Assessment of the potential of residuary microalgae from a stabilization pond for the production of biofuel

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Abstract

Biomass with high lipids are necessary to the biodiesel industry since the demand for this biofuel is increasing in Brazil and the main oilseed crop used for producing biodiesel in Brazil will not be able to aid the production of biodiesel without arable lands destined only for that purpose. Thus, the microalgae emerge as a potential biomass and they are being used as a source of products for biofuel production because of their high productivity and rapid growth. However, the high cost of microalgal biomass production is the main factor that prevents the use of its products on a large scale. Thus, this work is aimed at using microalgal biomass already present in effluents from aerobic and anaerobic lagoon ponds used for processing the effluent of the UASB reactor; to obtain products and it is proposed to use these as a biofuel. Initially, a qualitative and quantitative characterization of the microalgae species present in the aerobic anaerobic lagoon was performed. The biomass was recovered from the effluent using a vacuum filtration system and sun-dried. For the extraction of total lipids a mixture of polar and apolar solvents was used. The extract was analyzed using FTIR and GC/MS. The results showed that, in quantitative terms, the taxa Cyanobacteria and Euglenophyceae were predominant. In relation to the species found, the species with the largest number of individuals per milliliter were *Lepocinclis salina* Frits., *Plankthotrix isothrix* Bory and *Euglena* sp. The extract obtained presented values of 16 wt%. The infrared spectrum showed the presence of ester and alkanes just like the GC/MS analysis. The microalgae

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identified are producers, predominantly, of lipids with saturated fatty acids and hydrocarbon. The high presence of microalgae in a natural watercourse can bring environmental problems. Thus the obtainment of microalgal biomass aerobic and anaerobic from a lagoon pond might provide an economic and environmental gain.

Keywords: effluent from aerobic and anaerobic lagoon, lipids, hydrocarbon, biodiesel from microalgae, environmental impacts.

1 Introduction

Biomass with high lipids have been the target of research to ensure the supply of triglycerides for biodiesel plant (simple alkyl esters of fatty acids), since the demand for this biofuel is increasing in Brazil (3.6% per year) and it is observed that the main oilseeds used to obtain biodiesel in Brazil (soybeans, serving 75% of biodiesel production) will not be able to aid the production of this biofuel without the allocation of agricultural lands solely for that purpose, due to the low productivity of biomass (0.2–0.4 tons per hectare). Therefore, it is preferable that the biomasses used provide an optimum productivity in lipids with the use of a smaller surface area of land [1–3].

As a promising source of lipids for the biodiesel production chain, microalgae appear as a biomass that have high quantities of lipid (20%–50%), which is obtained naturally from the conversion of atmospheric carbon dioxide by the use of photosynthesis and assisting in the removal of CO_2 from the atmosphere [1]. These lipids are composed of glycerol, sugars or esters of saturated or unsaturated fatty acids (12 to 22 carbon atoms) [4], with its content and composition influenced by factors such as light, temperature, concentration and source of nitrogen and concentration of CO_2 [5] and can be obtained by mechanical (press) and/or the use of solvents, as it is done with traditional biomass [1]. What is observed is that the lipid content of microalgae and their productivity is much higher than some oilseed traditionally used to produce biodiesel.

The microalgal biomass is mainly obtained from cultivation in the laboratory or industrial closed systems (photobioreactors), or aerated ponds fed with culture medium [6]. In these, there is a predominance of one species by the use of microalgae cultivated by autotrophic and/or heterotrophic processes. In a way to minimize the costs of the final product (microalgae oil), microalgae can be obtained directly from a system where they are already available naturally, due to the provision of optimal environmental conditions for its development. The microalgae fed by effluent in a stabilization pond system, for example, can be used to obtain cheaper oil when compared to those obtained by a cropping system [7]. This will lead to saving water and nutrients as well as serve to remove nutrients from wastewater, such as nitrogen and phosphorus.

The aerobic and anaerobic lagoon stabilization pond is rich in nutrients in the aerobic zone in a pond of this system is the development of a species of microalgae. These, when performing the process of photosynthesis, provide oxygen for respiration of the bacteria present in this system [8, 9]. In addition, the effluent from the pond with high macroalgal biomass concentration is usually released into water, and it may cause impacts on the characteristics of areas fed by the water [9, 10]. Therefore, this medium presents itself as a potential supplier of microalgal biomass.

The high cost of oil from microalgae is the main factor that hinders its widespread use. This is related to spending on infrastructure (construction of tanks and photobioreactors, for example) for cultivation, use of media, and separation processes of microalgae from the medium liquid. Thus, in order to contribute to reducing the value of the oil from the microalgae and remove the large amount of biomass released into natural water, after treatment of the effluent in aerobic and anaerobic lagoon, we intend to use the microalgal biomass (called residual microalgae) already present in the effluent from aerobic and anaerobic lagoon ponds of the Wastewater Treatment Plant (WWTP) of Union Village, in Palmas-TO, to obtain lipids and propose the use for biodiesel production.

2 Methodology

2.1 Characteristics of the WWTP Union Village, Palmas-TO

The WWTP Union Village is located in the northern sector of the city of Palmas, state of Tocantins and belongs to the Sanitation Company of the state of Tocantins, consisting of pre-treatment (grating, box of sand and grease trap), an UASB (Upflow Anaerobic Sludge Blanket) reactor followed by an aerobic and anaerobic pond (Length: 256m, Width: 110m, Area: 28.160 m^2; Volume: 42.240 m^3; Height: 1.5m) for a post-treatment of that effluent. The aquatic environment receiving the effluent from the WWTP is the Stream Agua Fria whose mouth is the reservoir of Luis Eduardo Magalhães Hydroelectric Plant, where the release point of the effluent is located near the mouth of the stream. The population makes use of it for various purposes (recreation, fishing and doing their laundry) [11].

2.2 Collection of microalgae

For the qualitative and quantitative analysis of microalgae, the aerobic and anaerobic lagoon sample was collected of the effluent (100 mL) in an amber bottle with the aid of a collecting vessel and a funnel, both made of plastic. The sample was stored and transferred for analysis. Then, a volume of 10L was collected to obtain biomass. The collection was gathered at the outlet of the pond. After collecting the effluent, it was transported to a laboratory to be made into the concentration of microalgal biomass.

2.3 Qualitative characterization of the microalgae from the aerobic and anaerobic pond

The methodology used for the qualitative analysis of the microalgae from the aerobic and anaerobic pond was described by Marques [12], which consists of the taxonomic approach and the population structure, using optical microscopy (using a 40x objective, corresponding to the range 10μm to 25.30cm) binocular Olympus MX41, equipped with digital color camera and ocular micrometer, the organisms were observed in frontal, apical and lateral view, aiming to identify, measure and capture images. The characteristics (morphological and morphometric of the vegetative and reproductive life) of taxonomic value of the species were analyzed according to specialized bibliographies.

2.4 Quantitative analysis of the microalgae from the aerobic and anaerobic lagoon

Quantitative analysis was performed according to the method described by Uthermöhl [13], which is based on the random distribution of individuals in the bottom of the sedimentation chamber. The supernatant volume was 10 ml, in function of the sample concentration. The count was performed on an inverted microscope whose counted fields were distributed in parallel vertical transects, covering almost the entire area of the chamber, drawn randomly. The sedimentation time was at least 3 hours for each inch of height of the camera [14]. In calculation of the phytoplankton, it was considered as an individual unicellular organism, filaments, trichomes, colognes and cenobio. The number of fields counted was the necessary amount to reach 100 individuals of the more frequent species, or stabilizing the number of species added per field (minimum area of compensation). Lund *et al.* [15] considered the counting error less than 20% at a significance level of 95%. The count results were expressed as individuals per ml, calculated according to Nogueira and Rodrigues [16]. Both the quantitative and taxonomic analysis was performed in duplicate.

2.5 Harvest of microalgal biomass

The concentration of microalgal biomass was carried out using a vacuum filtration system which consisted of a Kitassato, Büchner funnel and vacuum pump (Prismatec model 131B) using, for the retention of microalgae, a quantitative paper filter (CAAL Nº1540). After the filtration, the concentrated biomass was dried in direct sunlight, open air (outdoors), and temperature and humidity were measured using a thermo-hygrometer TFA. The concentration of microalgal biomass was performed in triplicate.

2.6 Lipids extraction

The extraction of lipids from the biomass was performed according to Folch *et al.* in Lourenço [4], with adaptations. Initially, the dried samples (343.07 ± 5.44 mg) were put into a beaker and to this was added 60mL of a chloroform mixture: methanol (2:1 v/v). The mixture was stirred (with the aid of mechanical stirrer Fisatom model 713D) in the chapel, and macerated for 5 minutes. Then, a vacuum filtration was made, where the cell debris remained in the filter paper and the medium liquid was collected, its volume was measured, and transferred to a separated funnel. To this was added a solution of KCl 0.88% (corresponding to ¼ of the volume of the mixture contained in the funnel). The mixture was stirred manually three times and after this, the medium was put to sit for 10 minutes. The separation was made of the phases (top and bottom), and the upper phase was discarded. The lower phase, dense and with dissolved lipids, was separated and added to a mixture of methanol: distilled water (1:1 v/v) corresponding to ¼ of the solution volume. Again the system was agitated three times and left to stand for 10 minutes. The lower phase was separated and dried with anhydrous Na_2SO_4. Then the mixture was filtered in a vacuum. The solvent present in the mixture was removed by rota-evaporation using a rota-evaporation apparatus Fisatom model 802. The concentrate was measured and its mass was stored in an amber bottle and refrigerated.

2.7 Analysis of the extract by Fourier transform infrared (FTIR) and gas chromatography with mass spectroscopy (GC/MS)

The infrared spectra covering the region of 4000 - 400cm^{-1} were obtained in FTIR Spectrophotometer, Thermo Nicolet model Nexus 470, from the analysis of 1mL of sample extract obtained in the previous step. The spectra were obtained at room temperature in solid pellets, and were acquired with a resolution of 4cm^{-1} and 32 scans min^{-1}.

2.8 Obtaining and analysis of biodiesel

To obtain the biodiesel 180.4mg of lipid extracted from the microalgae collected in the WWTP pond were used. The methodology used was described by Xu *et al*. [17]. The biodiesel was analyzed by FTIR, as it was done for the lipid extract. The chromatographic analysis was performed according to Xu *et al*. [17], using equipment of GC/MS Varian Saturn 2200.

3 Results and discussions

3.1 Qualitative characterization of the microalgae from the aerobic and anaerobic lagoon

Descriptive species of the phytoplankton of the aerobic and anaerobic lagoon belong to the groups Bacillariophyceae, Cyanobacteria, Chlorophyceae, Cryptophyceae, Euglenophyceae, Dinophyceae and Zygnemaphyceae. Two taxa were identified belonging to Bacillariophyceae division, 9 taxa of Cyanobacteria division, 9 taxa of Chlorophyceae division, one of the Cryptophyceae, 9 of Euglenophyceae, 7 Zygnemaphyceae and 1 taxon of Dinophyceae division.

3.2 Quantitative analysis of the microalgae from the aerobic and anaerobic lagoon

The largest number of individuals per milliliter was microalgae belonging to Cyanobacteria and Euglenophyceae groups in the two collections. This may be related, according to Soldatelli [18], the characteristics of the culture medium (secondarily treated effluent by anaerobic UASB), rich in nitrogen compounds, enabling the environment for the proliferation of these groups of microalgae.

Fifteen taxa were identified at generic, specific and intraspecific levels. The genera numbers for different groups of algae were: 3 Cyanobacteria, 5 Chlorophyceae, 1 of Cryptophyceae, 5 Dinophyceae and 1 Euglenophyceae. In relation to the species found, the *Lepocinclis salina* Frits. the *Plankthotrix isothrix* Bory, *Euglena* sp. and *Phacus longicauda* (Ehr.) Duj. were the species with the highest number of individuals per milliliter.

3.3 Harvest of microalgal biomass

The amount of microalgal biomass obtained by filtration of the effluent from the aerobic and anaerobic pond was 821.77 ± 266.40mg. The effluent, after filtrated, had its apparent color changed (from green to transparent), as shown in fig. 1, confirming the recovery of microalgal biomass and improving the effluent to be released in the receiving body, since, according to Petry [19], when the receiving body has a combination of algae and halogenated compounds, trihalomethanes can be produced. Borges in Petry [19] analyzed samples of water containing chlorophyceae algae and cyanobacteria and noted that trihalomethanes were formed in these waters, even in small quantities compared with waters rich in humic acids. He noted also, by filtration or not of water containing algae, that the algae as well as its extracellular products act as precursors of trihalomethanes, where the water with algae in suspension has a higher risk for formation of these byproducts. According to Naval *et al*. [11], the WWTP Union Village generates a lot of negative impacts in various environments (water, soil and air) and the population that uses the stream water that receives the effluent from the WWTP. In respect the elevated presence of microalgae in the effluent of the WWTP, these authors show that, when thrown into the stream, algal biomass might generate impacts arising from the presence of toxin producing microalgae, might lead to a reduction of the photic zone of the stream and might alter the aquatic flora.

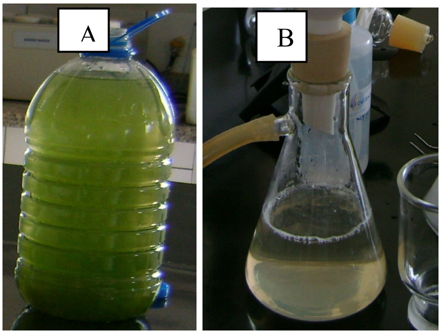

Figure 1: Effluent from the aerobic and anaerobic pond of the WWTP Union Village. Before (A) and after (B) filtration.

3.4 Lipid extraction

In percentage terms, an extract with values of 16wt% was obtained, with an average of 55.6 ± 1.044mg. Thus, considering only 0.50 m deep of the aerobic and anaerobic lagoon (area of greater light intensity, according to Von Sperling [9]), the volume of 14,080m³ would be obtained and, using the least amount of biomass obtained (594.2mg), recovered algal biomass would be approximately 858.086Kg and an amount, considering a 16% content, of approximately 127.39Kg in lipid.

The concentrate obtained had a greenish color and a waxy appearance, as seen in fig. 2. According to Thompson [20], some microalgae have an alternative form of lipids storage. The specie *Euglena gracilis* (Euglenophyceae), depending on the environmental condition of light in which is found, can store wax composed of fatty acids from 12 to 13 carbon atoms, with a greater quantity of wax when the cells are grown in an anaerobic environment. Besides wax, many species identified in the effluent of the aerobic and anaerobic pond in Union Village are producing saturated fatty acids, providing a solid consistency to the extract obtained, when present in the triglyceride structure.

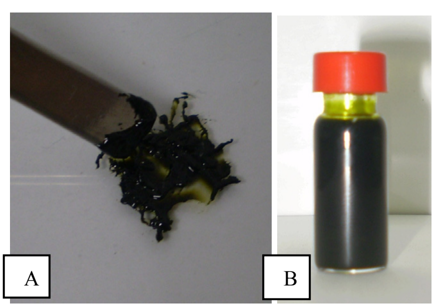

Figure 2: Lipid extract obtained of microalgae from the WWTP Union Village pond. Concentrate (A) and diluted in hexane (B).

3.5 Analysis of the extract by Fourier transform infrared (FTIR)

According to Yang *et al*. [21], most of the absorption bands representing the functional groups of triglycerides can be observed around 2937cm^{-1} (C-H asymmetric axial deformation), 2856cm^{-1} (axial symmetric deformation of C-H), 1749cm^{-1} (axial deformation of C=O group), 1454cm^{-1} (angular deformation such as scissoring of C-H), 1166cm^{-1} (angular deformation of C-O and C-H) and 709cm^{-1} (angular deformation such as rocking of C-H).

Several absorption bands were obtained from the FTIR analysis of the obtained extract, as shown in fig. 3. The numbers 1, 2 and 3 highlight the absorption bands characterizing the presence of esters [22].

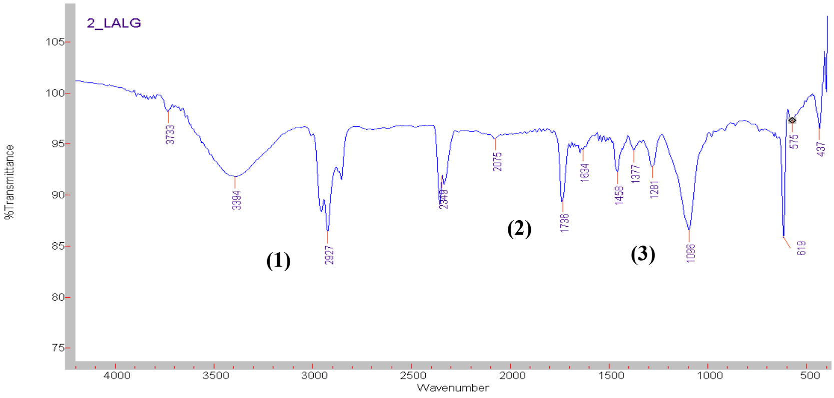

Figure 3: Infrared spectrum of the extract obtained from the microalgae of the WWTP Union Village and diluted in hexane. Axial deformation of C-H of alkanes (1) ($2927cm^{-1}$), axial deformation of C=O of saturated aliphatic esters (2) ($1736\ cm^{-1}$), axial deformation of O-C-C of esters (3) ($1096\ cm^{-1}$).

3.6 Analysis of the biodiesel by Fourier transform infrared (FTIR) and gas chromatography with mass spectroscopy (GC/MS)

Fig. 4 shows the FTIR spectrum of the lipid sample obtained from the microalgae collected in the pond and transesterified.

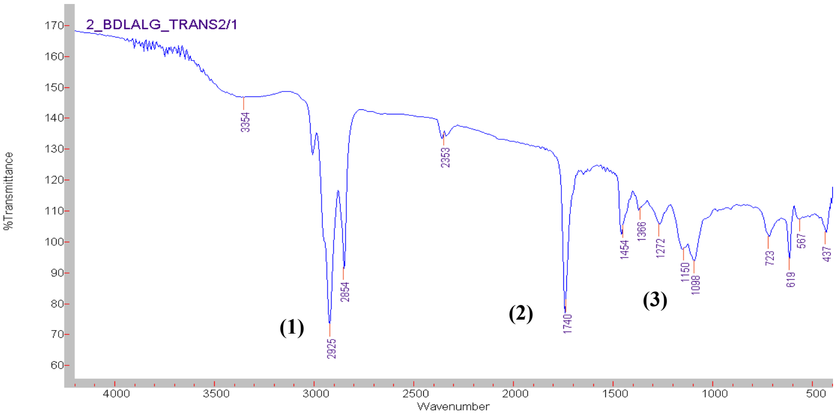

Figure 4: Infrared spectrum of the biodiesel from extract. Axial deformation of C-H of alkanes (1) ($2925\ cm^{-1}$), axial deformation of C=O of saturated aliphatic esters (2) ($1740\ cm^{-1}$), axial deformation of O-C-C of esters (3) ($1150\ cm^{-1}$).

Several absorption bands were obtained from the FTIR analysis of the obtained biodiesel, as shown in fig. 4. The numbers 1, 2 and 3 highlight the absorption bands characterizing the presence of esters [22]. The spectrum of the biodiesel sample from the microalgae obtained lipid showed some similarities with the spectrum of this lipid, but with differences such as the reduction of the O-H group band at 3354cm^{-1}.

The compounds observed by the analysis of GC/MS were Eicosane ($C_{20}H_{42}$) (42,801%) (1), Hexadecanoic acid methyl ester ($C_{17}H_{34}O_2$) (8,831%) (2), 12,15-Octadecadienoic acid, methyl ester ($C_{19}H_{34}O_2$) (8,803%) (3), 6-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$) (15,679%) (4), Hexanedioic acid, bis(2-ethylhexyl) ester ($C_{22}H_{42}O_4$) (10,004%) (5) and 1,2-Benzenedicarboxylic, diisooctyl ester ($C_{24}H_{38}O_4$) (13,852%) (6), as shown in the fig. 5.

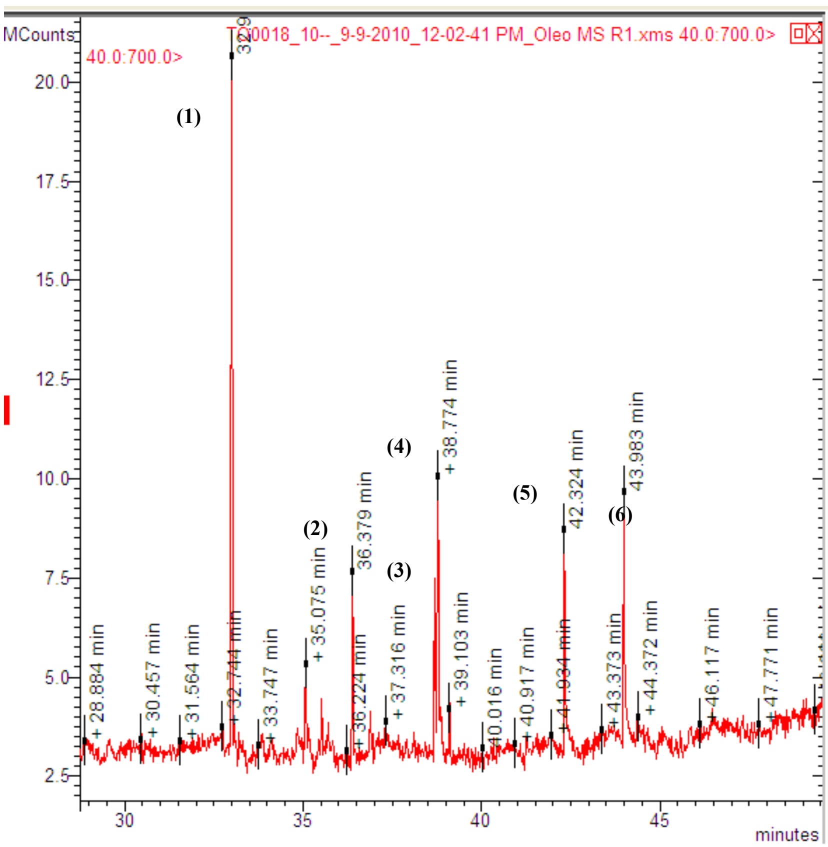

Figure 5: Spectrum of GC/MS of the extract from the microalgae after being derivatized.

4 Conclusion

The effluent of the aerobic and anaerobic lagoon of the WWTP Union Village is composed of different species. The taxa Cyanobacteria, Chlorophyceae, Euglenophyceae and Zygnemaphyceae present the highest number of species. However, with regard to the number of individuals per milliliter, species belonging to taxa Cyanobacteria and Euglenophyceae are predominant. The identified microalgae are producing lipids and fatty acids, which can be used to obtain biodiesel.

In the recovery of microalgal biomass, the filtration process can be used for the microalgal biomass concentration considering a bench scale. Biomass recovered from the effluent of the WWTP Union Village through this process has a lipid content of 16%, resembling the lipid obtained from microalgae grown by conventional processes, and has potential for use in obtaining lipid to biodiesel production.

From the standpoint of economic and environmental, the obtaining of microalgal biomass directly from the aerobic and anaerobic lagoon shows an alternative to the traditionally used procedures (photobioreactors and aerated tanks) to obtain algal biomass, to use the biomass for biofuel production and, simultaneously, to minimize environmental impacts of the elevated presence of this material in the environment.

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