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The role of biochemical engineering in the production of biofuels from microalgae

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ABSTRACT

Environmental changes that have occurred due to the use of fossil fuels have driven the search for alternative sources that have a lower environmental impact. First-generation biofuels were derived from crops such as sugar cane, corn and soybean, which contribute to water scarcity and deforestation. Second-generation biofuels originated from lignocellulose agriculture and forest residues, however these needed large areas of land that could be used for food production. Based on technology projections, the third generation of biofuels will be derived from microalgae. Microalgae are considered to be an alternative energy source without the drawbacks of the first- and second-generation biofuels. Depending upon the growing conditions, microalgae can produce biocompounds that are easily converted into biofuels. The biofuels from microalgae are an alternative that can keep the development of human activity in harmony with the environment. This study aimed to present the main biofuels that can be derived from microalgae.

1. Introduction

Many analyses have been carried out about the future possibility of exhausting the planet's resources and its ability to sustain its inhabitants. This century will see great changes, and fossil fuels will be partially replaced by new energy sources that can fulfill current needs, but which have a lower environmental impact.

Nature has developed photosynthesis, a very efficient light harvesting and conversion system, which uses sunlight to synthesize chemical energy carriers such as carbohydrates, lipids and proteins. Over millions of years nature has collected the sun's energy and stored it as fossil fuels such as oil, coal and natural gas. Besides the properties of photosynthetically produced fossil fuels, the potential of current photosynthetic systems as a provider of clean CO_2-neutral fuels, so called biofuels, has been recognized (Rupprecht, 2009).

Microalgae are photosynthetic organisms with relatively simple requirements for growth, when compared to other sources of biomass. The carbon source necessary for the cultivation of microalgae represents up to 60% of the costs with nutrients. Through the process of photosynthesis, the algae convert water, carbon dioxide (CO_2) and light into oxygen and biomass. The nutritional requirements for growing these microorganisms may be available in industrial waste, turning what is considered a problem into raw material for obtaining products with high added value, such as biofuels (Becker and Venkataraman, 1984; Vonshak, 1997).

Algal biomass has the potential to be converted into biofuel, yielding a CO_2-neutral energy carrier comparable to biofuels produced from other biomass sources. Biomass can be converted by use of thermo-chemical or biological methods. Thermo-chemical methods include direct combustion providing electricity, heat and mechanical power. Biological conversion methods include fermentation of the biomass to produce energy carriers like hydrogen, ethanol and biogas, or extraction of oils from the biomass for biodiesel production. All use of biofuel will re-emit CO_2, which can be re-captured from biofuel fired plants (Skjanes et al., 2007).

Hydrogen is a naturally occurring molecule, which is a clean and efficient energy carrier (Brennan and Owende, 2010). Microalgae are candidates for such a process since they exhibit hydrogen production under certain conditions and may use closed systems that allow the capture of hydrogen gas (Benemann, 1997). Microalgae possess the necessary genetic, metabolic and enzymatic characteristics to photoproduce hydrogen gas (Brennan and Owende, 2010). The photobiological production of biofuel hydrogen to microalgae can be increased according to the carbon content in biomass.

The fatty acids that microalgae produce can be extracted and converted into biodiesel, which is a renewable, biodegradable, non-toxic and environmentally friendly fuel. Biodiesel has the following advantages: 78% reduction in the emission of carbon dioxide when burnt, 98% reduction in sulfur emissions and 50% reduction of particulate matter (Brown and Zeiler, 1993). Several researches focused on the isolation of high lipid content microalgae that could be cultivated in large scale open pond cultivation for biodiesel production, and capturing CO_2 from coal-fired power

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plants as biological emission control process (Brennan and Owende, 2010).

Microalgae can produce various compounds by photosynthesis, and also by the catabolism of endogenous carbohydrates via respiration under dark aerobic conditions and via fermentation under dark anaerobic conditions. Although endogenous storage compounds are completely decomposed to carbon dioxide by aerobic respiration, various end-products are formed by anaerobic fermentation. End-products how ethanol are obtained to fermentation process. The ethanol being one of the attractive chemicals and an energy source (Ueno et al., 1998). Application of high carbohydrate-producing marine microalgae can generate an alternative biomass resource for ethanol production. The production of ethanol by fermentation from biomass consisting of carbohydrates was composed of two processes such as saccharification and fermentation. Saccharification requires enzymes to hydrolyze carbohydrates prior to fermentation (Matsumoto et al., 2003).

Biogas is the product of anaerobic digestion of organic matter and can be obtained from terrestrial biomass, such as domestic sewage, animal excrement and solid waste, such as weeds, fruit and vegetable leftovers, leaves or plants, or from aquatic biomass such as macro and microalgae or marine plants (Omer and Fadalla, 2003; Gunaseelan, 1997). The type of digestion that uses microalgal biomass can eliminate the process of harvesting and drying of biomass and the associated costs (Vonshak, 1997). In the latter case, this biomass can be burned for energy, since the calorific value of these microorganisms is higher than certain coals.

The objective of this study was to present the main biofuels that can be derived from microalgae.

2. Microalgal biotechnology

Biotechnology is an inter and multidisciplinary area of science, which means it is essential that there is a collaboration of effectively integrated professionals working in different fields of knowledge, such as biochemistry, physiology, genetics, microbiology, virology, botany, zoology, ecology and engineering.

Biotechnology is the body of knowledge, techniques and methods, with scientific or practical basis, that allows the use of microorganisms as an integral and active part of industrial production of goods and services. Biotechnology products range from modified foods and beverages, to other industrial products such as solvents, organic acids, esters, amino acids, polysaccharides, enzymes, vitamins, antibiotics, hormones and biofuels.

The use of microorganisms and their metabolic products by humans is one of the most significant fields of biotechnology activities. The knowledge of the activity of microorganisms in the conversion of certain substances into others is of great importance, as is the possibility of using a wide variety of substrates for viable products and subproducts, which enables a rational and balanced use.

In Mexico, the ancient Aztecs collected algae of the genus *Spirulina* from alkaline lakes for food consumption. The commercial cultivation of microalgae started many years ago, when the main species were *Chlorella*, *Spirulina*, used as a food, *Dunaliella salina* for the extraction of β-carotene, *Haematococcus pluvialis* for the extraction of astaxanthin and various other species used as feed in aquaculture (Borowitzka, 1999).

Microalgae represent the prokaryotic and eukaryotic (true algae) photosynthetic microorganisms that live in an aquatic environment (Vonshak, 1997). Over the past 30 years, microalgal biotechnology has developed and diversified significantly. The cultivation of microalgae is being applied in the production of pharmaceuticals, biochemicals, fertilizers, health food, animal feed and more recently has been proposed as a source of biofuels (Fig. 1). Microalgae can be combined to produce methane (biogas), ethanol extraction, to produce hydrogen photosynthetically, and some of them accumulate lipids from which fatty acids can be extracted giving rise to biodiesel (Scragg et al., 2003).

The commercial production of *Spirulina* is based on raceway-type open tanks, although some companies use a closed tubular

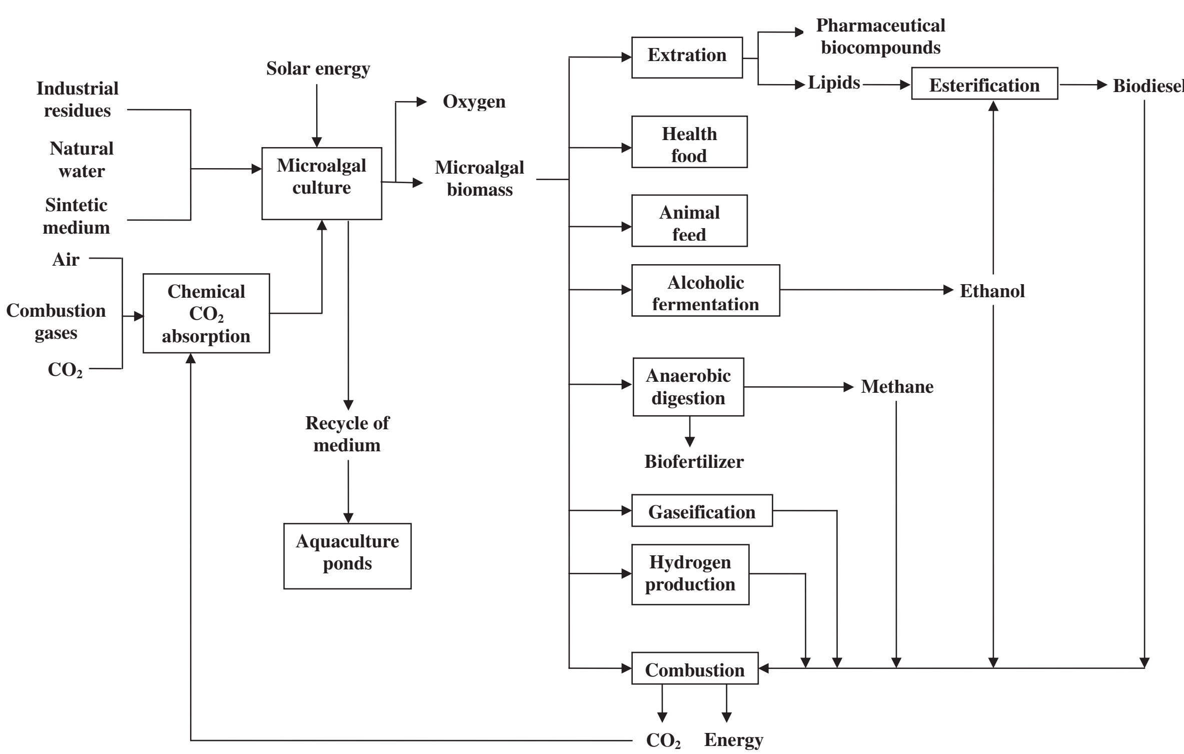

Fig. 1. Flow diagram of biomass microalgae potentialities.

bioreactor (Jiménez et al., 2003). Cultivation in raceway-type tanks are made in Israel, the United States and China. Circular tanks are used in Japan, Taiwan and Indonesia. In China, one company has an annual production of 200 tons of algae, which corresponds to 25% of national production and 10% of world production. The largest plant for microalgae cultivation in the world is located in Calipatria (CA, USA) in an area that is 440,000 m^2 (Spolaore et al., 2006).

Many companies sell nutraceuticals developed with microalgae. In Yangon (Myanmar), tablets, chips, creams and liquid extract of *Spirulina* are sold. In Kona (Hawaii, USA), Spirulina powder is marketed (Spolaore et al., 2006). In India, Inner Mongolia (China), Tianjin (China), Mexico, Cuba, Iran, Taiwan and Japan, industries produce *Dunaliella* microalgae to obtain β-carotene (Lee, 2001). The largest producers of Haematococcus on a commercial scale in the world are located in Kailuai-Kona (USA) and Chennai (India) (Dufossé et al., 2005).

In Brazil, the Laboratory of Biochemical Engineering (LEB), at the Federal University of Rio Grande (FURG) develops technologies for the cultivation of microalgae. Among the technologies studied and under study, the following stand out: discontinuous and semi-continuous cultivation (Reichert et al., 2006), cultivation in open and closed photobioreactors (Morais and Costa, 2007a,b), the effect of factors such as temperature, illuminance, aeration, replacement rate, cut-off concentration (Radmann et al., 2007), the profile of fatty acids in microalgae (Colla et al., 2004), the production of biofuels from microalgae, sources of alternative nutrients, such as Mangueira Lagoon water and CO_2 (Costa et al., 2003), production costs, simultaneous cultivation of *Spirulina* with toxigenic microalgae (Costa et al., 2006), the hypocholesterolemic potential of *Spirulina platensis*, the isolation of a native strain of *Spirulina* (Morais et al., 2008), the isolation of microalgae with potential for CO_2 biofixation (Morais and Costa, 2007c), the mathematical modeling of the growth of *Spirulina* (Costa et al., 2002) and development of nanofibers using microalgal biomass (Morais et al., 2010).

Since 1998, the LEB has been developing a project studying the cultivation of *Spirulina* on a pilot scale on the edge of Mangueira Lagoon (Morais et al., 2009), for addition to meals for local children. Products that are easy to prepare, conserve and distribute have been developed. These products include: instant noodles, flan, powdered mixture for cakes, cookies, chocolate powder, instant soup, isotonic sports drinks, gelatin powder and cereal bars.

The LEB, along with the President Medici Power Plant (UTPM), operated by the Society of Thermal Electricity Generation (CGTEE) since January 2005, has carried out the cultivation of microalgae for the biofixation of CO_2 that is emitted in the combustion of coal at UTPM. Due to this project, the partners joined the International Network for Biofixation of CO_2 and Greenhouse Gas Abatement with Microalgae (Morais and Costa, 2007a,b,c).

With the problems that world is now facing in terms of global warming due to burning of fossil fuels and depleting resources there is a serious renaissance of interest in renewable energy from biological sources (Rupprecht, 2009).

3. Applications of microalgae as biofuels

Microalgae are microscopic organisms that typically grow suspended in water and are driven by the same photosynthetic process adopted by higher plants. However, unlike higher plants, algae do not require a vascular system to transport nutrients, because as every cell is photoautotrophic, they can directly absorb the dissolved nutrients (Pelczar, 1993). Conventional terrestrial plants are relatively inefficient in capturing light, converting less than 0.5% of the solar energy received at typical midlatitudes into plant into plant biomass; in contrast, the photosynthetic efficiency of microalgae potentially can exceed 10.0% (Smith et al., 2009).

Through the process of photosynthesis, microalgae convert water, carbon dioxide and light into oxygen and biomass (Alberts et al., 2008). The carbon source that is required for the cultivation of microalgae represents 60.0% of the costs for the nutrient. The use of alternative sources such as CO_2 emitted from the burning of coal in power plants, as well as minimizing the problems caused by the emission of this gas, such as global warming, reduces costs with this nutrient and generates carbon credits that can be traded with countries that need to reduce emissions of greenhouse gases (Hughes and Benemann, 1997).

The production of 100 tons of microalgal biomass fixes 183 tons of CO_2 (Chisti, 2008). Microalgae can be grown on land unsuitable for agriculture and farming, or on inhospitable land such as deserts, using brackish water and/or wastes from the desalination process that, depending upon their composition, the cultivation medium may be added to the crop limiting nutrients. As fresh water is an increasingly scarce resource, the cultivation of microalgae improving water quality through the economical and environmentally-appropriate use of it (Rupprecht, 2009; Groom et al., 2007; Vonshak, 1997).

Microalgae can double their biomass in mean times that range from 2 to 5 days, achieving large yields, without the need for the application of pesticides, herbicides or fungicides. Doubling the biomass of terrestrial plants, genetically modified or not, takes months, and doubling the concentration of animal protein can take years. The production of protein from microalgae consumes three times less water than producing the same amount of protein from soy, which is the plant that is richest in this nutrient (Rupprecht, 2009; Dismukes et al., 2008; Costa et al., 2006).

Energy production from microalgae, when compared to other traditional forms of energy such as wind, hydro or from other biomass such as plants, household and industrial waste, has the advantage of simultaneously fixing large amounts of carbon dioxide. The search for solutions to energy problems requires understanding what the alternatives are and choosing the most appropriate ones. These choices may affect the local patterns of consumption and quality of life of people.

The choice of biofuels or raw material used for their production should take into account the conservation of sensitive ecosystems and biodiversity, competition for land with food and settled populations, the preservation of water resources and the impact of

Table 1
Biofuel content of some microalgae species.

Microalga	Biofuel	Photobioreactor	Productivity of biofuel	References
Dunaliella sp.	Ethanol	Erlenmeyer flask	11.0 mg g^{-1}	Shirai et al. (1998)
Chlorococum sp.	Ethanol	Bag photobioreactor	3.83 g L^{-1}	Harun et al. (2010a)
Neochlorosis oleabundans	Biodiesel	Polyethylene bags	56.0 g g^{-1}	Gouveia and Oliveira (2009)
Chlorococum sp.	Biodiesel	Bag photobioreactor	10.0 g L^{-1}	Harun et al. (2010a)
Chlamydomonas reinhardtii	Hydrogen	Roux bottle	2.5 mL h^{-1}	Ghirardi et al. (2000)
Spirulina platensis	Hydrogen	Erlenmeyer flask	1.8 μmol mg^{-1}	Aoyama et al. (1997)
S. platensis UTEX 1926	Methane	Glass sealed bottle	0.40 m^3 kg^{-1}	Converti et al. (2009)
Spirulina Leb 18	Methane	Open raceway	0.79 g L^{-1}	Costa et al. (2008)

fertilizers for pest control. The composition and rates of photosynthesis and growth of these organisms are strongly dependent on growing conditions, so that manipulation of these can result in a greater production of metabolites that are of interest. At the end of the process, according to the characteristics of the microalgal biomass obtained, it can be used to produce biodiesel, ethanol, hydrogen, biogas or direct burning (Table 1) and are not precursors of problems caused by fossil fuels and renewable energies (Kruse, 2009; Rupprecht, 2009).

4. Hydrogen photosynthetically produced by microalgae

World consumption of hydrogen is around $10^{12}\,m^3\,year^{-1}$. Around $250 \times 10^9\,m^3$ is used in the production of ammonia and another $250 \times 10^9\,m^3$ is divided between the petrochemical industry and other applications.

The use of hydrogen as a fuel causes less environmental impact, which is why many studies have been carried out on this element, so that it can play a more significant part in the energy consumption of many countries (Benemann, 2000). When burned as a conventional fuel, whether in stationary engines, gas turbines, automotive vehicles, or boilers and heaters, the use of hydrogen leads to the emission of practically just one type of pollutant, NO_x.

Microalgae have the genetic, metabolic and enzymatic characteristics for photoproduction of hydrogen (Brennan and Owende, 2010). Its capacity for synthesis is linked to the exposure of the microalgal cultivation to certain conditions, and closed systems that facilitate the capture of hydrogen gas can even be used. The photobiological production of hydrogen can be increased according to the biomass's carbon content (Benemann, 1997).

In the late 19th century it was reported that a natural bloom of the cyanobacterium *Anabaena*, when placed into a glass jar, rapidly started to evolve hydrogen gas. The first scientific investigation of H_2 evolution by microalgae demonstrated that after a period of dark anaerobic 'adaptation', the green alga *Scenedesmus obliquus* produces H_2 in the dark at low rates, with H_2 production greatly stimulated in the light, though only for relatively brief periods. Other noteworthy observations were that uncouplers and low CO_2 concentrations stimulated light driven H_2 production in green microalgae (Wunschiers and Lindblad, 2002; Benemann, 2000).

Under anaerobic conditions, the eukaryotic microalgae produce hydrogen as an electron donor in the process of CO_2 fixation. During photosynthesis, the microalgae convert water molecules into hydrogen ions (H^+) and oxygen. The hydrogen ions are then converted into H_2 by the enzyme hydrogenase (Hahn et al., 2004; Ghirardi et al., 2000). The photosynthetic production of O_2 results in rapid inhibition of the enzyme hydrogenase and the production of H_2 is inhibited. Therefore, cultivation of microalgae for the production of hydrogen must take place under anaerobic conditions (Brennan and Owende, 2010; Wunschiers and Lindblad, 2002; Benemann, 2000).

Two methods can be used for the photosynthetic production of hydrogen. In the first method, the hydrogen production takes place in two stages, where the synthesis of hydrogen and oxygen occur partially separated. In the first stage, the algae grow photosynthetically under normal cultivation conditions. During the second stage, the microalgae are exposed to anaerobic conditions and sulfur is limited. With this process system, no toxic products are generated, and compounds with high added value can be produced as a result of the microalgal cultivation.

The second method involves the simultaneous production of oxygen and hydrogen. In this method, the electrons that are released by the photosynthetic oxidation of water are used by the hydrogenase. The production of hydrogen is theoretically superior in the two-stage method, while the simultaneous production is rapidly inhibited by the action of oxygen (Brennan and Owende, 2010). Melis and Happe (2001) carried out the two-stage photosynthetic production of hydrogen and obtained a yield of $198\,kg\,ha^{-1}\,day^{-1}$.

According to Benemann (1993), in the first stage to produce hydrogen by microalgae, the CO_2 fixation into storage carbohydrates would be carried out in large (several hectares) unlined, paddle wheel mixed, raceway-type ponds. And such pond systems are used in both wastewater treatment and for commercial microalgae production. The algal culture would then be concentrated 10- to 20-fold by settling, and held in a large anaerobic fermenter (small, deep, covered lagoons) to induce the hydrogenase enzyme and initiate H_2 production, along with other metabolites, such as acetate. It was assumed that one-third of the H_2 stored in the carbohydrates would be released by anaerobic fermentations. Then the culture would be transferred to closed photobioreactors that expose the cells to light, converting acetate and remaining carbohydrates to H_2. Finally the depleted cells would be recycled to the open ponds to repeat this cycle, for a total of ten times in this conceptual analysis (Benemann, 2000).

A small pilot plant was operated in Japan for several years, using two $2\,m^2$ of open ponds for producing a green algal (*Chlamydomonas reinhardtii*) biomass and about $2\,m^2$ of closed photobioreactors, with an intermediate dark fermentation stage. In an impressive demonstration of the largest and longest-duration microalgal biohydrogen production project carried thus far, these researchers successfully operated this process on a continuous basis, producing several liters of H_2 per day (Benemann, 2000; Ikuta et al., 1998). They demonstrated the ability to recycle algal cultures repeatedly through the process, which included a separate dark fermentation stage and a hollow fiber ultrafiltration unit to separate the algal fermentation products and feed these to the photosynthetic bacterial photobioreactors (Benemann, 2000).

5. Biodiesel production from microalgae

Biodiesel is a potential substitute for conventional diesel fuel. This biofuel can be obtained from various raw materials by a process called transesterification or esterification. The transesterification reaction can be using super critical fluids, enzymatic, acid-catalysed or alkali-catalysed (Fukuda et al., 2001). In these processes occurs the conversion stage of lipids, in ethyl or methyl esters of fatty acids, using methyl alcohol or ethyl, respectively (Xuan et al., 2009).

Transesterification reactions occur in the presence of a catalyst, for which sodium hydroxide and potassium hydroxide can be used, among others (Xuan et al., 2009). After the transesterification reaction, the final reactional mass is made up of two phases, which are separated by decanting or centrifuging. The heavier phase consists of crude glycerine, containing excess alcohol, water and impurities inherent in the raw material. Due to the low solubility of glycerol in the esters, this separation generally occurs quickly.

The phase that contains water and alcohol is subjected to an evaporation process, which eliminates these volatile constituents from the crude glycerine, by liquefying these vapors in an appropriate condenser. The esters are centrifuged and dehumidified, which results in biodiesel that should have characteristics which match the specifications of the technical norms established for biodiesel for use as a fuel in diesel cycle engines (Ma[a] and Hanna, 1999). For the past decade, biodiesel has been gaining worldwide popularity and research interest in utilization in vehicle engines and other kinds of internal combustion (IC) engines as an alternative energy source to petro-diesel (Sgroi et al., 2005).

The raw materials from which lipids are currently extracted for biodiesel production consist mainly of oily seeds such as soybeans, palm, castor bean, peanut, sunflower, corn, rapeseed and cotton. However, the cultivation of plants depends on factors that may limit or even preclude the production of biodiesel, such as the need for large available areas, suitable soil, high quality water, seasonality, among others. With this type of cultivation, natural resources are not replaced and it may cause demineralization, salinization, desertification, soil erosion and depletion of water sources. Pesticides may also be required, which can cause an even greater negative environmental impact (Gerpen, 2005; Knothe, 2005; Krawczyk, 1996).

One of the biotechnological processes that have received increasing interest from companies and researchers is the cultivation of microalgae, which are an excellent source of organic compounds such as fatty acids (Johnson and Wen, 2009; Colla et al., 2004). The fatty acids that are produced by microalgae can be extracted and converted into biodiesel (Brown and Zeiler, 1993). Microalgae exhibit a great variability in lipid content. Among microalgae species, oil contents can reach up to 80%, and levels of 20–50% are quite common (Powell and Hill, 2009). The microalga *Chlorella* has up to 50% lipids and *Botryococcus* has 80%. The variations are due to different growing conditions and methods of extraction of lipids and fatty acids.

One of the main factors that influences the lipid and fatty acid content of microalgae in terms of cultivation is the CO_2 concentration. In areas where microalgae are grown for biodiesel production alongside fossil fuel power stations, CO_2 release can be significantly reduced and the lipid content increased (Sawayama et al., 1995; Brown and Zeiler, 1993).

Large growths of microalgae occur in many Chinese lakes such as Lake Chaohu, Lake Taihu and Lake Dianchi. This results in the pollution of water, the death of fish and illnesses that affect the lakeside residents. The reduction of these blooms has become an urgent project and one alternative is to use this large amount of biomass for biofuel production (Miao et al., 2004).

The analysis of the saturated fraction of biofuel from *Chlorella protothecoides* demonstrated that the alkanes chain reaches 10–30 carbons, while the alkanes chain of the saturated fraction of biofuel from *Microcystis aeruginosa* presented 10–28 carbons. When these results are compared to the chromatography of mineral diesel, the alkanes chain distribution is very similar to that of microalgae (Miao et al., 2004).

The carbon and hydrogen content of microalgal biofuel is greater than in plant biofuel, although the oxygen content is lower. The H/C and O/C mean molar ratio of microalgal biofuel was 1.72 and 0.26, while the H/C and O/C molar ratio of plant biofuel was 1.38 and 0.37, respectively. Microalgal biofuel is characterized by lower oxygen content and a higher H/C ratio than biofuel from plants, sunflower and cotton (Miao et al., 2004).

The oxygen content of microalgal biofuel compared to the oil of higher plants is important because high oxygen content is not attractive for the production of transportation fuels. Biofuel from *C. protothecoides* and *Microcystis aeruginosa* has a calorific value of 30 MJ kg^{-1} and 29 MJ kg^{-1} due to their high carbon and hydrogen content and low oxygen content, which also makes the biofuel from microalgae more stable than biofuel from plants.

The high hydrogen content of microalgal biofuel is due to chlorophyll and proteins. Compared with plant biofuel, microalgal biofuel has a high calorific value, low viscosity and low density. These physical properties of microalgae make them more suitable for biofuel than lignocellulosic materials (Miao et al., 2004).

The mean annual productivity of microalgal biomass in a tropical climate region is 1.53 kg m^{-3} day^{-1}, with a mean 30.0% of lipids extracted from the biomass, the concentration per hectare of total area is around 123.0 m^3 for 90.0% of the 365 days of a year, since the remaining 10.0% are used for maintenance and cleaning of the bioreactors.

Thus, the yield of biodiesel from microalgae is 98.4 m^3 ha^{-1}, so for the production of 5.4 billion m^3 of biodiesel required to transport, the microalgae an area of approximately 5.4 Mha must be cultivated, which represents only 3.0% of the area currently used for cultivation of plants for biodiesel production. This would be a possible scenario even if the concentration of lipids in the microalgal biomass was 15.0% of dry weight (Chisti, 2008).

To meet the required demand and add 5.0% biodiesel (B5) to mineral diesel oil, one would have to increase the production of vegetable oils by 50.0–100.0%. This is a difficult goal is achieve with these oils alone, since it represents a proportional increase in arable land with oil crops, and the current agricultural productivity has reached values that are difficult to increase.

As the concentration of fatty acids and productivity of microalgae are much higher than that of plants, this effort to increase oil production would not be so great (Ma[a] and Hanna, 1999). As an example, biodiesel output per required land area has been estimated to be – corn: 145.0 kg oil ha^{-1}, soybeans: 375.0 kg oil ha^{-1}, palm oil: 5000 kg oil ha^{-1}, algae: 80,000 kg oil ha^{-1} (Skjanes et al., 2007). The amount of land needed for the corresponding production using microalgae would be around 100.0–200.0 times less.

6. Fermentation of microalgal biomass for ethanol production

Alcoholic fermentation is one of the destinations for pyruvate at the end of the glycolytic pathway, and consists in its anaerobic conversion to ethanol and CO_2, in two steps. In the first, pyruvate is decarboxylated by the enzyme pyruvate decarboxylase, releasing CO_2 and forming acetaldehyde, which is then reduced to ethanol by the enzyme alcohol dehydrogenase (Lehninger et al., 2004). The fermentation can be represented by the equation:

$$C_6H_{12}O_6 + 2ADP + 2Pi \rightarrow 2C_2H_5OH + 2CO_2 + 2ATP + 2H_2O$$

The benefit of using ethanol as a fuel is that it reduces levels of lead, sulfur, carbon monoxide and particulates. In addition, there is the global benefit of reducing CO_2 emissions. With the increasing demand on fossil fuel replacements the USA, Europe and other states are using or considering the substitution of petrol with ethanol (Willke and Vorlop, 2004). Since the 1980s ethanol has been an established alternative to fossil fuels in Brazil. It is produced mainly from sugar and starch (sugar cane, corn) (Rupprecht, 2009). In Brazil, the use of ethanol as a gasoline substitute avoids the emission of 9.56×10^6 tons of carbon per annum (about 15.0% of Brazil's total emissions).

Traditionally, the ethanol is produced by fermentation of biomass sources, varying from agriculture energy plants to organic wastes (Xuan et al., 2009). The energetic yield of converting sugar into ethanol is estimated at 40.0%. The conversion of cereals into ethanol consumes more energy compared to the conversion of sugar into alcohol, and can even result in negative yields, which precludes the use of cereals from an energy point of view. In 2008, the US produced 600 million liters of alcohol from cereals for the production of beverages.

The largest bioenergy program that has taken place in the world was that of Brazilian sugar cane ethanol (Proálcool), which began in 1976. Under pressure from the rising cost of oil imports that threatened the balance of payments, the Brazilian government encouraged the production of ethanol from sugar cane and the adaptation of motors to the Otto cycle to run on "pure" ethanol (hydrated alcohol, with 96.0% ethanol and 4.0% water) or gasohol (78.0% gasoline and 22.0% anhydrous ethanol). The alcohol added increased the gasoline's octane and meant that the highly toxic tetraethyl lead additive could be removed.

Ethanol has a calorific value of 22.0 MJ L^{-1}, while gasoline has a value of 33.0 MJ L^{-1}. However, the higher octane rating of ethanol and the adjustments to the engines and injection systems mean that the technical equivalence of ethanol per liter of gasoline is about 1.15. Several questions about the environmental sustainability of bioenergy have become important after the growth of ethanol in the world market. One of the main ones is the competition for agricultural land for food. The value of agricultural commodities reached an unprecedented high in 2006, mainly those of grains. Price fluctuations tend to occur frequently, but the current uptrend is widespread, leading to inflation and discussions on future directions of agriculture and how to provide food to the poorer segments of the population. Thus, identifying alternative sources of bioethanol feedstock is of a high priority (Xuan et al., 2009).

Microalgae are a potential source of fermentable substrate since, according to the growing conditions, they may have high levels of carbon compounds in their composition, directly available for fermentation or after pre-treatment. Several microalgae produce ethanol when fermented, such as *Chamydomonas* sp., *Chlorella* sp., *Oscillatoria* sp., *Cyanothece* sp. and *S. platensis* (Ueno et al., 1998). Bacteria, yeasts or fungi are also used to ferment carbohydrates to produce ethanol under anaerobic conditions. However, alongside ethanol they produce other compounds such as CO_2 and H_2O. The theoretical maximum is 0.51 kg of ethanol and 0.49 kg CO_2 per kg of glucose.

The principle of ethanol production by microalgae consists in the cultivation of microorganisms, harvesting of cells, preparation of biomass, fermentation and extraction process of ethanol. The preparation of the biomass can be carried out through mechanical equipment or enzymes that break down the cell walls, making the carbohydrates more available, as well as breaking down large molecules of carbohydrates. When cells are broken down, the yeast *Saccharonmyces cerevisiae* is added to the biomass and fermentation begins. In this way, the sugar is converted to ethanol by yeasts. Distillation is used to purify the ethanol (Amin, 2009).

Ethanol produced by microalgae can be purified and used as fuel and the CO_2 can be recycled by using it as a nutrient for the cultivation of microalgae or use of residual biomass in the process of anaerobic digestion (Harun et al., 2010b).

The *Chorella vulgaris* microalga can be used as a source of ethanol due to its high carbohydrate content, with conversion efficiency above 65.0%. Ueno et al. (1998) obtained the maximum formation of ethanol from *Chlorella* sp. cultivated at 30.0 °C of 448.0 μmol g^{-1} in dry weight. When the temperature was reduced to 20.0 °C the ethanol concentration was 196.0 μmol g^{-1} in dry weight. Ethanol production decreased when temperature was increased to 35.0 °C and was completely inhibited at 45.0 °C. The determination of enzyme activity at 35.0 °C was lower than at 25.0 °C, indicating that enzymes are activated at low temperatures.

7. Microalgal biomass how substrate to produce biogas

Biogas is the product of anaerobic digestion of organic matter, consisting primarily of 55.0–65.0% methane (CH_4), 30.0–45.0% carbon dioxide (CO_2), traces of hydrogen sulfide (H_2S) and water vapor (Cooney et al., 2007; Kapdi et al., 2005), and may contain small amounts of H_2 and CO (Bailey and Ollis, 1986). The energy content of biogas produced through anaerobic digestion is 16,200–30,600 kJ m^{-3} depending on the nature of the source of biomass (Chisti, 2008).

Several bacteria act during the anaerobic process, which initially involves hydrolytic bacteria (mainly cellulolytic and proteolytic) for the degradation of raw material, the acidogenic bacteria, hydrogenated and sulfate-reducers. Organic acids, gases, salts and oxidized organic matter (among others) are formed from these microorganisms. In a final stage, the methanogenic bacteria form methane, CO_2 and reduced organic compounds (Omer and Fadalla, 2003; Cooney et al., 2007).

According to the substrate used, optimal conditions are defined for biogas production, such as temperature from 30.0 to 35.0 °C, pH 6.8–7.5, a C/N ratio from 20 to 30, total solids from 7.0% to 9.0%, and time of 20–40 days (Omer and Fadalla, 2003). After digestion, the sludge can be used as a biofertilizer, incinerated or used in animal feed.

The anaerobic digestion process is optimized when one uses compounds with moisture content between 80.0% and 90.0%, and the use of microalgal biomass is highly appropriate (Brennan and Owende, 2010). In southern Brazil, the biomass from *Spirulina* LEB 18 grown in an 18.0 m^3 raceway-type photobioreactor under environmental conditions was used as a substrate for biogas production in a semi-continuous anaerobic bioreactor. The feed rate of microalgal biomass was 0.1 day^{-1} and the initial concentration of *Spirulina* LEB 18 was 7.2 g L^{-1}. The biogas content obtained in the methane was 77.7% (Costa et al., 2008).

The process of anaerobic digestion occurs in three sequential stages: hydrolysis, fermentation and methanogenesis. The hydrolysis of complex compounds is the breakdown of carbohydrates into soluble sugars. Afterwards, the fermentation carried out by bacteria converts this biomass into alcohols, acetic acid, volatile fatty acids and gas containing H_2 and CO_2, which is primarily metabolized in CH_4 (60.0–70.0%) and CO_2 (30.0–40.0%) by methanogenesis. The conversion of microalgal biomass into biogas even recovers energy through the extraction of lipids that can be used for biodiesel production (Brennan and Owende, 2010).

Microalgae can produce a very high proportion of proteins that result in lowering the C/N ratio, which affects the efficiency of anaerobic digestion. This problem can be solved by co-digestion with products containing a high C/N ratio (Brennan and Owende, 2010). Yen and Brune (2007) achieved an increase in biogas production with the addition of residues from paper recycling to microalgal biomass. In this study, biogas production doubled (0.57 mL L^{-1} day^{-1}), using a waste/biomass ratio of 1:1, when compared to anaerobic digestion containing only algal biomass.

The high content of protein in algal biomass can result in increased production of ammonia, which inhibits anaerobic microorganisms. Sodium ions can also be toxic for some anaerobic microorganisms, which can be minimized by pre-adapting the microorganisms that are going to be used (Brennan and Owende, 2010).

Comparing microalgal biomass with the plant biomass that is traditionally used for the production of biogas, the former have the advantage that they grow in a liquid medium, and include a third dimension that is lacking in the latter, where the space available for cultivation is two-dimensional. In addition, the microalgal biomass (unlike plant biomass) does not contain lignocellulose, so no pre-treatment is required for further processing. The anaerobic digestion of microalgae is very interesting because it combines the recovery of CO_2, which is a compound produced from biogas (Harun et al., 2010b).

8. Combustion of biomass for energy

In the process of direct combustion, biomass is burned in the presence of air to convert the chemical energy stored in biomass into energy, usually in a boiler or steam turbine, at a temperature above 800.0 °C. It is possible to burn various types of biomass, but the combustion is only viable for biomass containing less than 50.0% moisture in dry weight (Brennan and Owende, 2010).

The combustion of biomass for heating or energy can be applied for domestic use or for a large scale industrial process for the production of 300.0 MW. The conversion of energy by direct combustion of biomass generally requires pre-treatment such as drying and grinding, which require an additional energy demand and increase costs.

The efficiency of converting biomass into energy from combustion is comparable to that of thermoelectric plants, but may have a high cost due to the high moisture content of the biomass. The combination of power generation and heat increases the overall efficiency of the plant.

The efficiency of net energy conversion by burning biomass is between 20.0% and 40.0%, with higher efficiencies obtained on a large scale (greater than 100.0 MW) or when the biomass undergoes co-combustion in thermoelectric power plants. When microalgae are used for direct combustion they have lower GHG emissions and air pollution compared to the burning of coal (Brennan and Owende, 2010).

9. Conclusion

Biofuels can efficiently replace the petroleum fuels and are associated with widespread availability, affordability, accessibility of technology, ease of transport, storage, versatility in use in engines and socio-economic and environmental benefits.

The advantages of using microalgae to produce biofuels are their continuous production, simple cell division cycle, acquisition of organic compounds through photosynthesis, tolerance to varying environmental conditions, use of waste or brackish water, use of land not used for agriculture and, when subjected to physical and chemical stress, they can be induced to produce high concentrations of specific compounds.

In addition, the application of microalgae for biofuels helps reduce carbon dioxide, the main greenhouse gas, thus reducing climate change that will affect the basic elements of human life: water, food, health and the environment, which affects millions of people through famine, drought and floods.

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