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Review Article

Review: Biofuel production from plant and algal biomass

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ABSTRACT

Biofuels are the promising alternative to exhaustible, environmentally unsafe fossil fuels. Algal biomass is attractive raw for biofuel production. Its cultivation does not compete for cropland with agricultural growing of food crop for biofuel and does not require complex treatment methods in comparison with lignocellulose-enriched biomass. Many microalgae are mixotrophs, so they can be used as energy source and as sewage purifier simultaneously. One of the main steps for algal biofuel fabrication is the cultivation of biomass. Photobioreactors and open-air systems are used for this purpose. The formers allow the careful cultivation control, but the latter ones are cheaper and simpler. Biomass conversion processes may be divided to the thermochemical, chemical, biochemical methods and direct combustion. For biodiesel production, triglyceride-enriched biomass undergoes transetherification. For bioalcohol production, biomass is subjected to fermentation. There are three methods of biohydrogen production in the microalgal cells: direct biophotolysis, indirect biophotolysis, fermentation.

Contents

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Introduction

To maintain all vital processes at the proper level human organism needs about 100 J per second (Fig. 1a). Human body receives the energy from food. About 30 times more energy is used on average to make our life more comfortable [1,2]. It puts challenges for humanity: search of energy sources, development of energy production techniques, and construction of infrastructure to facilitate energy use.

It is possible to define three general areas of energy consumption (Fig. 1b):

- Maintenance of electrical devices;
- Fuel for vehicles or motorized instruments;
- Space heating, maintenance of cooking systems.

Contemporary energy faces with problem.

World population is rising. In addition, the number of power devices per head is rising, too. Hence, worldwide

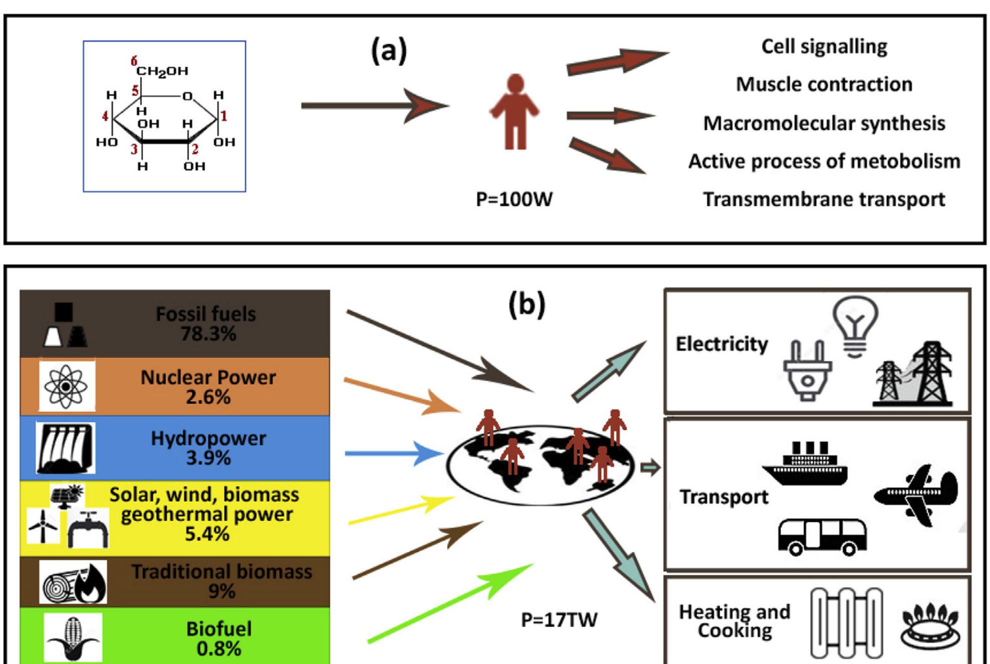


Fig. 1 – External energy sources and its scopes of application for (a): the human body; (b): the human community. P – power consumption. Data from Refs. [1,2].

energy demands grow. Intensities of energy raw production and processing and energy use grow with energy demand growing. Using traditional energy resources to meet contemporary energy demands is associated with environmental risks. The main energy source for the humanity is fossil fuel (coal, oil and natural gas) which is the result of many centuries of living organisms' residues decomposition.

The usage of this energy source has several disadvantages:

- Conversion of this fuel into electricity, fuel combustion or engine work on its derivatives is accompanied with significant CO_2 emission to the environment, that is the cause of climate change. In real conditions, there is no final fuel combustion, and besides CO_2, significant amount of other harmful gases is released into the atmosphere [3,4].
- Some harmful compounds, carcinogens and poisons are released to the atmosphere while using petroleum fuels.
- Fossil fuel is an exhaustible resource (the rate of its formation in natural processes is incomparable with the rate of its consumption) [5–7]. This problem looks different on the background of expected increase of energy consumed: the energy demand will increase by 50% between 2020 and 2030 years [8].

Almost 80% of consumed energy all over the world came from fossil fuels. Of this part, about 58% is accounted for the transportation sector [9].

In the field of electrification, hydraulic power industry (Canada, USA, and China) and nuclear power engineering (USA, France) significantly compete with fossil fuels [2]. The former, in spite of its seeming environmental friendliness and resource renewability, negatively affects the environment: while constructing the hydropower plant, flooding of big land areas occurs, and that perniciously affects the whole biocenosis. Nuclear power engineering is connected with considerable risks of ecological and humanitarian disasters in case of probable failure of any of the reactors.

Nowadays, all over the world the attention is focused on the alternative energy sources due to the above-mentioned energetic problems. As the global and national level, scenarios of alternative energy sources use are developing. There are several ways to obtain energy quite different in its nature: geothermal engineering, solar power engineering, wind power engineering, and bioenergetics [2].

Biofuel production is promising trend of alternative energy, exactly in transport sector.

Biofuel is a substance with large value of heat of combustion (biohydrogen, biodiesel, bioethanol, biomethanol) obtained from biomass [8,10].

All the organic material on the planet arising from the life-sustaining activity of organisms is called biomass. This concept includes wood, agricultural crops, water plants, forests, vegetable and animal remnants, microbial cultures. Proteins, lipids and carbohydrates are the main components of dry biomass [11]. Correlation of these components depends on the type of organism, the part of body, and the conditions of cultivation [11,12]. Not all the biomass is used for fuel production.

Three main components of biomass are the molecular precursors of biofuel [12]:

a) vegetable oils or animal fats (triglycerides);
b) starch and oligomeric sugars;
c) lignocellulose.

Fats are necessary mostly for biodiesel synthesis. Alcohols and biohydrogen are obtained from starch and sugars via fermentation process. Also, in accordance with treatment technique, alcohols and biodiesel can be produced from lignocellulose [12].

People have learned to use biomass to satisfy their energy needs long time ago. Wood burned in bonfires for heating or thermal processing of food belongs to the so-called traditional biomass [2]. The usage of biofuel in engines has been considered since the beginning of mechanical engineering: instead of petroleum diesel, biodiesel has been the first fuel for diesel engines [13]. However, at the beginning of XX century biofuel production could not compete with the cheap fossil fuels, which was easy to extract. Petrochemistry became the main direction in fuel power industry, which influenced the development of mechanical engineering. Now, due to the increased attention to the problems of fossil fuels, the opinion that environmentally friendly biofuels are a good substitute for fossil fuels is becoming more popular [14].

Biofuels has three general advantages in comparison with fossil fuels:

- Biofuel is renewable resource [8–10];
- Fewer toxic compounds are released into the atmosphere during the combustion of biofuel [9,10];
- There is no CO_2 emission into the atmosphere observed: organisms, which produce biomass, then absorb the greater part of released CO_2 [8–11].

The latter point should be explained. During the combustion of fuel, the greenhouse gases emission is rather intensive process. CO_2 assimilation by photosynthetic organisms occurs considerably slower. This fact should be taken into account while producing and using biofuels [11].

In addition, switching to liquid biofuels does not require significant changes in fuel infrastructure, unlike the transition to electric vehicles [15]. Biofuel can serve either as an addition to the traditional engine fuels or main fuel in motors [2]. For example, in Brazil the method of mixing the gasoline with ethanol produced from sugar biomass is widely used [11–14].

Further, attention will be focused generally on plant or algal biomass. Exactly photosynthetic organisms (plants, algae, and some bacteria) are of great interest for biofuel production. It is associated with the fact that the photosynthetic organisms are less demanding than the autotrophic organisms. The former organisms increase the biomass owing to atmospheric CO_2, while the latter ones need carbon substrate. Plant biofuels production (so-called biofuels of first and second generation), have a one big obstacle: competition with agriculture for cropland [9]. In addition, plant harvesting takes place not more than two or four times per year. It implies the limitation of plant biofuel production. Also plant biomass growth requires ensuring of optimal condition, and its further processing can include energy-intensive methods.

Algal biomass does not have this obstacle. Also, the rate of the microalgae biomass increasing is higher than that of plants. However, the growing of algal biomass requires the special complex cultivation system, bioreactors [10]. So far, the very difficult work is needed to optimize algal fuel production. On the one hand, it involves finding a stable and efficient algal strains or mutant strains creation. Such strains should give the maximum yield of the desired product in the shortest growing season. They should have a maximum resistance to stress factors. On the other hand, there should be identified the optimal conditions for culturing and processing of algal biomass. Bioreactors, must allow maintaining such conditions at the proper level. Eventually, the entire life cycle of algae must be directed solely to the production of the desired product. Nevertheless, algal energy has considerable potential. Now there are some technical and scientific obstacles for its development, but they can be overcome.

This article presents a current state of plant and microalgal energy. Different kinds of biofuel (alcohol, diesel, hydrogen) and methods of their fabrication are considered.

Biofuel generations

According to the way of biomass usage, biofuels can be divided to the primary and the secondary ones [16] (Table 1). The primary biofuel is the biomass without any additional treatment: wood, wood chips, animal fats, residues of forest and agricultural crops. The fuel of that kind is usually used for heating, cooking and agricultural needs and is widespread in Third World countries. Primary biofuels are also called traditional biomass [2]. On the one hand, this fuel does not require resource expenses for its processing; on the other hand, the field of its application is rather small. For the year 2013, energy obtained from the traditional biomass has reached about 9% of all energy consumed in the world. The secondary biofuel is produced from biomass (the primary biofuel) by extraction of the most energy-intensive substances (biohydrogen, biomethanol, biodiesel), which can be used to substitute ubiquitous fossil fuel (Table 1).

In its turn, the secondary biofuel can be divided into three generations: the first, the second and the third [9,16,12]. This division is based on the general feedstock for fuel production, the processing methods, and historical sequence of the fuel's appearance on the world energy market.

The first generation

Biomass of food crops enriched with sugars, starch (stems of sugar cane, sugar beet) and oils (soybeans, sunflower seeds, rapeseeds) is a feedstock for production of bioalcohols and biodiesel of the first generation (Table 1). The simple and comparatively cheap method of treatment – microbial fermentation – is the advantage of the production of this fuel. But there are several problems concerning this biofuel [12]:

- Low productivity of cultivated lands: only insignificant part of grown biomass is used for biofuel production.
- Competition with food processing industry for the usage of lands.

Table 1 – Classification of biofuels. Adapted from Refs. [9,16].

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

The second generation

Biofuels of the second generation are produced from lignocellulosic biomass (Table 1). Lignocellulose is highly presented in plant biomass in comparison with oils and starch; it is the main component of cell walls. Lignocellulose consists of three components: cellulose (40–50%), lignin (15–20%), and hemicellulose (25–35%) [12]. All of these components are of certain value for people, but the process of extraction of particular component, especially of cellulose, is rather difficult.

Lignin is an amorphous polymer, which consists of phenylpropane structures. It provides cellulose stiffness and stability against many hydrolytic enzymes. Lignin can be removed by dissolution in alkaline-alcohol solutions. Lignin per se can be burned to obtain heat or electricity; also, it is possible to extract useful chemical compounds from it by chemical treatment.

Hemicellulose is an amorphous polymer containing several different types of sugars: D-xylose, L-arabinose, D-galactose, D-glucose, D-mannose. It is possible to hydrolyze hemicellulose to obtain monomeric sugars for following fermentation of them to alcohols.

Cellulose is a straight glucose polymer with certain rigidity that impedes its hydrolysis [12].

Advantages of lignocellulose-enriched biomass:

- The efficiency of land use: the major part of biomass is used for biofuel synthesis compared to biofuel of the first generation.
- The impact on food sector is lower at the expense of usage of non-food crops.

The difficulty of treatment techniques is the disadvantage of this type of biomass [10].

The third generation

The third generation of biofuel is connected with algal biomass (Table 1). The use of algal biomass for fuel synthesis is relatively new direction of bioenergetics. According to the data of different investigations, algal biomass can accumulate considerably high amount of lipids in comparison with biomass of oil plants. This fact allows considering algae as a good source for biodiesel production [17,18]. The main difference between algal bioenergetics and plant bioenergetics is in the technology of biomass up-building. Plants do not require additional methods, besides growth techniques already known from agriculture, and a special effort for creation of certain conditions. On the other hand, plant bioenergetics requires the usage of valuable resource (arable lands), and provides relatively low yield in a ratio of the organic feedstock mass to the mass of biofuel synthesized. Microalgae can grow in conditions, which are unsuitable for plant growth: saline soils, waste water. So the usage of microalgae is being considered as an interesting potential feedstock for biofuels production [19,20]. Nevertheless, algae require special systems of growth – bioreactors, their construction depends on the species of alga grown.

Algal biomass

In this article, we generally focus on the usage of algae as a source of biomass.

Algae

Algae are the group of organisms, which are similar in their morphological and physiological features (ability of photoautotrophic metabolic pathways, existence predominantly in water, the body is presented by thallus – the body without a clear division between the organs) [9,10]. Algae constitute the polyphyletic group of organisms, i.e. this group connects the species relatively distant from each other: eukaryotes (red algae, green algae, brown algae, diatoms) and prokaryotes (cyanobacteria) [21].

Different algae perform various ways of metabolism. Several algae are capable of switching the metabolic pathways under the changing of environmental conditions. The metabolic pathway is determined by three factors: the source of carbon for the synthesis of sugars, the source of energy for ATP synthesis, and the source of electrons for the biologically important redox reactions. Organisms capable of assimilating the carbon from the atmospheric CO_2 (autotrophy) and from organic molecules (heterotrophy) in accordance with environmental conditions are called mixotrophs. Many of algae are mixotrophs [10,22]. The pathway of ATP synthesis can be phototrophic or chemotrophic depending on the source of energy.

In the former case, the organism absorbs the energy of electromagnetic waves. In the latter case, the organism obtains the energy while splitting high-energy compounds. There are heterotrophic organisms, which are capable of absorbing the energy of the sun, – photoheterotrophs; and there are autotrophic organisms, which obtain the energy from redox reactions, – chemoautotrophs.

The algal ability of mixotrophy could be useful for the producing of the third generation biofuel. Microalgae are able to fix CO_2 not only from the atmosphere, but also from exhaust fumes and soluble carbonates. This fact positively characterizes them in terms of biomass cultivation convenience. Also, microalgae convert light energy into chemical bonds energy more efficiently than higher plants do [23].

Different algae have a different proportion of proteins/carbohydrates/fats. For example, *Spirulina maxima* has 60–71%w/w of proteins, *Porphyidium cruentum* has 40–57%w/w of carbohydrates, *Scenedesmus dimorphus* has up to 40%w/w of lipids [10]. Some species of green algae such as *Botryococcus braunii* and *Chlorella protothecoides* also contain high levels of terpenoid hydrocarbons and glyceryl lipids which can be converted into shorter hydrocarbons as major crude oil [24]. Also, composition of biomass depends on the growing conditions. Depending on the prevalence of lipids or sugars, algae biomass can be a good raw material for the production of biodiesel or bioalcohol respectively. Therefore, microalgae can be a good source of biofuel under certain conditions [25].

It is possible to determine the following advantages of microalgae as a feedstock for biofuel synthesis [10]:

- Microalgae synthesize large amount of neutral fats (20–50%) from the dry mass;
- The products from microalgae can be obtained throughout the year in contrast to terrestrial plants;
- Microalgae require less water than terrestrial plants;
- Microalgae absorb a lot of CO_2 (1 kg of dry mass absorbs approximately 1.83 kg of CO_2) that suggest to grow them near the thermal power stations and other sources of greenhouse gases;
- Microalgae absorb ammonium ions NH_4^+, nitrate anion NO_3^-, and phosphate anion PO_4^- from the environment, that allows to consider them as the wastewater purifiers;
- Many of algae are possible to be grown in conditions unfavorable for terrestrial plants, so they do not compete for the arable lands with food crops [10];
- It is possible to extract various chemical compounds from algal biomass, which are useful for different fields of human activity [23].

The biofuel production at the expense of algae includes following steps:

1) Microalgae cultivation increase of algal biomass under special conditions in bioreactors or under natural conditions in open ponds [26];
2) Biomass collecting using various techniques of biomass filtration from the medium [26];
3) Mechanical or chemical pretreatment of biomass (drying, disintegration) [26];
4) Thermal or chemical biomass-to-biofuel conversion;
5) Purification of the biofuel.

Cultivation

One of the most important stages of development of algal biomass from an object of investigations to the competitive product is the design of cheap and effective systems of microalgae cultivation. The obligatory element of such system is the medium, in which the organisms are developing and reproducing. For the efficient biomass increase of the desirable content, it should contain sufficient sources not only of carbon, but also of various necessary mineral elements: nitrogen and phosphorus in significant amounts, and magnesium, calcium, chlorine, silicon, manganese, iron in smaller amounts. Generally, nitrates are the source of nitrogen, but it is possible to use ammonium and urea [10].

The systems of microalgae cultivation should have the following parameters:

- high productivity;
- low cost of production and maintaining;
- simplicity in parameters control (temperature, pH, O_2, turbulence);
- reliability.

The systems of microalgae cultivation can be roughly divided in two large common types: open-air systems (ponds) and close systems (photobioreactors) [10].

Open-air systems

The largest industrial systems of algae cultivation are included in open-air systems. It is connected with the simplicity and cheapness of their designing and maintenance; duration of their usage and efficiency of biomass increase is higher than in many types of bioreactors. In all types of open-air systems excluding natural ponds, mechanism of medium stirring is important. For example, paddle wheel and pivoted agitator are the integral elements in a raceway pond and a circular pond, respectively (Fig. 2).

There are different types of open-air systems [10]:

1) Natural and artificial ponds. They can be used for microalgae cultivation only in case of proper climatic conditions and in the presence of necessary elements in water in terms of certain salinity and pH. For example, in the lakes near the Lake Chad the yield of cyanobacterium *Arthospira* reaches 40 tons per a year [27].
2) Raceway ponds, a system of several loopback ponds, each of them reminds a raceway in shape. In the special place there is a paddle-wheel that causes the flow of water [10] (Fig. 2a).
3) Circular ponds, artificial cylindrical shallow ponds with the system of stirring at the expense of central rotor. They have a certain limitation in size: the pond of a big diameter no longer pays back because of energy expenses for rotor rotation [28] (Fig. 2b).
4) Inclined ponds (cascade system), inclined surface, at which a thin layer of medium comes down. Stirring is created by turbulence caused by the gravity [10].

The main disadvantages of open-air system are the sensitivity to weather, season and time of day; temperature control, which is difficult to implement; illumination; pollution with heterotrophic organisms, which decreases the efficiency; and the lack of opportunities to monocultural cultivation. As a result, obtained biomass is heterogeneous [10].

Photobioreactors

Photobioreactors are the close systems, which enable to grow certain species of microalgae under specific constant conditions. Frequently, these conditions differ from environmental ones; the energy is necessary for its maintenance. While

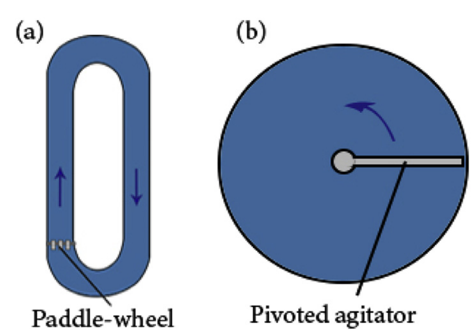


Fig. 2 – Open-air cultivation systems with stirring mechanism. (a): raceway pond with paddle-wheel; (b): circular pond with pivoted agitator.

constructing bioreactors, attention should be paid to the following [10]:

a) Available light for photosynthetic algae, which is sufficient for optimal photosynthesis and do not cause photoinhibition;
b) Supply of CO_2, which is maximal for biomass synthesis, but do not lead to CO_2-inhibition effects. Its concentration ranges approximately from $2.3*10^{-2}$M to $2.3*10^{-4}$ M.
c) Providing of necessary medium stirring. It increases the supply of nutrients and light to all the cells. Without the stirring, cells near the surface would absorb more light, and cells at the center would experience light starvation;
d) Reliability of the construction.

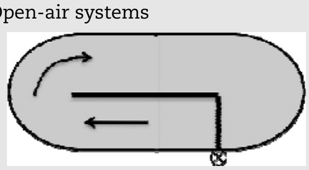
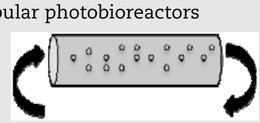
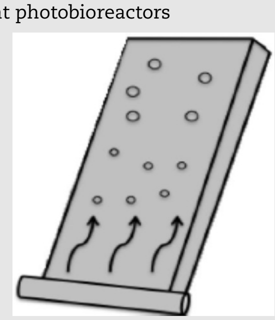
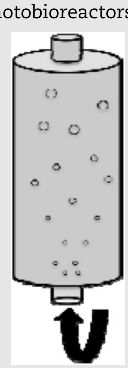
The advantages of bioreactors in contrast to open-air system [10] (Table 2):

- The control of almost all biotechnologically important parameters (pH, temperature, atmosphere content);
- Decrease of the risk of pollution;
- The opportunity of growing microalgae of one particular species without any impurities;
- flexible technical design

There are following types of bioreactors:

1) Tubular PBR – the system of transparent or opaque (with interior illumination) pipes, in which the medium flows with the help of pump. The pipes are connected with the systems of gas and heat exchange. In accordance with the form of pipes, bioreactors can be horizontal, serpentine, vertical, inclined, conical-shaped and helical. The largest photobioreactor of that type (about $700M^3$) is situated in Germany. Nevertheless, the maximal productivity of 25 g/

Table 2 – Comparison of advantages and disadvantages of different culture systems. Adapted from Ref. [10].

Culture systems	Advantages	Disadvantages
Open-air systems 	• Relatively economical; • Easy to clean up; • Easy maintenance; • Utilization of non-agricultural land; • Low energy inputs.	• Little control of culture conditions; • Poor mixing, light and CO2 utilization; • Difficult to grow algal cultures for long periods; • Poor productivity; • Limited to few strains; • Cultures are easily contaminated.
Tubular photobioreactors 	• Relatively cheap; • Large illumination surface area; • Suitable for outdoor cultures; • Good biomass productivities.	• Gradients of pH, dissolved oxygen and CO_2 along the tubes; • Some degree of wall growth; • Requires of large land space; • Photoinhibition.
Flat photobioreactors 	• Relatively cheap; • Easy to clean up; • Large illumination surface area; • Suitable for outdoor cultures; • Low power consumption; • Good biomass productivities; • Good light path; • Readily tempered; • Low oxygen build-up; • Shortest oxygen path;	• Difficult scale-up; • Difficult temperature control; • Some degree of wall growth; • Hydrodynamic stress to some algal strains; • Low photosynthetic efficiency.
Column photobioreactors 	• Low energy consumption; • Readily tempered; • High mass transfer; • Good mixing; • Best exposure to light–dark cycles; • Low shear stress; • Easy to sterilize; • Reduced photoinhibition; • Reduced photo-oxidation; • High photosynthetic efficiency.	• Small illumination surface area; • Sophisticated construction materials; • Shear stress to algal cultures; • Decrease of illumination surface area upon scale-up; • Expensive.

m^2d was obtained in serpentine bioreactor, which is rather small (10 m^3) [29].

2) Flat PBR. The first bioreactors were of that type. Advantages: the largest area of illumination, and, as a result, maximal photosynthetic activity of organisms. Here, the thin layer of medium flows through the flat transparent surface [30]. The surface can be vertical or inclined. But these bioreactors have low capability of temperature control compared with tubular ones.
3) Column PBR. Transparent vertical columns with medium are aerated from the bottom [31]. It is easy to control the conditions of growth in such photobioreactors.

Table 2 lists the advantages and disadvantages of different types of photobioreactors.

Energy conversion process

After the stage of cultivation and preparation of biomass, the stage of actual conversion of biomass into biofuels comes. Regardless of the biomass nature, several techniques of its conversion to the biofuel can be outlined. In general, it is possible to divide them into three groups different in their cost, purity of end-product and environmental friendliness [10,19]:

a) Biochemical approaches (hydrolysis, fermentation). They are operating with chemical processes occurring in living cells;
b) Chemical approaches (hydrolysis, transesterification), which means the providing of the certain reactions in the presence of catalyst aimed to obtain desired product;
c) Thermochemical approaches (pyrolysis, gasification, liquefaction), which means the treatment of feedstock under the high temperature and pressure in order to obtain the compounds with the low molecular weight and low O_2 content;
d) Direct combustion in order to obtain the heat or to convert the heat energy to electroenergy.

This division is also applicable to the biofuel production from algal biomass (Fig. 3).

Biological treatment of biomass is usually more complex, expensive and has longer reaction time, but it is selective: quite small amounts of chemical compounds are obtained during biological processing. The production of some compounds is not possible with thermochemical methods, but only at the expense of chemical or biochemical methods. During thermochemical treatment, crude mixture of a large number (up to several hundred) of different compounds with different power characteristics is produced. Reaction times of thermochemical processing are less than those of biochemical processing [10,12].

In the next chapters, we will look at the biochemical and chemical methods of producing hydrogen, bioalcohol and biodiesel. In this chapter, we briefly describe thermochemical methods.

Pyrolysis is an anaerobic decomposition of biomass at the temperatures from 650 to 800 K. The substance proceeds in a vapor phase, and then condenses into the mixture, which contains more than 350 various components of low molecular weight: organic acids, aldehydes, alcohols, sugars, esters, ketones, aromatic compounds. This mixture is called bio-oil. Pyrolysis is an effective pathway of lignocellulose biomass decomposition [12].

Gasification is a treatment of biomass for temperatures above 1000 K. During the gasification, the gas mixture is formed including CO_2, CO, H_2, CH_4, N_2 and other compounds in its content. From this mixture, it is easy to obtain the syngas – the mixture of CO with H_2 [12]. An important characteristic of syngas is the ratio of the concentration of carbon monoxide and of molecular hydrogen, respectively. This ratio may vary in the presence of water in the water–gas shift reaction [32]:

$$CO + H_2O \leftrightarrow CO_2 + H_2 \quad (1)$$

Known technology of further conversion of syngas is the Fischer–Tropsch synthesis. The final products of the Fischer–Tropsch synthesis (equation (2) and (3)), a mixture of alkanes and alkenes, are called Fischer–Tropsch liquids. From

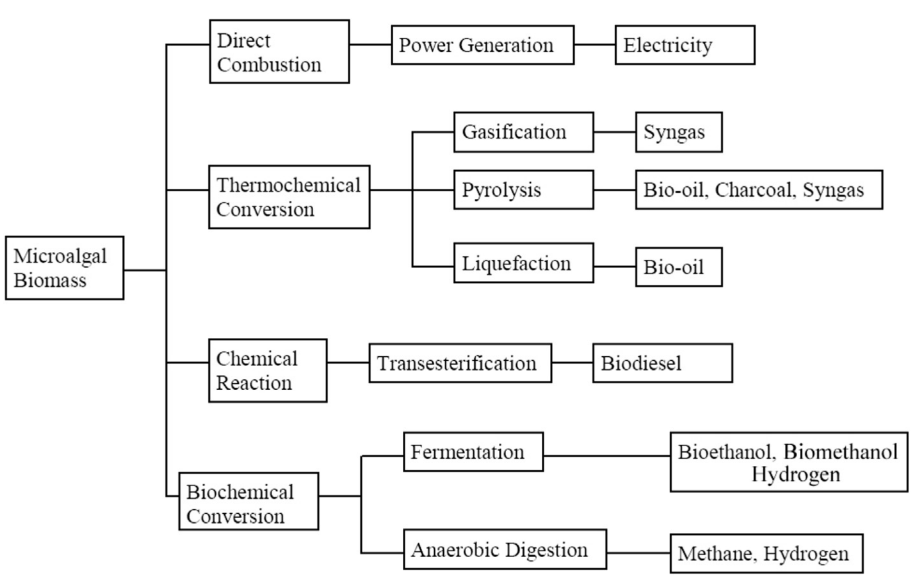


Fig. 3 – Methods of processing microalgae biomass. Adapted from Ref. [19].

them one can get the traditional fuel: jet fuel, clean diesel, gasoline. It is possible to use conventional refining methods, such as cracking and reforming. Fischer–Tropsch synthesis can be expressed in two equations [33]:

$$n\cdot CO + (2n+1)\cdot H_2 \rightarrow C_nH_{2n+1} + n\cdot H_2O \tag{2}$$

$$n\cdot CO + 2n\cdot H_2 \rightarrow C_nH_{2n} + n\cdot H_2O \tag{3}$$

Liquefaction is the catalytic thermal decomposition of biomass to unstable small components, which are then combined to bio-oils. For liquefaction the temperature used is lower in comparison with pyrolysis, but the pressure is higher (5–20 atm). This reaction needs water and basic catalyst [12].

The thermochemical treatment is convenient to distinguish the following four stages: pretreatment, thermal processing, purification and upgrading (Fig. 4). During the pretreatment biomass drying and hydrolytic molecular weight reduction occurs. The resulting raw material is subjected to thermochemical treatment. The resulting crude fuels need to be further purified. Produced chemical fuel can be modified to increase the power intensity and stability (chemical treatment, during which the octane rating of the fuel increases) [12].

Liquid biofuel productions

Nowadays, bioalcohol and biodiesel are considered to be the most likely non-fossil alternative fuels. Bioalcohols are produced from the biomass by fermentation of starches and sugars isolated from corn, wheat, sugarcane, sugar beets, molasses, potato, fruit waste and also from microalgae [9]. Biodiesel consists of monoalkyl esters derived from different oil-rich sources via transesterification process [34]. Production of biodiesel and bioethanol is most developing shares of bioenergy: in year 2012 global production of bioethanol and biodiesel was about 83.1 billion and 22.5 billion liters respectively [35].

Bioalcohol

Bioethanol, biomethanol, biobutanol and biopropanol are the main bioalcohols produced. The most common bioalcohol is bioethanol. The fermentation reactions are caused by microorganisms, which feed on sugar-enriched feedstock [36].

The application of ethanol has been considerably expanded due to the development of purification methods. Ethanol can be used as the substitute of gasoline to reduce CO_2 emission. Furthermore, during combustion, it is possible to reduce the emission of some toxic gases (CO, NO) due to the presence of oxygen in its molecular form [16].

For bioethanol production, there are several feedstocks: sucrose and bagasse from sugarcane, starch from corn, lignocellulosic materials from wheat straw [37] (Table 2). Bagasse can be used for ethanol production, because its production reaches the amount of several hundred kilograms per ton of sugarcane [38,39]. The corn rich in starch is also used as a source of bioethanol via fermentation and distillation reactions.

Moreover, algae being a good source for biohydrogen production can be another source of bioethanol as a substrate for fermentation processes. In this process, bacteria, yeast or fungi can use microalgal carbohydrates (glucose, starch) and

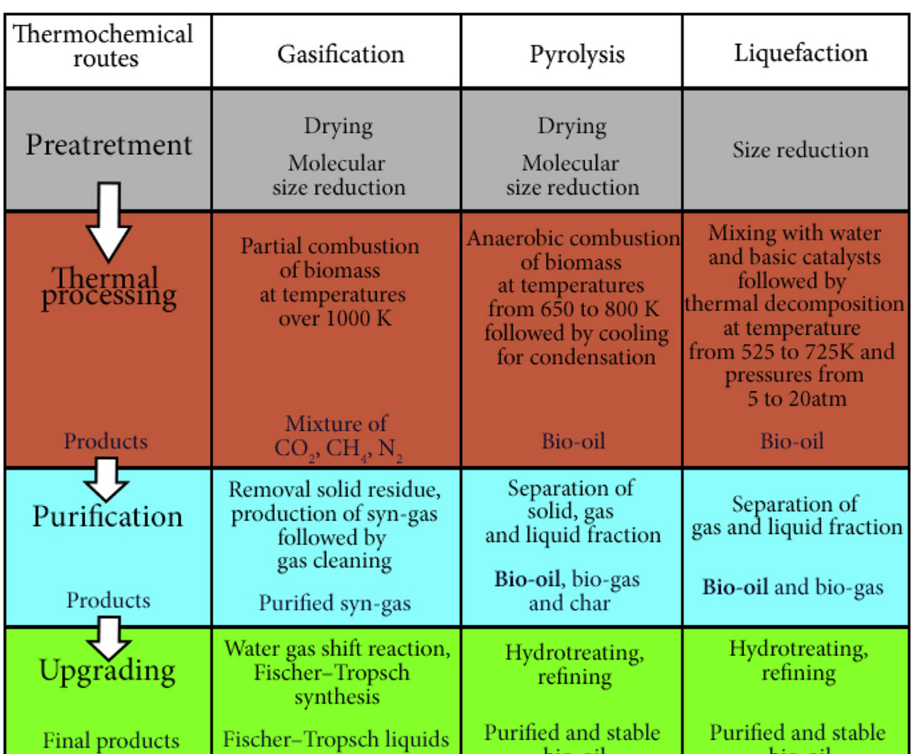


Fig. 4 – The sequence of steps of thermochemical processing of biomass and description of each of these stages for gasification, pyrolysis and liquefaction [12].

proteins as carbon sources [40]. For example, some microalgae, such as *Chlorella*, *Chlamydomonas*, *Dunaliella*, *Scenedesmus* and *Spirulina* were found to accumulate significant amounts (>50%) of starch and therefore considered to be the potential feedstock for bioethanol production [34,41,42]. Microalgae can assimilate cellulose, which can be further fermented to bioethanol [34,43]. Also, microalgae can perform self-fermentation processes to produce bioethanol. As it has been reported, the marine green algae *Chlorococcum littorale* managed to produce 450 μmol(ethanol)*g^{-1} at 30 °C via dark fermentation [44].

The process of fermentation requires less consumption of energy. Moreover, CO_2 produced as by-product during this process can be used as carbon sources for microalgae in cultivation process and therefore reduce the emission of greenhouse gases [10].

The bioethanol production via fermentation of microalgal biomass has several advantages [34]:

- It can use the whole biomass or microalgal leftover from oil extraction;
- It occurs in an aqueous medium; therefore, the biomass requires no drying and can be concentrated simply by settling.

Besides that, the cell disruption methods can at the same time disrupt complex sugars necessary for yeast fermentation [34].

High carbohydrate content, cellulosic cell walls and starch based cytoplasm make algal biomass a good feedstock for bioethanol production. Since lignin removal is a rate-limiting step for lignocellulosic substrate, an absence of lignin in algal biomass reduces the costs, time and difficulty of the saccharification process [45]. Methanol is one of the most industrially significant alcohols that can be used as a clean fuel or as an addition to gasoline [15]. Currently, the methanol production is based on the chemical process, which converts natural gas into methanol. This process proceeds in three stages: steam reforming, synthesis and distillation process. Moreover, methanol can be easier recovered than ethanol, which forms an azeotrope with water. An azeotrope is a mixture of two or more liquids with unchanged composition under distillation. The ethanol-water azeotrope cannot be purified without adding to the mixture an entraining agent – benzene, cyclohexane, heptane [46]. Only then, at low pressure an absolute ethanol can be distilled from the ethanol-water mixture. But these procedures are rather time-consuming and expensive in contrast with the methanol production. Additionally, biomethanol can be produced by gasification and partial oxidation of carbohydrates with O_2 and H_2O [15] and by oxidation reactions in biomass [47].

Biomethanol can be produced by fermentation and distillation of the plant and algal crops containing sugars and starch (Fig. 5). Besides that, biomethanol can be produced from biomass and biodegradable wastes, which can be considered as a potential fuel for gasification and syngas production [48]. The conversion of the biomass into the biomethanol occurs by adding of sufficient amount of hydrogen to the synthesized gas [49].

Also, process of gasification occurs in some microalgae, for example, *Spirulina* sp. In addition, there is an abundance of proteins and carbohydrates in microalgae biomass, which can be treated for methanol production [50]. Nevertheless, biomethanol received less attention than bioethanol due to its corrosive and toxic properties [51].

Biodiesel

Biomass enriched with triglycerides is common crude material for biodiesel production [12]. During transesterification reaction of triglycerides, hydrolysis of ester bond between glycerol and fatty acid chain occurs followed by esterification with methanol. Fatty acid methyl esters (FAME) are produced during transesterification reaction. FAME is major constituent of biodiesel [34]. This reversible reaction is depicted in equation (4).

$$\text{Triglyceride} + 3CH_3OH \xleftrightarrow{\text{catalyst}} C_3H_8O_3(\text{Glycerine}) + 3\text{FAME} \quad (4)$$

Biodiesel can be the way to substitute petroleum diesel fuels. Chemically, biodiesel is close to mineral diesel, but it has lower volumetric heat capacity. Therefore, it is possible to replace the mineral diesel by biodiesel. Also, the viscosity of biodiesel is close to mineral diesel [16,9].

Transesterification of vegetable oils and animal fats with methanol or ethanol is used for biodiesel production [52]. The most commonly used oils are from rape seeds and soybeans. Also, tropical plant *Jatropha curcas* has been considered as potential source for biodiesel production due to its rapid growth and high seed productivity. The seeds of *Jatropha*

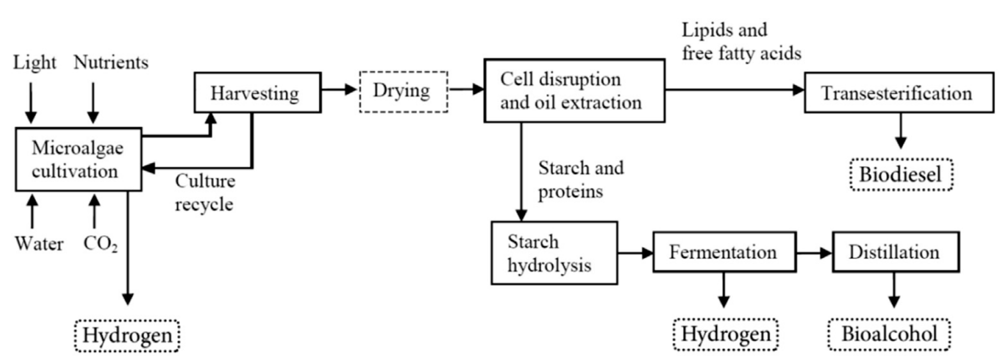


Fig. 5 – Production steps of hydrogen, biodiesel and bioalcohol production from algal biomass. "Drying" is highlighted by a dotted line because in some cases it does not necessary. Adapted from Ref. [10].

contain 38% of fat that makes them a good source of oil [53]. The biodiesel quality depends on the type of feedstock used.

There are several advantages of biodiesel:

- Its production is cheap;
- It is renewable;
- It has reduced toxicity because of biodegradables used for its production and does not produce poisonous fumes [54–56].

Also, biodiesel helps to reduce the risk of global warming due to reducing CO_2 emissions to the atmosphere. Biodiesel releases CO_2 to the atmosphere when burned, but crops used to produce biodiesel take up CO_2 from the atmosphere for their growth processes. It was determined that biodiesel reduces CO_2 emissions by 78.5% compared with petroleum diesel fuel [57].

Algae are the most promising source for biodiesel production. As it has been shown, some species of algae can yield up to 60% of their weight in the form of oil, which can be converted to biodiesel [18]. In this regard, the algae can produce more biodiesel from one kilogram of biomass compared to some plants [58]. For algae growth, it is possible to use either high rate algal ponds or photobioreactors with controlled specific conditions.

Production of biodiesel using algae is shown in Fig. 5. First, under special conditions algae produce biomass. Then, this biomass is subjected for pretreatment, which is followed by transesterification reaction of lipid fractions with alcohols. At this step, the addition of some catalyst is necessary. The substance obtained after this reaction is then purified and finally converted into biodiesel.

As it has been estimated, microalgae are capable of producing biodiesel 200 times more efficiently than traditional crops [10]. After cultivation, microalgae can yield from several hours to ten days [10,59]. Thus, it is possible to obtain large amount of microalgae biomass for biofuels production easier and faster than that of crops. To achieve that, big areas of agricultural land and addition of great amount of herbicides and pesticides are not necessary [60].

As a significant source for biodiesel production, microalgae have some important features. For example, microalga *C. protothecoides* may contain 55% of lipid when growing under nitrogen limitation [61], *Chlorella minutissima* yields larger amount of lipids at 25 °C in basic medium [62]. Also, green colonial microalga *B. braunii* 765 can produce biodiesel under 25 °C [63].

Furthermore, as it has been shown, microalgal biodiesel has similar physical and chemical characteristics to petroleum diesel and biodiesel from oil crops [9,10].

Photobiological hydrogen production

Nowadays, there is a great demand for hydrogen in the world. According to the data for the year 2011, the consumption of hydrogen has reached 900 billion m^3. For the present day, 96% of all hydrogen is obtained from fossil fuels: 48% from natural gas, 18% from coal, and 30% from other hydrocarbons-containing fossils. In the future, the application of hydrogen as an alternative for fossil fuels could be of significant importance, since the reserves of fossils are being exhausted [64].

One of the ecologically friendly ways of renewable energy obtaining is the production of molecular hydrogen using microorganisms [65–67]. There are several microorganisms commonly used for this process: algae, bacteria and archaea. In this article, the attention will be focused on the usage of photosynthetic microorganisms for hydrogen production.

The clear advantage of photosynthetic hydrogen generation is zero emission of greenhouse gases; therefore this approach is rather promising [68]. Nevertheless, there are several factors limiting hydrogen photoproduction yield:

- Light absorbance of phototrophic organisms is low under regular solar radiation [69–71];
- The high amount of ambient oxygen has an inhibitory effect on hydrogen production enzymes [72–74];
- The rate of photosynthetic CO_2 assimilation is rather low.

Many photosynthetic organisms are able to produce hydrogen. In the late 1990s, A. Melis et al. discovered that during photosynthesis the culture of green algae *Chlamydomonas reinhardtii* could switch from the production of oxygen to the hydrogen production under conditions of sulfur deprivation in the medium [75]. They found that the function of the special enzyme responsible for this reaction (hydrogenase) was impaired in the presence of oxygen, but depleting the amount of sulfur in the medium interrupted internal oxygen flow. This condition allowed the hydrogenase to react, and the algae could produce hydrogen [75].

Thus, specific conditions are necessary for the cultures of photosynthetic microorganisms to produce hydrogen efficiently: full or partial anaerobiosis [76,77], nutrient starvation [75,78,79] and also some other factors leading to the enhancement of respiration, fermentation and a partial loss of photosynthetic activity.

There are several approaches of biohydrogen generation:

- Splitting water to hydrogen and oxygen by green algae and cyanobacteria – direct biophotolysis [80] (Fig. 5);
- Photodecomposition of the accumulated biomass enriched by carbohydrates during the process of photofermentation by photosynthetic bacteria – indirect biophotolysis [81,69];
- Dark fermentation of organic compounds (Fig. 5).

Direct biophotolysis

As it has been demonstrated, the green alga *Scenedesmus obliques* is able to release hydrogen during the direct biophotolysis [82], filamentous cyanobacteria *Anabaena cylindrica* can simultaneously produce both H_2 and O_2 while treated in argon atmosphere [83].

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

$$H_2O + 2Fd_{ox} \rightarrow 2H^+ + \frac{1}{2}O_2 + 2Fd_{red} \tag{5}$$

$$2H^+ + 2Fd_{red} \leftrightarrow H_2 + 2Fd_{ox} \tag{6}$$

The first reaction is common for all oxygenic phototrophs. For the second reaction, it is necessary to provide anaerobic or microaerobic conditions. To produce hydrogen, the reaction should be catalyzed by the bidirectional enzyme – hydrogenase.

There are three types of hydrogenases:

- [FeFe]-hydrogenase.

Hydrogenases of that kind are generally found in algae. Photosynthetic ferredoxin (PetF) is the electron donor for this reaction [67,84];

- [NiFe]-hydrogenase.

Cyanobacterial cells contain such hydrogenase [67]. In this case, both ferredoxins and flavodoxins could be the donors of electrons [85];

- [Fe]-hydrogenase.

[Fe]-hydrogenase was found in archaea [86].

The direct water biophotolysis usually occurs when cultures are exposed to the light after a period of dark anaerobic adaptation [82,87]. It has very high initial rates (up to 300 μmol H_2/(mg(Chl)*h)). However, due to the fast inactivation of hydrogenases by O_2 the direct biophotolysis has short duration [88]. This problem should be overcome to make this process more effective. Apart from hydrogenase, nitrogen-fixing enzyme – nitrogenase – also can produce hydrogen, in certain conditions.

Indirect biophotolysis

Microalgae and cyanobacteria produce hydrogen from stored glycogen and starch in case of indirect biophotolysis [89–91]. This process proceeds in two steps. At first, the synthesis of carbohydrates occur under the light. At second, the hydrogen is produced from carbohydrates via photofermentation (eq. (3)) [89,92–94]:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \tag{7}$$

However, the degradation of carbohydrates is often accompanied with accumulation of other fermentation end-products. Thus, species which perform the indirect water biophotolysis have special mechanisms to separate O_2 and H_2 evolution either in space or in time. For example, filamentous heterocystous cyanobacteria have heterocysts – specialized cells for photoproduction of hydrogen, and the process is driven by the nitrogenase system extremely sensitive to O_2 [95]. Here, the production of hydrogen as a by-product of nitrogen fixation depends on carbohydrates provided by vegetative cells. In the absence of nitrogen, nitrogenase catalyzes only the reduction of H^+ to H_2, and the highest rate of hydrogen production can be observed. For unicellular N_2-fixing cyanobacteria, performing photosynthesis during the day time and N_2-fixation at night is the way to separate these two processes in time [96]. Cyanobacteria *Cyanothece* can demonstrate the rate of hydrogen production of 465 μmol(H_2)/(mg(Chl)*h) [97]. In green algae *Chlamydomonas*, both direct and indirect water biophotolysis pathways are possible. The pathway depends on the physiological state of the cells.

Hydrogen production through fermentation

Along with the direct biophotolysis of water, hydrogen can be generated via photofermentation (by photosynthetic bacteria) as a second stage of indirect biophotolysis mentioned above, or via dark fermentation (by anaerobic organisms, e.g. anoxygenic bacteria) from organic substrates (Fig. 6), mostly carbohydrates and organic acids [98]. During photofermentation, purple photosynthetic bacteria decompose organic compounds such as acetate, malate, lactate and butyrate using the sunlight as a source of energy; the only products of such process are H_2 and CO_2 [99]. Purple bacteria cannot split water, but they are capable of using the near infrared region of light spectrum. Due to that feature, it is possible to use purple bacteria for wastewater treatment and simultaneous hydrogen production [100]. Another great advantage of photofermentation is the fact that, despite the utilization of organic wastes, photosynthetic bacteria can be used for CO_2 removal from exhaust gases of thermal power plants and other industries [99]. Moreover, purple non-sulfur bacteria are able to fix nitrogen [101]. They use mainly nitrogenase system for hydrogen production, and the reductants are provided by the photoassimilation of organic acids. Recently, another method of hydrogen production by using

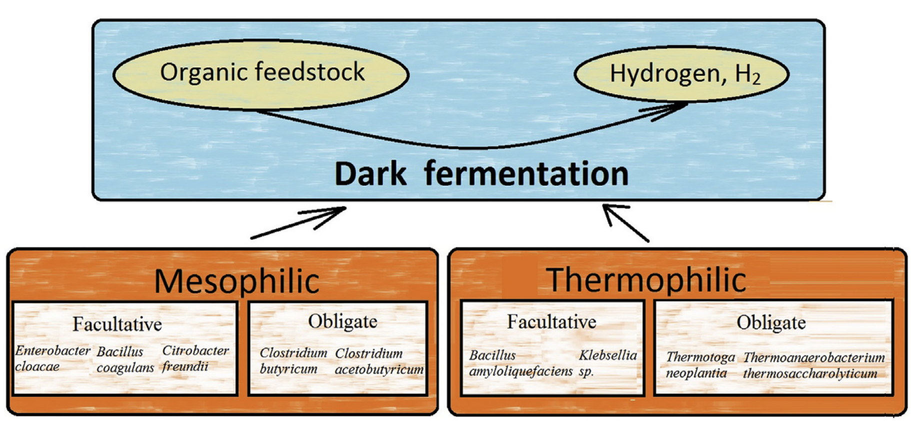


Fig. 6 – Examples of different microorganisms which capable to dark fermentation. Adapted from Ref. [34].

both algae and bacteria was reported [99]. The algae are grown in special ponds for starch accumulation. Then, hydrogen can be produced from the starch via bacterial fermentation. For this process, it is possible to use thermo-tolerant bacteria with high H_2-production yield. In addition, there is no inhibition of the reactions caused by oxygen, as O_2-sensitive algal hydrogenase is not involved in the process [99].

The photofermentative H_2-production can also be increased by addition of some micronutrients to the growth medium: Fe, Mo for *Rhodobacter* [102–104], and Ni, Mg for *Rhodopseudomonas*, respectively [105].

Hydrogen production can be performed via the fermentation of carbohydrate-enriched feedstocks by some microorganisms under anaerobic conditions and in the absence of light (Fig. 2). This process is known as dark fermentation [106]. Fermentative microorganisms use multiple hydrogenases: *Escherichia coli* utilizes [NiFe]-hydrogenase, *Clostridium* species utilize [FeFe]-hydrogenases.

In addition, different bioreactors can be used for hydrogen production. Bioreactors with bacteria immobilized on hollow fibers have been reported to be the way of hydrogen generation via dark fermentation [99]. Immobilization is considered to increase the physiological functions of bacteria. Hollow fibers can be of different sizes, but all of them are made of semipermeable membranes. Hollow-fiber bioreactors have several advantages [99]:

1. The products of fermentation are automatically separated from the cells of the microorganisms;
2. Hollow fibers increase the bioreactor surface, it is possible to make such systems more compact;
3. Attached cultures of microorganisms provide the high stability.

Hollow-fiber bioreactors were successfully tested for hydrogen production from glycerin by use of the bacteria *Enterobacter aerogenes* [107].

Dark fermentation has been considered to be the less expensive approach in comparison with photofermentation [108]. Nevertheless, it is important to take into account several factors such as pH of medium, and concentration of nutrients [109]. In addition, not only hydrogen can be produced by dark fermentation, but some other biofuels also can be obtained [110,111].

Many works are related to the creation of various mutant strains of microalgae [112–116]. Their metabolism is optimized for the production of hydrogen. One of the promising mutant strains are the D1 protein mutants, like *C. reinhardtii* L159I-N230Y, [115,116]. Hydrogen production rate was achieved 11.1 mL(H2)/(L*h) for this strain [116].

Conclusion

Presently, much work has been carried out to improve the effectiveness and efficiency of processes of biofuel production from algae biomass. The third generation biofuel must be without drawbacks of the first two generations [9,10,18,62].

Many of the works are aimed at optimizing of algae cultivation system: open-air system and photobioreactor. Other works are related to the search for the most productive species of microorganisms [117,118] or genetic modifications [116,119–123]. Work on using co-cultures as a source of biomass [124] is also interesting. A model organism for the work on the generation of bioalcohol is *Saccharomyces cerevisiae* [125–128].

The third generation biofuel is not competitive in the energy market for now. It is at the development stage. Algal biomass has not yet become a popular raw material for the generation of engine fuels, although there are many intensive bioreactors. Algae attract attention not only as energy source, but was a source of a number of pharmacological and cosmetically significant compounds. The lack of mechanisms for cheap cultivation impedes commercialization of third generation biofuel.

The fact that a large number of diverse works is carried out in this area indicates that currently algal energy is intensively developing in all directions: increase in growth rate, improving of harvesting methods, the genetic engineering of crops, optimization of chemical and thermal methods for producing biofuels. This is due to the urgency of the energy problem: the environmental risks are associated with the use of fossil fuels and disadvantages of first and second generation biofuels. But a lot of work on improving of algae cultivation and processing mechanisms must be done to ensure commercialization of microalgal biofuels. Nevertheless, we can expect that in the future biofuels can meet the demands for energy. It will be eco-friendly and low-cost solution of energy problems.

Acknowledgments

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