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BIOGAS PURIFICATION THROUGH MICROALGAE CULTIVATION IN PHOTOBIOREACTOR

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Abstract. Climatic changes of the last two centuries driven by the emission of greenhouse gases are currently an indisputable fact. One way to reduce greenhouse gas emissions is the production of energy from renewable sources. The use of biogas as an energy source shows promise especially in agricultural regions, where there is a wide availability of waste that can be turned into energy, reducing production costs and environmental impacts. One alternative for improving the utilization of biogas is to eliminate the impurities of its composition as hydrogen sulphide (H_2S) and carbon dioxide (CO_2) emissions. The method of CO_2 microalgae fixation presents as an important alternative. In this study, we established a model of microalgae photobioreactor Air-lift, with the goal of capturing the CO_2 present in the biogas. A biogas purification process was applied in order to increase its calorific value. For this system, the growth profiles of microalgae cultivated with concentrations of H_2S, CH_4 and CO_2 were evaluated, as well as physical-chemical and biological process. The maximum yield of biomass obtained in the culture aerated whit biogas was 1.23 g/L. The maximum cell concentration obtained was 125 x 10^6 cells/mL, using 25% of biogas and 75% of atmospheric air. This result demonstrates a high efficiency of microalgae cultures to upgrade biogas. In the present study, the calorific value of the biogas was estimated taking into account the existing percentage of methane in biogas. We estimated an average increase of the calorific value of 6104.904 kcal/m^3 to 7767.268 kcal/m^3, representing an increase of approximately 27% in the calorific value of the biogas after purification with microalgae cultivations, and thus approaching to the calorific value of pure methane.

Keywords: Greenhouse gases, Biogas, Microalgae

1. INTRODUCTION

Global warming induced by increased concentrations of greenhouse gases in the atmosphere and the increasing demand for energy is one of the major problems facing humanity today. According to IPCC (2007), the global emissions of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) increased 70% from 1970 to 2004, primarily as a result of human activity.

One way to reduce greenhouse gas emissions is the production of energy from renewable sources such as biofuels and biogas. This issue has received much attention mainly due to the depletion of natural fossil fuels (Sachs, 2007). The biogas from the anaerobic digestion of organic waste is a mixture of methane (CH_4), carbon dioxide (CO_2), hydrogen sulfide (H_2S) and several smaller hydrocarbons. Presents itself as a potential fuel for power generation and when purified can be used with the same standards as fossil natural gas. The high concentration of H_2S in biogas can

corrode engines, pipelines and storage structures. Thus, chemical methods and biological and chemical are used for the removal of H_2S (Abatzoglou and Boivin, 2009).

However, after the initial processes of removing hydrogen sulfide, biogas can still exhibit high CO_2 content, which lowers its heating value and increases the emission of carbon monoxide and hydrocarbons into the atmosphere when held in its burning engines. The presence of high concentrations of CO_2, turn the biogas more costly to be compressed and transported in comparison with the natural gas. Also, the capture of carbon dioxide in biogas can improve the efficiency of the engine. Several research strategies on the sequestration of CO_2 have been performed, including physical, chemical and biological.

Carbon dioxide can be biologically converted to organic matter by microorganisms such as photosynthetic microalgae, and that matter can be transformed into high-value products such as bioethanol, biodiesel and hydrogen (Pulz and Gross, 2004;. Skjanes *et al.,* 2007). The process of CO_2 fixation by microalgae presented as an alternative for the sequestration of carbon dioxide. In comparison with terrestrial plants microalgae have higher rates of carbon dioxide fixation. Thus, these microorganisms can utilize the CO_2 present in the biogas for the production of biomass (Costa *et al*., 2000). The lipid fraction of microalgae biomass produced can be extracted and transesterified for biofuel production (De Morais and Costa, 2007a). The products present in biomass as proteins, pigments and enzymes can be marketed in order to increase the commercial viability of projects related to the production of microalgae.

In this scenario, the use of a biological process for the fixation of CO_2 from biogas is presented as a technology to be evaluated. To determine the application of this technology were developed in pilot scale photobioreactors, model Air-lift cultivation of microalgae for in order to fix the CO_2 present in the biogas produced in the anaerobic digestion of agro-industrial effluents. The biogas purification process was applied in order to increase the calorific value of biogas, thus increasing its capacity to connect it to a pipeline for the distribution network. In this study the growth profiles of cultivated microalgae CH_4 and CO_2 concentrations were measured.

Thus, this study evaluated the process of purification of biogas derived from sewage treatment process by autotrophic cultivation of microalgae, aiming to improve the performance of the process as a whole, by reducing the emission of greenhouse gases and generating a source of biomass for biofuel production through a biotechnological process.

2. METHODOLOGY

2.1. MICROALGAE

A mixture of microalgae collected in a eutrophic lake in Curitiba-Paraná was maintained at 2 L Erlenmeyer flask with 1.6 L of liquid medium Chu (Chu, 1942). The culture was maintained at 17 ± 2 ° C under constant illumination with aeration of the cultures by the addition of compressed air (5 $L.min^{-1}$). After several Microalgae cultivations predominantly observed the presence of microalgae of the genus *Scenedesmus* and this material was used in the experiments proposed. It is proposed the use of wild species mainly by its lower sensitivity to climatic conditions and contaminants. Moreover, previous experiments performed in the laboratory suggest that microalgae excellent capacity for wastewater treatment by reuse of nutrients present in the culture medium, and low sensitivity to temperature changes.

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2.2. MICROALGAL CULTURES MEDIUM AND CHEMICALS

This study used secondary effluent treatment by digestion of pig manure, collected on a farm in the town of Irati in the State of Paraná, and added to the culture at a ratio of 10%. The manure was stored in plastic pumps 200 L truck and transported to the laboratory in Curitiba - PR.

2.3. PHOTOBIOREACTORS

The diagram of experimental apparatus used in this study is described in Figure 01. The photobioreactors were built with acrylic tubes of 50 mm diameter, height 300 cm and nominal volume of 11 L. The air/gas used in the equipment is supplied through an inlet at the bottom of the reactor, passing through the dispersion system in the center of the column. Thus, the airflow runs through the riser causing the volume of the outer tube down, circulating the system.

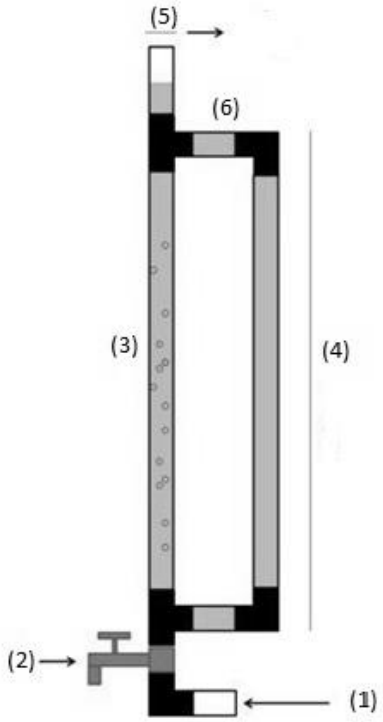

FIGURE 01 - Experimental equipment. Photobioreactor Model Air-Lift - (1) Input air/gas, (2) Out-collection sampling, (3) Column ascending; (4) External Column Down, (5) Output of air/gas, (6) Degasser.

2.4. AERATION OF CULTURE "MIX *Scenedesmus"* WITH BIOGAS

The photobioreactors used in this work were constructed on the premises of the Núcleo de Pesquisa e Desenvolvimento de Energia Sustentável (NPDEAS) of Universidade Federal do Paraná (UFPR). The working volume of the reactors was 10 - 11 liters as the volume of air/gas injected to the aeration system. The biogas used in the experiments was produced by the anaerobic digestion of sewage waste from the laboratory itself. The biogas is desulfurizado by chemical absorption through filters containing iron filings, resulting in the concentration limit of 100

ppm. After the desulfurization process, biogas balloon was stored in storage and then compressed for use in the cultivation of microalgae as shown in Figure 02.

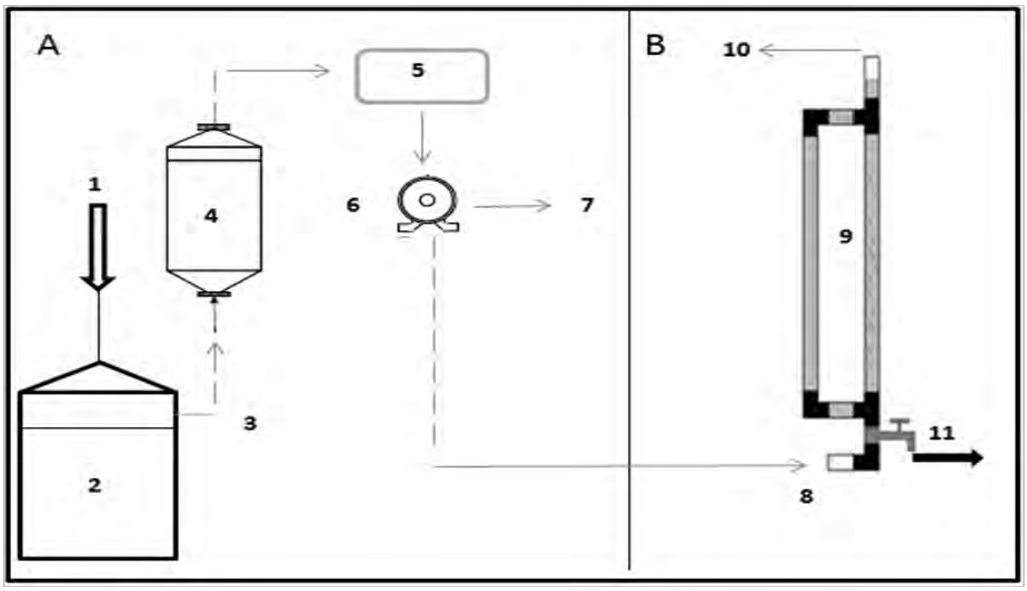

FIGURE 02 - Diagram of experiment: (A) Anaerobic digestion of waste and (B) autotrophic cultivation of microalgae in photobioreactors for removal of CO_2 present in the biogas. The overall operation consisted of the following daily events: 1 - pulse power waste in the digester; 2 - anaerobic digestion, 3 - production of biogas in the digester; 4 - biogas filtration for removal of hydrogen sulfide; 5 - Accumulation of biogas produced balloon; 6 - Compression and storage of biogas; 7 - sampling for analysis of biogas; 8 - injection of biogas accumulated daily in the photobioreactor; 09 - Growth of autotrophic microalgae; 10 - Sampling biogas enriched after treatment photosynthetic; 11 - sampling *Scenedesmus* suspension. Black lines refer to the flow of the liquid phase, while the gray lines and broken lines for streams of gaseous phase.

The aeration of the growing air and biogas was controlled with flowmeter, a flow rate of 2 L/min during the period of maximum radiation of sunlight (10am – 14am) at a concentration of 25% biogas and 75% atmospheric air, which leads to a concentration of 2 - 5% CO_2 in gas/air mixture injected into the cultivation. The configuration of the reactor used for CO_2 capture is shown in Figure 03.

Variations in cell density of microalgae cultivation throughout the development, were monitored daily by counting cells under a microscope (x400) with the aid of a Neubauer chamber (Improved Chamber). The cell density was expressed as number of cells per milliliter of culture (Cell.ml^{-1}) an average of the three counts. With the experimental cell density were prepared according to the time charts (day) (Soares, 2009). The biomass of the cultures was measured during the experiment to calculate the maximum productivity using equation (1) and the end of the experiment recovered by centrifugation at 5000 rpm for 5 minutes at 4° C. After separation the quantification of lipids by the method Bligh & Dyer (1959).

PRODUCTIVITY (P)

$$P\ (g.L^{-1}.day^{-1}) = \frac{Dry\,weight_{final} - Dry\,weight_{initial}}{Time_{final} - Time_{initial}} \quad (1)$$

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FIGURE 03 - Air-Lift photobioreactors for cultivation of microalgae and CO_2 capture in biogas produced from the anaerobic digestion of waste.

2.5. GROWING MICROALGAE IN PHOTOBIOREACTORS FOR CO_2 CAPTURE AND BIOGAS PURIFICATION.

To read the removal of CO_2 present in the biogas, the culture was aerated only biogas with a flow rate of 2 L/min, and the sample biogas input and output of microalgae cultivation for analysis. Samples of gases were performed with the "Biogas Analysis Kit", a device developed by Embrapa Suinos and the company Alfakit Ltda, which is the determination of carbon dioxide (CO_2) by adapting the Orsat method, in which a basic solution reacts with CO_2 making him rush to measure its concentration. The result of methane gas (CH_4) is obtained indirectly by the difference of income and CO_2 concentrations are obtained in percentage (%) with accuracy of ± 5%. The efficiency of CO_2 capture $E_{CapCO2}(\%)$ was calculated by Equation 2:

$$E_{CapCO2}(\%) = \frac{\text{Influent } CO_2 - E\text{ffluent } CO_2}{\text{Influent } CO_2} \times 100 \qquad (2)$$

The capacity for CO_2 fixation followed the method reported by Devinny *et al.*, (1999) and Jacob-Lopes *et al.*, (2009).

Following the same pattern for the analysis of the purification capacity of biogas through cultivation of microalgae, a sample was collected in biogas input and output of microalgae cultures to determine the biogas composition before and after passing through the culture. The efficiency of biogas enrichment $E_{EnrCH4}(\%)$was calculated by Equation 3:

$$E_{EnrCH4}(\%) = \frac{\text{Effluent } CH_4 \text{ - Influent } CH_4}{100 \text{ - Influent } CH_4} \times 100 \quad (3)$$

The pH of the cultures was analyzed daily during the experimental period.

3. RESULTS AND DISCUSSION

3.1. Initial phase

This research began with the study of the composition of the biogas produced in the digester stage that precedes its power to photobioreactors. During this phase, the digester showed high concentrations of methane in the biogas (Table 01). The percentage of methane in the biogas ranged 65a in the range of 80%, while the percentage of carbon dioxide between 20 and 35%. The concentration of methane in the biogas was similar to that described in the literature (65 to 85%) (Taleghanie Kia, 2005).

3.2. AERATION OF CULTURE MIX *Scenedesmus* WITH BIOGAS

In this paper an experiment was conducted to determine growth capacity of microalgae using CO_2 presents in biogas as a source of carbon. The "Mix" of *Scenedesmus* was grown in photobioreactor Air-Lift with 11 Liters of workload. The gas was prepared in a percentage by volume of air (75%) and biogas (25%). Samples were collected daily to assess microalgae culture growth by cells count. Figure 04 shows the growth potential of microalgae culture aerated with concentrations of atmospheric air and biogas.

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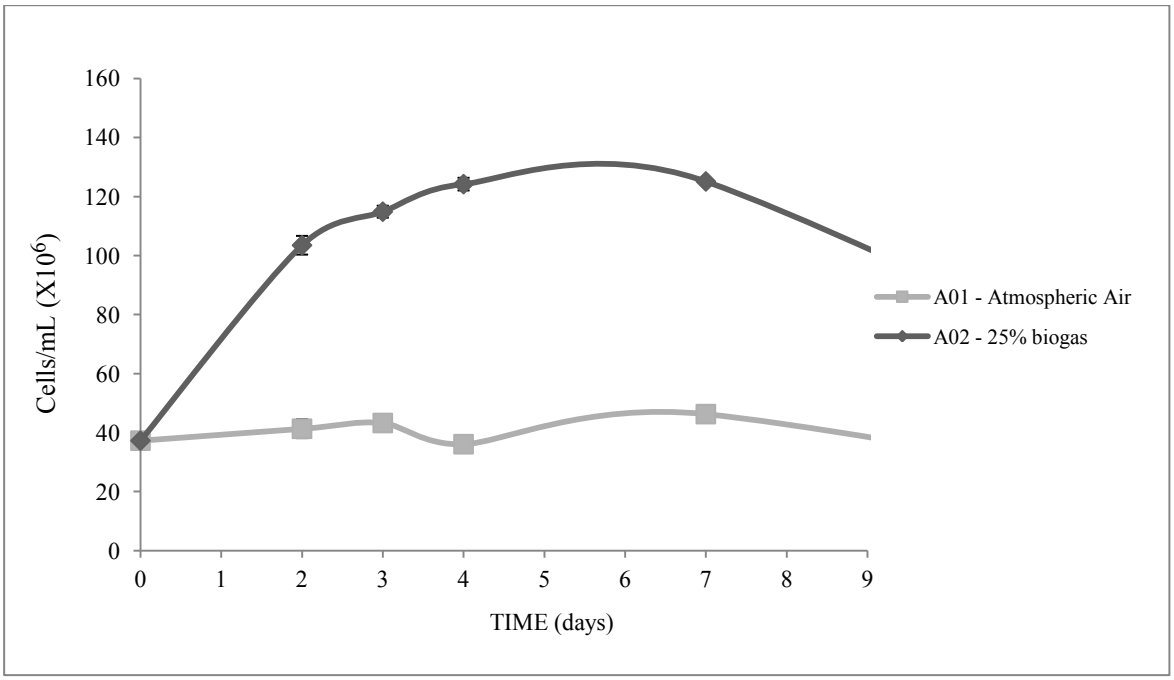

Figure 04 - Profile of growth of "Mix" of *Scenedesmus* grown in photobioreactor Air-Lift bubbled with air (A01) and Biogas 25% (A02). The initial concentration in both cultures was 40 x 10^6 cells/mL. Microalgae cells were cultured for 7 days with aeration constant of 2 L/min. Samples were collected every 24 hours for the determination of growth by cell counting. Error bars correspond to standard deviation.

To evaluate the performance of the growth of microalgae using CO_2 from biogas, a system was installed next to the digester (Figure 03). Biogas produced in the anaerobic digester was introduced in the cultivation of microalgae compression together with the atmospheric air also compressed. Figure 04 shows the growth curves that resulted in different final concentrations in cells/mL. Comparing the growth profile of microalgae, realizes high efficiency cell growth aerated cultivation with biogas. After two days of adaptation of cultures, cultivation with biogas showed high cell growth coming a12.500 x 10^4cells/mL on the fourth day of cultivation.

The purpose of this study was to demonstrate that a photosynthetic system can be effectively deployed for recovery of biogas through CO_2 biofixation, increased its calorific value. The results shown in Figure 05, show that the microalgae *Scenedesmus* was able to grow using this source of CO_2, reaching maximum biomass concentration of 1.23 g/L. The maximum yield achieved was 0.2715 $g.L^{-1}.day^{-1}$ for growing aerated with 25% biogas in the fourth day of culture. In this work the experiment was stopped on day 7 after the stabilization of cell growth. One possible cause of the rapid stabilization of culture growth is the initial concentration of nutrients found in the culture medium, which was used at a concentration of 10% of pig slurry and 90% distilled water and the high initial concentration of cells.

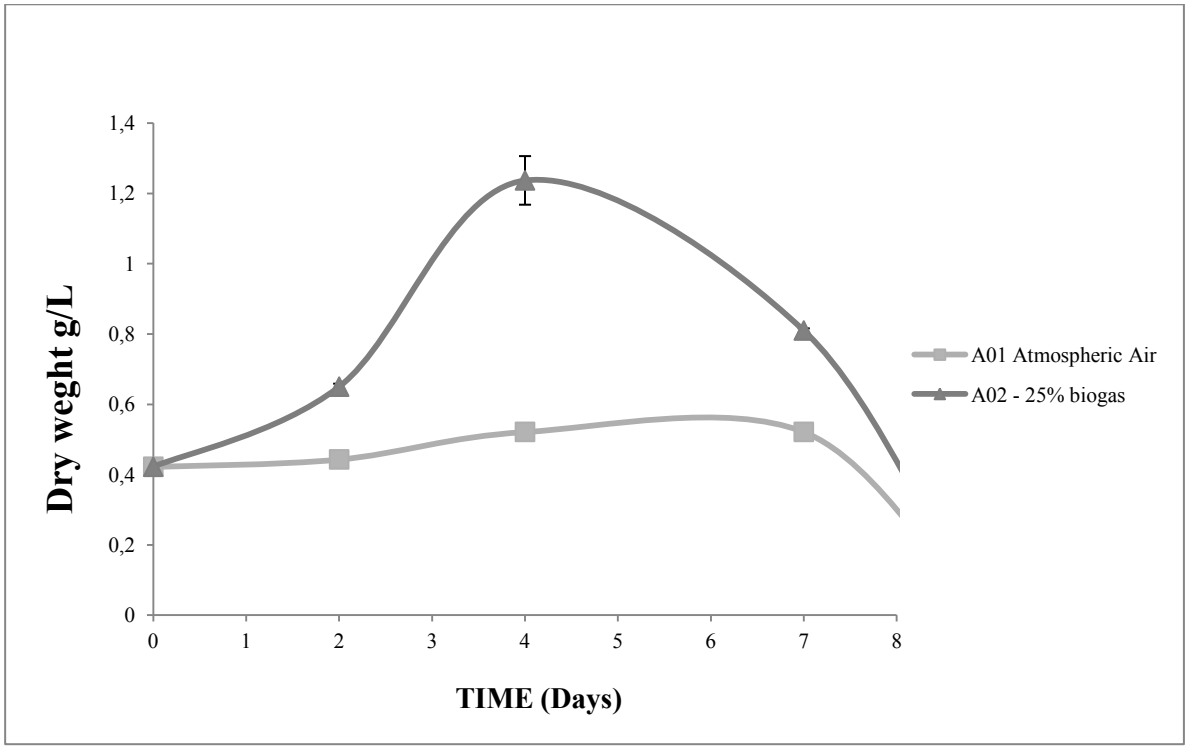

Figura 05 - Dry weight (g/L) - Microalgae cultivation aeration with atmospheric air – (A01) and aeration with 25% of biogas – (A02).

The potential growth of Microalgae cultivations with biogas is significantly higher than that using only atmospheric air. These results show that the strategy of aeration using biogas as a source of carbon may enhance the growth of microalgae. Particularly, the microalgae Scenedesmus showed great growth under ambient conditions using this carbon source.

3.3. **GROWING MICROALGAE IN PHOTOBIOREACTORS FOR CO_2 CAPTURE AND BIOGAS PURIFICATION**

Of photosynthetic organisms on the planet, microalgae present as the most efficient in the use of CO_2 and can fix much larger amounts of CO_2 by land area when compared to terrestrial plants. (Brown and Zeiller, 1993). The combination of increased proliferation and cell biomass should be taken into account as base for the development of efficient production microalgae. A use these bodies using waste as a source of nutrients presents an important alternative for the production of biomass and removal of organic pollutants and inorganic nutrients, mostly nitrogen and phosphorus. (Martinez *et al,.* 1999; Proulx *et al,.* 1994). The use of microalgae offer great advantages compared to conventional techniques primarily by treatment environmental issue, since large loads to reduce pollution by removing harmful substances and heavy metals present in the effluent. Thus, different microalgae have been used successfully for this purpose (de-Bashan *et al.,* 2002, Perez - Jimenez *et al.,* 2004; Voltolina *et al,.* 2005).

In this work, initially biogas was produced by anaerobic digestion of waste from the sewage system of the laboratory itself. After filtration of contaminants (H_2S), the biogas was compressed and subsequently transferred to the cultivation of microalgae in photobioreactor Air-Lift with aeration of 2 L/min. Thus, the CO_2 present in the biogas can be used as carbon source for the photoautotrophic growth of microalgae.

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Through the analysis of the components present in the biogas (methane, CO_2, H_2S and ammonia) was established to change the composition of biogas during the experiment, as shown in Table 01.

TABLE 01 - Variation of biogas composition in % during the period of the experiment and calorif power.

COMPONENT	1º	3º	5º	6º	8º
Methane % in Influent Biogas- CH_4 (a)	75%	65%	80%	75%	65%
Calorific Value in Influent Biogas - CH_4 (kcal/m^3)	6377,19	5493,46	6783,22	6377,19	5493,46
Methane % in Effluent Biogas - CH_4 (b)	85%	95%	92,5%	95%	90%
Calorific Value in Effluent Biogas - CH_4 (kcal/m^3)	7213,15	8072,99	7858,03	8072,99	7619,18
Efficiency of CH_4 enrichment % (d)	40%	85.7%	62.5%	80%	71.5%
Carbon Dioxide % in Influent Biogas Affluent - CO_2 (a)	25%	35%	20%	25%	35%
Carbon Dioxide % in Effluent Biogas - CO_2 (b)	15%	5%	7,5%	5%	10%
CO_2 removal efficiency % (c)	40%	85,7%	62.5%	80%	71,5%
Hydrogen sulphide (H_2S) ppm	< 0,002	< 0,002	< 0,002	< 0,002	< 0,002
Ammonia (NH_3) ppm	0,0015	0,0015	0,0015	0,0015	0,0015

(a) Influent CO_2 (%) and CH_4 (%) determined from the load before the biogas effluent from the aeration in the cultures;

(b) Effluent CO_2 (%) and CH_4 (%) determined from the load biogas effluent after the aeration cultivation;

(c) CO_2 capture efficiency (%) was determined by the following formula:

$$E_{CapCO2}(\%) = \frac{\text{Influent } CO_2 - E\text{ffluent } CO_2}{\text{Influent } CO_2} \times 100$$

(d) The efficiency of biogas enrichment (% methane) was determined by the following formula:

$$E_{EnrCH4}(\%) = \frac{\text{Effluent } CH_4 - \text{Influent } CH_4}{100 - \text{Influent } CH_4} \times 100$$

The table shows that the initial values of carbon dioxide decreased their concentration up to 5% in biogas analyzed after injection in Microalgae cultivations. The carbon assimilation is explained by the process of photosynthesis by microalgae, which use sunlight to absorb carbon dioxide. Periods of low light may contribute to lower photosynthetic rate and following low CO_2 assimilation by microalgae. Even so, the biogas after passage through the culture of *Scenedesmus*, we detected a significant reduction of CO_2 and a higher proportion of methane. Compared to baseline, the percentage of methane in biogas purified increased to values of 95% at ambient temperature and luminosity. The other substances present in the biogas (NH_3 and H_2S), showed no significant concentration can be tolerated by the cultures of microalgae (Abatzoglou and Boivin, 2009).

The concentration of the substances present in the biogas varies according to the characteristics of the waste used, and also the operation of the process of digestion. The use of biogas as a source of energy depends on the identification of chemical composition and calorific value. These parameters determine the potential of biogas as well

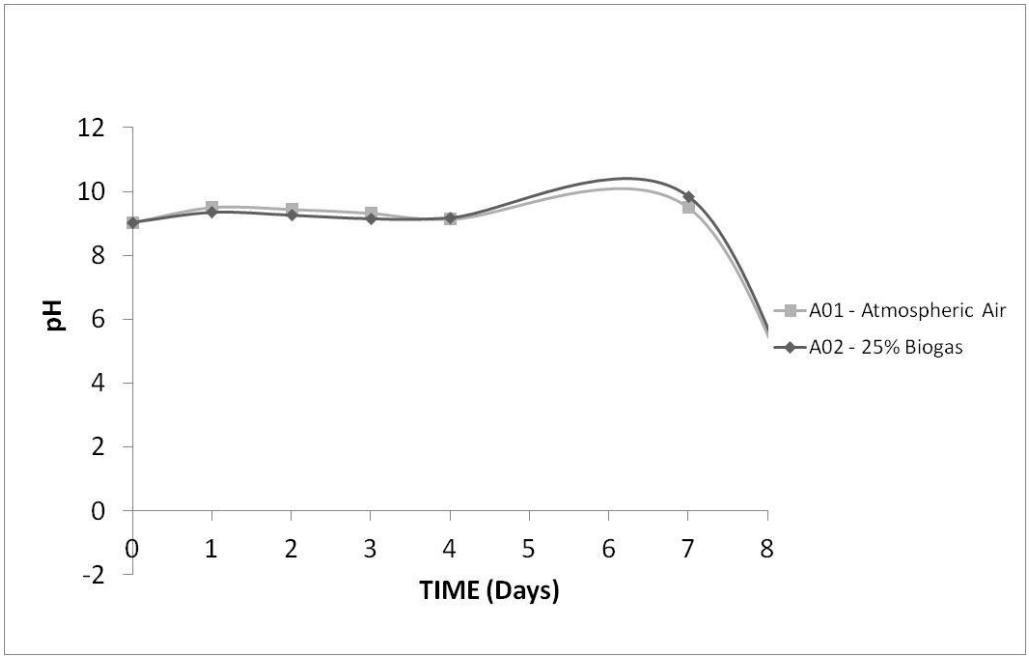
as allowing the design of purification processes in order to increase the calorific value and avoid possible damage to equipment and power generators. The main component of biogas is methane, a colorless, highly combustible. According to the percentage that participates in methane biogas composition, the calorific value of this can range 5000-7000 kcal/m^3. This calorific value can reach 12,000 kcal/m^3 once removed carbon dioxide and other contaminants from the mixture. The calorific value of biogas depends on the percentage of methane (CH^4) therein.

The pure methane under normal conditions (101.325 kPa pressure and a temperature of 0 º C), has calorific value equivalent to 8512.47 kcal/m^3 (CCE, 2000). The biogas with methane content ranging between 50% and 80%, has lower calorific value, between 4270.56 and 6792.78 kcal/m^3. The biogas with methane content ranging between 50% and 80%, has lower calorific value between 4270.56 and 6792.78 kcal/m^3. Thus, for each 10% CO_2 gas mixture of biogas, this corresponds to approximately 859.85 kcal/m^3 less in its calorific value (Inácio, 1995).

In the present study, the calorific value of the biogas was estimated taking into account the existing percentage of methane in biogas. We estimated an average increase of the calorific value of 6104.904 kcal/m^3 to 7767.268 kcal/m^3, representing an increase of approximately 27% in the calorific value of the biogas after purification with microalgae cultivations, and thus approaching to the calorific value of pure methane.

3.4. pH

In this study, we sought to monitor the pH of the cultures with daily readings during the experiment. Figure 06 shows the pH of cultures grown microalgae photobioreactor. According to the figure, the pH remained stable for both cultures.



3.5. LIPIDS

To investigate the lipid content, a sample was collected from each Microalgae cultivation at the end of the experiment for the extraction of lipids. One of the most effective extraction procedure is the method of Bligh & Dyer (1959), a simplified version of classical procedure using chloroform-methanol proposed by Folch *et al.*, (1957) An advantage of the presented methods based on the binary mixture and chloroform methanol is the ability to extract neutral lipids so as polar lipids. In this work, the culture enriched with CO_2 (2 - 5%) from the biogas had the highest

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concentration of lipids compared to cultivation only aerated with air (Table 02). However, no significant differences between cultures. These results indicate that the "Mix *Scenedesmus*" cultivated with biogas can efficiently grow CO_2 capturing and enriching biogas without negative effects on the productivity of lipids.

Table 02 - Concentration of lipids Microalgae cultivation A01 (Atmospheric Air) and A02 (Biogas 25%).

	A01- Atmospheric Air	A02 – Biogás 25%
Lipids (%)	9,7 ± 0,24	11,53 ± 0,73

4. CONCLUSION

The present study aimed to evaluate the use of a system of photobioreactors for the purpose of purifying biogas through cultures of microalgae. This system shows promise forward current technologies biogas purification, it helps to decrease the concentration of CO_2 in biogas concomitantly producing microalgae biomass. The "Mix Scenedesmus" was able to use CO_2 from biogas produced from the anaerobic digestion of wastewater from the facility's own laboratory. These results indicate that cultivation of microalgae can efficiently grow and capture CO_2 from biogas, promoting enrichment. In this work we obtained an increase of 27% in the calorific value of the biogas after purification with microalgae cultivations.

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