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MICROALGAE BIOMASS CULTIVATED WITH BIODIGESTED WASTE FOR BIOFUEL PRODUCTION

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Abstract. *The concern and care for the quality of water is a matter of extreme importance today. The laws have become stricter and industries are looking for improved and efficient alternatives to treat their effluents. The biodigestion is used for processing for organic waste, but even after this process, these wastes still have a high content of organic load. One technique which has proven effective for treatment of various wastes is the use of these materials for the cultivation of microalgae. These microorganisms are widely used for the production of alternative energy, such as biodiesel. This work proposes the use of a biodigester effluent, which has high levels of nitrogen and phosphorous (compounds present in the standard culture mean and required for the growth of microalgae), at different concentrations to the growth of the microalgae Scenedesmus sp. Therefore, it is possible evaluate to the consumption of nutrients present in the residue, the cell multiplication, and also the growth of microalgae. This work is very important because in addition to treating an effluent with high pollution load, adds value to the process, since it allows the use of microalgae for oil obtention and biodiesel production*

Keywords: *wastewater, microalgae, biodiesel, bioremediation*

1. INTRODUCTION

Much of the energy consumed worldwide is derived from petroleum, considered a non-renewable source of energy and with finite reserves (Posten & Schawb, 2009; Lam & Lee, 2012). In addition, the burning of fossil fuels has raised numerous environmental questions, including the emission of gases that contribute to global warming. Against this background, alternative energy sources are extremely important and thus receive privileged attention during the last decades in order to achieve sustainable energy in future (Fukuda, Kondo e Noda, 2001; Chisti, 2007; Lam & Lee, 2012).

Biofuels are a promising alternative for the replacement of petroleum fuels, especially when taking into account the growing demand for fuel which is related to the number of cars, which grows with the economy (Posten & Schwab, 2009).

Among the main biofuels is biodiesel, a fuel that can be derived from various sources, such as soybean, corn, canola, microalgae, and other various oilseeds. Comparing the different raw materials, it can be highlighted the microalgae because these microorganisms have high productivity per area of cultivation. Furthermore, its production does not compete with agricultural areas and, therefore, do not affect food crops. In addition, wastes can be used as a source of nutrients for the growth of microalgae as swine and cattle manure, even after anaerobic digestion. The biodigestion eliminates pathogens and reduces the organic load, but the levels of nitrogen and phosphorus are still high, and thus, can be used as a mean of alternative culture. Based on this, the objective of this study was to use sewage effluent biodigested for the growth of microalgae *Scenedesmus sp*. and evaluate the capacity of this genus grow in this manure.

2. LITERATURES REVIEW

Biofuels, especially those produced from plants, can replace part of the demand for fossil fuels (Perlack *et al.,* 2005). However, the use of plants for obtaining fuels worries since it competes directly with food crops, and consequently causes an increase in food prices (Fargione *et al.,* 2008). Furthermore, algae can produce 20 times more oil per hectare compared to land crops such as soybean and canola (Sheehan *et al.,* 1998; Chisti, 2007; Benemann, 2008).

Chisti (2007) conducted a research that indicates that the replacement of all fuel consumed in the united states for biodiesel would require 0.53 billion cubic meters of this biofuel per year, considering the pace of consumption in 2007. It also states that the use of cooking oil or oil from other oleaginous can not meet this demand, taking into account that for obtaining palm oil (an oilseed crop with high yield) 24% of all U.S. acreage would be required to meet 50 % of the country's demand. When considering microalgae as raw material, only 1-3% of the total sown area would be enough to satisfy the same 50%. Thus, it can be considered that the productivity of microalgae is above of some oilseeds commonly used for production of biodiesel (Tab. 1).

Table 1. Comparison of some sources of biodiesel.

Crop	Oil yield (L/ha)	Land area needed (M ha)[a]	Percent of existing US cropping area[a]
Corn	172	1540	846
Soybean	446	594	326
Canola	1190	223	122
Jatropha	1892	140	77
Coconut	2689	99	54
Oil palm	5950	45	24
Microalgae[b]	136900	2	1.1
Microalgae[c]	587000	4.5	2.5

[a] For meeting 50% of all transport fuel needs of the United States;
[b] 70% oil (by weight) in biomass;
[c] 30% oil (by weight) in biomass. Source: Chisti (2007)

Another advantage of microalgae cultivation is the need of a smaller amount of water when compared to earth cultures (Li *et al.,* 2008; Clarens *et al.,* 2010). In contrast, the culture mean commonly used for cultivation are composed of different synthetic nutrients and salts necessary for the growth of microalgae. Thus, the synthetic culture mean enhances the value of the product (Chisti, 2007). Wastewater have some of the nutrients required and thus provide a suitable alternative to artificial means (Chen *et al.,* 2009).

Benemann & Oswald (1996) suggest that the cultivation of microalgae in large-scale targeting only biofuel production is not economically advantageous, emphasizing the importance of integrating biofuel production to wastewater treatment and CO_2 supplementation, as also suggested Oswald & Golueke (1960). Chisti (2008) states that the cost of biodiesel production from microalgae should fall to nearly 10 times to be competitive with petroleum. Apart from biodiesel production, the biomass of microalgae grown in wastewater can be converted to biofuel in other ways, such as anaerobic digestion and biogas (methane and carbon dioxide) and fertilizer.

When the algae cultivation is combined with the use of the wastewater, the costs of the artificial mean significantly reduces (Chen *et al.,* 2009). Wastewater usually contains a high concentration of nitrogen that causes eutrophication of water bodies when improperly discarded. Such wastes are usually submitted to anaerobic digestion, resulting in the reduction of organic matter. However, even after digestion, levels of nutrients such as nitrogen and phosphor are not completely reduced (Park *et al.,* 2010). The concentration of these compounds are similar to the concentrations found in culture media used for the growth of microalgae.

3. MATERIALS AND METHODS

The cultures were performed on algae of the genus *Scenedesmus sp.* in laboratory scale in triplicate, and also airlift type reactor in duplicate, using the mean CHU modified as standard (Tab. 2). The alternative mean was tested was sewage biodigested (Fig. 1) of the Core of Research and Development of Energy Self-Sustaining (NPDEAS) diluted in distilled water at three concentrations: 10%, 15% and 25%.

Table 2. CHU modified mean compostion

Reagent	Formula	Amount ($g.L^{-1}$)
Sodium nitrate	$NaNO_3$	0.25
Calcium chloride dihydrate	$CaCl_2.2H_2O$	0.025
Magnesium sulfate heptahydrate	$MgSO_4.7H_2O$	0.075
Potassium phosphate dibasic	K_2HPO_4	0.075

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Monobasic potassium phosphate	KH_2PO_4	0.175
Sodium chloride	NaCl	0.025
Tríplex III/EDTA	$C_{10}H_{14}N_2Na_2O_8.2H_2O$	0.05
Potassium hydroxide	KOH	0.031
Ferrous sulfate heptahydrate	$FeSO_4$	0.00498
Boric acid	H_3BO_3	0.01142
Zinc sulfate heptahydrate	$ZnSO_4.7H_2O$	$8.82\ 10^{-6}$
Manganese chloride tetrahydrate	$MnCl_2.4H_2O$	$1.44\ 10^{-6}$
Sodium molybdate	$NaMoO_4.2H_2O$	$1.19\ 10^{-6}$
Copper sulphate pentahydrate	$CuSO_4.5H_2O$	$1.57\ 10^{-6}$
Cobalt nitrate hexahydrate	$Co(NO_3)_2.6H_2O$	$0.49\ 10^{-6}$

The production cost of 1000 liters of culture mean CHU is R$ 28.02. The reagents listed were solubilized in distilled water according to the concentrations of tab. 2.

Figure 1. NPDEAS biodigester. Source: The author

For the laboratory scale cultivations were used 2 L Erlenmeyer flasks with a working volume of 1.8 L and made in triplicate. The cultures were kept under constant illumination (2 lamps 40 W), continuous aeration by air injection system with a flow rate of 2 liters.minute^{-1} and temperature of 22 °C (Fig. 2). The cultivation in airlift reactor was carried out in external environment, only with the dilution of 25%, without control lighting and temperature, with a working volume of 11 L and aeration with atmospheric air at a rate of approximately 4 L.min^{-1} (Fig. 3).

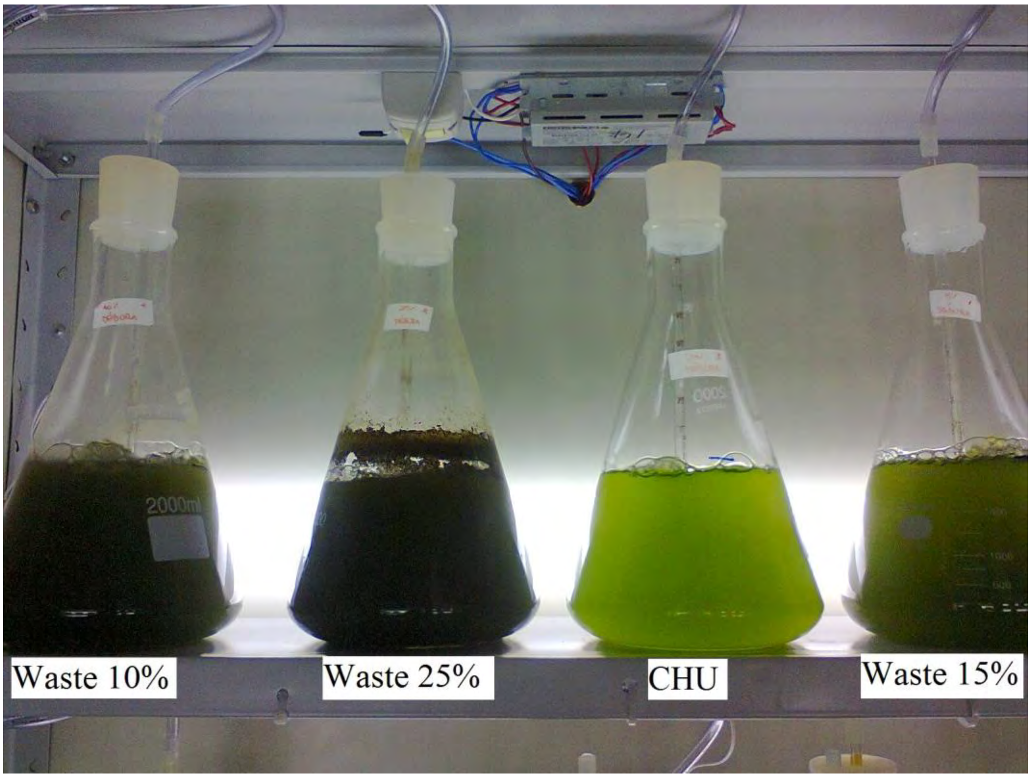

Figure 2. Arrangement of bottles in cultivation at laboratory scale. From left to right: 10%, 25%, CHU and 15%. Source: The author

Figure 3. Airlift Reactor. Source: The author

In none of the cultivation was pH correction. To monitor the growth daily counts of the number of cells were realized with the aid of a Neubauer chamber, and quantified the dry biomass of the algae. At the end of cultivation in airlift reactor, the percentage of lipids was determined by the method of Bligh & Dyer (1959). All analyzes were performed in triplicate. It was used, in the construction of graphs, error bars representing the confidence interval calculated from the standard deviation around the means, assuming a confidence level of 95% ($\alpha = 0.05$).

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4. RESULTS AND DISCUSSION

The dilutions of 10% and 15% (Fig. 3 and 4) had a rapid growth in laboratory scale. However, dilution of 25% (Fig. 5) had a longer period of adaptation to the manure. One possibility for such behavior is that the dark color of the manure hinders the penetration of the light. As the microalgae grow, the color intensity drecreases and light incides more easily on the cultivation, facilitating the process of photosynthesis. The three concentrations obtained a growth very similar to the standard mean. CHU, 10%, 15% and 25% cultivations obtained final cell concentration of 1480 $x10^4.mL^{-1}$, 1404 $x10^4.mL^{-1}$, 1014 $x10^4.mL^{-1}$, 1573 $x10^4.mL^{-1}$ respectively (Tab. 3), proving the possibility of replacing the standard means by alternative mean suggested. This mean is attractive because it is a waste with necessity of treatment before final disposal, and reduces costs in the process as a whole.

Table 3. Final cell concentration in laboratory scale

Mean	Cell concentration ($x10^4.mL^{-1}$)
CHU	1480
10% manure	1404
15% manure	1014
25% manure	1573

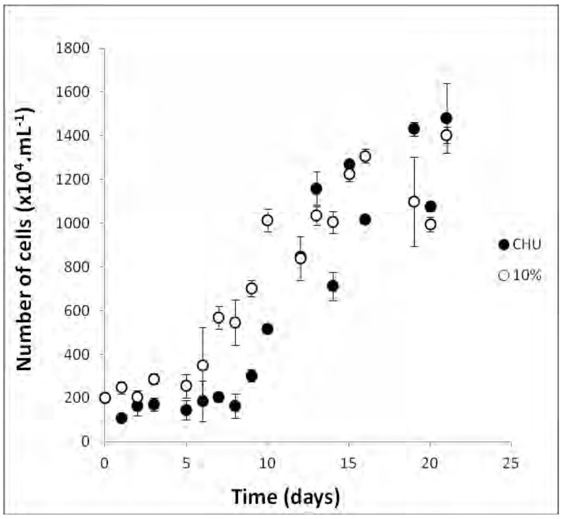

Figure 3. Graph comparing mean CHU cell growth with 10% manure (Laboratorial scale). Bars represent confidence interval with α = 0.05

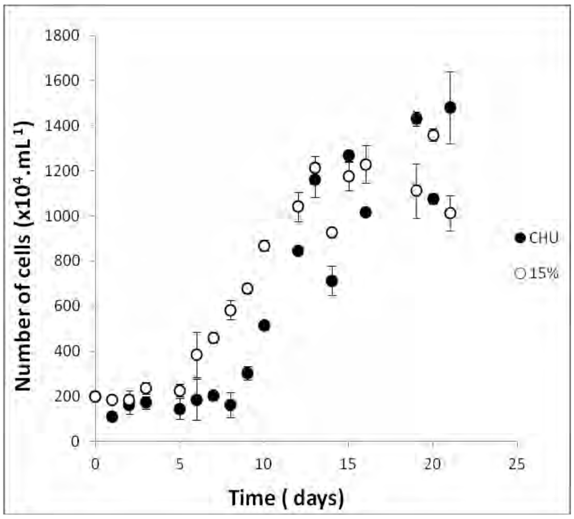

Figure 4. Graph comparing mean CHU cell growth with 15% manure (Laboratorial scale). Bars represent confidence interval with α = 0.05.

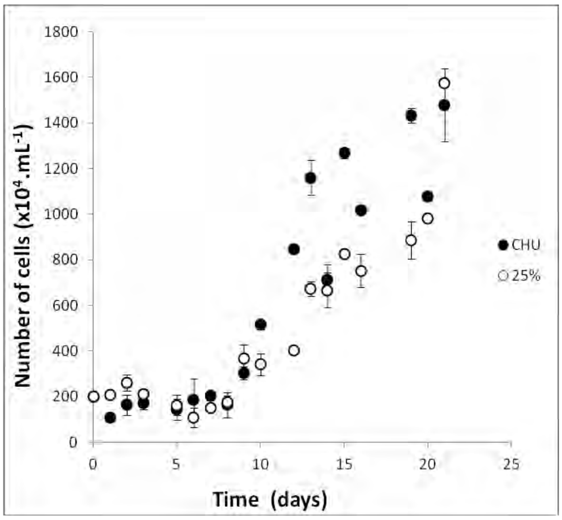

Figure 5. Graph comparing mean CHU cell growth with 25 % manure (Laboratorial scale). Bars represent confidence interval with α = 0.05.

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In addition to the increase of the cell density could also be observed the increase of microalgal biomass. To represent this data, the value of the initial biomass was discounted everyday in all dilutions. At day 21 of cultivation in laboratorial scale the levels of dry biomass were 0.41 $g.L^{-1}$ in the CHU mean, 0.37 $g.L^{-1}$ with 10% manure (Fig. 6), 0.54 $g.L^{-1}$ with 15% (Fig. 7) and 0.46 $g.L^{-1}$ 25% of the residue (Fig. 8).

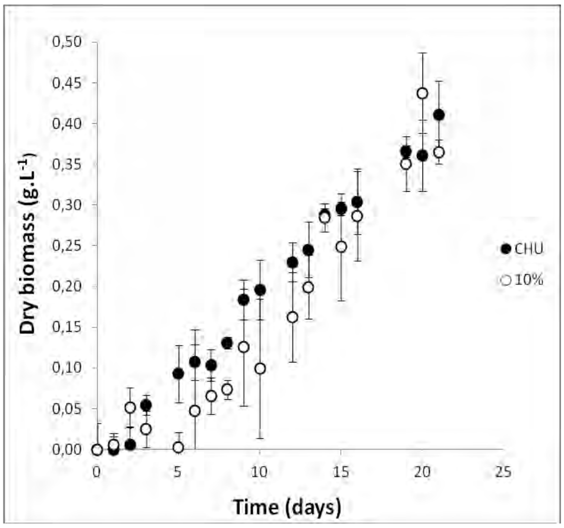

Figure 6. Graph comparing mean CHU dry biomass with 10% manure (Laboratorial scale). Bars represent confidence interval with α = 0.05.

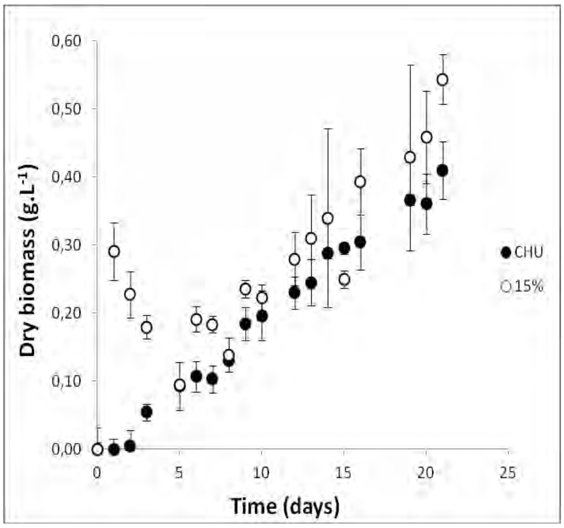

Figure 7. Graph comparing mean CHU dry biomass with 15% manure (Laboratorial scale). Bars represent confidence interval with α = 0.05.

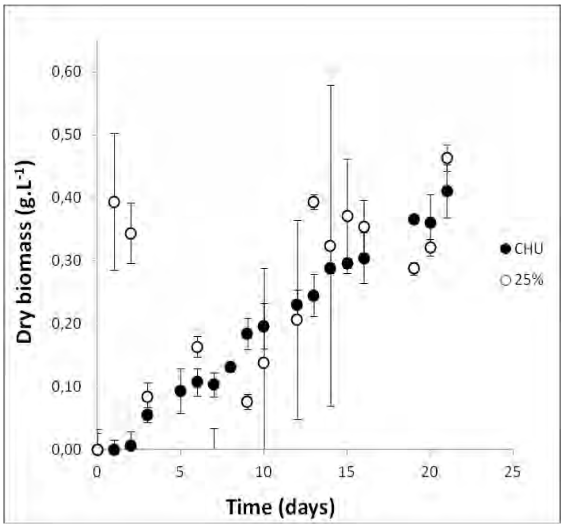

Figure 8. Graph comparing mean CHU dry biomass with 25% manure (Laboratorial scale). Bars represent confidence interval with α = 0.05.

The large error bars observed in the graphs of dry biomass are due to the difficulty of applying this method to the manure in question, because it has particulates.

Based on these preliminary data, considering that both dilutions were very close to the growth of the standard mean (CHU), it was opted for the highest concentration (25%) for the realization of cultivation on a larger scale in the airlift type reactor. The performance of this kind of reactor was superior due to its aeration, that is more efficient when compared to the laboratory scale cultures. In this cultivation it was observed that the mean with manure diluted to 25% grew very similarly to the standard mean CHU. Furthermore, it was obtained a higher cell number at the end of the 13 days of cultivation in airlift reactor, equivalent to 3542 $x10^4.mL^{-1}$ against a cell concentration of 2183 $x10^4.mL^{-1}$ in

CHU mean cultivation (Fig. 9). The final biomass of microalgae grown in manure was 0.50 g.L^{-1}, discounting the initial biomass, as the cultives on smaller scale. The final lipids percentage was 11.71%.

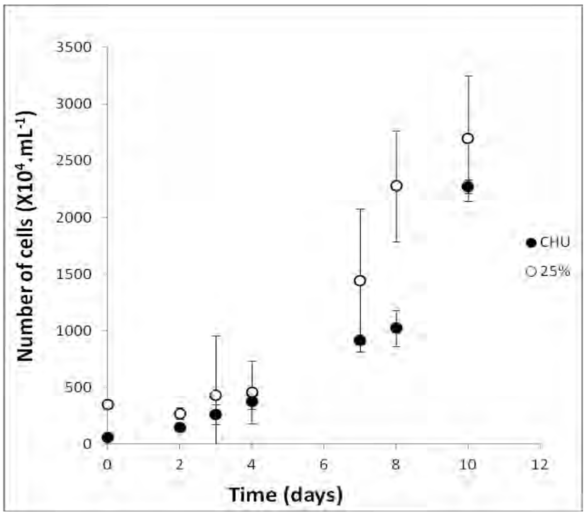

Figure 9. Graph comparing mean CHU cell growth with 25% manure (Airlift reactor). Bars represent confidence interval with α = 0.05.

Experiments with swine manure in NPDEAS (data not shown) suggest that nitrogen and phosphate decrease with the growth of microalgae, using them as a source of nutrients (Taher *et al.,* 2012). Based on these data it is believed that this is a possibility for the treatment of human sewage in two steps: first biodigesting and eliminating pathogens and lastly using it as culture mean for microalgae. Besides the treatment of the manure, these two steps, integrated, generate biofuels such as biogas (formed in anaerobic digestion), and the accumulation of microalgae biomass to the production of biodiesel. Biogas can be used to generate heat or electricity, and can be reused as a source of CO_2 in the cultivation of microalgae.

5. CONCLUSION

Through this work, it can be observed that the residue from the NPDEAS biodigester can be used as an alternative to the growth of microalgae and is a promising trend in the production of biofuels. The alternative mean is interesting not only for its low cost, but mainly for their environmental profile, as if discarded improperly entail environmental problems. This study demonstrates that it is possible to use this residue for the growth of algae, adding value to the process and final product. To grow, these microorganisms use the nutrients in manure. While the microalgae remove the nutrients, the residue is properly treated. For more accurate results are still required more analysis such the residue characterization before and after the cultivations (confirming the removal of nutrients), lipid profile of the produced oil and nutrient removal curve over the days of cultivation.

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