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Full Length Research Paper

Sustainable production of biodiesel by microalgae and its application in agriculture

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According to estimates of the intergovernmental panel on climate change, the continued use of oil based-energy is responsible for more than two thirds of the anthropogenic emissions of greenhouse gases in the atmosphere. In Brazil, the National Program for use and biodiesel production has looked for the diversification of feedstock for biodiesel production. Among several alternative sources of energy, microalgae biomass shows great potential to be used as raw material for producing biodiesel. Rich in lipids and fatty acids, the oil yield per hectare in some strains of microalgae is considerably higher than the most conventional oilseed crops such as palm, *Jatropha*, soybean and sunflower. The commercial production triggered strong interest at the 1960s, with the development of a series of technologies to cultivate microalgae in open ponds and photobioreactors. Industrial or agricultural wastes such as vinasse previously treated in anaerobic digesters, for example, can be recycled and reused through the cultivation of microalgae, besides the application in the fertirrigation of sugar cane crop. This would also qualify the cultivation of microalgae as a clean development mechanism to reduce the levels of greenhouse gases.

Key words: Biodiesel from microalgae, clean development mechanism, greenhouse gases, large-scale production, oil yield, vinasse.

INTRODUCTION

Technical reports of the Intergovernmental Panel on Climate Change (IPCC, 2007) have shown that the use of oil over decades as main energy matrix is responsible for more than two thirds of the anthropogenic emissions of greenhouse gases (GHG) in the atmosphere, such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O).

In Brazil, the National Program for Use and Biodiesel Production (PNPB) has been searching the diversification of feedstock for biodiesel production. Among several alternative sources of energy, microalgae biomass shows potential to be used as raw material for producing biodiesel, which will allow the total replacement of diesel oil (Danielo, 2005; Teixeira and Morales, 2007). Currently Brazil occupies a privileged position and leadership in the development of new technologies in biofuels.

Microalgae are usually microscopic, prokaryotic or eukaryotic, and uni- or pluri-cellular organisms. Among the photosynthetic organisms, microalgae are the most efficient in the absorption of CO_2 and their growth is directly related to the reduction of GHGs, since they require large quantities of CO_2 as carbon source (Chisti, 2007). Fatty acids and lipids are present in the composition of cell membranes as well as in storage compounds, metabolites and sources of energy (Banerjee et al., 2002).

Although microalgae cannot immobilize carbon for long periods like the trees in a forest, for example, microalgae biomass can be cultivated together with power-plants that generate CO_2 excess. As the CO_2 resulting from industrial processes can be used by microalgae cells to carry out the photosynthesis, it is interesting that microalgae culture is fertilized with CO_2, instead of it be released into the atmosphere. Microalgae require large quantities of CO_2 as nutrient; with potential to function

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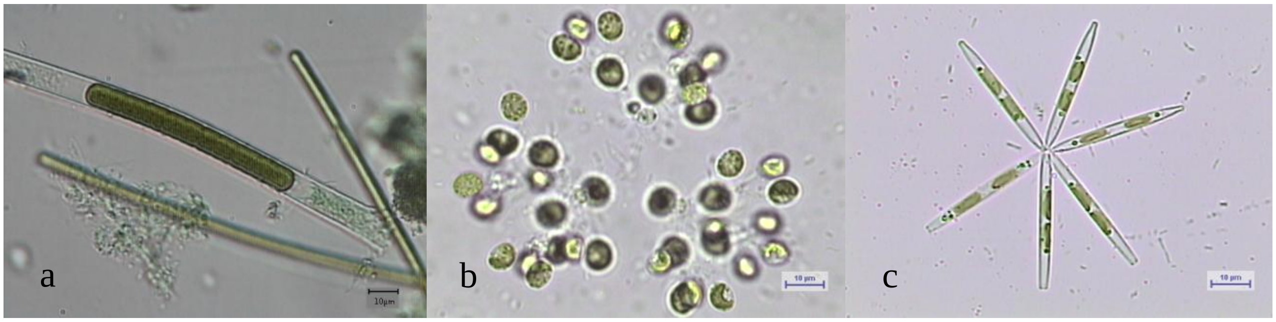

Figure 1. Photomicrography of microalgae. (a). *Lyngbia* sp. (Cyanobacteria), (b) *Dictyosphaerium pulchellum* H. C. Wood (Clorophyta), and (c) *Synedra* sp. (Bacillariophyceae). Scale bar: 10 μm.

as carbon sink.

The consumption of microalgae for food is a tradition which dates back to very old ages, where native peoples from Asia consumed some species of the genus *Nostoc*, and other people such as the Aztecs in Mexico, and the Kanembous in Africa, consumed algae of the genus *Spirulina*. The winds pushed and clustered algae biomass at the margins. Then the biomass was dried, mashed and cut into small slabs (Durand-Chastel, 1980; Dillon et al., 1995). However, the commercial production of microalgae triggered strong interest in the twentieth century, starting at the 1960s, with the development of a series of technologies to produce biomass for cultivation of microalgae in open farm ponds and photobioreactors (Wagner, 2007).

Microalgae cultivation as a source of renewable biomass can be applied to the production of biodiesel in replacement to oil. Global warming is currently associated with increases of CO_2 in the atmosphere.

Microalgae: A source of energy and biomass for biodiesel production

Microalgae are a generic term without taxonomic value, covering organisms of well-varied morphology and cell structure: phylogenetically they are prokaryotic or eukaryotic, unicellular, colonial or filamentous. In this review it was considered the prokaryotic microalgae – Cyanobacteria (Cyanophyceae), and the eukaryotic microalgae – green algae (Chlorophyta), and diatoms (Bacillariophyceae). They can be found in seawater, brackish, freshwater and in the soil (Figure 1).

Under the name microalgae are species that have chlorophyll a, using a photosynthetic process similar to the higher plants, and other pigments (Pérez, 2007; Patil et al., 2008). Estimates of the number of known species vary widely according to different locations around the world. The Algal Collection of the U.S. National Herbarium, located in the Smithsonian National Museum of Natural History, is represented by over 219,548 accessioned and inventoried specimens (Smithsonian National Museum of Natural History, 2011). But only a small number of microalgae strains maintained in culture collections are cultivated on an industrial scale (Becker, 2004). Such genetic diversity reflects in the biochemical composition of species, which is why there is today an unlimited quantity of high-value compounds.

The biochemical composition of microalgae is not only determined by the nature of each species or strain, but also is strongly influenced by factors such as light intensity, temperature, pH, nutrients and agitation of the culture medium. According to Becker, 2004 microalgae exhibit high levels of proteins and lipids, reaching values of 71 and 22% (by dry mass) respectively, depending on the species. The interaction of these factors can contribute significantly to optimize the growth of microalgae. The pH is also important in the cultivation of biomass, ranging from neutral to alkaline for most species of microalgae (Pérez, 2007).

Algal cultivation is gradually increasing worldwide (Sheehan et al., 1998; Dayananda et al., 2005; Spolaore et al., 2006). The biomass is intended to be used for many purposes such as production of single cell protein, carotenoids, chlorophyll, enzymes, esters, antibiotics, vitamins, hydrocarbons, extraction of pigments, animal feed, food supplement (Kay, 1991; Banerjee et al., 2002; Lorenz and Cysewski, 2003; Shimizu, 2003; Spolaore et al., 2006), and in bioremediation of contaminated areas (Kalin et al., 2005; Munoz and Guieysse, 2006). Biotechnology researches indicate that the main application of microalgae biomass is for production of food supplements, but the cultivation has been restricted to few species belonging to the genera *Chlorella*, *Dunaliella*, *Scenedesmus* (Chlorophyta) and *Spirulina* (Cyanophyceae) (Becker, 2004).

The production of microalgae biomass in large-scale is one of the issues concerning the oil supply for use as biodiesel. Rich in lipids and fatty acids, the oil yield per hectare in some strains of microalgae is considerably higher than the most conventional crops such as oil palm, *Jatropha*, soybean and coconut (Table 1). Oils found in microalgae cells show some physical and chemical properties similar to those of vegetable oils, and therefore,

Table 1. Potential sources of biodiesel.

Crop	Oil yield (L ha^{-1})	Land area needed (M ha^{-1})
Zea mays (corn)	172	1540
Glycine max (soybean)	446	594
Brassica napus (canola)	1190	223
Jatropha curcas (Jatropha)	1892	140
Cocos nucifera (coconut)	2689	99
Elaeis guianeensis (palm)	5950	45
Microalgae specie[a]	136,900	2
Microalgae specie[b]	58,700	4.5

Source: Adapted from Chisti, 2007._[a]:70% oil (by dry weight) in biomass; [b]: 30% oil (by dry weight) in biomass.

Table 2. Oil contents found in some species of microalgae.

Microalgae	Division	Oil content (% dry mass)
Botryococcus braunii	Chlorophyta	25 – 75
Chlorella sp.	Chlorophyta	28 – 32
Crypthecodinium cohnii	Dinophyta	20
Cylindrotheca sp.	Heretorokonthophyta	16 – 37
Dunaliella primolecta	Chlorophyta	23
Isochrysis sp.	Haptophyta	25 – 33
Nannochloris sp.	Chlorophyta	20 – 35
Nannochloropsis sp.	Heretorokonthophyta	31 – 68
Neochloris oleoabundans	Chlorophyta	35 – 54
Nitzschia sp.	Heretorokonthophyta	45 – 47
Phaeodactylum tricornutum	Heretorokonthophyta	20 – 30
Tetraselmis sueica	Chlorophyta	15 – 23

Source: Adapted from Chisti, 2007.

can be considered a potential raw material for the production of biodiesel (Chisti, 2007).

Biodiesel from microalgae is a renewable energy source whose use does not contribute to increase the GHG in the atmosphere, since its production and use represents a closed cycle of CO_2. Moreover it is biodegradable, non-toxic, can be safely handled, and contains no sulfur, benzene and other aromatic compounds (Wagner, 2007). The cultivation of microalgae provides a number of other economic advantages such as relatively low costs for harvesting and transportation, lower cost of water, use of areas of infertile soils as support for the system of cultivation, high efficiency of CO_2 photosynthetic fixation by area, and the ability to grow in saline simple media (Danielo, 2005).

The use and advantages of various genera of microalgae for production of biodiesel have been widely discussed in literature (Sheehan et al., 1998; Sawayama et al., 1999; Danielo, 2005; Metzger and Largeau, 2005; Chisti, 2007; Ranga et al., 2007; Wagner, 2007; Chisti, 2008). Among the genera reported, *Botryococcus* (Trebouxiophyceae, Chlorophyta) is described as one of the most efficient producers of oil (Ranga et al., 2007) (Table 2). *Botryococcus braunii* Kűtzing is a green colonial microalga found in lakes and reservoirs of fresh and brackish water in the world. This species has attracted great scientific and commercial interest because of its ability to accumulate high amounts of lipids, which can be converted into biodiesel, jet-fuel, gasoline and other important chemicals (Metzger and Largeau, 2005).

It is well described that species of *Botryococcus* spp. produce various types of hydrocarbons, of which botryococens are the most important because they are produced in larger quantities (Dayananda et al., 2005). Depending on the type of hydrocarbon produced, *B. braunii* (Figure 2) is classified among strains A, B or L. Algae belonging to race A produce primarily oil and n-alkadiene and triene hydrocarbons, numbered C_{23} - C_{33}; the strain B produces triterpenoid hydrocarbons, C_{30} - C_{37} botryococcens (Metzger et al., 1985) and methyl squalene C_{31} - C_{34} (Achitouv et al., 2004), while strain L produces a single type of triterpenoid hydrocarbon,

Figure 2. Morphology of Botryococcus braunii. Arrows show matrix of lipids. Source: Culture Collection of Autotrophic Organisms (CCALA).

named licopedien (Metzger et al., 1990). Also hydrocarbons, *B. braunii* species synthesize fatty acids, triacil glicerols and sterols (Dayananda et al., 2005).

Oil extraction of microalgal biomass: Transesterification

The National Renewable Energy Laboratory (NREL) and the Department of Energy's Office of Fuels Development (DOE) in the United States were pioneers in 1970 to start research with strains of microalgae for use in biofuels, given the context of possible shortages of oil in the country (Wagner, 2007). From 1978 to 1996 DOE established a program for the sustainable production of oil from algae, whose original mission for the algae project was CO_2 mitigation (Sheehan et al., 1998).

During the first years of study researchers figured out that some species of algae were able to produce about 50% or more of their weight in lipids, under proper conditions. Since then the main focus of the program, known as Aquatic Species Program (ASP), has been the production of biodiesel from algae with high contents of lipids in ponds, utilizing waste CO_2 from coal burning plants. Located in Golden, Colorado, NREL is a consortium of research laboratories aiming the production of oil from microalgae. Currently NREL has selected about 300 species of marine and freshwater microalgae, mainly belonging to the group of diatoms (genera *Amphora*, *Cymbella*, *Nitzschia*, etc.), and green algae (especially the genus *Chlorella*) (Wagner, 2007).

According to scientists from NREL, the oil yield in the algae biomass is at least 30 times higher than in oil crops such as palm, sunflower or peanut, commonly used in the production of oil. The advantage of microalgae to grow in liquid medium allows them greater access to water, CO_2 and nutrients. Another important aspect to be considered is the surface area exposed to the sun and not exactly the volume where they grow. Thus microalgae productivity is measured in terms of biomass (kg of algae or oil) per day, per unit area exposed to the sun, which allows comparisons of their data with the data from terrestrial plants (Danielo, 2005).

Biodiesel is a clean-burning fuel produced from grease, edible vegetable oils (soybean, rapeseed, sunflower), non-edible vegetable oils (*Jatropha*, castor, pongamia), non-edible microalgae lipids and animal fats. Its chemical structure is of triglycerides molecules, that are composed of three fatty acids (R–COOH) and one glycerol [$C_3H_5(OH)_3$] molecule (Vasudevan and Briggs, 2008). The transesterification reaction consists of transforming triglycerides into fatty acid alkyl esters, in the presence of an alcohol, such as methanol or ethanol, and a catalyst, such as an alkali or acid, with glycerol as byproduct (Figure 3). It is the main chemical process used to solve the high viscosity of triglycerides, but maintaining the cetane number and heating value closer to the diesel fuel (Canakci and Sanli, 2008).

When compared with other thermochemical processes used to transform biomass into liquid fuel such as pyrolysis, gasification or Fischer-Tropsch synthesis, transesterification requires lower energy and economic requirements to convert plant oils into biodiesel. It is desirable to have higher contents of oil in order to minimize the energetic and economic costs (Vasudevan and Briggs, 2008).

Large-scale production of biodiesel from microalgae

The integrated production of microalgal biodiesel requires large quantities of biomass that is harvested, and pre-treatments are used to reduce water content and increase the energy density in the algae paste (Patil et al., 2008). The oil is then separated from the paste wither by a chemical process or by pressing in a high pressure device such as a screw press. The finished product is algae oil in a form that is then suitable for use in the transesterification reaction to make biodiesel fuel (Figure 4) (Canakci and Sanli, 2008).

According to Hossain et al. (2008) microalgae can provide renewable biofuels from different sources and processes, such as: methane produced by anaerobic digestion of the algal biomass; biodiesel derived from microalgal oil, and photobiologically produced biohydrogen.

Photobioreactors

In summary there are basically two ways for cultivating microalgae: photobioreactors and the open ponds. The

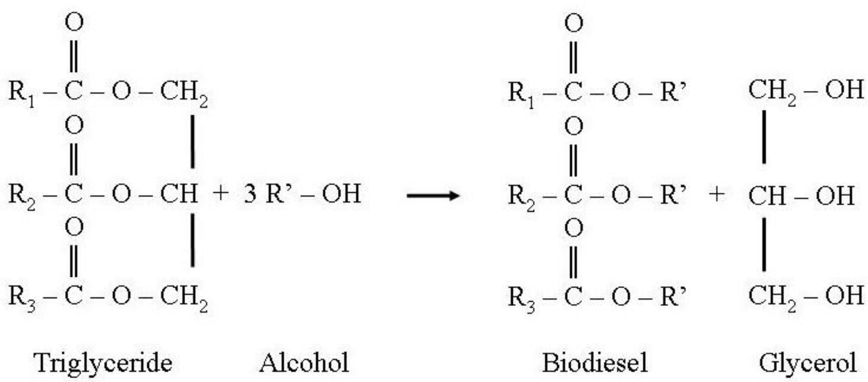

Figure 3. Schematic representation of the transesterification reaction.

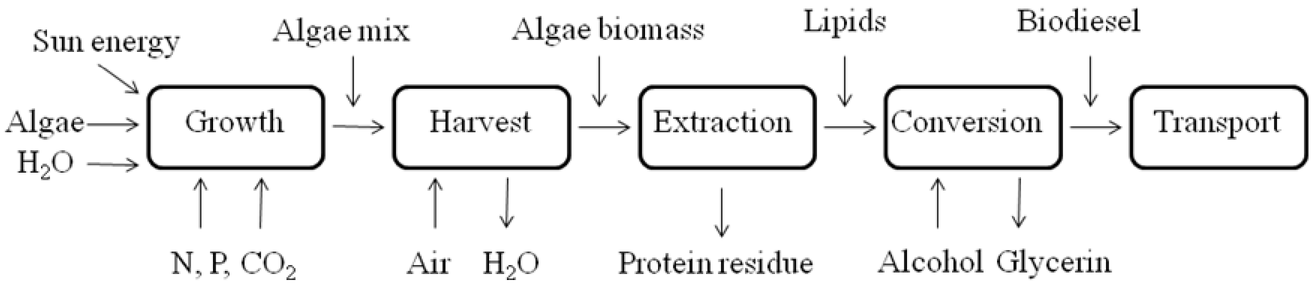

Figure 4. Integrated system to the production of biodiesel from microalgae.

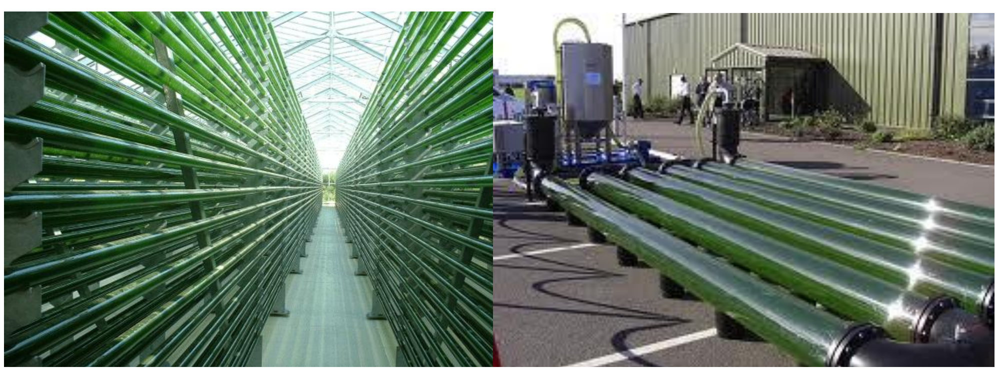

Figure 5. Technology for production of microalgae in tubular photobioreactors. Source: Calpoly (2011).

photobioreactors are constructed from glass tubes, plastic or polycarbonate, and it is possible to control some parameters like pH, temperature, light, nutrient concentration, etc. The costs for adjustment and operation of a photobioreactor are higher than that of the open ponds. A photobioreactor consists basically of a bioreactor coupled to a specific type of light (Figure 5) (Danielo, 2005).

According to data from Dr. Isaac Berzin, chief´s technology officer of the American GreenFuel Technologies Corporation in Cambridge, Massachusetts, the GreenFuel´s bioreactor is a triangular structure made of polycarbonate tubes with 2 to 3 long meters and 10 to 20 cm in diameter. The hypotenuse of the triangle is exposed to sunlight while the other sides are in opposite direction to the sun. The gas containing 13% CO_2 is injected into the tubes in order to obtain maximum growth of algal biomass, GHG sequestration and oil production (Danielo, 2005). Photobioreactors using microalgae as feedstock for capturing CO_2 and NOx gases are increasing in countries like Israel, United States, Portugal, Australia, among others (Sheehan et al., 1998).

Figure 6. Cultivation of microalgae species in open farm ponds. Source: La Monica (2008).

Photobioreactors require much smaller surface area than the raceway ponds, which helps minimize the concerns with the use of lands to produce fuel, instead of food. The production of biomass and oil are also significantly higher than in the open ponds (Pérez, 2007). Chisti, 2007 calculates that for an annual production of 100 t of biomass and consuming the same amount of CO_2 for cultivating an microalga with high oil yield (70% by wt oil in biomass), photobioreactors provide higher oil yield per hectare (136.9 m^3 ha^{-1}) than the raceway ponds (99.4 m^3 ha^{-1}).

Raceway ponds

Microalgae cultivation in open ponds, called as "raceway ponds", is held in a large bowl with about 30 cm depth and continuous water flow pumped by a motorized paddle wheel. Carbon dioxide is also added to the tank. Algae cultivation will grow continuously with all necessary nutrients being provided, especially the CO_2, sun energy and water (Figure 6) (Sheehan et al., 1998).

The biggest advantage by using the open ponds is its relative simplicity in structure, with biomass yields at low production costs and operational management. However the efficiency and oil yield of the raceway ponds are significantly lower than the photobioreactors (Chisti, 2007; Pérez, 2007). Not all species of algae are capable of growing in open ponds, due to easy contamination by other algae and bacteria. This is why the number of microalgae species capable of growing in the open ponds is still small. Another disadvantage is some difficulty to control the temperature and the amount of light in the ponds (Sheehan et al., 1998).

Taking into account synthetic culture mediums the high costs of nutrients can be a limiting factor for microalgae cultivation. But for alternative culture mediums such as industrial or agricultural wastes, the possible limiting factors are related to light intensity, temperature variation, nutrient concentration and agitation bath (Barrocal et al., 2010). Thus it is fundamental to adequate such parameters in order to provide ideal growth conditions that every species of microalga requires to reach maximum efficiency in biomass and oil production.

Alternative treatment for vinasse and cultivation of microalgae

Vinasse is a byproduct generated at approximate rates of 13 L vinasse / L alcohol during distillation of fermented sugarcane juice for ethanol production. The composition of vinasse is well-varied, due mainly to the composition of the juice used in the alcoholic fermentation. Whatever is the fermentation process, the predominant residues are water, organic matter and the minerals: potassium (K) and sulfur (as sulfate) (Parnadeau et al., 2008).

At all stages of the sugar cane cycle there are GHG emissions. The principal gases released to the atmosphere are CO_2, CH_4 and N_2O, which show different global warming potentials (CO_2 = 1, CH_4 = 23 and N_2O = 296). The global warming potential of the other gases is calculated in accordance with their values of equivalent CO_2 (Ceq) (IPCC, 2007). As examples it can be mentioned the planting, cultivation, harvesting, reed reformation and the production and disposal of residues (vinasse and bagasse).

The treatment of vinasse in anaerobic digesters to produce CH_4 can prevent the emissions of GHGs to the atmosphere, and consequently reduce the “carbon footprint” of ethanol production from sugar cane. Biological treatments can be used to clean the vinasse previously treated in anaerobic digesters, with potential of it to be reused safely.

Vinasse digestion is an alternative method not so widely used and studied that consists in the reduction of its biological oxygen demand (BOD) and chemical oxygen demand (COD) (Table 3), through anaerobic reactors. The main residue is biogas, a mixture of CH_4 and CO_2 gases (55 and 45%, respectively) produced through oxidation of organic matter in the absence of oxygen, by the methanogenic bacteria. It also shows advantages such as: low power consumption, small scale production of sludge for disposal, high efficiency in reducing the organic load and lower pollution potential, since the biogas produced can be used for co-generation

Table 3. Physico-chemical characteristics of *"in natura"* and digested vinasse.

Chemical attributes	"*In natura*" vinasse	Digested vinasse
pH	4	6.9
COD (g L^{-1})	29	9
N_{total} (mg L^{-1})	550	600
N_{amon} (mg L^{-1})	40	220
P_{total} (mg L^{-1})	17	32
Sulphate (mg L^{-1})	450	32
Potassium (mg L^{-1})	1,400	1,400

Source: Biometano – São Martinho Mill.

power (Rocha et al., 2010).

The digested vinasse maintains its use for fertirrigation of the sugar cane crop, since only part of the organic carbon load is removed during the fermentation performed by the methanogenic bacteria. It is a potential culture medium for many microorganisms that, in turn, can produce economic and attractive products. A promising alternative is cultivating microalgae in the digested vinasse.

In order to use vinasse as complete culture medium for microalgal growth it is very important to know its chemical composition, and based on such information comparing them with the nutritional requirements in terms of N, P and S to the metabolism of microalgae. Based on data available in the Table 3 it becomes feasible to evaluate the potential of vinasse as cultivation medium and nutrients source to the microalgae.

Because of CO_2 needs to carry out the photosynthesis, it is interesting that microalgae biomass is "fertilized" with CO_2, which does not spread quickly in liquid medium. The injection of CO_2 produced by burning of the CH_4 that is released in the anaerobic digestion of the vinasse can be used as carbon source to the photoautotrophic microalgae, instead of being emitted into the atmosphere.

Thus the production of biodiesel derived from the microalgae biomass may play a significant role to the increasing world demand for fuel oil.

Perspectives

According to estimates of the Intergovernmental Panel on Climate Change (IPCC, 2007) the use of petroleum-based energy is the main responsible for more than two thirds of the emissions of GHGs in the atmosphere and, consequently, the current global climate change concerns.

In Brazil the National Program for Use and Biodiesel Production (PNPB) has developed research aiming to test different feedstock for production of renewable biofuels. Among several alternative sources of energy, microalgae show potential to be used as raw material to produce biodiesel, ethanol, and to reduce the carbon emissions from power-plants (Danielo, 2005; Teixeira and Morales, 2007).

Considering the photosynthetic organisms, microalgae are the most efficient in the absorption of CO_2 and their growth is directly related to the reduction of GHGs, since they require large quantities of CO_2 as carbon source. Given optimal conditions microalgae can double its volume within hours. By comparing species of microalgae (up to 70% oil by dry weight) with the oleaginous crop of higher oil production – palm tree – they show a benefit of approximately 23 times in oil yield (Chisti, 2007). *B. braunii* is a species of microalga described as one of the most efficient producers of oil.

Microalgae are becoming a very promising source of biodiesel, whose use and production represents a closed cycle of CO_2. Industrial or agricultural wastes such as the vinasse previously treated in anaerobic digesters, for example, can be reused and recycled through the cultivation of microalgal species, besides its application in the fertirrigation of the sugar cane crop. This would also qualify the cultivation of microalgae as a Clean Development Mechanism (CDM), or technological alternatives for development of clean energy sources which do not emit CO_2, or that may reduce the levels of the GHGs.

The fast-growing rates of microalgae favor the extraction of oil in large-scale although some barriers can be overcome, which are: select the right algae species, create the photobiological formula for each species and build a low cost-effective photobioreactor that can induce a highly efficient microalgal growth (Patil et al., 2008). The global interest in clean and sustainable technologies is ensured by a search of how to identify oil-rich algae and develop processes for extracting algae oil and other products economically.

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