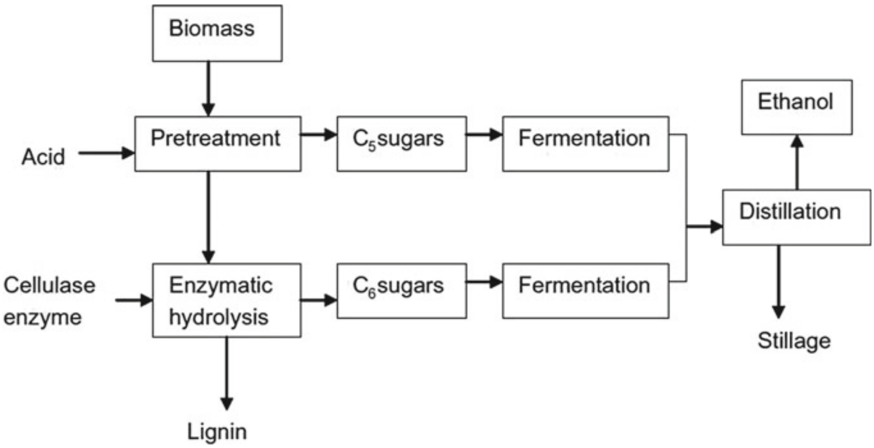


Fig. 5 Process of enzymatic hydrolysis (Demirbas et al. 2011)

gae can produce high levels of carbohydrates rather than lipids—like backup polymers. One such type of algae of importance is blue–green algae, including *Spirogyra* and *Chlorococum* sp. which contain accumulated polysaccharides at high levels in their complex cell walls or as a starch. Such a starch accumulation can be used to produce bioethanol—at high concentrations the blue–green algae of the *Chlorococum* sp. can produce ethanol which is 60% rich. It can be produced from samples that are extracted before the proportion of lipids versus those that are still dry whole cells (Harun et al. 2010; Eshaq et al. 2011).

Microalgae in particular are a potential feedstock for producing bioethanol since they can accumulate starch at levels of about 37% (Hirano et al. 1997). Microalgae used to produce bioethanol through drying is grown in an appropriate aquatic environment. Subsequently the algae is milled and its hydrolyzed mass fermented and distilled (Demirbas and Demirbas 2010). The first step in producing ethanol using microalgae is the use of a mechanical machine or enzyme to release the microalgal starch from the cells. When the cells start to degrade, the fermentation of biomass is initiated by the addition of yeast such as *Saccharomyces cerevisiae* (Nguyen and Vu 2012). The output product of fermentation is ethanol. The mechanical process uses the ethanol is discharged from the cistern and pumped into a chamber to feed the distillation unit. Ethanol is produced by microalgal photosynthesis and intracellular anaerobic fermentation (Pimentel and Patzek 2005; Demirbas 2011). Crop residues of 1 ha can produce 10–100 times more than any other source of oil crop. The cycle of oil crops takes between a few months to 2 or 3 years for full production. However, algae can begin to produce harvestable oil after 3–5 days (Nguyen and Vu 2012).

4.2.2 Ethanol Production from Macroalgae

Seaweed is categorized into three types: red, green, and brown. These types contain different glucans, many polysaccharides made up of glucose, which are considered to be biomaterials with great potential. These seaweeds have low concentrations of lignin (Yanagisawa et al. 2011).

4.3 Energy Extraction from Macroalgal Biomass

4.3.1 Energy Plucking-Out Methods Needed for Dry Macroalgae

Direct Combustion

Direct combustion is the oldest major technique used to obtain energy from dry biomass. This method can provide heat and/or steam, which is used domestically and industrially or for electricity production. High moisture content in the biomass can decrease the heat energy released when compared to the heat generated using dry biomass (20% MC) (Demirbas 2001). The direct combustion of biomass is "possible" only for biomass which has a moisture content of less than 50% (Varfolomeev and Wasserman 2011).

Pyrolysis

Pyrolysis is an alternative thermolytic technique used to convert biomass into fuel. This can be broadly defined as the thermal decomposition process of biomass by heating without interference from air during processing (McKendry 2002; Saidur et al. 2011; Li et al. 2013). Pyrolysis processes are categorized by their temperatures and length of process time, that is, slow, fast, and flash (Ghasemi al. 2012; Li et al. 2013). The pyrolysis process is characterized by long dwelling times at very low heating rates and low reactor temperatures (Milledge et al. 2014), along with the production of char rather than fuel products, whether gaseous or liquid (Brennan and Owende 2010; Ghasemi et al. 2012). Slow or fast pyrolysis shelters are ranging from modern technologies that work at temperatures greater than 500 °C and slow process of vapor retention times for a few seconds or less (Brennan and Owende 2010; Li et al. 2013).

Pyrolysis can produce large amounts of fuel materials relative to the amount of biomass used. The process of pyrolysis can be improved in favor of bio-oil production (a liquid product whose structure depends on the feedstock and pyrolysis procedure used), syngas, or solid char (Ghasemi et al. 2012) depending on the product phase required.

Gasification

The process of gasification is the transformation of organic matter to a combustible gas mixture (syngas) at a high temperature (800–1000 °C) by partial oxidation (Demirbas 2001; Saidur et al. 2011). This process includes the following stages. First the paralysis occurs in a response producing char. Thereafter, the process of gasification occurs, in the presence of a gasifying agent like O_2 or H_2O, which produces syngas. Importantly, the syngas produced by char gasification is much higher than that produced by conventional pyrolysis (Ahmed and Gupta 2010). The gas has a calorific value of about 4–6 MJ m^{-3} (McKendry 2002), and is comprised of a mixture of gases like carbon monoxide (20–30%), hydrogen (30–40%), ethylene (1%), methane (10–15%), nitrogen, and water vapor (Saidur et al. 2011). Syngas can be burned to produce heat or be converted to electricity (Demirbas 2001; McKendry 2002). The syngas produced from gasification can be used to produce methanol and hydrogen forms of fuel (Saidur et al. 2011).

4.4 Energy Plucking-Out Methods Needed for Wet Macroalgae

4.4.1 Hydrothermal Treatments

The process of liquefaction occurs at high pressures and low temperatures. Biomass in a hydrothermal process is converted to hydrocarbon fuel in the presence of hydrogen and a catalyst (McKendry 2002). It is then fermented to produce bioethanol (via anaerobic digestion). The hydrothermal process is considered a pressurized aqueous pyrolysis process (Marcilla et al. 2013). It produces a biofuel by using lower oxygen and moisture contents than the pyrolysis process (Neveux et al. 2014).

Biofuel productivity from hydrothermal liquefaction of microalgae reaches 41% (as percentage mass of original dry microalgae biomass) for *Spirulina* microalgae (Jena and Das 2011), about 45% for *Scenedesmus* microalgae (Vardon et al. 2012), 37% for *Dunaliella* (Minowa et al. 1995), 56% for *Enteromorpha prolifera* sp. (Zhou et al. 2010), 63% for *Laminaria saccharina* (Anastasakis and Ross 2011), and over 49% for *Desmodesmus* (Alba et al. 2012).

4.4.2 Macroalgal Anaerobic Digestion

Seaweeds are comprised of mostly biomass which is suitable for anaerobic digestion (AD) (Sutherland and Varela 2014). In fact, in the 19th century, biogas from algae was used as a source of lighting in an iodine production factory (Milledge et al. 2014). Recently, Tokyo Gas stated that about 20 m^3 of methane gas could be generated from 1 ton of seaweed, and when mixed with natural gas could produce 9.8 kW of

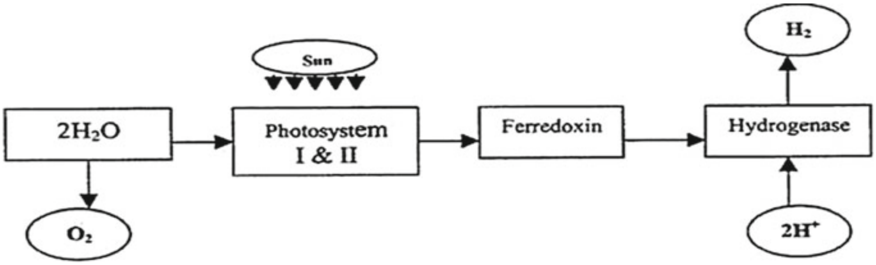


Fig. 6 Mechanism of biophotolysis (Shaishav et al. 2013)

power (Huesemann et al. 2010). The environmental importance of producing biogas from seaweed is based on its ability to decrease greenhouse gas emissions to 42% as compared to the 82% from natural gas (Milledge et al. 2014). In addition, digestion (the material left after the anaerobic process) contains compounds which contain nitrogen and phosphorus, making it a potential fertilizer for seaweed and biological feedstock, thereby providing additional income streams to seaweed anaerobic processing (Milledge et al. 2014).

5 Biohydrogen

The diversity of biofuel sources is very important in energy production (Saifuddin and Priatharsini 2016). Biohydrogen carries the promise of being a clean fuel for use in the future due to increased pollution from fossil fuels and the continued decline in the availability of fossil fuel quantities. Biohydrogen produced from algae is an alternative to the depleting sources of gasoline and is also a clean source of energy (Shaishav et al. 2013).

5.1 Various Processes Used for the Production of Hydrogen from Algae

5.1.1 Direct Biophotolysis

The separation of water molecules under sunlight in the presence of microalgae forms the basis of direct biophotolysis. Microalgae have genetic, metabolic, electron, and enzymatic transport mechanisms in order to produce hydrogen gas. Oxygen and hydrogen is produced by converting a readily available substrate, water, through biophotolysis using solar energy as illustrated in Fig. 6 (Shaishav et al. 2013).

The general reaction for biophotolysis is described by:

$$2H_2O + \text{light} = 2H_2 + O_2$$

Green algae like *C. reinhardtii* produce hydrogen under anaerobic processes, and in addition to hydrogen production, the hydrogen is used as an electronic donor (Happe et al. 1994). The conversion of hydrogen-generated ions to hydrogen gas occurs in the medium of electrons by the enzyme hydrogenase which is found in the cells. The energy of light which is absorbed by photosystem I is used to generate electrons that are transferred to ferredoxin by photosynthesis II (Shaishav et al. 2013).

5.1.2 Indirect Biophotolysis

Indirect biophotolysis processes produce hydrogen through blue–green algae like cyanobacteria. The following reactions demonstrate hydrogen formation from water by cyanobacteria (Pinto et al. 2002):

$$12H_2O + 6CO_2 + \text{lightenergy} = C_6H_{12}O_6 + 6O_2$$

$$C_6H_{12}O_6 + 12H_2O + \text{lightenergy} = 12H_2 + 6CO_2$$

Photosystem II works on the extraction of electrons from water molecules using the energy of sunlight during the process of photosynthesis. The resulting electrons are transferred from water oxidation to Fe-S proteins in ferredoxin on the reduced side of photosystem I. Hydrogenase in the algal stroma allows the electrons to reduce ferredoxin and donate it to two protons to produce a single hydrogen molecule (Shaishav et al. 2013). For the production of photosynthetic hydrogen, cyanobacteria have been identified as perfect candidates. They can produce, in the presence of N_2 and CO_2 in the air, water and mineral salts followed by incubation under argon light and a CO_2 atmosphere that is rapidly becoming a source of energy (Pinto et al. 2002).

5.1.3 Dark Fermentation

Dark fermentation means producing hydrogen in a dark environment in the absence of oxygen, sunlight, and water. Fermentative microorganisms hydrolyze complex organic polymers into monomers that are transformed into a combination of low-molecular-weight organic acids and alcohol by producing bacteria (Schara et al. 2008; Das and Veziroglu 2008). The features of dark fermentation for hydrogen production are: a lack of light; use of different sources of carbon; and production of by-product acids like lactic, butyric, and acetic. However, the disadvantages are: producing a gas mixture which contains carbon dioxide; having the ability to be separated; and having relatively lower hydrogen yields (Saifuddin and Priatharsini 2016).

5.1.4 Photo-Fermentation

Sunlight as an energy source is used for fermentative conversion of organic substrates to H_2 and CO_2 (Sharma and Arya 2017). The reaction of photo-fermentation is demonstrated in the following equation:

$$CH_3COOH + 2H_2O + light = 4H_2 + 2CO_2$$

Purple non-sulfur (PNS) bacteria are used to produce electrons, protons, and CO_2 using sunlight, whereas oxidization of the organic acid substrates occurs by utilization of the tricarboxylic acid cycle (Akkerman et al. 2002; Manish and Banerjee 2008). The advantages of this method include producing organic acids by dark fermentation, removal of environmental pollutants, and use of industrial waste. The disadvantages include the pretreatment of industrial effluent due to the fact that it may be toxic and the need for nitrogen-limited conditions (Mathews and Wang 2009).

6 Conclusions

Biofuel can be obtained from various raw materials—provided it contains cellulose and lignins—as oils, algae, grass, and wood. Microalgae are considered one of the best raw materials for biofuel production. Microalgae such as *C. reinhardtii, D. salina*, and various *Chlorella* species can be used to extract biodiesel; *Chlorococum* species can be used to extract bioethanol; and finally *C. reinhardtii* can be used to extract biohydrogen. The common methods used to extract biofuel are direct combustion, pyrolysis, gasification, and fermentation. The perfect extraction, that is, the easiest, safest, and fastest method is a biochemical technique (fermentation and pyrolysis), which includes photo-fermentation or pyrolyzation of a biomass in the presence of an inert gas.

References

Abdelaziz AEM, Leite GB, Hallenbeck PC (2013) Addressing the challenges for sustainable production of alga biofuels: II. Harvesting and conversion to biofuels. Environ Technol 34:1807–1836

Ahmed II, Gupta AK (2010) Pyrolysis and gasification of food waste: syngas characteristics and char gasification kinetics. Appl Energy 87:101–108

Akkerman I, Janssen M, Rocha J, Wijffels RH (2002) Photobiological hydrogen production: photochemical efficiency and bioreactor design. Int J Hydrogen Energy 27:1195–1208

Alba LG, Torri C, Samori C, Van der Spek J, Fabbri D, Kersten SRA, Brilman DWF (2012) Hydrothermal treatment of microalgae: evaluation of the process as conversion method in an algae biorefinery concept. Energy Fuels 26:642–657

Anastasakis K, Ross AB (2011) Hydrothermal liquefaction of the brown macro-alga *Luminaria saccharina*: effect of reaction conditions on product distribution and composition. Bioresour Technol 102:4876–4883

Branyikova I, Marsalkova B, Doucha J, Branyik T, Bisova K, Zachleder V, Vitova M (2011) Microalgae—novel highly efficient starch producers. Biotechnol Bioeng 108:766–776

Brennan L, Owende P (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev 14:557–577

Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, Markager S, Olesen B, Arias C, Jensen PD (2011) Bioenergy potential of UlvaLactuce: bio-mass yield, methane production and combustion. Bioresour Technology 102:2595–2604

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25(3):294–306

Daroch M, Geng S, Wang G (2013) Recent advances in liquid biofuel production from algae feedstocks. Appl Energy 102:1371–1381

Das D, Veziroglu TN (2008) Advances in biological hydrogen production processes. Int J Hydrogen Energy 33:6046–6057

Demirbas A (2001) Biomass resource facilities and biomass conversion processing for fuels and chemicals. Energy Convers Manag 42:1357–1378

Demirbas A (2007) Progress and recent trends in biofuels. Prog Energy Combust Sci 33:1–18

Demirbas MF (2011) Biofuels from algae for sustainable development. Appl Energy 88:3473–3480

Demirbas A, Demirbas MF (2010) Algae energy: algae as a new source of biodiesel. Springer Science & Business Media, Berlin

Demirbas MF, Balat M, Balat H (2011) Biowastes-to-biofuels. Energy Convers Manag 52(4):1815–1828

Eshaq FS, Ali MN, Mohd MK (2011) Production of bioethanol from next generation feed-stock alga *Spirogyra* species. Int J. Eng. Sci. Technol. 3:1749–1755

Ghasemi Y, Rasoul-Amini S, Naseri AT, Montazeri-Najafabady N, Mobasher MA, Dabbagh F (2012) Microalgae biofuel potentials (Review). Appl Biochem Microbiol 48:126–144

Hall J, Payne G (1997) Factors controlling the growth of field population of *Hydrodictyon reticulatum* in New Zealand. J Appl Phycol 9:229–236

Happe T, Mosler B, Naber JD (1994) Induction, localization and metal content of hydrogenase in the green alga *Chlamydomonas reinhardtii*. Eur J Biochem 222:769–774

Harun R, Danquah MK, Forde GM (2010) Microalga biomass as a fermentation feedstock for bioethanol production. J Chem Technol Biotechnol 85:199–203

Hirano A, Ueda R, Hirayama S (1997) CO_2 fixation and ethanol production with microalga photosynthesis and intracellular anaerobic fermentation. Energy 22:137–142

Hossain S, Salleh A (2008) Biodiesel fuel production from algae as renewable energy. Am J Biochem Biotechnol 4(3):250–254

Hossain NB, Basu JK, Mamun M (2015) The production of ethanol from microalgae Spirulina. Procedia Eng 106:733–738

Huesemann M, Roesjadi G, Benemann J, Metting FB (2010) Biofuels from microalgae and seaweeds. In: Biomass to biofuels. Blackwell Publishing Ltd.: Oxford, UK, pp. 165–184

Jena U, Das KC (2011) Comparative evaluation of thermochemical liquefaction and pyrolysis for bio-oil production from microalgae. Energy Fuels 25:5472–5482

John RP, Anisha GS, Nampoothiri KM, Pandey A (2011) Micro and macroalga biomass: a renewable source for bioethanol. Bioresour Technol 102(1):186–193

Lang X, Dalai AK, Bakhshi NN, Reaney MJ, Hertz PB (2002) Preparation and characterization of biodiesels from various bio-oils. Bioresour Technol 80:53–62

Li L, Rowbotham JS, Greenwell CH, Dyer PW (2013) An introduction to pyrolysis and catalytic pyrolysis: versatile techniques for biomass conversion. In: Suib SL, Elsevier Ed (eds) New and future developments in catalysis: catalytic biomass conversion. Amsterdam, The Netherlands, pp 173–208

Liau BC, Shen CT, Liang FP, Hong SE, Hsu SL, Jong TT, Chang CM (2010) Supercritical fluids extraction and anti-solvent purification of carotenoids from microalgae and associated bioactivity. J Supercrit Fluids 55:169–175

Manish S, Banerjee R (2008) Comparison of biohydrogen production processes. Int J Hydrogen Energy 33(1):279–286

Marcilla A, Catalá L, García-Quesada JC, Valdés FJ, Hernández MR (2013) A review of thermochemical conversion of microalgae. Renew Sustain Energy Rev 27:11–19
Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications. Renew Sustain Energy Rev 14:217–232
Mathews J, Wang G (2009) Metabolic pathway engineering for enhanced biohydrogen production. Int J Hydrogen Energy 34:7404–7416
McKendry P (2002) Energy production from biomass (part 3): Gasification technologies. Bioresour Technol 83:55–63
Medipally SR, Yusoff FM, Banerjee S, Shariff M (2015) Microalgae as sustainable renewable energy feedstock for biofuel production. Bio Med Res Int 2015:519–513
Metzger P, Largeau C (2005) *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. Appl Microbiol Technol 66:486–496
Milledge JJ, Smith B, Dyer PW, Harvey P (2014) Macroalgae—derived biofuel: a review of methods of energy extraction from seaweed biomass. Energies 7:7194–7222
Minowa T, Yokoyama S, Kishimoto M, Okakura T (1995) Oil production from alga cells of *Dunaliella tertiolecta* by direct thermochemical liquefaction. Fuel 74:1735–1738
Neveux N, Yuen AKL, Jazrawi C, Magnusson M, Haynes BS, Masters AF, Montoya A, Paul NA, Maschmeyer T, De Nys R (2014) Biocrude yield and productivity from the hydrothermal liquefaction of marine and freshwater green macroalgae. Bioresour Technol 155:334–341
Nguyen THM, Vu VH (2012) Bioethanol production from marine algae biomass: prospect and troubles. J Viet Environ 3(1):25–29
Nigam PS, Singh A (2011) Production of liquid biofuels from renewable resources. Prog Energy Combust Sci 37:52–68
Pimentel D, Patzek TW (2005) Ethanol production using corn, switchgrass, and wood; biodiesel production using soybean and sunflower. Natural Resour Res 14:65–76
Pinto FAL, Troshina O, Lindblad P (2002) A brief look at three decades of research on cyanobacterial hydrogen evolution. Int J Hyd Ener 27:1257–1264
Rösch C, Skarka J, Wegerer N (2012) Materials flow modeling of nutrient recycling in biodiesel production from microalgae. Bioresour Technol 107:191–199
Saidur R, Abdelaziz EA, Demirbas A, Hossain MS, Mekhilef S (2011) A review on biomass as a fuel for boilers. Renew Sustain Energy Rev 15:2262–2289
Saifuddin N, Priatharsini P (2016) Developments in bio-hydrogen production from algae: a review. Res J Appl Sci Eng Technol 12(9):968–982
Schara V, Maeda GT, Wood TK (2008) Metabolically engineered bacteria for producing hydrogen via fermentation. Microb Biotechnol 1(2):107–125
Scott SA, Davey MP, Dennis JS, Horst I, Howe CJ, Lea-Smith DJ, Smith AG (2010) Biodiesel from algae: challenges and prospects. Curr Opin Biotechnol 21:277–286
Shaishav S, Singh RN, Satyendra T (2013) Biohydrogen from Algae: Fuel of the Future. Int Res J Environ Sci 2(4):44–47
Sharif ABMH, Nasrulhaq AB, Majid HAM, Chandran S, Zuliana R (2007) Biodiesel production from waste cooking oil as environmental benefits and recycling process. A review. Asia Biofuel Conference Book. Dec 11–13, Singapore
Sharma A, Arya SK (2017) Hydrogen from alga biomass: a review of production process. Biotechnol Rep 15:63–69
Shuba ES, Kifle D (2018) Microalgae to biofuels: 'Promising' alternative and renewable energy. Review. Renew Sust Energy Rev 81:743–755
Sijtsma L, Swaaf ME (2004) Biotechnological production and applications of the w-3-polyunsaturated fatty acid docosahexaenoic acid. Appl Microbiol Biotechnol 64:146–153
Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96
Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresour Technol 83:1–11

Sutherland A, Varela J (2014) Comparison of various microbial inocula for the efficient anaerobic digestion of Laminaria hyperborea. i, 14. https://doi.org/10.1186/1472-6750-14-7
Topare NS, Rauta SJ, Renge VC, Khedkar SV, Chavan YP, Bhagat SL (2011) Extraction of oil from algae by solvent extraction and oil expeller method. Int J Chem Sci 9(4):1746–1750
Vardon DR, Sharma BK, Blazina GV, Rajagopalan K, Strathmann TJ (2012) Thermochemical conversion of raw and defatted alga biomass via hydrothermal liquefaction and slow pyrolysis. Bioresour Technol 109:178–187
Varfolomeev SD, Wasserman LA (2011) Microalgae as source of biofuel, food, fodder, and medicines. Appl Biochem Microbiol 47:789–807
Voloshin RA, Rodionova MV, Zharmukhamedov SK, Veziroglu TN, Allakhverdiev SI (2016) Review: biofuel production from plant and algae biomass. Int J Hydrogen Energy 41:17257–17273
Wang B, Li Y, Wu N, Lan CQ (2008) CO_2 bio-mitigation using microalgae. Appl Microbiol Biotechnol 79:707–718
Yamada T, Sakaguchi K (1982) Comparative studies on Chlorella cell walls—induction of protoplast formation. Arch Microbiol 132:10–13
Yanagisawa M, Nakamura K, Ariga O, Nakasaki K (2011) Production of high concentrations of bioethanol from seaweeds that contain easily hydrolysable polysaccharides. Process Biochem 46(11):2111–2116
Zhou D, Zhang L, Zhang S, Fu H, Chen J (2010) Hydrothermal liquefaction of macroalgae Enteromorpha prolifera to bio-oil. Energy Fuels 24:4054–4061