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Review

Processing and culturing of microalgae in biofuel production

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Microalgae can provide several different types of renewable biofuels. These include methane produced by anaerobic digestion of the algal biomass; biodiesel derived from microalgal oil and photobiologically produced biohydrogen. This review presents the current classification of biofuels, with special focus on microalgae and their applicability for the production of biodiesel. The paper considered issues related with the processing and culturing of microalgae, for not only those that are involved in biofuel production, but as well as the possibility of their utilization in environmental pollution control, especially with relation to greenhouse gas emissions and the process of sewage purification. The paper gives also a characterization of microalgae used in the production of biofuels and of their advantages relative to other raw materials used in fuel production.

Key words: Biodiesel, biofuels, microalgae, photobioreactors, environmental applications.

INTRODUCTION

The rapid economic growth that took place in the second half of the 20th century caused a re-orientation in the manner of utilization of energy raw materials. A new model of the world economy has developed mainly on the basis of petroleum and natural gas, with a declining importance of hard coal (Ryan et al., 2006; Mata et al., 2010). However, it appeared that the resources of those raw materials deplete fast and their use causes a number of unfavorable effects, such as acid rains or global warming with the resultant climate changes (Demirbas, 2007; Somerville, 2007). Non-uniform distribution of resources of energy raw materials in the world has contri-buted to the dominant, often even dictatorial position of certain privileged countries in international politics. The dependence of the world economy on oil is such that speculations concerning the exhaustion of the raw material may result in a crisis in the world market. Such a phenomenon happened thrice in 1973, at the turn of 1980 and 1981 and in 2008, when the price of oil soared to the level of 146.14 dollars per barrel (Huang et al., 2010). Apart from this, transport and the energy producing industry are the primary anthropogenic sources of greenhouse gas emissions in the European union that are responsible for more than 20 and 60%, respectively, of that emission (Mata et al., 2010).

The ongoing changes and the economic uncertainty cause the necessity of searching for a source of energy that would permit a reduction in the consumption of petroleum. One of such sources is biofuels. Application of such materials involves the use of ecologically clean energy and the possibility of the production of fuels in countries that do not have their own energy resources, which would make them independent on supplies of petroleum. However, the challenge that has become apparent in the production of biofuels is the competition between fuels and food production, the effect of which has been an increase in food prices (Somerville, 2007; Li et al., 2008a; Rude and Schirmer, 2009).

A solution to that dilemma may lie in the application of microalgae for the production of biofuels. In the aspect of alternative fuels, microalgae is a miniature factory that in the process of photosynthesis, transform carbon dioxide and light into biomass rich in mineral components

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(Banerjee et al., 2002; Lorenz and Cysewski, 2003; Spolaore et al., 2006). Additionally, those photosynthesizing microorganisms are useful in bioremediation of polluted environments (Kalin et al., 2005; Munoz and Guieysse, 2006) and play an important role as "biofertilizers", through binding atmospheric nitrogen (Vaishampayan et al., 2001).

This review presents the current classification of biofuels, with special focus on microalgae and their applicability for the production of biodiesel. The paper considers issues related with the processing and culturing of microalgae for not only those that are involved in biofuel production, but as well as the possibility of their utilization in environmental pollution control, especially with relation to greenhouse gas emissions and the process of sewage purification. The paper gives also a characterization of the microalgae used in the production of biofuels and their advantages relative to other raw materials used in fuel production. Furthermore, the paper presents the current state of knowledge on the culturing, growth, harvest and processing of those microorganisms.

BIOFUELS: DEFINITION, CLASSIFICATION AND CHARACTERIZATION

Biofuels are fuels obtained from biomass (organic matter such as plant and animal organisms and microorganisms). In Europe, biodiesel is produced mainly from sugar beet and cereals and in the USA and Brazil it is produced from maize and sugar cane. Industrial, agricultural, forestry and household wastes may also be a source of renewable energy used in biofuel production. Examples of this may be straw, waste wood, sewage sludge, compost, garbage or remnants of food. Plant biomass from which biofuels are produced constitutes storage of solar energy (Somerville, 2007; Stephanopoulos, 2007; Babu, 2008; Hodaifa et al., 2008).

The use of biofuels is a method of reducing the imports and consumption of fossil fuels and reducing carbon dioxide emissions to the atmosphere, even by 90%. This is possible thanks to the closed circulation cycle of carbon dioxide that is emitted during the combustion of biofuels, but also absorbed by plants in the process of photosynthesis. Biofuels are classified as solid, liquid and gaseous. Solid biofuels include such materials as straw (in the form of bales, pellets or briquettes), specific tree species, such as basket willow, *Sida hermaphrodita*, but also granulated sawdust or straw (pellets), hay or other plant species. Liquid biofuels are obtained mainly through alcohol fermentation of carbohydrates to ethanol, butyl fermentation of biomass to butyl alcohol or from vegetable oils (rapeseed oil) esterified to biodiesel. Gaseous biofuels (biogas) are formed through anaerobic fermentation of liquid and solid wastes from agricultural animal production, such as liquid manure or farmyard manure (FYM). They can also be produced in the process of biomass gasification (wood gasification), from which generator gas (so-called wood distillation gas) is obtained (Demirbas, 2007; Demibras, 2009).

Biofuels can also be classified into 1st, 2nd and 3rd generation biofuels. First generation biofuels are those produced from organic matter that can be used for the production of food or fodder. That organic matter includes primarily starch, sugars, animal fats and vegetable oils. The sources of those materials are potatoes, cereal grain, rapeseed, soybean, maize, or sugar cane. First generation biofuels are produced using conventional methods that do not require high energy inputs, such as fermentation or esterification. The use of raw materials such as sugar cane, maize, wheat or sugar beet, that can also be used to produce human food or animal feed, shows that if too much fuel is produced from such materials the food prices may rise drastically, which may be a challenge for some countries (Somerville, 2007; Brennan and Owende, 2010).

As raw materials used in the production of first generation biofuels are in competition with food production, there are ongoing search for such raw materials for biofuels that would not create this kind of conflict. An ideal solution is provided by cellulose products, such as wood, straw, tall perennial grasses or wastes from the wood processing industry. Fuels produced from such raw materials are called second generation biofuels. At present, they are still not very popular due to the high costs of production, but research in this area has permitted a notable reduction of the costs involved. It is assumed that in future, such fuels will make first generation biofuels obsolete. Second generation biofuels may contribute to an alleviation of the problem partially to satisfy the requirements for fuels in a sustainable, inexpensive and environment-friendly manner. The advantage of second generation biofuels is the possibility of using the whole plant (including the stem, leaves and husks) and not just a part of it (grain) as is the case with raw material for first generation biofuels. Second generation biofuels can also be produced from plants of which no part is edible, such as *Jatropha curcas*, cereals with very low yield of grain, wastes from the wood processing industry, fruit skins or pulp from fruit processing. Such plants can grow in marginal areas and use salt water for their growth, which is an obvious advantage (McKendry, 2002; Schenk et al., 2008).

Third generation biofuels are primarily fuel cells, using hydrogen as the primary source of energy. At present, algae are the main raw materials from which such biofuels can be produced at high efficiency levels and at low investment. Algae are a material that is cost-effective and provides a relatively high yield of biofuel. Their undoubted advantage is the fact that, they are not a burden on the environment and that they are biodegradable. The culture of such algae as *Botryococcus braunii* and *Chlorella vulgaris* is relatively easy, but the extraction of oil from their biomass is already a fairly major problem (Chisti, 2007; Huang et al., 2010; Mata et al., 2010).

CHARACTERIZATION OF MICROALGAE AND THEIR INDUSTRIAL SIGNIFICANCE

Microalgae are prokaryotic or eukaryotic photosynthesizing microorganisms that are characterized by rapid growth; most often live in acid environment and have a unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (*Cyanophyceae*) and of eukaryotic microalgae is green algae (*Chlorophyta*) and diatoms (*Bacillariophyta*) (Li et al., 2008b; Li et al., 2008c).

Microalgae occur in all ecosystems on earth, not only aquatic but also in soil ecosystems and are characterized by being adapted to living in an extremely broad spectrum of environmental conditions. It is estimated that, there are 50 thousand species of algae in the world, but only about 30,000 algal species have been identified and examined so far. Algae are a group of thallophytic organisms, most frequently autotrophic, typically living in aquatic environments or in wet habitats. The body of alga is homogeneous or built of little-varied cells thallus, with sizes from several microns to several meters. The thallus may assume shapes similar to those of leaves or stems, the function of which is to absorb food from the environment. The organisms occur in fresh and salt waters, cool or warm. They live in all the geographic zones, but are the most populous in the northern hemisphere, where their annual production amounts to about 1.5 million tons. Most frequently gathered and used algae include the following: green algae, containing green chlorophyll, yellow xanthophyll and orange carotene; red seaweeds, with red phycoerythrin, blue phycocyanin and green chlorophyll; brown algae, whose pigment corpuscles are filled with brown fucoxanthin, next to chlorophyll and xanthophylls.

Those algae found the most extensive application in certain Asian countries, mainly as a food and fodder component and as a fertilizer. In most of the developed countries, this method of utilization of algae is still treated with caution. Algae that provide many valuable chemical compounds have found an application in the cosmetics and pharmaceutical industries, where they are used to obtain extracts and meals. The extracts are usually used in creams, tonics and shampoos, while the meals find an application in beauty masks and slim-down baths. However, it may soon turn out that the already notable scope of utilization of algae will be considerably expanded if the hopes related with their use in power generation and in industry (biomass production for power generation and for biodiesel production) come true (Metting, 1996; Spolaore et al., 2006).

Many researchers have reported the advantages of microalgae in biodiesel production when compared with other materials used for the purpose (Chisti, 2007; Li et al., 2008a; Khan et al., 2009; Huang et al., 2010). Algae are easy to culture, characterized by rapid growth and are able to grow in waters unsuitable for human consumption. Microalgae transform solar energy into chemical energy in the process of photosynthesis, increasing their mass within a few days. Moreover, they can grow anywhere, provided they have access to sunlight and simple nutrients, though their rate of growth depends also on the availability of an addition of certain specific compounds and appropriate aeration (Aslan and Kapdan, 2006; Verma et al., 2010).

Various species of microalgae can adapt to living in various environmental conditions. Therefore, it is possible to find the most specific species of algae and to grow them under local conditions, which is not possible in the case of other biodiesel raw materials (like soybean, rapeseed or palm seed oil). Microalgae are also characterized by higher rate of growth and productivity compared to the yielding of traditional crops and require significantly smaller growing areas than other substrates of biofuels of agricultural origin, therefore, in the case of algae grown for energy, the competition for arable soils with other crops, especially those grown for food, is greatly limited (Mata et al., 2010).

Microalgae can be used for the production of various energy carriers, including the following: biomethane produced through anaerobic digestion of algal biomass (Spolaore et al., 2006); biodiesel made of oil obtained from algae (Roessler et al., 1994; Banerjee et al., 2002; Gavrilescu and Chisti, 2005; Deng et al., 2009); biohydrogen produced photobiologically (Ghirardi et al., 2000; Melis, 2002; Fedorov et al., 2005; Kapdan and Kargi, 2006) and bioethanol (Fortman et al., 2008; Mata et al., 2010).

The idea of using microalgae as a source of fuel is not new (Chisti, 1980; Nagle and Lemke, 1990), but now, in the “world” of alternative fuels, biofuels from algae draw an increasing interest. The growing of algae for energy reduces the threat of global warming, as it contributes to the limitation of consumption of fossil fuels and uses large amounts of CO_2 for its production (Gavrilescu and Chisti, 2005). Biodiesel produced from algae does not contain sulphur, due to which it plays a role in the reduc-tion of emissions of CO, hydrocarbons and SO_X, though it may increase the level of emission of NO_x when com - pared with other engine types (Johnson and Wen, 2010).

The use of microalgae for biofuel production may also have other aspects. Explained further, is a list of possibilities that could be considered for practical utilization: elimination of CO_2 produced by industry through bio-binding by microalgae, which would reduce the emission of greenhouse gases by factories and use them for biodiesel production (Wang et al., 2008); purification of sewage through elimination of NH_4^+, NO_3^-, PO_4^{3-} and utilization of the polluted waters for growing algae (Wang et al., 2008); transformation of algal biomass remaining after oil extraction, into ethanol, methane, animal fodder, organic fertilizer with a high N:P ratio or simple combustion for co-generation of energy (electric power, heat) (Wang et al., 2008); the combination of algae ability to grow under difficult conditions and the limitation of the availability of nutrients show that they can be grown on soils that cannot be used for farming, with waste waters used as a medium for their growth, with no need to use clean waters (Mata et al., 2010); microalgae can also be used in other industries, including the production of chemical products such as fats, polyunsaturated fatty acids, oils, natural dyes, sugars, pigments, antioxidants, highly bioactive compounds and other chemical compounds (Raja et al., 2008; Mata et al., 2010); with relation to their derivatives, with high biological activity and a broad spectrum of potential commercial applica-tions, microalgae may revolutionize a large number of biotechnologies, including biofuels, the cosmetics and pharmaceutical industries, food supplements and pollution control (Hu et al., 2008; Raja et al., 2008).

MICROALGAE-CULTURE METHODS AND PRODUCTION OF BIOMASS

At present, microalgae appear to be a good substrate for biodiesel production. They are considered as substrates for second generation biofuels, together with other sources of biomass, such as lignin-cellulose materials, organic wastes and dedicated energy crops that are characterized by high yielding potential and are not used as a source of human food (Schenk et al., 2008).

Biomass production from algae is more complicated than the cultivation of those crops. Their growth requires light, carbon dioxide, water and mineral salts. Temperature at which algae are grown must oscillate within the range of 20 to 30°C. To minimize the costs of the biomass production, the production must be based on easily available sunlight. The substrates for algae cultures must supply mineral components required by algal cells. These include mainly nitrogen, phosphorus, iron and in some cases, silicon. The minimum nutrient requirements must be determined based on a suitable molecular formula that for the biomass of microorganisms is as follows: $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$. The formula was developed by Grobbelaar (2004). Biogens such as phosphorus must be supplied at a significant overdose. Phosphorus forms complexes with iron ions and after its addition to the substrate, is not wholly available to microorganisms. Substrates used in algal culturing are too expensive, therefore the substrate most commonly used is sea water, rich in natural compounds of phosphorus and nitrogen as well as other microelements (Molina Grima et al., 1999).

Algal biomass contains on average 50% of carbon in its dry matter. The carbon comes from carbon dioxide necessary for algal growth (Sanchez Miron et al., 2003). To produce 100 mg of biomass, algae need approximately 183 mg of CO_2. The advantage of biodiesel production from algae is the fact that those microorganisms absorb and transform carbon dioxide and other substances emitted to the atmosphere. Apart from that, algal growth requires nitrates and phosphates, which often contributes to environment protection against their excessive levels. Development of algal production close to coal power stations that emit large amounts of CO_2 to the atmosphere or close to sewage treatment plants could help in solving two major problems of contemporary world atmospheric and soil environment pollution (Chisti, 2007).

Large-scale production of algal biomass is usually conducted in continuous cultures and that requires the use of artificial lighting. In this method, the substrate is inoculated with a constant dose of microalgae in suspension that has to be continuously stirred to prevent biomass settlement (Molina Grima et al., 1999).

Practical methods of growing algae on a large scale include open ponds (Molina Grima, 1999) and photobioreactors (Sanchez Miron et al., 1999). Ponds for algae production are built as a closed recirculation loop forming a canal with depth of ca. 0.3 m. The stirring and circulation are provided by a turbine that enforces the motion of the suspension. The turbine operates continuously, preventing sedimentation of algae. Algal biomass is drained behind the turbine, at the end of the recirculation loop. The ponds are lines with white plastic. Such ponds are cheaper to build and maintain than photobioreactors, but biomass production under such conditions is notably lower than in photobioreactors (Chisti, 2007).

Photobioreactors permit the production of large amounts of biomass. They are built of translucent materials and permit growing of exactly those microalgae species that are required (Molina Grima, 1999; Pulz, 2001; Carvalho et al., 2006). Generally, one can distinguish the 3 types of photobioreactors: vertical column photobioreactors, cylindrical photobioreactors and flat or panel-type photobioreactors.

Light is the fundamental parameter determining the growth of microalgae. They require controlled access of light that is usually sunlight, but can be replaced with artificial light sources. Inside the photobioreacto, the light zone close to the source of light and the dark zone, far from the illuminated surface can be distinguished. The existence of the dark zone is due to light absorption by the microorganisms and their auto-obscuration. That phenomenon shows that in the reactor, the following layers of algae are formed: an outer layer of algae, exposed to excessive light intensity that may cause photoinhibition; a central layer, with perfect illumination and an inner layer of algae, with light deficit, where respiration processes occur at high rates (Molina Grima et al., 1999; Molina Grima et al., 2001).

To ensure proper light conditions for algae, some bioreactors employ special lighting panels emitting light in the red range. Proper location of the light source and suitable gas-liquid thermodynamics determine both the growth of the microorganisms and the biomass production

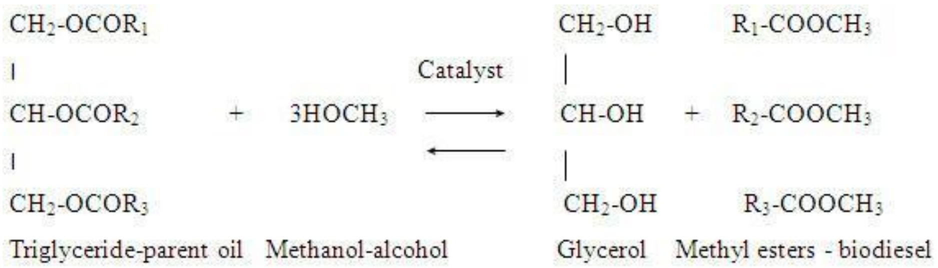

Figure 1. Transesterification of oil to biodiesel. R_1-R_3-hydrocarbon groups.

(Sanchez Miron et al., 1999). Also, important in photobioreactors is the rate of aeration or the medium's circulation. Combining proper geometry of illumination with medium circulation, the cells can be made to circulate between the light and dark zones at a certain frequency and at regular time intervals (Molina Grima et al., 2000; Molina Grima et al., 2001).

Biomass sedimentation in photobioreactors is limited by continuous turbulent flow enforced by mechanical or aeration pumps. Mechanical pumps may cause damage to the biomass (Chisti, 1999; Sanchez Miron et al., 2003), but they are easy to install and ale operated. Aeration pumps are less popular, as they require proper maintenance, periodic cleaning and disinfection of the bioreactor (Chisti, 1999). Suspension stirring through blowing air in at the bottom of the reactor facilitates gas exchange and temperature equalization in the highly turbulent upper zone (Molina Grima et al., 1999; Molina Grima et al., 2000).

Oxygen is produced in the process of photosynthesis. In a typical tubular photobioreactor, the maximum amount of released oxygen may be about 10 g O_2 m^3 min^{-1}. Excessive levels of dissolved oxygen inhibit photosynthesis and cause the reaction of photo-oxidation, which leads to damage to algal cells (Molina Grima et al., 2001). The suspension circulating in the photobioreactor uses CO_2, which leads to increase in pH (Camacho Rubio et al., 1999). Sometimes it is necessary to inject carbon dioxide to prevent excessive increase of pH (Molina Grima et al., 1999). Another problem is biomass losses caused by the respiration of the organisms during the night. Such losses could be reduced through controlled lowering of temperature in the photobioreactor (Chisti, 2007).

The selection of a suitable method of production of algae for the production of biodiesel as well as biomass requires a comparison of the two presented methods, that is, ponds and photobioreactors. Calculations show that, both methods are comparable in terms of the level of biomass production and in terms of consumption of CO_2. However, the production of algae in photobio-reactors permits greater amounts of oil to be obtained (by ca. 1/3) compared to algae culturing in ponds (Molina Grima, 1999; Lorenz and Cysewski, 2003; Spolaore et al., 2006).

The separation of algal biomass from the culture suspension can be effected through its filtration or centrifuging (Molina Grima et al., 2003). Recent studies (Beer et al., 2009; Brennan and Owende, 2010) have been focused on the application of genetic engineering in microalgae breeding, aimed at the acquisition of organisms characterized by high productivity and energy level, with relation to the full utilization of their capabilities.

STAGES OF BIODIESEL PRODUCTION AND TRANSESTERIFICATION

The processes of biodiesel production from microalgae proceed at the following stages: production of biomass through the growth of algal cells, isolation of the cells to the culturing medium, followed by the extraction of lipids. Next, biodiesel or other biofuels are produced in accordance to related technologies and processes used for other biofuel substrate (Mata et al., 2010).

Until now, biodiesel production is based on vegetable or animal fats. Oil production from algae (at an industrial scale) is the matter of a near future (Chisti, 2007). Biodiesel is a proven fuel and the technology of biofuel production and use has been known for over 50 years (Knothe et al., 1997; Barnwal and Sharma, 2005; Felizardo et al., 2006; Meher et al., 2006; Enweremadu and Alamu, 2010). At present, biodiesel is mainly produced from soybean, rapeseed and palm seed oil, (crops used for food) (Felizardo et al., 2006). The typical process of commercial production of biodiesel proceeds in several stages. The parent oil used in biodiesel production is mainly composed of triglycerides (Figure 1) in which 3 molecules of fatty acid are esterified by glycerol molecules. In biodiesel production, triglycerides enter into a reaction with methanol which results in the formation of transesterification, methyl esters of fatty acids, biodiesel and glycerol as a waste product. The reaction proceeds in stages: triglycerides are first transformed into diglycerides, then to monoglycerides and next to glycerol. According to the stechiometric notation of the reaction, subjecting 1 mole of triacylglycerol to methanolysis, will result in the use of 3 moles of methyl alcohol and 3 moles of methyl esters of fatty acids and 1 mole of glycerol will be obtained. As the reaction of methanolysis is an equilibrium reaction, an excessive amount of one of the substrates

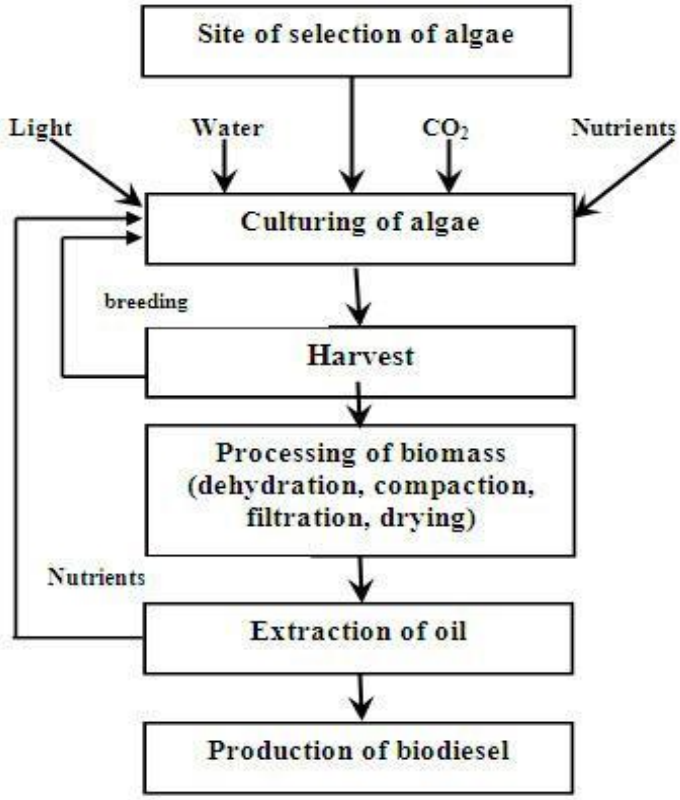

Figure 2. The stages of biodiesel production from microalgae.

should be used (usually alcohol) or the reaction should be performed in stages, with the waste product (glycerol) removed after each stage (Chisti, 2007). In the industrial process, 6 moles of methanol are used per each mole of triglyceride (Fukuda et al., 2001). This high excess of methanol guarantees that the reaction will shift in the direction of methyl esters, towards biodiesel. Under such conditions, the amount of methyl esters is above 98% of the base weight (Fukuda et al., 2001; Bamgboye and Hansen 2008).

Transesterification is catalyzed by acids, alkali (Fukuda et al., 2001; Meher et al., 2006) and lipolytic enzymes (Sharma et al., 2001). Alkaline catalysis of transesterification is about 4000 times faster than the acid-catalyzed reaction. Commonly used alkaline catalysts include sodium and potassium hydroxides at concentration of 1% with relation to the weight of the oil. Of course, lipolytic enzymes can also be used for the purpose, but at present that method is not used due to the relatively high costs of such catalysis (Fukuda et al., 2001). In the industrial practice, the process of transesterification is most frequently conducted at temperatures of 60 to 70°C in the presence of an alkaline catalyst. To achieve a high degree of conversion of the esters (triacyloglycerols), a high excess of methanol is applied that after the termination of the process of transesterification, is distilled out and returned to the process. Under such conditions the reaction proceeds for about 90 min. Higher temperatures can also be applied, at higher pressures, but that is a costly process. Oil used in the reaction of methanolysis must meet specific requirements and in particular, it should be well dehydrated and devoid of free fatty acids causing the formation of soaps that reduce the level of the catalyst and cause problems with the isolation of the glycerin and ester fractions. Due to the poor solubility of methanol in oil and relative ease in the water phase, it is important that the reaction system be stirred vigorously, especially in the initial phase of the reaction, which improves the contact between the alcohol and tiracyloglycerol (Chisti, 2007).

Future processes of biodiesel production from algae may be based on similar principles. The production of methyl esters or biodiesel from oil extracted from algae is presented in a study by Belarbi et al. (2000), though in that case, the end product was meant for use in the pharmaceutical industry. The process described by those authors also preceded in stages, that is, extraction trans-esterification of fatty acids from algae biomass, followed by fractioning on chromatographic columns (Belarbi et al., 2000).

Currently, numerous studies (Lee et al., 2002; Chiu et al., 2008; Mata et al., 2010; Yoo et al., 2010) are concerned with the culturing of algae and with the individual stages of their processing, as well as with the determination of the final profitability of those processes. Figure 2 presents the stages of biodiesel production from microalgae.

In the selection of a site for the culturing of algae, the following criteria should be followed; availability of water, salinity and chemical properties of the water, topography, geology and land ownership, climatic conditions, that is, temperature, isolation, evaporation and access to sources of nutrients and of carbon.

Microalgae can live in a broad spectrum of environmental conditions, especially under nutrient deficit and in other unfavorable conditions; algae can be grown with the use of industrial sewage. Before starting a culture of algae for the production of biodiesel, the following criteria should be taken into account (Mata et al., 2010): growth rate, measured by the total content of biomass accumulated in a unit of time in a unit of volume; level of lipids, measured not by the total content but by the content of free fatty acids and triglycerides; resistance to changes in environmental conditions, especially in temperature, level of nutrients, light, competition with other algae and bacteria; availability of nutrients, especially carbon dioxide; ease of isolation and processing of biomass; and ease of obtaining other required chemical compounds.

Proper method of selection of desired species of algae and development of optimum photobiological formula for each species are the key issues for achieving low-cost algae cultures, irrespective of the geographic situation (Chojnacka and Marquez-Rocha, 2004).

Algae are characterized by various types of metabolism

Table 1. Comparison of some sources of biodiesel (mean values).

Crop	Oil yield (L ha^{-1})	Land area needed (M ha)
Corn	169	1545
Soybean	443	589
Coco nut	2679	95
Palm oil	5938	41
Microalgae-70% oil in biomass	136	2
Microalgae- 30% oil in biomass	58	4.5

(autotrophic, heterotrophice, mixotrophic and photoheterotrophic) and can utilize various types of metabolism in response to changes in environmental conditions. Listed further are a few examples of growth of various microalgae.

Photoautotrophic

They use light as the sole source of energy that is transformed into chemical energy in the process of photosynthesis.

Heterotrophic

Heterotrophic uses only organic compounds as a source of carbon.

Mixotrophic

This group is capable of autotrophic or heterotrophic feeding depending on environmental conditions, intensity of light, presence of organic nutrients and substrates for photosynthesis or chemosynthesis.

Photoheterotrophic

This is also known as photoorganotrophs with metabolism using sunlight and organic compounds as a source of carbon.

ESTIMATION OF THE POSSIBILITY OF THE USE OF BIODIESEL FROM MICROALGAE

At present in the USA, the annual consumption of biodiesel is about 530 million m^3. To substitute fossil oil with vegetable oil would require the sowing of 111 million hectares with oil-bearing plants (Chisti, 2007). In Poland, it is estimated that in 2010 the consumption of fuels may reach even 20 million tons, that is, 30% more than in 2004. A particularly high increase of consumption will be observed in the case of diesel fuel, as diesel engines are the standard in heavy transport vehicles. The continuously growing demand for liquid fuels causes increased demand for petroleum, which may result in a continuous rise of its prices. An additional problem is the fact that most of the world petroleum resources are located in countries that are now politically unstable, which does not guarantee continuity of supplies. It is therefore, necessary to search for new kinds of fuels and alternative fuels that due to their lower cost or lower emissions of toxic components of exhaust gases would, in a time perspective, become substitutes for petroleum-based products. Such alternative fuels include liquid fuels (biodiesel and bioethyl gasoline) and gas fuels (biomethane), produced from biomass, as well as biohydrogen from biomass gasification. Biomass is a term covering solid or liquid substances of plant or animal origin that undergo biodegradation. They can originate from products, wastes, as well as residues from agricultural and forestry production and also from industries processing such products. Recently, a lot of interest has been focused also on bioenergy from the combustion or processing of algae (Felizardo et al., 2006).

Table 1 presents some crop plants used for biodiesel production and the required land areas required to meet the projected demand. In Table 1, algae are shown as one of the sources of biodiesel which totally disqualify fuels of organic origin. As opposed to oil -bearing plants, microalgae grow extremely fast and have much higher oil content. Normally, microalgae double their biomass within 24 h. The time required for doubling the biomass in experimental cultures, under optimum conditions, may even be reduced to only 3.5 h whereas, the oil content of algae may even exceed 80% of their dry mass (Spolaore et al., 2006). For these reasons, algae cultures may be an unlimited source of oil-rich biomass.

Many species of microalgae are capable of accumulating notable amounts of lipids, which contributes to high oil yields. The average content of lipids varies within the range of 1 to 70%, but under certain stress conditions some algal species may achieve dry matter lipid content of up to 90% (Dunahay et al., 1996; Ratledge and Wynn, 2002; Guschima and Harwood, 2006; Yoo et al., 2010). The productivity of oil obtained from algae depends on the rate of their growth and on the level of biomass oil content. Microalgae characterized by high oil productivity are especially desirable for biodiesel production. Table 2 lists a number of algae species that have oil content in the thallus in the range of 20 to 60%. Algae produce many different kinds of lipids, hydrocarbons and other complex compounds and not every species is suitable for biodiesel production (Banerjee et al., 2002; Guschina and Harwood, 2006). Various environmental conditions, nutrients, culture conditions and growth phase may affect the content and composition of fatty acids in algae. For example, nitrogen deficit and salinity stress induce the accumulation of C18:1 in all algal species, and of C20:5 in *B. braunii* (Thomas et al., 1984). Other authors

Table 2. Oil content of some microalgae (mean values).

Microalgae	Oil content (% dry matter)
B.braunii	50
Chlorella sp.	30
C. cohnii	20
Cylindrotheca sp.	26
D. primolecta	22
Isochrysis sp.	29
M. salina	>20
Nannochloris sp.	30
Nannochloropsis sp.	50
N. oleoabundans	44
Nitzschia sp.	46
P.tricornutum	25
Schizochytrium sp.	63
T. sueica	19

(Pratoomyot et al., 2005; Hu et al., 2008; Gouveia and Oliveira, 2009) also reported differences in the fatty acid composition of various species of microalgae. The advantage of using algae for the production of biofuels is the fact that they do not constitute any competition on the food market. Also, with relation to algae, the introduction of genetic modifications increasing oil yield raises less controversy (Chisti, 2007).

Potentially, instead of microalgae, oil could also be produced by heterotrophic microorganisms growing on natural sources of organic carbon (Ratledge and Wynn, 2002). However, those microorganisms are less efficient when compared with photosynthesizing microalgae.

PROFITABILITY OF THE USE OF BIODIESEL FROM MICROALGAE

For biodiesel produced from microalgae to be accepted by the population, it must meet common standards. Oil obtained from microalgae is rich in polyunsaturated fatty acids with 4 and more double bonds, such as the eicosapenztaenoic and decosapentaenoic acid. Methyl esters of fatty acids, as well as fatty acids that contained in their carbon chains unsaturated bonds undergo transformations such as hydrolysis, autooxidation or polymerization. The storage of biofuels, whether based on algal or vegetable oil, such as rapeseed or soybean oils, raises certain difficulties such as, during extended storage, one can observe the development of microorganisms and the formation of sludge substances. This causes their viscosity changes and they may fork sediments in storage tanks and in the fuel tanks of motor vehicles. For these reasons, long-term storage of biofuels is not possible (Belarbi et al., 2000; Chisti, 2007).

Genetic engineering can be applied to improve the economics of biodiesel production from microalgae (Dunahay et al., 1996; Roessler et al., 1994). In particular, genetic engineering could be used to achieve the following: to improved efficiency of the process of photosynthesis to permit increase in biomass production; increased rates of multiplication of the microorganisms; increased levels of oil in the biomass; improved temperature tolerance of the microorganisms and limiting the level of losses caused by temperature drops; reduction of photoinhibition and reduction of sensitivity of photooxidation causing the destruction of cells (Zhang et al., 1996; Chisti, 2007).

OTHER APPLICATIONS OF MICROALGAE

Biodiesel production from microalgal and other bioproducts can be more environmentally sustainable, cost-effective and profitable, if combined with processes such as wastewater and flue gas treatments. According to Zeiler et al. (1995) green alga *Monoruphidium minutum* can efficiently utilize simulated flue gas containing high levels of carbon dioxide, sulphur, nitrogen oxides as a feedstock to produce biomass. For that reason, it is beneficial if microalgae are tolerant to high CO_2 concentrations in order to be used for its fixation from flue gases.

Aquaculture systems involving microalgae production and wastewater treatment with high levels of amino acids, enzymes seem to be promising for the growth of microalgae combined with biological cleaning. Microalgae can use organic compounds that contain nitrogen and phosphorous from manufacturers wastewater. Additionally, microalgae can mitigate the effects of sewage effluent and industrial sources of nitrogenous waste such as those originating from water treatment (Mata et al., 2010).

Different species of microalgae can contain high-level chemical compounds. Depending on microalgae species that may be extracted from different pigments: antioxidants, -carotenes, triglycerides, fatty acids, vitamins and others chemicals. Compounds extracted from microalgae can be used in cosmetics, foods and pharmaceutical industry (Brennan et al., 2010; Mata et al., 2010).

Specific species of microalgae are suitable for the preparation of animal feed supplements. Algae such as *Chlorella*, *Scenedesmus* and *Spirulina* have beneficial aspects including improved immune response, improved fertility, better weight control and healthier skin (Brennan et al., 2010). Current efforts and business investment are driving attention and marketing efforts on the promises of producing algal biodiesel and superior production systems.

CONCLUSION

Biodiesel derived from microalgae appear to be the only current renewable source that can potentially completely substitute fossil fuels. There are many challenges in biodiesel production. The limiting factors for the utilization of microalgae as a raw material for the production of biofuels include the harvesting and the processes of oil extraction and the supply of CO_2 for high efficiency of microalgae production. Also, the availability of light, nutrients and levels of CO_2 and O_2 must be monitored carefully during the culturing of algae to ensure optimum conditions for high levels of oil and biomass of algae. The biggest challenge is that, microalgal biodiesel are not economically competitive with fossil fuels at today's energy prices.

Investment in the development of technologies based on algae cultures for the production of biofuels is necessary for the development of a technology that will be economically viable. Such efforts should be supported financially and preceded by political and economic plans and strategies. The primary strategy is to identify algae species that have a high oil content that will also grow quickly to produce biodiesel. The second one is to develop photobioreactors that allow large-scale culture of microalgae.

Further research in the field of biodiesel production from microalgae should be focused on the reduction of the costs of small and large-scale systems. Moreover, the reduction of costs through maintenance of purity of waters and nutrients, the utilization of sewage and the nutrients contained in it and utilization of CO_2 emitted by the industry, are all related with the protection of the natural environment in its broad sense. Algae can also be utilized in other branches of human activity such as agriculture, medical sciences and in the chemical, cosmetics and pharmaceutical industries.

Despite the earlier mentioned challenges, microalgae are promising feedstock for biodiesel production. Research on microalgae based biodiesel production should be continued and commercial-scale use of microalgae for biodiesel production would require massive investments in production facilities.

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