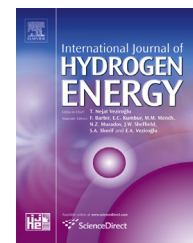


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## Review Article

## Biofuel production: Challenges and opportunities



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## ABSTRACT

It is increasingly clear that biofuels can be a viable source of renewable energy in contrast to the finite nature, geopolitical instability, and deleterious global effects of fossil fuel energy. Collectively, biofuels include any energy-enriched chemicals generated directly through the biological processes or derived from the chemical conversion from biomass of prior living organisms. Predominantly, biofuels are produced from photosynthetic organisms such as photosynthetic bacteria, micro- and macro-algae and vascular land plants. The primary products of biofuel may be in a gas, liquid, or solid form. These products can be further converted by biochemical, physical, and thermochemical methods. Biofuels can be classified into two categories: primary and secondary biofuels. The primary biofuels are directly produced from burning woody or cellulosic plant material and dry animal waste. The secondary biofuels can be classified into three generations that are each indirectly generated from plant and animal material. The first generation of biofuels is ethanol

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Hydrogen  
Bioethanol  
Biomethanol

derived from food crops rich in starch or biodiesel taken from waste animal fats such as cooking grease. The second generation is bioethanol derived from non-food cellulosic biomass and biodiesel taken from oil-rich plant seed such as soybean or jatropha. The third generation is the biofuels generated from cyanobacterial, microalgae and other microbes, which is the most promising approach to meet the global energy demands. In this review, we present the recent progresses including challenges and opportunities in microbial biofuels production as well as the potential applications of microalgae as a platform of biomass production. Future research endeavors in biofuel production should be placed on the search of novel biofuel production species, optimization and improvement of culture conditions, genetic engineering of biofuel-producing species, complete understanding of the biofuel production mechanisms, and effective techniques for mass cultivation of microorganisms.

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## Introduction

The “biofuels” in this review are referred to the energy-enriched chemicals generated through the biological processes or derived from the biomass of living organisms, such as microalgae, plants and bacteria. The increasing global population requests more energy supplies for improving the quality of life. Biofuels can be one of the sources to fulfill the global energy demand. Fossil fuels are being used as a main source of energy for many years; however, the usage of them is unsustainable and causes environmental issues related to fossil fuel combustion [1–3]. Hence, this challenge may allow fossil fuels to be substituted by renewable energy sources such as biofuels that is environmentally friendly [4].

The recent approaches for microbial biofuel production are well explored and recognized [5,6], and a possibility of microalgal cultivation strategies for direct energy conversion to produce biofuels has been recommended. For example, biofilm cultivation of microalgae or cyanobacteria could be the new platform of biomass production pathways that ultimately explored for biofuel processing pathway. The approach of biofilm cultivation is promising for biofuel production by microalgae or cyanobacteria [5,6]. For the past several decades, the best-known source of biofuels is the plant biomass. Currently, the increasing evidences showed that algal biomass as a favorable source for biofuel production [4]. A main feature distinguishing plants and algae from other sources is their ability to photosynthesize. Photosynthesis is the process of sugars formation from atmospheric carbon dioxide by solar

energy [3]. It is the distinctive way of carbon fixation of plant and green algae in nature, which demonstrates that photosynthesis is exclusive process of growth of plant and algal biomass which is raw material for biofuel production.

Sugar is the basic molecular substrate for the production of bioethanol and biomethanol [7]. The use of photosynthetic organisms as a source of biofuel is cheap and feasible, i.e. atmospheric CO<sub>2</sub> serves as source of carbon and sunlight serves as an energy source. Photosynthesis proceeds in two stages: a light-dependent and light-independent [3]. During the light-dependent stage, light energy is absorbed, converted into a charge separation and ultimate converted into the synthesis of ATP and NADPH. Main photosynthetic pigments involved in the absorption of light are chlorophyll in plants and bacteriochlorophyll in bacteria. During the light-independent stage, the energy and electrons from ATP and NADPH, respectively, are used to produce sugars.

An essential element of photosynthetic apparatus is the electron transport chain through a sequence of electron carriers embedded in thylakoid membrane. Electron transfer is triggered by light and achieved via electron carriers within large membrane complexes that are coupled via that mobile electron carriers such as plastoquinone, plastocyanin, cytochrome c or ferredoxin [3]. The NADP<sup>+</sup> molecules are reduced to NADPH by the electrons from the water and the ferredoxin:NADP oxidoreductase. In case of oxygenic photosynthesis, water acts as an electron source. Molecular oxygen and protons are evolved as a result of the water decomposition. Under certain conditions, protons come upon hydrogenase, not upon ferredoxin:NADP oxidoreductase, and molecular hydrogen is

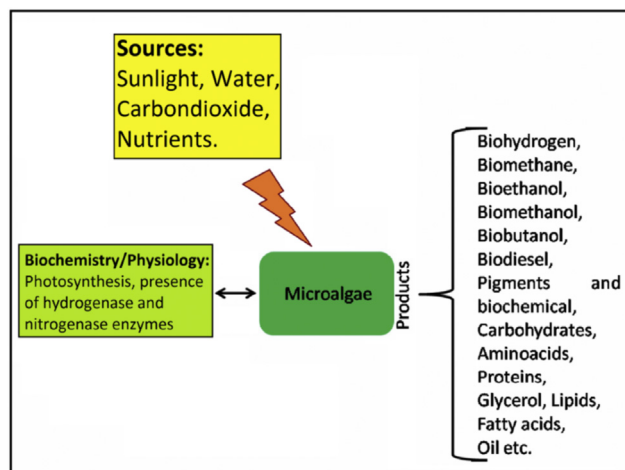
generated. This phenomenon indicates a mechanism for biohydrogen production in the photosynthetic apparatus as [8].

On one hand, photosynthesis is a process of biomass accumulation. Plant biomass is a raw material for synthesis of bioalcohol, biodiesel, and fermentation-derived biohydrogen. On the other hand, under certain conditions photosynthetic apparatus serves as a source of biohydrogen. Although plants and algae have a similar photosynthetic apparatus, algae have several advantages over higher plants with regard to biofuel production. As we know, biodiesel, triglycerides, fatty acids, lipids, carbohydrates, ethanol, alcohols, cellulose or the biomass of organisms are the major biofuels, and they can be produced by several species of algae, bacteria or yeast [9].

Based on current knowledge, the usage of microalgae is being considered as an attractive feedstock for biofuels production [10]. Depending on species and cultivation method microalgae can produce biohydrogen [9], biomethanol, bioethanol, biodiesel [11], or carbohydrates, proteins or other compounds that are being used in pharmaceutical companies [12]. The algal derived biofuels production requires only sunlight, CO<sub>2</sub> and water and generates multiple renewable energy products (Fig. 1). Algal-based biofuels production is about hundred times higher than that of higher plants. The algal biomass can be further processed to produce biofuels during fermentation by microorganisms. Currently the several microorganisms have been discovered to produce biofuels efficiently: (a) genetic engineering of cyanobacteria to enhance hydrogen production [13]; (b) optimization of hydrogen production and metabolic engineering for biofuels production in bacteria [14,15]; (c) dark fermentation by bacteria to convert carbohydrates to biohydrogen and other biofuels; (d) photo-biological methods to produce biohydrogen by microalgae [16]; (e) genetic engineering of the yeast to increase ethanol production by tolerating high alcohol concentration; (f) genetic engineering of microorganisms that can ferment carbohydrates to increase bioethanol and biobutanol production; (g) screening the microalgae that can produce more oil for biodiesel production; and (i) fermentation of plant cell wall carbohydrates by yeast or other microorganisms to produce biofuels.

The production of biofuels can be achieved by several species of algae and has the highest potential to produce alternative sources of energy. Presently, ethanol, alcohols, biodiesel, triglycerides, fatty acids, lipids, carbohydrates, cellulose and the biomass of organisms are considered as the major biofuels sources. Global attempts have been made for the identification of desired strains of algae. Several species of algae that have the capacity of high amount biomass production (in terms of carbohydrates, proteins, lipids) can be used as an alternative source of bioenergy. For example, in dry weight *Spirulina maxima* has 60–71% w/w of proteins, *Porphyridium cruentum* has 40–57% w/w of carbohydrates, *Schizochytrium* species have 50–77% w/w of lipids. Hence, different microalgae can be utilized as the preferred source of different biomass under certain conditions [17].

In addition, microalgae can produce higher amounts of biodiesel than cotton or palm plants [18]. For example, the ketoacid decarboxylase gene in the cyanobacteria *Synechococcus elongates* PCC 7942 improved the production of



**Fig. 1 – The working mechanism of microalgae in the energy sector.**

isobutyraldehyde and butanol [19]. Some species of green algae, such as *Botryococcus braunii* and *Chlorella protothecoides*, also contain high levels of terpenoid hydrocarbons and glyceryl lipid, which can be converted into shorter hydrocarbons as major crude oil [20]. Those algae also have great potential for the production of petroleum fuels such as bioethanol, triterpenic hydrocarbons, isobutyraldehyde, and isobutanol [17].

Even genetic engineering of many bacteria species such as *Escherichia coli* and *Bacillus subtilis* also produced higher amounts of bioalcohol, isoprenoids and fatty acids derivatives. Additionally, some species of bacteria have unique properties to be the source of biofuels. *Clostridium acetobutylicum* and *Clostridium beijerinckii* have been used for the production of biofuels by acetone-butanol-ethanol fermentation [21]. Some species of bacteria, such as *Bacillus* and *E. coli*, produce lactic acid and glutamic acids as a source of some chemicals [22]. It has been identified that several species of bacteria have the ability to produce ethanol.

However, it is noted that the production of biofuels and hydrogen varies according to the bacteria. Genetic studies have shown higher amount of hydrogen production and lower amount of ethanol production in *Caldicellulosiruptor*, *Thermococcus*, *Pyrococcus* and *Thermotoga* species. This fact indicates the important role of gene regulation in the bacterial cell biochemical pathways and also relationship between these pathways and production of biofuels [14]. Recently, co-culture of bacteria also played significant role in production of biofuels. The co-culture of *Thermoanaerobacter* species with cellulolytic organisms has been found effective for ethanol production [23]. It has been considered, that the popular microorganism *Saccharomyces cerevisiae* is a model organism for the efficient production of ethanol and lipid by fermentative process [24–26].

Biofuels may be classified into two categories: primary and secondary biofuels. The primary biofuels are the natural biofuels directly produced from firewood, plants, forest, animal waste, and crop residue. The secondary biofuels are directly generated from plants and microorganisms and may be further divided into three generations (Table 1). The first generation of biofuels is the production of ethanol from starch

**Table 1 – Classification of biofuels (adapted from Ref. [4]).**

| Biofuels                                                                             |                                                                                                                                                                                                                                                                   |                                                                                                                                                                                                                                                                                    |                                                                                                                     |
|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------|
| Primary                                                                              | Secondary                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                    |                                                                                                                     |
|                                                                                      | First generation                                                                                                                                                                                                                                                  | Second generation                                                                                                                                                                                                                                                                  | Third generation                                                                                                    |
| Firewood, wood chips, pellets, animal waste, forest and crop residues, landfill gas. | Bioethanol or butanol by fermentation of starch (from wheat, barley, corn, potato) or sugars (from sugarcane and sugar beet.)<br>Biodiesel by transesterification of oil crops (rapeseed, soybeans, sunflower, palm, coconut, used cooking oil, and animal fats.) | Bioethanol and biodiesel produced from Conventional technologies but based on novel starch, oil and sugar crops such as <i>Jatropha</i> , cassava or <i>Miscanthus</i> ;<br>Bioethanol, biobutanol, syndiesel produced from lignocellulosic materials (e.g. straw, wood and grass) | Biodiesel from microalgae<br>Bioethanol from microalgae and seaweeds<br>Hydrogen from green microalgae and microbes |

rich food crops like wheat, barley, corn, potato, sugarcane, or biodiesel from soybean, sunflower and animal fat. While the second generation of biofuels is the production of bioethanol and biodiesel from several species of plants such as *jatropha*, *cassava*, *miscanthus*, straw, grass and wood. The third generation of biofuels is the production of biodiesel from microalgae and microbes [4,27].

## Biohydrogen production

Production of molecular hydrogen by the culture of photosynthetic microorganisms is one of the most promising approaches for generation of renewable energy [28–30]. The process occurs under ambient temperatures, and requires sunlight, water and minimal amounts of macro- and micro-nutrients. Since generation of hydrogen through photosynthesis results in zero emission of greenhouse gases and other environmental pollutants, it is anticipated that the future efforts may lead to the engineering of the ecologically friendly platform for industrial production of renewable energy [8]. Biohydrogen can be used as directly in internal combustion engines or also used to power fuel cells for electricity, however in both cases the only byproduct is water.

However, the challenges and difficulties are that the available systems for sustainable biohydrogen production still have the low yields and low efficiencies. Light driven hydrogen production by microalgae has some problems due to liberation of molecular oxygen during photosynthesis which can irreversibly inhibit hydrogenases. Apart from this, the production cost of biohydrogen is not competitive to replace the hydrogen production from fossil fuel [31]. In summary, the major limiting factors for hydrogen photoproduction are (a) the different efficiency of light utilization by phototrophs under different solar light intensities [32–35], (b) the high level of ambient oxygen that impairs the hydrogen production enzymes and the pathways in cells [36–38], and (c) the rate of photosynthetic CO<sub>2</sub> assimilation necessary for an efficient accumulation of cell biomass and its further conversion to biohydrogen is low [32,39].

Many photosynthetic organisms are capable of hydrogen evolution, and the list of hydrogen producers includes species from different genera of both prokaryotes and eukaryotes, in particular, the species of photosynthetic bacteria, cyanobacteria and green algae [40,41]. Photosynthetic bacteria cannot use water as the electron donor. In contrast, cyanobacteria and

green algae are capable of splitting water to molecular oxygen and protons in the light. In both cases, the solar energy is converted into the chemical energy in chemical compounds, such as ATP and NADPH, which are further used for driving metabolic activity of the cells, building the biomass, and storing the carbohydrates and other energy-enriched molecules [3]. Although the above-mentioned activities are common for all phototrophs, the H<sub>2</sub>-producing species are also capable of breaking down their organic compounds to produce hydrogen. Since the release of molecular hydrogen is always the loss of energy for the cells, the efficient and continuous production of H<sub>2</sub> in the cultures of photosynthetic microorganisms occurs only under specific conditions, such as full or partial anaerobiosis [42,43], nutrient starvation [44–46], and some other factors, which lead to a partial loss of photosynthetic activity, enhancement of respiration, and fermentation.

There are two main approaches to generation of biohydrogen. The first approach, known as indirect process [32,39], utilizes the potential of photosynthesis to build biomass. The accumulated biomass, especially carbohydrates, is further converted to biohydrogen through fermentation and/or photofermentation. This approach involves two distinct stages, which are separated either by reaction spatially in two different bioreactors or by alternating the photosynthetic and fermentation periods. The second approach, which is the major subject of this section, seeks to harness photosynthesis to split water to hydrogen and oxygen via either direct or indirect water biophotolysis processes. The H<sub>2</sub> evolution by green alga *Scenedesmus obliquus* in the light biophotolysis has been a subject of applied R&D for several decades [47]. It is also reported that simultaneous H<sub>2</sub> production and O<sub>2</sub> evolution were demonstrated in filamentous cyanobacteria *Anabaena cylindrica* exposed to argon (Ar) atmosphere [48].

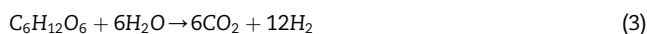
In green algae and some cyanobacteria, direct water biophotolysis proceeds in two steps:



The first reaction occurs commonly in all oxygenic phototrophs, but the second reaction requires anaerobic or microaerobic conditions. The H<sub>2</sub> production reaction is catalyzed by the bidirectional hydrogenase enzyme. There are two types of hydrogenase enzymes. The algal bidirectional hydrogenases

are [Fe–Fe] enzymes, whereas cyanobacterial hydrogenases belong to the class of [Ni–Fe]-hydrogenases [30]. The direct water biophotolysis process usually occurs when cultures are exposed to the light after a period of dark anaerobic adaptation [47,49]. It has high initial rates, up to 300  $\mu\text{mol H}_2/(\text{mg Chl}^*\text{h})$ . However, due to the high sensitivity of bidirectional hydrogenases to oxygen, especially [Fe–Fe] enzymes, the direct biophotolysis lasts from few seconds to a few minutes. Taking into account fast inactivation of hydrogenases by  $\text{O}_2$  co-produced during photosynthesis by oxygenic phototrophs [9], significant efforts have been taken to overcome this problem [37,50,51].

In case of indirect biophotolysis, microalgae and cyanobacteria produce hydrogen from stored carbohydrates, such as glycogen and starch [52–54]. In contrast to direct biophotolysis, indirect biophotolysis consists of two stages. In the first stage, carbohydrates are synthesized in the presence of light, and in the second stage they are utilized to produce hydrogen through photofermentation [52,55–57]:



The degradation of carbohydrates is not complete, and often accompanied by accumulation of other fermentation end products. Thus, in the indirect water biophotolysis process  $\text{O}_2$  evolution and  $\text{H}_2$  production stages are separated from each other either spatially or temporally. In filamentous heterocystous cyanobacteria, oxygen evolution is carried out in the vegetative cells and  $\text{H}_2$  photoproduction is localized in specialized cells, heterocysts, and hydrogen photoproduction is driven by the nitrogenase system. Heterocysts insure a microaerobic environment for the nitrogenase, which is extremely  $\text{O}_2$ -sensitive [51]. The thylakoids in heterocysts also lack PSII, which prevents oxygen evolution further lowering oxygen levels. Here, hydrogen is evolved as a by-product of nitrogen fixation and its production depends on carbohydrates supplied by vegetative cells. The highest rate of  $\text{H}_2$  production is observed in the absence of nitrogen, when nitrogenase exclusively catalyzes reduction of  $\text{H}^+$  to  $\text{H}_2$ . Unicellular  $\text{N}_2$ -fixing cyanobacteria realize a strategy of temporal separation of photosynthesis and nitrogen fixation. They perform photosynthesis during the day time and  $\text{N}_2$  fixation at night [58]. The energy generated by photosynthesis is stored in glycogen granules. At the onset of the dark period, high rates of respiration rapidly create a microoxic intracellular environment, which facilitates  $\text{N}_2$  fixation and  $\text{H}_2$  production. In wild-type of *Cyanothece*, the rate is 465  $\mu\text{mol (H}_2)/(\text{mg Chl}^*\text{h})$  [59].

Photofermentation and dark fermentation are two useful methods of  $\text{H}_2$  production from different organic substrates, mostly carbohydrates and organic acids. In photofermentation, photosynthetic bacteria use sunlight as a source of energy and convert organic compounds, such as acetate, lactate and butyrate, into  $\text{H}_2$  and  $\text{CO}_2$ . Unlike the green algae and cyanobacteria, purple bacteria could not split water. However, they are capable of utilizing light in the near infrared region ( $>800\text{ nm}$ ). These properties make them unique players in the integrated system for wastewater treatment with simultaneous  $\text{H}_2$  production [30]. The contribution of nitrogenases and hydrogenases into the process may vary depending on the experimental and physiological

conditions. Purple non-sulfur bacteria belong to the most active nitrogen fixers [60] and thus produce  $\text{H}_2$  mainly through the nitrogenase system. Coupling of nitrogen fixation to anoxygenic photosynthesis allows them to produce  $\text{H}_2$  at very high rates that are increased in the absence or deficiency of nitrogen. The reductants required for  $\text{H}_2$  production by nitrogenase are supplied by the photoassimilation of organic acids. Extensive studies were done on the optimization of  $\text{H}_2$  production in the cultures [61].

It has been shown that the micronutrient supplementations with iron (Fe) and molybdenum (Mo) in the growth medium can increase the photofermentative  $\text{H}_2$  production [62–64] some *Rhodobacter* species. The role of other nutrients such as nickel (Ni) and magnesium (Mg) has also been demonstrated in *Rhodospseudomonas* sp [65].

Under anaerobic conditions, many microbes are capable of producing  $\text{H}_2$  during the fermentation of carbohydrate-enriched feedstocks (Fig. 2). The process occurs without light and therefore, is known as dark fermentation [66]. Fermentative microbes are found to encode multiple [Ni–Fe]- and [Fe–Fe]-hydrogenases. In *E. coli*, for example,  $\text{H}_2$  production is linked to [NiFe]-hydrogenase via the pyruvate-formate-hydrogenlyase reaction, whereas *Clostridium* species produce  $\text{H}_2$  via [Fe–Fe]-hydrogenases linked to the pyruvate-ferredoxin oxidoreductase reaction. Fermentative  $\text{H}_2$  production here may proceed at very high rates, with reported values of up to 15  $\text{LH}_2/(\text{Lh})$  using sucrose as a substrate [67].

Taking into account several aspects, dark fermentation is less energetically costly than photofermentation [69]. The process occurs in facultative or obligate anaerobic bacteria that consume organic substance [31]. It is found that multiple environmental factors, such as pH of the medium and metal cofactor availability, are important in dark fermentation [70]. Dark fermentation not only produces hydrogen but also produces some other types of biofuels [71,72].

## Other biofuels production

Even bioalcohol has been used as a source of fuel for several decades by applying traditional methods. Nowadays, bioalcohol is considered as a non-fossil alternative transport. To date the main source of bioalcohols is plant material containing abundant starch and sugars including grain crops and sugarcanes, however more recently attention has shifted to perennial grasses such as switchgrass and *Miscanthus*. These crops do not compete with food usage yet the fermentation and distillation processes requires prior conversion of the cellulosic biomass into sugars. Ethanol is the most common bioalcohol, while biopropanol and biobutanol are the less common. These alcohols are produced by fermentation of carbohydrate-enriched feedstock by microorganisms [73]. Amount of plant biomass depends on efficiency of plant photosynthetic activity and cultivation conditions.

Traditionally, ethanol was used for production of alcohol-containing drinks. However, with the development of purification techniques, the use of ethanol has been largely extended to other purposes. For example, gasoline can be replaced by bioethanol to reduce carbon dioxide emission. Due to presence of oxygen in its molecular form, relatively low

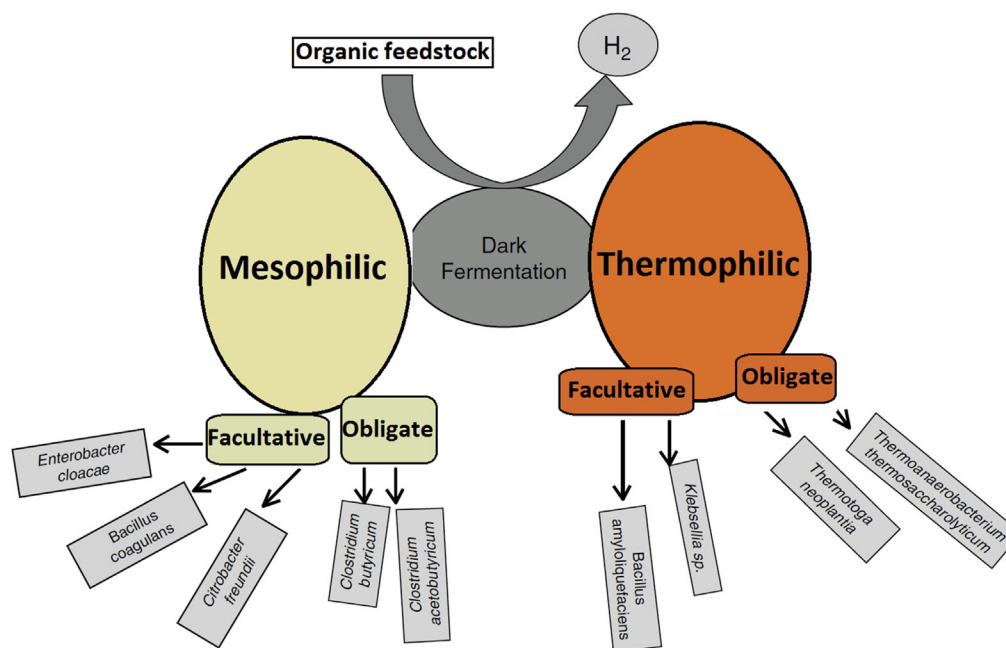


Fig. 2 – Hydrogen production via dark fermentative (adapted from Ref. [68]).

temperature combustion is possible with reducing the emission of several toxic gases such as carbon monoxide (CO), nitric oxide (NO) and volatile organic compounds.

There are several sources for bioethanol production, such as agricultural wastes, lignocellulosic biomass, rice straw, and sugarcane. Feedstocks, such as sucrose from sugarcane, sugar, beet, starch from corn, wheat or lignocellulosic materials from straw, wood and bagasse (dry pulpy residue of sugarcane stems left after the extraction of juice), are commonly used for bioethanol production [7] (Table 2). Sugarcane produces high amount of bioethanol when the waste product of bagasse is utilized. This cellulosic material is produced at the level of around several hundred kilograms per a ton of sugarcane that can be used for ethanol and electricity generation [74,75]. In addition, the crop plants containing in starch, such as corn, are also used as feedstocks for conversion to sugars through hydrolysis and finally to bioethanol through fermentation and distillation.

Algae are another source of bioethanol production, although they can be used for biohydrogen production. Recently, it has been reported that algae are a potential feedstock for biofuel generation, because algae contain ~50% of lipids for production of biodiesel and rest component sugar and proteins for bioethanol production.

Currently, the methanol is mainly produced from fossils. Methanol production is based on the chemical process, which converts natural gas (CH<sub>4</sub>, methane) into methanol by three stages, i.e. steam reforming, synthesis, and distillation process. Methanol is considerably easier to be recovered than the ethanol, as ethanol forms a conjunction with water (azeotrope) that is expensive to purify. In case of methanol, it's recycling is easier because it does not form an azeotrope. It is the reason why biomethanol is preferred for biodiesel, despite its toxicity [77]. In addition, biomethanol production by gasification and partial oxidation of carbohydrates with O<sub>2</sub> and

H<sub>2</sub>O has been depicted [77]. Not only by partial oxidation of carbohydrates but also oxidation reaction in biomass yields biomethanol [78].

Biomethanol can be produced either by fermenting or by distilling the crops containing sugars and starch. In this process, starch is converted into simple sugars and finally to bioethanol and biomethanol. Biomethanol can be produced from biomass and biodegradable wastes. Biomass can be considered as a potential fuel for gasification and further syngas production and methanol synthesis [79]. With addition of enough amount of hydrogen to the synthesized gas, all the biomass is converted into biomethanol [80]. Microalgae are unique and the fastest growing photosynthetic organisms that are able to produce several bio-oils. Up to 50% of algae's weight is comprised of oil and therefore, the usage of algae to produce biomethanol can be considered. Microalgae such as *Spirulina* sp. can also produce biomethanol by the process of gasification. Bioethanol has received more attention than biomethanol due to its corrosive and toxic properties [81].

Table 2 – Ethanol production from different feedstock (adapted without modification from Ref. [76]).

| Source        | Ethanol yield (gal/acre) | Ethanol yield (L/ha) |
|---------------|--------------------------|----------------------|
| Corn stover   | 112–150                  | 1050–1400            |
| Wheat         | 277                      | 2590                 |
| Cassava       | 354                      | 3310                 |
| Sweet sorghum | 326–435                  | 3050–4070            |
| Corn          | 370–430                  | 3460–4020            |
| Sugar beet    | 536–714                  | 5010–6680            |
| Sugarcane     | 662–802                  | 6190–7500            |
| Switch grass  | 1150                     | 10,760               |
| Microalgae    | 5000–15,000              | 46,760–1,402,900     |

Biodiesel is an alternative to fossil fuels in the past decades and increasingly receives the attention worldwide. It can be produced from renewable biological materials and can substitute the petroleum diesel fuels. Biodiesel fuels are produced through transesterification of various animal fats and vegetable oils usually with methanol or ethanol [82]. It can be produced from many seed oils, but the most common ones are rapeseed and soybean oils. The quality of biodiesel depends on the natural characteristics of feedstocks used for its production. Biodiesel is important due to several reasons: (a) it can provide a cheap and local fuel for rural economies; (b) it is sustainable and renewable; and (c) production has little toxic waste with largely biodegradable input and outputs [83].

In addition, biodiesel is environmental friendly [84] and can be used to improve the engine performance [85]. It is made of non-toxic chemicals and combustion does not release sulfur and nitrogen rich flue gas as does as petrochemicals. Production of biodiesel is a simple process in two steps (Fig. 3). The first step consists of the extraction of fats or oils from animal or plant tissues. The second step is transesterification

of lipid fraction with alcohols in the presence of catalysts to generate biodiesel.

Biodiesel is similar to crude oil-derived diesel in chain length, viscosity, energy density and can be a “drop in” fuel requiring little modification of existing internal combustion engines. The vegetable oil esters contain 10–11% of oxygen that can accelerate more combustion than hydrogen-based diesel. Additionally, biodiesel has only a slightly lower volumetric heat capacity than mineral diesel. The conversion of triglycerides into methyl or ethyl esters through the transesterification process reduces the molecular weight to one-third of the triglycerides, reduces the viscosity by a factor of about eight and increases the volatility marginally. Another attractive aspect of biodiesel is “recycling carbon dioxide.”

One of the economically important plant *Jatropha curcas* has been identified as potential source of biodiesel production. It can grow in tropical and subtropical zone of the world. Due to its rapid growth and high seed productivity, the plant has been considered as an appropriate candidate for biodiesel. The seeds of *J. curcas* are the primary source of oil because they

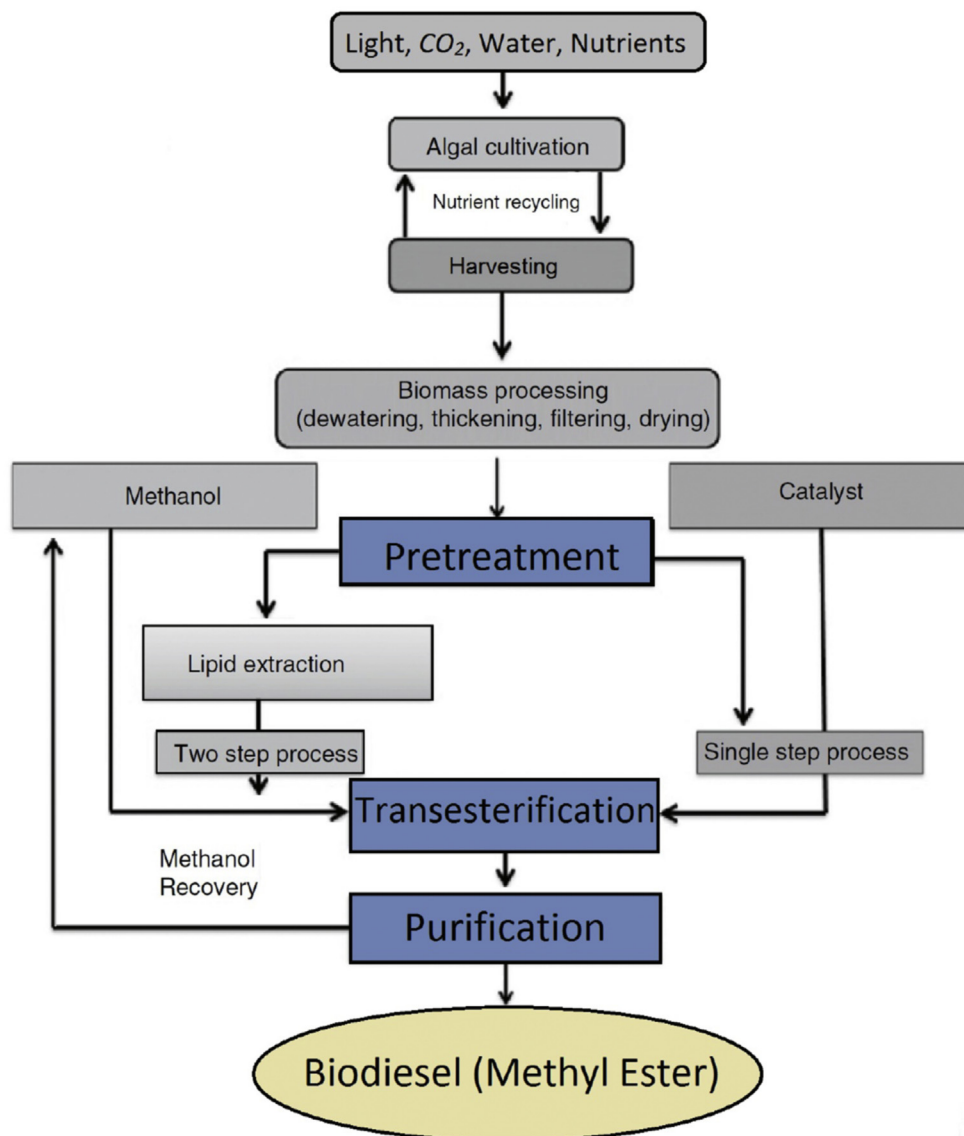


Fig. 3 – Biodiesel production by algal cultivation (adapted from Ref. [87]).

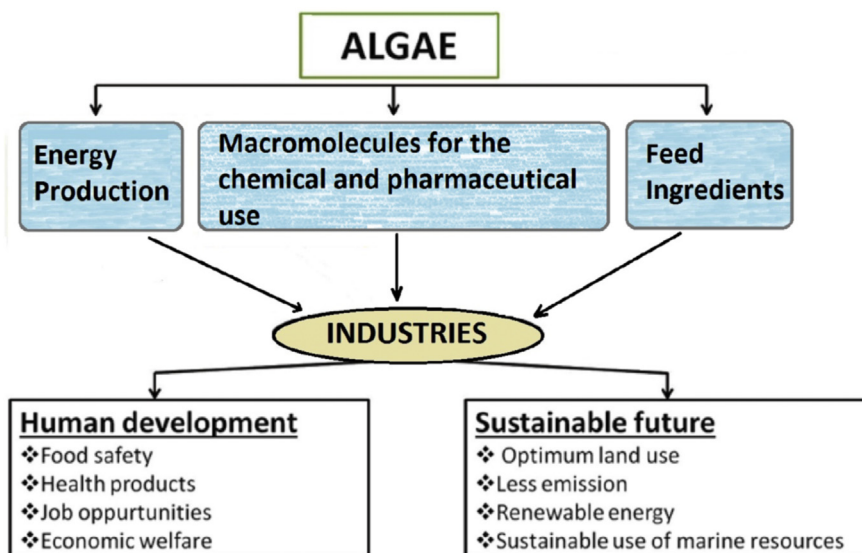


Fig. 4 – Potential applications of algae in a biorefinery concept (adapted from Ref. [99]).

contain; 6.2% of moisture, 18% of protein, 38% of fat, 17% of carbohydrates, 15.5% of fiber and 5.3% of ash [86].

The third generation of biofuels is microalgae, which is overcoming disadvantages of the first and the second generations of biofuels [4,88,89]. Nowadays, photosynthetic microalgae appear to be the best candidate to meet the global energy demand (Fig. 4). It has been estimated that microalgae have a capacity to produce biodiesel 200 times more efficiently than traditional crops, as microalgae can harvest from several hours to ten days of cultivation [4,90]. Harvesting of the microalgae is easier and faster process than that of land plants for biofuel production, and high quality agricultural land is not necessary for microalgae biomass production [91]. Microalgae use light energy to convert carbon dioxide into organic compounds more efficiently than higher plants [12], suggesting they are a superior source for production of biofuels [92]. Microalgae, such as *C. protothecoides*, may contain 55% of lipid when grown heterotrophically under nitrogen limitation [93]. Another green colonial microalga *B. braunii* 765 produces biodiesel, hydrocarbons and biocrude oil under 25 °C [94]. *Chlorella minutissima* produces more lipids at 25 °C when cultured in basic medium. The species of *C. minutissima* UTEX2341 is also a source of biodiesel because of the presence of C16 and C18 lipids in its body [95]. The process of biofuels production from microalgae is a complex process. It follows several steps such as cultivation, harvesting, drying, cell disruption, lipid extraction for biodiesel production and transesterification hydrolysis, fermentation distillation for bioethanol production [96]. Microalgae can be divided into five main groups [97]: blue-green algae (Cyanobacteria), green algae, Diatoms, Red algae, and Brown algae.

Among them, cyanobacteria are likely the most dominant for biofuels production. Many species of microalgae can convert lipid to biodiesel by transesterification. The biodiesel derived from microalgae is similar to petroleum diesel on the basis of viscosity and density [90]. These groups of microorganisms need the usage of chemicals unlike higher plants that need herbicides and pesticides for their better yield. Another

feature of microalgae is the abundance of proteins and carbohydrates that can be processed for methanol and alcohol fuel production [98].

## Conclusion

While the global energy demand is increasing, fossil energy has the continuing weaknesses with the significant risk to environment. The amount of fuel globally consumed as well as the demand is expected to grow rapidly, and the use of fossil energy causes significant problem and harmful impact to the environment on earth. The current global energy crisis has brought a significant attention in the world. Renewable energy sources are critical to solve the global energy issue. Biofuels are an excellent example of renewable energy that can be produced using biological organisms and reduce the dependence on fossil fuels. Photosynthesis is able to increase the amount of plant and algal biomass using atmospheric carbon dioxide on the large scale. Therefore, biofuels – biomass-derived fuels – are based on photosynthesis and might be the key to meet demands of energy being eco-friendly and cost-effective [100].

Recent report showed that targeted modification in central carbon metabolism, such as overexpression of isocitrate dehydrogenase and deletion of glutamate synthase, can effectively enhance ethylene production in *E. coli* [101]. Alkane production is observed in *Synechococcus* sp. PCC 7002 by heterologous expression of an acyl–acyl carrier protein reductase and an aldehyde decarbonylase [102]. Cultivation of biofuel-producing microalgae demands favorable environmental conditions, such as suitable light, temperature, nutrients, salinity, and pH [103]. Although applying environmental stress can increase the production of biofuel, it generally at the expense of decreased biomass yield. The balance between biomass accumulation and biofuel productivity is critical.

Currently it is still problematic and challenging for biofuel to be commercially competitive over fossil fuel. It is proposed that biofuel production may be combined with nitrogen-rich,

municipal waste water and CO<sub>2</sub>-rich flue gas treatment to be more sustainable and cost-effective [104]. To develop new strains with commercial potential, it is necessary to combine multiple genetic engineering approaches to optimize biofuel production [105]. To address the energy crisis, the global collaborative efforts are essential for transforming biofuels into our current energy system. These collaborative activities will facilitate the cultivation methodology development and technology innovation of biofuel production, including fundamental mechanistic research, cell growth facility construction, genetic engineering of algae strains, and biofuel production condition optimization. Comprehensive investigations of photosynthesis mechanisms for biofuel production under diverse conditions are vital as these efforts provide specific information on the optimization of growing the biomass of phototrophic organisms. Biofuel may provide an important role in transportation sector, although biofuels alone cannot fulfill the global demands. Based on current progress and advances in the field of biofuel production, we believe that the large-scale production of biofuels is urgent and achievable to meet our energy need in the future.

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