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MICROALGAE HETEROTROPHIC CULTURE FOR BIODIESEL PRODUTION

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Abstract. *Pollution generated in urban center due to the heavy use of fossil fuels, motivates many research groups to seek sustainable solutions to this problem. In Brazil, one of the partial solutions adopted consists in the use of biodiesel B5 in heavy vehicles, derived mainly soybean oil and animal fat. An alternative to diversification of raw materials for the manufacture of biodiesel is the use of fatty acids from microalgae. Thus, the objective was to maximize the production of fatty acids from microalgae by heterotrophic cultivation. This causes the microalgae cultivation not carry out photosynthesis, since they use glucose or equivalent substrate as carbon source. Through these experiments, it is expected dominate this cultivation technique to apply in future waste that, through enzymatic treatments may become alternative sources of carbon, not competing with the agricultural area and food production. Partial results show that Scenedesmus sp., microalgae used in NPDEAS – Center of research and development of self-sustaining energy, in heterotrophic cultivation presents productivity from 1.22 to 2.02 g.L*$^{-1}$ *at a cost of U.S. $ 0.96 to U.S. $ 1.60.Kg*$^{-1}$ *while in autotrophic cultivation reaches productivity of 1.55 g.L*$^{-1}$ *at a cost of U.S $ 110.57.Kg*$^{-1}$.

Keywords: *Polution, Biodiesel, Fatty acids, Microalgae, Heteretrophic.*

1. INTRODUCTION

The increase in the number of vehicles not only in capitals, as in all cities makes the generated pollution be an aggravanting of people health quality who lives in this urban centers (CETESB, 2012). According to Paes (2012) the buildings and paving, beyond of the automobile combustion pollution, increase the solar radiation absorbing surface and reduce the air relative humidity.

Air pollution has been a much discussed topic, especially after the industrial revolution, as it has a great impact on peoples living in large urban centers. Some of the complication caused by poor air quality are generated by incomplete combustion engine that releases CO_2, NO_2 and particulate matter, these substances can generate chronic respiratory diseases, reducing lung function, affecting heart rate, vascular tone and blood coagulation (Azevedo, 2010). With this, research and development of new energy, specially renewable sources is the focus of this work.

With this concern of generating renewable energy the NPDEAS - Center of research and development of self-sustaining energy, uses microalgae to obtain biomass and oil for biodiesel production that according to Law No. 11.097, of January 13, 2005 is a biofuel derived from renewable biomass for use in internal combustion engines with compression ignition or, in accordance with regulations, to generate another type of energy, which can partially or totally replace fossil fuels (Brazil, 2005). Being one of the goals of this group become feasible to produce large-scale microalgae for biodiesel production.

Microalgae are microorganisms that even deprived of nutrients can adapt to the medium, accumulating lipids using the available sources of carbon (Praveenkumar, 2012), besides having great potential both in the production of food sources such as the production of biodiesel, which may also be used for nitrogen fixation in bioremediation. The biodiesel derived by oilseeds and ethanol from sugar cane, contrary to what is said to be renewable causes a large impact on the environment because they use a large area that could be used for other agricultural products, this being another opportunity for microalgae, since they can be grown in photobioreactors, these devices allow a large use of the area. A practical example is using palm as comparison, which is a plant having increased yield and production of oil, 5950, liters per hectare. A country the size of the U.S. needs 0.53 billion m³ of biodiesel annually, so need 111 million hectares to meet the need of the country, this space corresponding to 61% of the arable area. Facts that can make the microalgae oil profitable (Chisti, 2007).

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2. MATERIALS & METHOS

The cultivation and analyzes were performed in the city of Curitiba in polytechnic campus of the Federal University of Paraná specifically in NPDEAS (Center of research and development of self-sustaining energy) which offer various experiments involving microalgae to obtain biomass, biodiesel and development of sewage treatment. However, the objective was to lower the cost of microalgae biomass production using glucose as carbon source, and the biodiesel production was not made.

2.1. Heterotrophic culture of *Scenedesmus sp.*

As in other photosynthetic organisms and plants in general, algae have simplified two stages, the part where photosynthesis capture the carbon monoxide and carbon dioxide and through photosynthesis process produces glucose, and the part in which the microorganism uses the glucose to generate the secondary metabolites, one of the metabolites of interest to our search is acetyl-coenzyme a, which grows the fatty acids production. Thus, as the cultivation doesn't need light source and don't perform photosynthesis, metabolism starts a step forward, producing secondary metabolites and also in under certain conditions can cause a change in the metabolic pathways to increase the fatty acids production.

The medium was initially made up of $90 g.L^{-1}$ glucose concentration determined by an earlier work made by the NPDEAS group. As a heterotrophic culture, all samples removed for analyzes was done with care to avoid the entry of light in the culture.

2.2. Cell count

The cell count was done daily at the same time with a Neubauer chamber. This method doesn't need formalin since the *Scenedesmus sp.* have no motion.

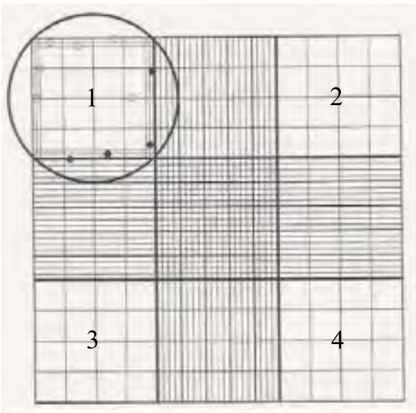

Figure 1. Neubauer chamber on microscope view showing the count areas 1, 2, 3 e 4

After counting the cells number in 1, 2, 3 and 4 fields the obtained value is multiplied by the correction factor which is 10^4, already known to this method.

2.3. pH measures

The culture pH is a very important information to know how the cells are growing and if there is some kind of bacterial contamination mainly competing with the algae (*Scenedesmus sp.*). In addition, the microalgae may also release basic components, which in excess may cease growing. The pH was measured daily at the same time and with equipment calibrated prior to testing.

2.4. Culture absorbance

The culture absorbance was accompanied daily at the same time with a 10 mL sample, cuvettes with 1 cm optical path and in a 540 nm wavelength in spectrophotometer.

2.5. Waste as carbon source

In addition to tests with commercial glucose, continuation of this research and completing this works objective, will also address the use of waste as a carbon source for the heterotrophic cultivation. The remaining residue broth will

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be used in the process, which treated with cellulase, amylase and protease will release nucleic acids and glucose, components that can be used by microalgae.

3. RESULTS AND DISCUSSIONS

In testes was observed a high growth in the first four days, compared to an autotrophic culture.

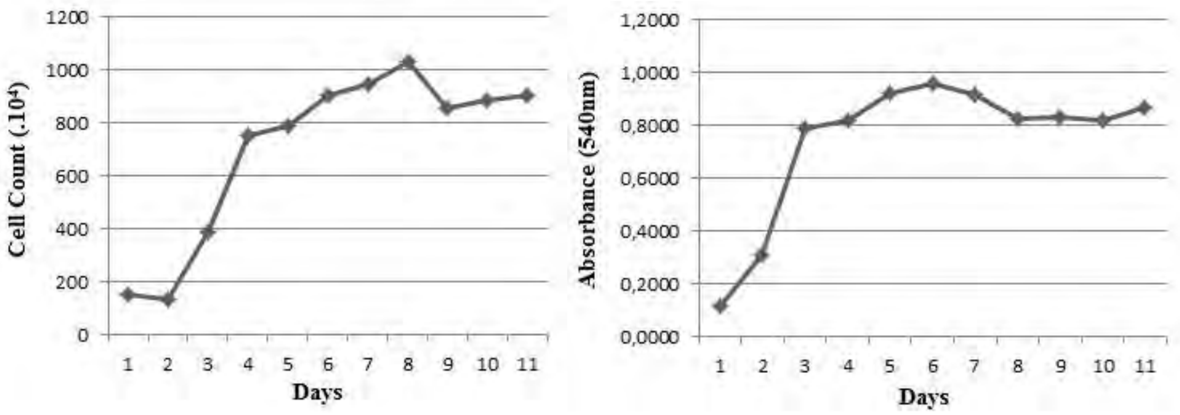

Figure 2. Microalgae growth in heterotrophic scheme using glucose as organic carbono source. Cells numbers x10^4 per mL

As can be seen in cell count figure and the absorbance growth curves are very similar, thus, a demonstration of the heterotrophic growing power mainly in the first days of cultivation can quadruple the number of cells. Another factor that can be observed in the table 2 is a decrease in the cell number between the first and second day of culture, this is due to the fact that algae have not previously been adapted to this growth medium, being this the adaptation period.

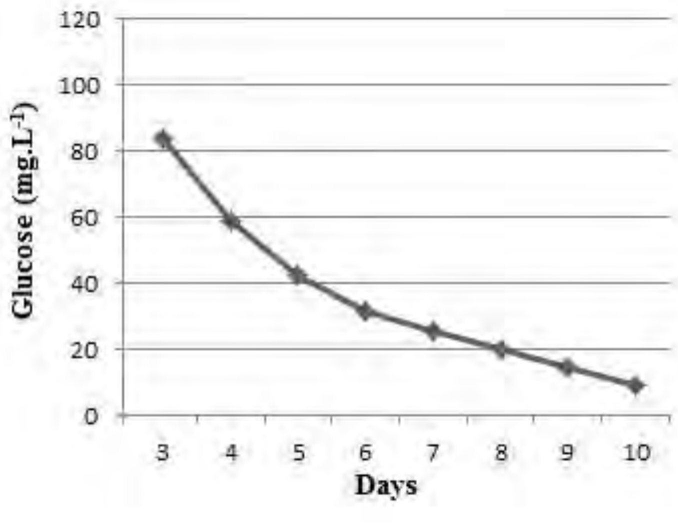

Figure 3. Graph of glucose consumption after the third day of culture

Considering that cultivation began with 9000 mg.L-1 it appears that the consumption of glucose in the early days is high, and after the second day for a continuous cultivation would require addition of glucose in the culture medium.

The culture medium pH apparently remained constant after the third day as shown in the figure below with varying pH in an acid medium according to the cells and the absorbance increased, a fact that may be due to the secondary metabolites production. In this pH range is still possible to work with the same microalgae even the ideal pH being 8.2 to 8.7 (Bersanti and Gualtieri, 2006).

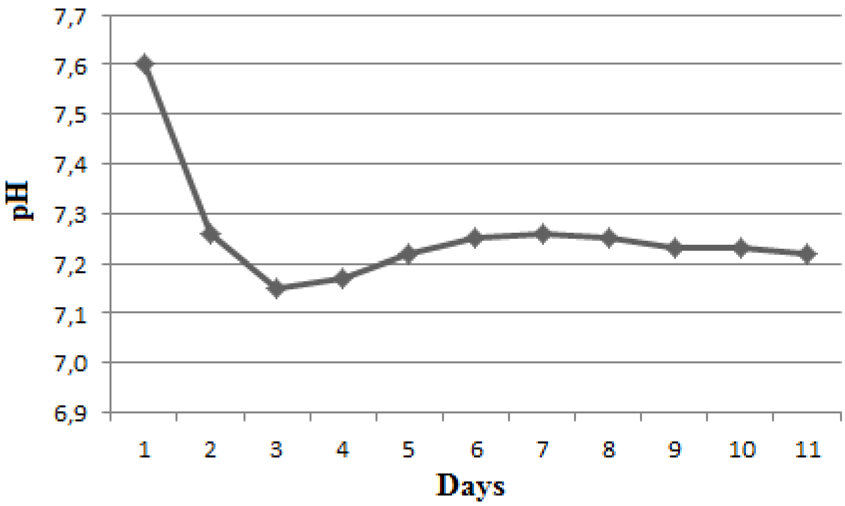

Figure 4. Heterotrophic culture pH graph

The heterotrophic culture has shown satisfactory values in four days since there is an increase of approximately 4 times the number of cells of the inoculum. It is possible to obtain 28 g.L^{-1} of biomass, result that shows a great performance against a growing autotrophic biomass concentration with is 1 g.L^{-1} approximately (Oliveira, 2012). Taking into account the composition of the CHU medium culture, with is mostly use for the production of autotrophic algae *Scenedesmus sp.*, reaches a value of U.S $ 110.57.Kg^{-1} for a yield of 1.55 g.L^{-1}. In the heterotrophic culture may be discarded prices of all reagents, as for it only need is a carbon source which can steel be obtained with residues, making the process even more cost low. Thus, with the use of a commercial carbon source arrives at an amount of U.S. $ 0.96 to U.S $ 1.60.Kg^{-1} with a quantity of biomass of 1.22 a 2.02 g.L-1, values which can be reduced, using waste enzymatically or chemically treated for the cultivation of microalgae.

The use of techniques such as heterotrophic cultivation can be an outlet for some kinds of pollution, as in the casee involving residues of agro-food products. After an enzymatic of chemical treatment, such residues can be utilized for cultivating heterotrophic microalgae causing the cultivation cost becomes even lower. In this experiment evaluated only the behavior of a heterotrophic cultivation, but new experiments with the use of waste will continue this work.

4. ACKNOWLEDGEMENTS

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