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Evaluation of marine algae as a source of biogas in a two-stage anaerobic reactor system

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ABSTRACT

The marine algae are considered an important biomass source; however, their utilization as energy source is still low around the world. The technical feasibility of marine algae utilization as a source of renewable energy was studied to laboratory scale. The anaerobic digestion of *Macrocystis pyrifera*, *Durvillea antarctica* and their blend 1:1 (w/w) was evaluated in a two-phase anaerobic digestion system, which consisted of an anaerobic sequencing batch reactor (ASBR) and an upflow anaerobic filter (UAF). The results show that 70% of the total biogas produced in the system was generated in the UAF, and both algae species have similar biogas productions of 180.4($\pm$1.5) mL g^{-1} dry algae d^{-1}, with a methane concentration around 65%. The same methane content was observed in biogas yield of algae blend; however, a lower biogas yield was obtained. In conclusion, either algae species or their blend can be utilized to produce methane gas in a two-phase digestion system.

1. Introduction

The utilization of current energy sources has been generating environmental pollution of air, water and soil through the years. These negative effects have increased interest in the development of new technologies to obtain clean energy, mainly through the utilization of renewable energy sources [1].

Several biological processes to convert biomass to energy, and thus provide a source of fuel, have been studied in recent decades [2,3]. One of the most important processes for this proposal is the anaerobic digestion of organic matter to obtain biogas (consisting mainly of methane and carbon dioxide) as a product of the metabolic action of methanogenic bacteria. Another reason to utilizing biomass to generate energy is that the solid remainder product from anaerobic degradation can be used as organic fertilizer [4].

Marine algae consist of polysaccharides (alginate, laminaran and mannitol), with zero lignin and low cellulose content, which make them an easy material to convert to methane by anaerobic digestion processes.

Only few works have evaluated the marine algae conversion by anaerobic biodigestion to methane production. The first studies evaluated algae species as *Macrosystis pyrifera*, *Tetraselmis*, *Gracilaria tikvahiae*, *Hypnea* and *Ulva*; these studies, in general, conclude that marine algae are good feedstocks for the anaerobic digestion process, due to their high conversion rates and efficiencies obtained [5–7].

The anaerobic biodegradation of marine algae is carried out by three groups of bacteria [8] (Fig. 1): (1) hydrolytic and

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fermentative bacteria, which hydrolyze polymers, and ferment the resulting monosaccharide to carboxylic acids and alcohols; (2) acetogenic bacteria, which convert these acids and alcohols to acetate, hydrogen and carbon dioxide; and (3) methanogenic bacteria, which convert the end products of acetogenic reactions to methane and carbon dioxide. Several studies have proposed the physical separation of these anaerobic phases in order to increase the degradation of organic matter, improve biogas production and attain better control of operating conditions [9–12]. The metabolic pathways of the two-phase anaerobic digestion process are the same as those of conventional digestion; however, they are physically separated in (i) hydrolytic and acetogenic phase and (ii) methanogenic phase (Fig. 1) [9,13].

The objective of this study was to evaluate the technical feasibility of biogas production utilizing marine algae *Macrocystis pyrifera* and *Durvillea antarctica* in a two-phase anaerobic digestion system at laboratory scale.

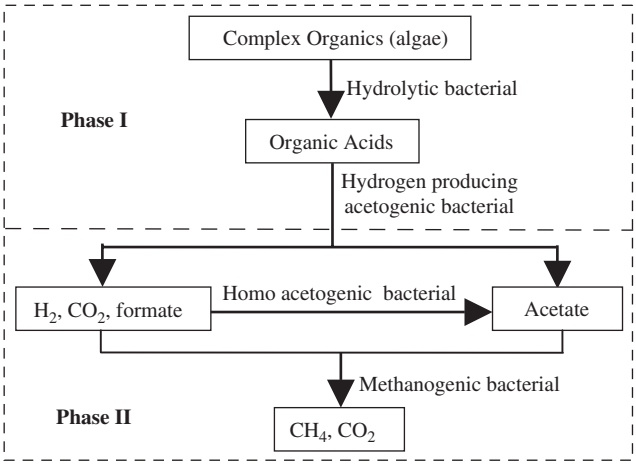

Fig. 1 – Biogas production process.

2. Materials and methods

2.1. Marine algae

M. pyrifera (algae A) and *D. Antarctica* (algae B) were collected in March of 2005 on Nigue beach (39°17′S and 73°13′W), south of Caleta Queule, Region IX, Chile. The algae collected were the material living at the time of collection in the inter-tidal zone. The algae species were dried and stored after 12 h of harvesting. A part of this dry material was stored during 2 months in a freezer at −15 °C and the other part was used immediately for the inoculum production.

The algae species were washed, dried at 70 °C for 24 h, then crushed and dissolved in 300 mL of distilled water before feeding into the bioreactors.

2.2. Experimental system

The methane generation system consists of two anaerobic bioreactors and a gas meter (Fig. 2). The first bioreactor was an anaerobic sequencing batch reactor (ASBR) of 2.5 L (New Brunswick scientific, Bioflo 2000), with a check valve biogas outflow, one feed valve and two valves to drain leachate and residual sludge. The pH in the ASBR was maintained between 5.5 and 5.7 with a solution of HCl (1 N). The leachate generated in the ASBR (300 mL d^{-1}) was pumped to a PVC (1 L) equalizer, where the pH was adjusted between 6.8 and 7.2 with a solution of NaOH (4 N), before it was added to the upflow anaerobic filter (UAF). The operation cycle of the ASBR was the following: (i) filling (10 min); (ii) digestion

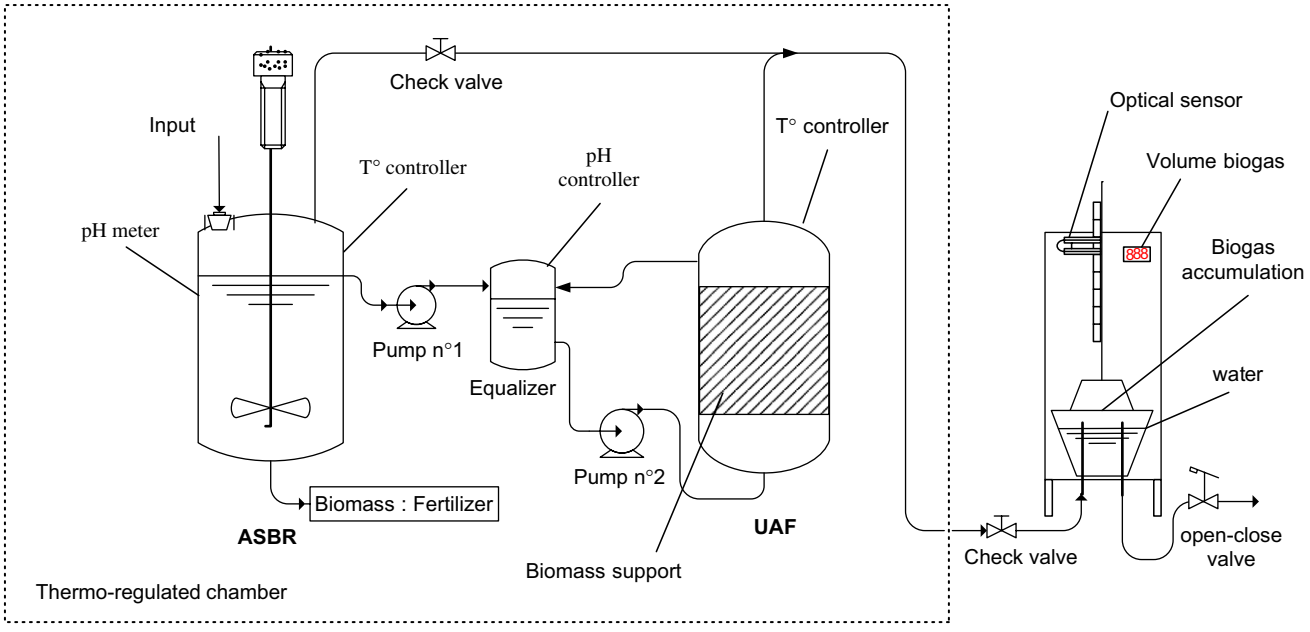

Fig. 2 – Schematic design of a two-phase anaerobic digestion system.

(23.3 h); (iii) sedimentation (15 min); (iv) leachate pumped to the equalizer (5 min); and (v) sludge drain (10 min).

The second bioreactor was a UAF (4 L) with a check valve biogas outflow and a valve to drain residual solids. The bioreactor was packed with PVC rings as microbial support and the hydraulic retention time (HRT) was 24 h. Both bioreactors were maintained at 37 °C in a thermo-regulated chamber during all the evaluation time.

The flow rate and volume of the biogas produced in the bioreactors were determined in a gas meter (2.5 L), equipped with an optical sensor, a volume indicator and an automatic discharge system (Fig. 2).

2.3. Adaptation of the microbial flora

Cow manure was utilized as inoculum for the bioreactors. Then, 600 mL of inoculum was mixed with 300 mL distilled water, and was filtered using Glass Fiber Advantec GC 50. The filtered liquid was collected in vessels of 1 L, which were maintained in anaerobic conditions at 37 °C and 100 rpm; 1 g glucose d^{-1} was supplemented as a carbon source for a period of 5 days. The inoculum obtained (1 L) was added to the bioreactors and the pH was adjusted to 5.0 and 7.0 for the ASBR and UAF, respectively. Then the ASBR was fed with 60 g glucose L^{-1} d^{-1} and 50 mL of yeast extract (30 g L^{-1}) every 2 days. The same feeding procedure was applied to the UAF, with further addition of 300 mL d^{-1} of the leachate produced in the ASBR. This feeding procedure was carried out until biogas production was observed in both bioreactors.

When biogas was detected in the bioreactors, algae (*M. pyrifera*) was fed in and glucose elimination started gradually for a period of 37 days according to the following procedure: (I) 3 g glucose, (II) 2.5 g glucose+0.5 g dry algae, (III) 2 g glucose+1 g dry algae, (IV) 1.5 g glucose+1.5 g dry algae and (V) 3 g dry algae. The feed change was realized every 7 days.

2.4. Biogas generation from algae

A series of four experiments was carried out in order to determine the biogas production rates of algae species in a two-phase digestion system. In the first experiment, the biogas generation in the ASBR and UAF systems was evaluated during a period of 15 days each using *M. pyrifera* (algae A). In the second, third and fourth experiments, the biogas yield of *M. pyrifera* (algae A), the blend 1:1 (w/w) of algae (A and B), and *D. antarctica* (algae B) were evaluated in the integrated system during a period of 31 days, respectively. In the four experiments a load of 3 g dry algae d^{-1} were fed in the system.

2.5. Analytical methods

Methane (CH_4) and carbon dioxide (CO_2) concentrations in the biogas were analyzed in a GC-FID (Perkin-Elmer Instruments, Clarus 500), equipped with a capillary column (SPB-1); nitrogen was used as carrier gas (6 mL min^{-1}). The temperatures of the injector, column and detector were 150, 215 and 260 °C, respectively.

Hydrogen sulfide (H_2S) and ammonia (NH_3) content in biogas were analyzed using gas detector tubes and a manual gas sampling pump (RAE system, Cole-Parmer).

Chemical oxygen demand (COD) and volatile solids (VS) were determined according to the standard method [14]. All the samples were previously filtered using Glass Fiber Advantec GC 50 filter paper.

The elemental composition (C, H, N and S) of algae, leachate and sludge was analyzed in a CE Instruments equipped with Eager 200 software. The combustion temperature and the oxygen pressure were 1000 °C and 150 kPa, respectively; helium (140 mL min^{-1}) was used as carrier gas.

3. Results and discussion

3.1. Adaptation to biodegradation of the algae

Fig. 3 shows the adaptation period of the consortium to algae degradation utilizing *M. pyrifera* (algae A). In the early adaptation period with glucose as the sole carbon source (phases I and II) an average biogas production of 673(±198) mL d^{-1} was observed. However, when the glucose concentration was replaced by algae A, a decreased biogas production was achieved (phases III–V) (Fig. 3). Nevertheless, from days 25 to 37 (phases IV and V), a stationary state production of biogas around 410(±96.7) mL d^{-1} was obtained. This result indicates that a biogas yield of 60.8% was attained when algae was utilized at a level between 50% and 100% (phases IV and V), in comparison with glucose as the sole carbon source (phase I) (Fig. 3). The low biogas production obtained with algae may depend on their lower carbon bioavailability, given their molecular structure (polysaccharides) in comparison with glucose.

3.2. Biogas generation in the ASBR, UAF and integrated system

During the evaluation of biogas production in the individual systems (ASBR and UAF) it was observed that approximately

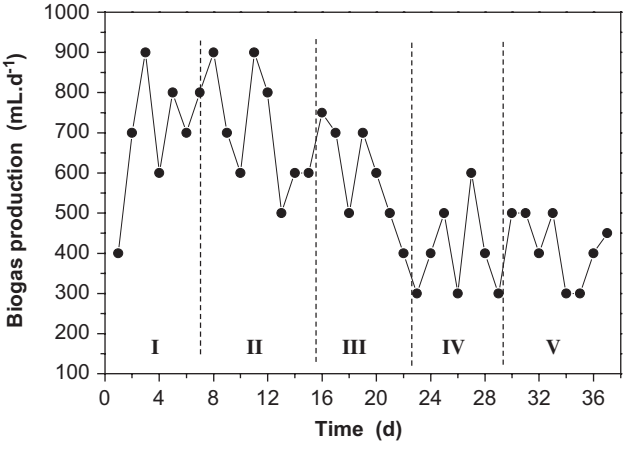

Fig. 3 – Adaptation periods of the microbial flora in the bioreactors utilizing *M. pyrifera* (algae A): (I) 3 g glucose, (II) 2.5 g glucose+0.5 g dry algae, (III) 2 g glucose+1 g dry algae, (IV) 1.5 g glucose+1.5 g dry algae, (V) 3 g dry algae.

30% of the total biogas was generated in the ASBR (hydrolytic and acidic phase), while 70% was produced in the UAF (methanogenic phase). These results show that biogas production occurs mainly in the UAF, where pH was maintained at 7.0. Nagamani et al. [15] mention that a pH between 7.0 and 7.2 is optimum for increased biogas yield, although gas production was also satisfactory between pH 6.6 and 7.6, while at a lower pH (<6) gas production is negatively affected.

For integrated system evaluation a total biogas accumulation of 16.9, 15.2 and 16.7 L was achieved during 31 days for algae A, A+B and algae B, respectively (Fig. 4). Although the blend of algae attained the lower biogas accumulation, its production rate was almost the same as pure algae. This indicates that algae A, algae B and their blend have similar biogas production rates (0.545, 0.492 and 0.540 L d^{-1}, respectively).

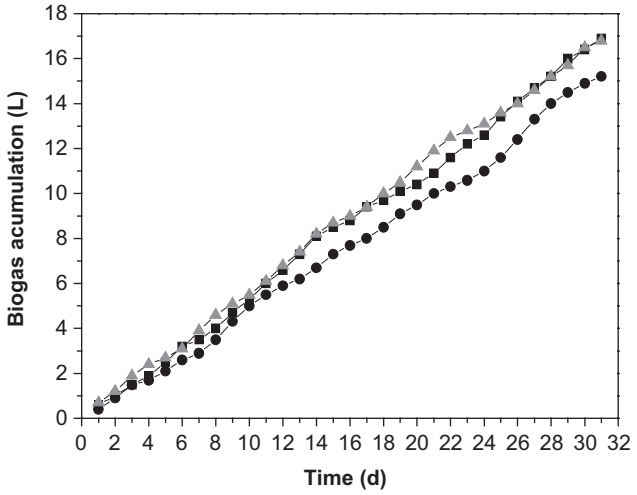

Fig. 4 – Biogas accumulation from the integrated system: experiment 2 (algae A) (■), experiment 3 (algae A+B) (●) and experiment 4 (algae B) (▲).

3.3. *Specific biogas production and methane content*

Fig. 5 shows the specific biogas production per gram of dry algae and its methane composition for algae A (experiment 2), algae A+B (experiment 3) and algae B (experiment 4) in the integrated system. The specific biogas production rate averages were 181.4(±52.3), 164.2(±54.9) and 179.3 (±80.2) mL g^{-1} dry algae d^{-1}, respectively. These results indicate that experiment 3 has the lowest biogas production, which represents 11% less biogas in comparison with the experiments where pure algae were used. According to Fig. 5, biogas yield was in a range of 95 to 260 mL g^{-1} dry algae d^{-1} for

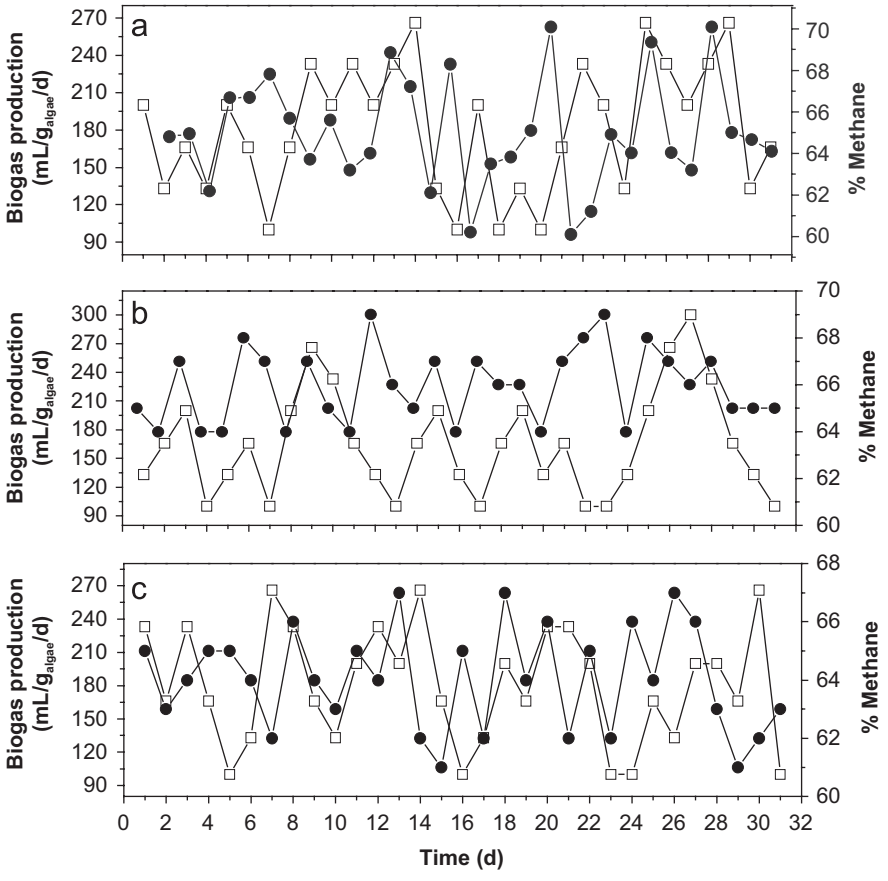

Fig. 5 – Development of the specific biogas production (□) and methane percentage (●) in an integrated system. (a) Algae A (experiment 2), (b) algae A+B (experiment 3) and algae B (experiments 4).

the three experiments during 31 days of evaluation. The maximum value observed in this range is similar to the biogas production of 250 mL g^{-1} dry algae d^{-1} achieved with *M. pyrifera* and *Laminaria* in a two-phase digestion system [16]. However, these values are lower than the biogas yield of 340 and 300 mL g^{-1} dry solid d^{-1} attained in one-phase systems with cow manure and sewage, respectively [17].

VS values for the UAF between 8 and 2 g L^{-1} and for the ASBR between 7 and 3 g L^{-1} were obtained in experiment 2. On the other hand, in experiments 3 and 4 the VS in the UAF were between 9 and 3 g L^{-1} and for the ASBR between 8 and 3 g L^{-1}. These VS results were similar to the 7 g L^{-1} reported previously by Chynoweth [16] for the biological gasification of marine algae. These VS variations in the operation ranges in each experiment correspond to the feeding of fresh algae (3 g dry algae d^{-1}) and later diminution of VS during one operation day, this variation staying during every day of operation.

The methane percentage in the biogas collected in experiments 2–4 (Fig. 5) was in the range of 60–70% during 31 days, with an average value of 64.70(±2.5)%, 64.4(±1.8)% and 65.3(±1.6)%, respectively. These results show that methane percentage is not a function of the algae species evaluated. Usually, biogas produced from marine algae contains between 50% and 65% of methane, although methane concentrations as high as 75% have been reported for *M. pyrifera* in a two-phase anaerobic digestion system [16]. The methane percentage obtained in the present study is comparable to the values of methane content (45–70%) generated during the stationary methanogenic phase in sanitary landfills, where a huge variety of organic municipal solid wastes are disposed of, consisting mainly of 45–60% cellulose and hemi-cellulose [18,19]. Similar methane percentages are also achieved in a two-phase anaerobic digestion system where organic wastes such as fruit, vegetables, spent tea leaves and cow manure were evaluated [4,13].

The average content of carbon dioxide in the biogas produced during the four experiments was around 18.3(±1.4)%; this indicates that the biogas produced has a CH_4/CO_2 ratio higher than 1, which is adequate for energy recovery. On the other hand, the percentages of hydrogen sulfide (H_2S) and ammonia (NH_3) in biogas were around 0.1% and 1%, respectively. The contents of these compounds in landfill gas are generally less than 1% for both compounds [20]. The high concentration of NH_3 observed in the biogas may be due to the nitrogen content in the marine algae, which generated a low C/N ratio in the residual sludge produced in the ASBR (Table 1). This low C/N ratio and the anaerobic condition in the ASBR suggest that the excess of nitrogen was converted to ammonia (NH_3) or ammonium (NH_4^+).

3.4. COD variation

Fig. 6 shows the variation of the COD for each bioreactor and experiment (algae A, algae A+B and algae B). The COD values in the ASBR were higher than the values registered in the UAF in the three experiments as it has been expected; this result suggests that the hydrolysis and the volatile fatty acids (VFA) formation were carried out in the ASBR, while the VFA were converted to methane in the UAF (Fig. 1). The average COD values for experiments 2 (algae A), 3 (A+B) and 4 (algae B) were 6803(±523), 6211(±541) and 6735(±516) mg L^{-1} to ASBR, while to UAF were 5397(±597), 5571(±577) and 5478(±521) mg L^{-1}, respectively. In this case the algae blend (experiment 3) achieved the lowest COD in the ASBR and the highest value in the UAF, which is related to its low biogas production (Fig. 4).

According to the literature, at sanitary landfills average COD values of 22,000 mg L^{-1} (range of 6000–60,000 mg L^{-1}) are attained during the acidic phase, while in the methanogenic phase average values of 3000 mg L^{-1} (range of 500–4500 mg L^{-1}) are obtained [19]. Changes of the COD values between these two phases in sanitary landfills generally occur at a time interval of several years due to the huge quantity of organic matter contained in municipal solid waste. Although in the present study, algae was used as sole carbon source in the two-phase digestion system, the COD values suggested that the methanogenic phase is the limiting step in the anaerobic degradation of algae, since only 16.7(±0.5)% of the COD was removed in the UAF. This low COD removal may be due to the low HRT in the UAF (1 day), which indicates that to increase the COD removal a HRT more that 1 day would be needed. Nagamani et al. [15] mention that a HRT of 14 days is an optimal value for biogas production from cow manure, while a lower HRT results in accumulation of VFA.

3.5. Elemental analysis

Table 1 shows the elemental analysis of algae A, algae B, leachate and residual sludge of ASBR and UAF. The contents of carbon, nitrogen, hydrogen and sulfur of *M. pyrifera* and *D. antartica* show that both algae species have similar

Table 1 – Elemental analysis of algae species, leachate and sludge of ASBR and UAF

Element	Algae (A) *M. pyrifera* (%)	Algae (B) *D. antartica* (%)	Leachate ASBR (%)	Sludge ASBR (%)	Sludge UAF (%)
Sulfur	0.83	0.88	1.86	0.64	0.96
Carbon	38.94	39.13	37.95	37.66	32.01
Hydrogen	5.22	5.53	3.85	5.59	2.13
Nitrogen	1.58	1.67	1.73	4.31	1.47

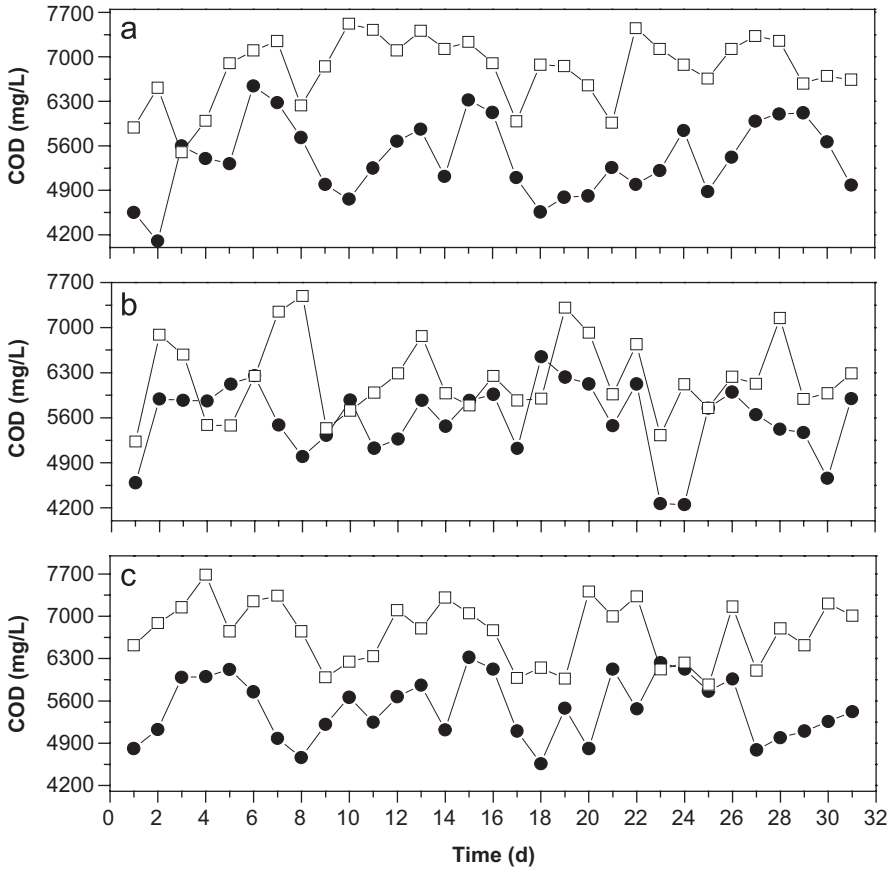

Fig. 6 – Development of chemical oxygen demand (COD) in the ASBR (□) and UAF (●). (a) Algae A (experiment 2), (b) algae A+B (experiment 3) and algae B (experiments 4).

elemental compositions. This explains the similarity of the results obtained in biogas production for algae A and algae B (Fig. 4).

The carbon balance in the ASBR shows that 32% of the total carbon inlet (algae) to the system was converted to VFA, 6% to biogas and 62% to residual sludge, while in the UAF, it was converted to biogas (45%) and residual biomass (55%). In addition to algae biogas production, Stevenson [21] mentions that the residual biomass can be utilized as biofertilizer, since it generally contains a high quantity of nutrients (vitamins and minerals) and a C/N ratio in a range of 20–30.

4. Conclusions

The marine algae species studied are a renewable energy source that can generate biogas with high methane concentration in a two-phase anaerobic digestion system.

M. pyrifera and *D. antartica* algae present practically the same biogas production per gram of algae due to their similar elemental compositions (C, H, N).

The COD values of the ASBR and UAF indicate that the methanogenic phase is the limiting step in the anaerobic degradation of algae at the operational conditions presented in this study.

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