



Biochemical methane potential of microalgae: Influence of substrate to inoculum ratio, biomass concentration and pretreatment

M.E. Alzate^a, R. Muñoz^a, F. Rogalla^b, F. Fdz-Polanco^a, S.I. Pérez-Elvira^{a,*}

^a Department of Chemical Engineering and Environmental Technology, University of Valladolid, Dr. Mergelina s/n, Valladolid, Spain

^b AQUALIA Gestión Integral del Agua, S.A, Spain

HIGHLIGHTS

- ▶ Anaerobic digestion of microalgae have highest productivities at S/I ratios of 0.5.
- ▶ Methane productivity is depended of microalgae specie.
- ▶ The microalgae concentration for anaerobic digestion must be greater than 10 gTS/kg.
- ▶ COD solubilization no imply an improvement on methane productivity.
- ▶ Thermal hydrolysis substantially improves methane productivity of microalgae.

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ABSTRACT

The anaerobic digestion of three microalgae mixtures was evaluated at different substrate to inoculum (S/I) ratios (0.5, 1 and 3), biomass concentrations (3, 10 and 20 gTS/kg) and pretreatments (thermal hydrolysis, ultrasound and biological treatment). An S/I ratio of 0.5 and 10 gTS/kg resulted in the highest final methane productivities regardless of the microalgae tested (ranging from 188 to 395 mL CH₄/gVS_{added}). The biological pretreatment supported negligible enhancements on CH₄ productivity, while the highest increase (46–62%) was achieved for the thermal hydrolysis. The optimum temperature of this pretreatment depended on the microalgae species. The ultrasound pretreatment brought about increases in CH₄ productivity ranging from 6% to 24% at 10,000 kJ/kgTS, without further increases at higher energy inputs. The results here obtained confirmed the lack of correlation between the solubilization degree and the methane enhancement potential and pointed out that anaerobic digestion of algae after thermal pretreatment is a promising technology for renewable energy production.

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1. Introduction

Microalgae as a feedstock for biogas production have been studied since the early fifties based on their large areal productivities compared to conventional crops (Chisti, 2007; Golueke et al., 1957). In the recent years, the need to seek for renewable energy sources to replace fossil fuels has triggered the research on microalgae anaerobic digestion. However, few systematic studies have been conducted to date to fully explore the maximum biodegradability of microalgae and to enhance their CH₄ productivity.

The biochemical methane potential (BMP) assay constitutes a useful tool to determine both the ultimate biodegradability and the methane conversion yield of organic substrates. In this context, the determination of the BMP of an organic residue can help in the design and economic evaluation of a biogas plant (Angelidaki et al.,

2009). In this assay, the substrate to inoculum (S/I) ratio is a key parameter affecting the result. In addition, the determination of the optimum S/I ratio for a specific residue could also help to establish a start-up protocol for continuous anaerobic digesters in order to optimize this critical operational stage (Fernández et al., 2001).

The concentration of substrate in the BMP assay also impacts on the final biodegradability and methane productivity. Hence, an excessive concentration of solids makes proper mixing more difficult and could generate inhibition by accumulation of fatty acids. On the other hand, too low substrate concentrations significantly increase the process heating costs and require larger digester volumes in order to avoid the wash-out of anaerobic biomass. Additionally, some studies have shown that the initial substrate concentration can modify the methane content in the biogas and the methane productivity (Fernández et al., 2001, 2008). In this regard, microalgal cultures have low biomass concentrations (<0.5–27 g/L) (Molina et al., 2003; Suh and Lee, 2003) and the need to concentrate this biomass before anaerobic digestion could increase

* Corresponding author.

E-mail addresses: frogalla@fcc.es (F. Rogalla), sarape@iq.uva.es (S.I. Pérez-Elvira).

the production cost of microalgae, which could eventually jeopardize the economics of the entire biofuel production process. Hence, in order to avoid extra biomass harvesting costs the determination of the optimal concentration for anaerobic digestion of microalgae is crucial.

Preliminary studies on anaerobic digestion of microalgae have shown low methane productivities (0.18–0.39 LCH₄/g VS) compared to municipal solid waste or fruit and vegetables waste (0.39–0.53 LCH₄/g VS) (Gunnaseelan, 1997; Mussnug et al., 2010). These experimental findings have been attributed to the strong cell walls of microalgae, which make them highly resistant to bacterial attack even when cells are not alive (Golueke et al., 1957). Recalcitrant compounds like polyaromatics, heteropolysaccharides, algaenan, sporopollenin, silica, uronic acid and lignine were found in the cell walls of microalgae that showed a low biodegradability (Gunnison and Alexander, 1975a,b,c; Sander and Murthy, 2009). In this context, a pretreatment step able to break up the cell wall of microalgae could increase their biodegradability and therefore their CH₄ productivity. Different pretreatments have been successfully applied to activated and primary sludge to enhance its CH₄ productivity. These pretreatments can be classified as mechanical (ultrasonic, lysis – centrifuge, liquid shear, collision plate, grinding, etc.), biological (aerobic, microaerophilic or anaerobic), thermal and chemical (oxidation, alkali treatments, etc.). For instance, CH₄ productivity enhancements of up to 100% have been recorded when using thermal hydrolysis and ultrasound in activated sludge (AS) (Carrère et al., 2010). Biological pretreatments based on increasing the bacterial hydrolytic activity have also enhanced methane productivity by 86% when applied to activated sludge (Carrère et al., 2010). Even though these pretreatments could also be used for microalgae, little information is available regarding the potential of microalgal pretreatments to enhance their anaerobic digestion of algal biomass.

In this study, the influence of different S/I ratios and biomass concentrations on the BMP was evaluated in three different microalgae. In addition, the potential of thermal hydrolysis, ultrasound and a biological treatment (hydrolytic activity) for increasing the biodegradability and CH₄ productivity in these microalgae was assessed.

2. Methods

2.1. Microalgae and inoculum

Microalgae A, a mixture of microalgae cultivated in a synthetic mineral salt medium in a tubular photobioreactor and free of bacterial contamination was kindly provided by Cajamar Foundation (Almería, Spain). This microalgae mixture was composed of 40% *Chlamydomonas*, 20% *Scenedesmus* and 40% of an unknown microalgae tentatively characterized as *Nannochloropsis*. The concentration of total solids as received was ≈180 gTS/kg. These microalgae were refrigerated at 4 °C prior to use.

Microalgae B and C were cultivated in a 180-L open photobioreactor operated in a continuous culture mode at 36 days of hydraulic residence time and artificially illuminated at the Department of Chemical Engineering and Environmental Technology at the University of Valladolid (Spain). The photobioreactor was fed with anaerobic digestion effluent and synthetic biogas. Microalgae B were harvested from the bottom of a settler located at the photobioreactor outlet with a concentration of ≈10 gTS/kg. The composition of this microalgae mixture was 58% *Acutodesmus obliquus*, 36% *Oocystis* sp., 1% *Phormidium* and 5% *Nitzschia* sp. Microalgae mixture C, mainly composed of *Microspora*, was harvested from the surface of the photobioreactor (autoflotation) at ≈40 g TS/kg. Both microalgae mixtures were refrigerated at 4 °C prior to use.

The anaerobic inoculum was collected from a pilot anaerobic digester treating activated sludge at 35 °C at the Department of Chemical Engineering and Environmental Technology at the University of Valladolid (Spain).

2.2. Anaerobic digestion batch tests

Three series of tests (BMP assays) were conducted to determine the influence of the S/I ratio, microalgae concentration and microalgae pretreatment on the BMP and microalgae biodegradability. The tests were performed in serum bottles of 160 ml filled with 80 ml of a mixture of anaerobic inoculum and microalgae (untreated or pretreated). To provide enough buffer capacity for anaerobic digestion, the anaerobic inoculum was supplemented with 5 g NaHCO₃/L. The bottles were closed with butyl septa, sealed with aluminum caps, purged with helium for 15 min and incubated in a thermostated room at 35 °C in a rotary shaker at 120 rpm. Control tests containing 80 mL of inoculum were carried out in order to determine the CH₄ production potential of the inoculum. The production of methane from the inoculum (obtained from the control tests) was subtracted from the total methane production to obtain the microalgae methane production.

2.2.1. Ratio test

The microalgae concentration was kept constant at 10 gTS/kg and three S/I ratios were tested: 0.5, 1 and 3 (VS_{microalgae}:VS_{inoculum}).

2.2.2. Concentration test

The S/I ratio was kept constant at 1 (VS_{microalgae}:VS_{inoculum}) and three microalgae concentrations were tested: 3, 10 and 20 gTS/kg approximately. This concentration range was selected to evaluate the BMP of microalgae harvested using low cost technology such as sedimentation, where microalgae concentrations reach concentrations of ≈1.6% TSS (Mohn, 1980).

2.2.3. Pretreatment test

Tests with pretreated microalgae (as described below) were conducted at a S/I ratio of ≈1 and microalgae concentrations of 9–10 gTS/kg, except for the microalgae subjected to thermal hydrolysis, which experienced a dilution during this pretreatment. Tests carried out with non-pretreated biomass were used as controls.

The anaerobic digestion process in the three series of tests was monitored by periodic measurements of the pressure of the headspace and biogas composition. The BMP assays were stopped when the daily methane production was less than the 1% of the total accumulated methane. All tests were carried out in duplicate. The methane production was expressed at a standard temperature and pressure (STP) of 0 °C and 1 atm, respectively. Microalgae biodegradability was calculated as the ratio of the empirical to the theoretical CH₄ production, the latter estimated assuming a theoretical production of 350 mLCH₄/g COD_{degraded}. The total nitrogen (TN), total ammonium (N–NH₄⁺), total solid (TS), volatile solid (VS), total and soluble chemical oxygen demand (COD_T–COD_S) concentrations were determined for the raw (initial) and digested microalgae.

2.3. Microalgae pretreatments

Microalgae were diluted (when necessary) with distilled water to a concentration of 9–10 gTS/kg prior to pretreatment. All pretreatments were carried out in batch mode. TN, N–NH₄⁺, TS, VS, COD_T and COD_S concentrations were determined before and after each pretreatment. Three different pretreatments were applied to the three target microalgae:

2.3.1. Thermal hydrolysis

Samples of 200 mL of microalgae were maintained during 15 min in a stainless steel vessel heated by direct 9 bar steam injection at $T_1 = 110 \pm 5 \text{ }^\circ\text{C}$ ($1.0 \pm 0.2 \text{ bar}$), $T_2 = 140 \pm 4 \text{ }^\circ\text{C}$ ($1.2 \pm 0.2 \text{ bar}$) and $T_3 = 170 \pm 3 \text{ }^\circ\text{C}$ ($6.4 \pm 1 \text{ bar}$). Due to the dilution of the microalgal sample as a result of steam condensation, the final volume of the pretreated samples was measured in a graduated cylinder at the end of the experiment and used for further calculations.

2.3.2. Ultrasound

This pretreatment was performed in a plastic beaker (not temperature control) containing 200 mL of microalgae using an ultrasound system (UP400S Ultrasonic Processor – Hillscher, Germany) with the ultrasonic probe immersed in the middle of the microalgal sample. The energy supplied (E) (kJ/kgTS) was a function of the average ultrasonic power (P) (Watts), ultrasonic time (t) (seconds), sample volume (V) (milliliters), and initial total solid concentration (TS) (gTS/kg):

$$E = \frac{P \times t}{V \times \text{TS}} \quad (1)$$

Four energies were tested: $U_1 = 10,000 \text{ kJ/kgTS}$, $U_2 = 27,000 \text{ kJ/kgTS}$, $U_3 = 40,000 \text{ kJ/kg TS}$ and $U_4 = 57,000 \text{ kJ/kg TS}$.

2.3.3. Biological pretreatment

The biological pretreatment was carried out microaerobically in 2 L glass bottles containing 500 mL of algal biomass with air as headspace atmosphere. The bottles were closed with butyl septa and aluminum caps, and incubated in the dark in a roller shaker at $55 \text{ }^\circ\text{C}$ during 12 h (E1) and 24 h (E2). Preliminary tests showed that the systems remained aerobic after 24 h of incubation.

The solubilization degree (S_D) (%) of COD, was calculated according to Eq. (2) in order to evaluate the efficiency of the pretreatments:

$$S_D = \frac{\text{COD}_S - \text{COD}_{S_0}}{\text{COD}_T - \text{COD}_{S_0}} \times 100 \quad (2)$$

Where, COD_S is the soluble COD after pretreatment, COD_{S_0} is the soluble COD in the raw microalgae and COD_T the total COD of the microalgae (Donoso-Bravo et al., 2011).

2.4. Analytical procedures

TS, VS, COD_T and COD_S concentrations were determined according to Standard Methods (2005). To obtain the soluble phase, samples were centrifuged at 5000 rpm for 10 min in a Kubota 5100 centrifuge (Kubota Corporation, Japan). The soluble TN was measured in a TNM-1 analyzer (Shimadzu, Japan). Ammonium was determined either colorimetrically according to the Nessler method using a Hitachi U-2000 Spectrophotometer (Hitachi Corporation, Japan) or using a Thermo Scientific – Orion 9512HPNWP Ammonia electrode. The pressure in the headspace of the serum bottles was measured with a pressure sensor PN 5007 (IFM, Germany), while the biogas composition was analyzed periodically using a gas chromatograph coupled with a thermal conductivity detector (Varian CP-3800, USA) according to Donoso-Bravo et al. (2011). Helium was used as the carrier gas.

3. Results and discussion

3.1. Ratios test

The maximum methane productivity, biodegradability, and the higher rate of methane productivity were reached at a S/I ratio of 0.5, regardless of the microalgae tested (Fig. 1 and Table 1). At this

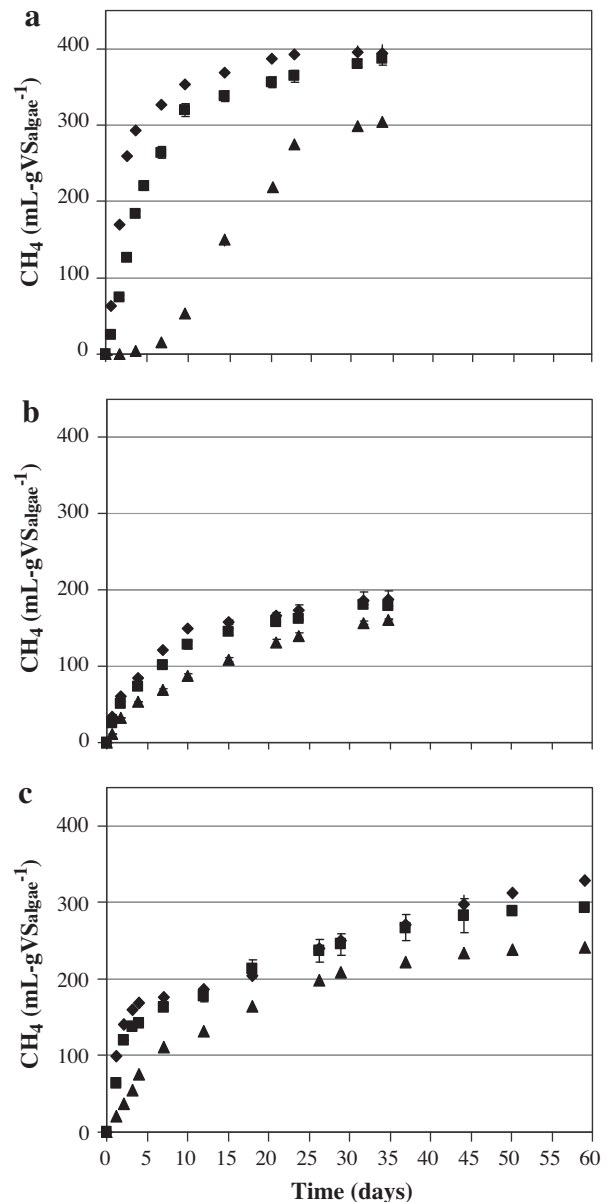


Fig. 1. Time course of methane productivity for microalgae A (a), B (b) and C (c) at a S/I ratio of 0.5 (◆), 1 (■) and 3 (▲) and a microalgae concentration of 10 gTS/kg. Vertical bars represent the standard deviation.

ratio, the final methane productivity was 395 ± 11 , 188 ± 11 and $329 \pm 12 \text{ mLCH}_4/\text{g VS}$ for microalgae A, B and C, respectively, which ranged between the values obtained by Mussnug et al. (2010) during the anaerobic digestion of 6 different microalgae (218–387 $\text{mLCH}_4/\text{g VS}$). By day 5, the CH_4 productivities for microalgae A, B and C accounted for 77%, 52% and 52% of the final productivities, respectively.

At a S/I ratio of 1 the final methane productivities and biodegradabilities were slightly lower (2–11% and 2–9%) than those obtained at a S/I of 0.5 (Fig. 1). At a S/I ratio of 3, the occurrence of a lag phase (4 days) in microalgae A tests, together with the lower rates and final CH_4 productivities in all microalgae tested suggest the potential inhibition of the methanogenic activity (Fig. 1). In this context, González-Fernández and García-Encina (2009); Zhou et al. (2011) reported an accumulation of VFAs (mainly acetic acid and propionic acid) at a high S/I ratio. The accumulation of these organic acids can overwhelm the reserve of bicarbonate alkalinity, which

Table 1

Influence of the S/I ratio and biomass concentration on the final ammonium concentrations, ammonium released and biodegradability for microalgae A (VS/TS = 0.79), B (VS/TS = 0.83) and C (VS/TS = 0.90).

Microalgae	S/I ratio	TS concentration (g/kg)	Total ammonium (mg-N/L)	Ammonium released (mg N/gVS _{algae} added)	Ammonium released (mg N/gVS _{algae} eliminate)	Biodegradability (%)
A	0.5	10	1159 ± 5	118 ± 1	170 ± 7	70 ± 2
	1.0	10	951 ± 7	102 ± 1	149 ± 5	68 ± 2
	3.0	10	697 ± 16	84 ± 3	156 ± 3	54 ± 1
	1.0	20	1485 ± 5	48 ± 0	79 ± 1	60 ± 0
	1.0	3	393 ± 40	80 ± 3	128 ± 8	62 ± 1
B	0.5	10	1079 ± 7	103 ± 3	344 ± 10	30 ± 2
	1.0	10	817	81	283	27 ± 2
	3.0	10	561 ± 47	64 ± 8	251 ± 31	26 ± 0
	1.0	20	1331 ± 21	31 ± 2	93 ± 7	33 ± 0
	1.0	3	374 ± 11	67 ± 4	346 ± 28	20 ± 0
C	0.5	10	1141 ± 33	64 ± 8	97 ± 9	66 ± 2
	1.0	10	848 ± 34	48 ± 7	79 ± 6	60 ± 4
	3.0	10	594 ± 23	50 ± 3	102 ± 6	50 ± 0
	1.0	20	1138 ± 8	19 ± 1	27 ± 1	70 ± 1
	1.0	3	333 ± 12	13 ± 4	22 ± 7	59 ± 0

Biodeg: Biodegradability.

may cause a drop in pH and therefore an adverse impact on both propionic- and acetic acid-utilizers (Speece, 2006). An accumulation of VFAs can also indicate that some other factors might be affecting the methanogenic bacteria (McCarty, 1964a). The accumulation of VFAs observed by the above mentioned authors was likely due to the higher availability of easily hydrolysable substrate at high S/I ratios, however, in any case the likely accumulation never resulted in the complete stop of the biodegradation process.

The final CH₄ content in the biogas was approximately constant for each microalgae and regardless of the ratio tested: 70 ± 3%, 72 ± 1% and 66 ± 0% for microalgae A, B and C, respectively.

3.2. Concentration tests

The changes in the TS concentration of the three microalgae at a constant S/I ratio showed significant differences on the final methane productivity of the microalgae. The reduction in the TS concentration from 10 to 3 gTS/kg on the microalgae A, B and C caused a reduction on methane productivity of 15%, 25% and 5%, respectively. Likewise, the increase in the TS concentration of microalgae A from 10 to 20 gTS/kg induced a reduction on its final CH₄ productivity of 9%, although the methane production at the 5th day was the same at both concentrations (221 ± 1 mLCH₄/g VS_{algae}). On the contrary, the increase in TS concentration from 10 to 20 gTS/kg for microalgae B and C increased their methane productivity by 15% and 10%, respectively. In addition, Fig. 2b and c show that the rate of methane productivity for microalgae B and C on the first five days was favored by the increase in the concentration of TS. Unexpectedly, when the TS concentration was reduced to 3 gTS/kg the percent of methane on the biogas increased from 69% to 75% in microalgae A, 73% to 78% in microalgae B and from 66% to 72% in microalgae C. On the contrary, when the concentration was increased to 20 gTS/kg, the percent of methane decreased to 64% for microalgae A, 68% for microalgae B and 63% for microalgae C.

No inhibition by high ammonium concentrations was recorded in any of the ratio and concentration tests conducted, based on the typical ammonium inhibitory range (1500–3000 mg/L) reported by McCarty (1964b) (Table 1). Overall, the highest N-NH₄⁺ concentrations (>1000 mg N-NH₄⁺/L) were obtained at a ratio of 0.5 and 10 gTS/kg and a ratio of 1 and 20 gTS/kg, while the lowest final concentrations corresponded to the tests carried out at 3 gTS/kg. The ammonium released per gram of VS added or eliminated (Table 1) was different for each microalgae. No a pattern of the effect of biomass concentration and S/I ratio on this parameter

was identified. In this particular study the values of ammonium per gram of VS added are in agreement with the theoretical ammonium released reported by Heaven et al. (2011) for different microalgae. The amount of N-NH₄⁺ released during anaerobic digestion will be of paramount relevance in the context of microalgae cultivation with nutrient recycling from microalgae digestion.

3.3. Pretreatment

The results here obtained clearly showed that all pretreatments increased the COD₅ in all microalgae tested, this increase being more significant the higher the energy input. Hence, the highest S_D values were reached with U4 and T3 pretreatments, regardless of the microalgae (Table 2). The S_D obtained for U4 and T3 were 32% and 32% for microalgae A, 60% and 63% for microalgae B and 62% and 40% for microalgae C, respectively. These results are in agreement with those reported by Samson and LeDuy (1983), who recorded S_D of 68% and 30% (estimated from the original data) for the ultrasonic and thermal (150 °C and 1 hour) pretreatment of *Spirulina maxima*. The lowest S_D was achieved for B1 and B2 pretreatment. The S_D achieved for B1 and B2 were 11% and 9%, 21% and 19% and 29% and 29% for microalgae A, B and C, respectively.

Overall, all pretreatments evaluated resulted in an increase in the N-NH₄⁺ and soluble TN concentrations (Tables 2–4). The mismatch between the increase in N-NH₄⁺ and soluble TN suggest that most of the nitrogen released remained as organic nitrogen, as experimentally observed by Donoso-Bravo et al. (2011) during activated sludge pretreatment.

The ultrasonic and thermal pretreatments supported an increase in both CH₄ productivity and biodegradabilities compared to the control tests, while the biological treatment mediated a decrease in both parameters (Table 2) in microalgae A. In the particular case of the ultrasonic pretreatment, despite the S_D increased at increasing ultrasound energies, both CH₄ productivities and biodegradabilities remained approximately constant. Average CH₄ productivities of 308 ± 3 mLCH₄/g VS_{algae}, 217 ± 7 mLCH₄/g VS_{algae} and 307 ± 7 mLCH₄/g VS_{algae} were obtained for microalgae A, B and C, respectively, which represent average increases of 13 ± 1%, 10 ± 4% and 20 ± 3% on CH₄ productivity compared to the control tests. This clearly confirms that higher microalgal COD solubilizations do not necessarily enhanced CH₄ productivities. In this context, Samson and LeDuy (1983) subjected *S. maxima* to ultrasound + mechanical disintegration for 10 min with even a slight decrease on CH₄ productivity (190 mLCH₄/g VS vs 170 mLCH₄/g VS) despite achieving a 68% S_D during microalgal-pretreatment.

Thermal hydrolysis of microalgae A and B supported higher increases on COD solubilization and CH_4 productivities at increasing steam temperatures. The maximum methane productivities for microalgae A and B were $398 \pm 8 \text{ mLCH}_4/\text{gVS}_{\text{algae}}$ and $307 \pm 4 \text{ mLCH}_4/\text{gVS}_{\text{algae}}$, respectively, at 170°C . These results are in agreement with those reported by Chen and Oswald (1998), who also reported an increase of methane production at increasing thermal pretreatment temperatures. In the case of microalgae C, the maximum methane productivity was $413 \pm 5 \text{ mLCH}_4/\text{g VS}_{\text{algae}}$ at 110°C and the minimum was $359 \pm 3 \text{ mLCH}_4/\text{g VS}_{\text{algae}}$ at 170°C despite that higher solubilization was reached at the highest temperature. González-Fernández et al. (2011) also showed that COD solubilization might not be a key factor in methane production. These authors showed that during the thermal hydrolysis of *Scenedesmus* in an open vessel, S_D of 5% and 6% (estimated from the original data) were obtained at 70 and 90°C , respectively, while the improvement on methane production accounted for 12% ($85 \text{ mLCH}_4\text{-gCOD}_{\text{in}}^{-1}$) and 123% ($170 \text{ mLCH}_4\text{-g COD}_{\text{in}}^{-1}$), respectively. Similarly, Samson and LeDuy (1983) subjected *S. maxima* to a 1-h thermal hydrolysis at 50°C and 100°C (water bath) and 150°C (steam sterilizer) achieving S_D of 12%, 12% and 30% respectively. The methane production under continuous mode (20 day of retention time and loading rate of $2.0 \text{ kgVS}/\text{m}^3 \text{ d}$) was 190 mL/g VS for the control tests and 200, 180 and 180 mL/g VS for the microalgae pretreated at 50, 100 and 150°C , respectively. Overall, the maximum increase on methane productivity for the three microalgae pretreated in this study was $54 \pm 8\%$ and superior to the 33% obtained by Chen and Oswald (1998) pretreating an unknown microalgae at 100°C for 8 h at a solid concentration of 3.7%.

The mechanisms underlying the pretreatments applied to the three microalgae are fundamentally different. Hence, the ultrasonic pretreatment breaks up the microbial cell by cavitation, releasing the intracellular material. Cavitation promotes chemical reactions due to the high local temperatures and pressures, creates extreme shear forces in the liquid and leads to the formation of highly reactive radicals (H^\bullet and OH^\bullet), which facilitates chemical reactions to destroy organic matter. This destruction of organic material accelerates the hydrolysis of the biomass and VFAs are more readily generated through acidogenesis and subsequently transformed into methane through methanogenesis (Appels et al., 2008). The thermal pretreatment employed in this study uses the heating and pressurization of the microalgae followed by a sudden decompression (steam explosion), which plays a key role in biomass hydrolysis. This combined heating-pressurization-decompression pretreatment destroys the cell walls and releases the intracellular cell content, which becomes accessible for biological degradation (Pérez-Elvira et al., 2006). At high temperatures, the H^+ and OH^- present in water facilitate acid- or base-catalyzed reactions, which promotes the further degradation of some organic compounds (Akiya and Savage, 2002). During thermal pretreatment, the formation of recalcitrant compounds could occur, which might inhibit anaerobic digestion (Fdz-Polanco et al., 2008). The highest CH_4 productivities recorded here for the microalgae pretreated with thermal hydrolysis, compared to those treated with ultrasound, suggests a higher degree of decomposition of organic material with thermal hydrolysis. In addition, the fact that microalgae C exhibited the highest biodegradability at 110°C suggests the formation of recalcitrant compounds at higher temperatures.

The biological pretreatments B1 and B2 applied to microalgae A caused a reduction on CH_4 productivity of 4% and 8%, respectively, and its biodegradability was reduced by 13% and 18%, respectively. When microalgae B was subjected to B1 and B2, its CH_4 productivity was reduced by 3% and 13%, respectively, while its biodegradability increased by 4% with B1 and decreased by 8% with B2. On the other hand, B1 increased both CH_4 productivity and biodegradability of microalgae C in 4% and 18%, while B2 increased both

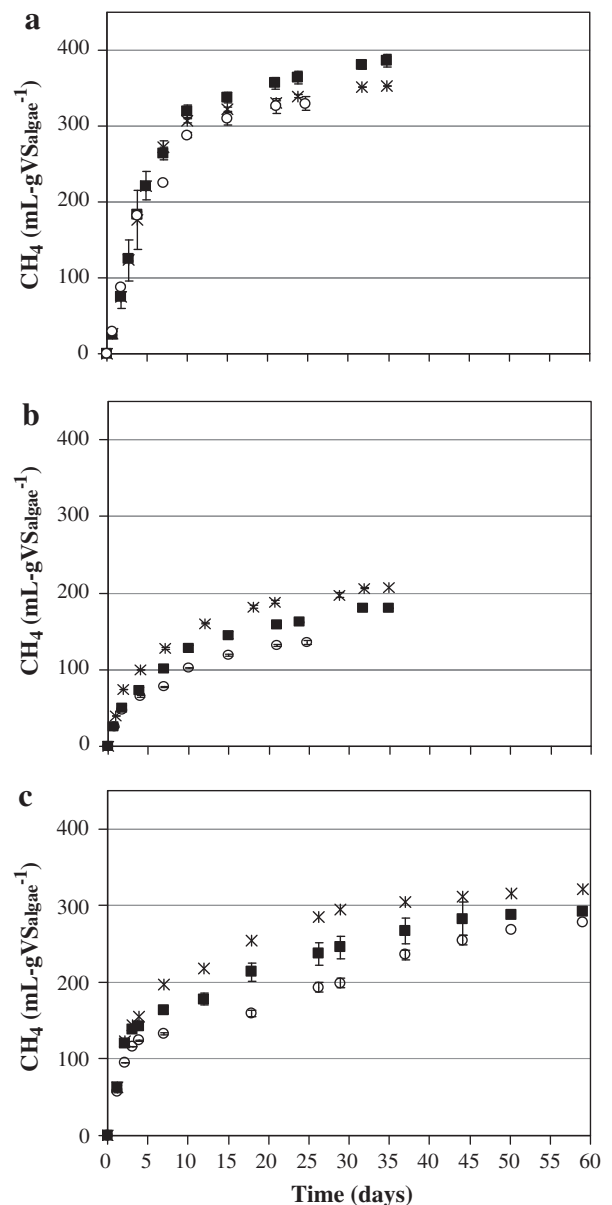


Fig. 2. Time course of methane productivity for microalgae A (a), B (b) and C (c) at S/l ratio = 1 and 3 gTS/kg; (o), 10 gTS/kg (■) and 20 gTS/kg (*). Vertical bars represent the standard deviation.

parameters in 5% and 17%, respectively. The microaerobic biological pretreatment applied in this study requires the presence of microorganisms capable of secreting the hydrolytic enzymes needed for the degradation of complex organic matter (Burgess and Pletschke, 2008). However, despite there being no studies reporting the release of extracellular enzymes by microalgae, the aerobic decomposition of microalgae is well documented. Hence, during the aerobic decomposition of microalgae the biodegradable fraction may be consumed by either endogenous respiration or by heterotrophic microorganisms (decomposers), which grow as a function of the quantity of this decomposed algal biomass (Jewell and McCarty, 1971). A study by Jewell and McCarty (1971) showed that seeded algal cultures were more easily degradable than axenic cultures of microalgae. In our particular study, the microalgae cultivated on synthetic medium and free of contamination by bacteria (microalgae A) might have undergone an oxidation by endogenous respiration, so the organic biodegradable fraction was reduced,

Table 2

Influence of microalgae pretreatment on the S_D , final aqueous $N-NH_4^+$ and TN concentrations after pretreatment and subsequent anaerobic digestion of microalgae A at S/I = 1 and 10 gTS/kg.

Pretreatment	Pretreated microalgae A			Digested microalgae A			
	S_D (%)	$N-NH_4^+$ (mg/L)	TN (mg/L)	CH_4 productivity mL/gVS _{algae}	Productivity increase (%)	Biodeg. (%)	Biodeg increase (%)
Control	0	7	112	272 ± 1		49 ± 0	
U1	14	5	239	310 ± 6	14	52 ± 1	7
U2	28	30	537	309 ± 1	14	53 ± 0	9
U3	30	32	361	309 ± 6	13	52 ± 1	5
U4	32	30	378	305 ± 6	12	54 ± 1	10
B1	11	57	233	262 ± 10	-4	42 ± 2	-13
B2	9	34	239	252 ± 5	-8	40 ± 1	-18
T1	9	6	191	323 ± 3	19	54 ± 0	10
T2	16	22	258	362 ± 0	33	59 ± 0	21
T3	32	25	433	398 ± 8	46	62 ± 1	27

Biodeg: Biodegradability.

Table 3

Influence of microalgae pretreatment on the S_D , final aqueous $N-NH_4^+$ and TN concentrations after pretreatment and subsequent anaerobic digestion of microalgae B at S/I = 1 and 10 gTS/kg.

Pretreatment	Pretreated microalgae B			Digested microalgae B			
	S_D (%)	$N-NH_4^+$ (mg/L)	TN (mg/L)	CH_4 productivity mL/gVS _{algae}	Productivity increase (%)	Biodeg. (%)	Biodeg increase (%)
Control	0	28	47	198 ± 9		34 ± 2	
U1	24	134		209 ± 7	6	37 ± 1	9
U2	48	54	333	214 ± 6	8	40 ± 1	17
U3	53	66	602	223 ± 5	13	40 ± 1	18
U4	60	55	634	223 ± 0	13	43 ± 0	25
B1	21	80	320	193 ± 5	-3	36 ± 1	4
B2	19	89	355	171 ± 8	-13	32 ± 1	-8
T1	22	28	254	219 ± 1	11	45 ± 0	30
T2	37	70	436	260 ± 1	31	46 ± 0	35
T3	63	42	594	307 ± 4	55	57 ± 1	66

Biodeg: Biodegradability.

Table 4

Influence of microalgae pretreatment on the S_D , final aqueous $N-NH_4^+$ and TN concentrations after pretreatment and subsequent anaerobic digestion of microalgae C at S/I = 1 and 10 gTS/kg.

Pretreatment	Pretreated microalgae C			Digested microalgae C			
	S_D (%)	$N-NH_4^+$ (mg/L)	TN (mg/L)	CH_4 productivity mL/gVS _{algae}	Productivity increase (%)	Biodeg. (%)	Biodeg increase (%)
Control	0	23	23	255 ± 2		47 ± 0	
U1	30	40	199	314 ± 8	24	70 ± 2	49
U2	56	26	420	301 ± 2	18	64 ± 0	38
U3	57	79	450	301 ± 9	18	64 ± 2	37
U4	62	100	514	310 ± 1	22	72 ± 0	55
B1	29	22	244	266 ± 4	4	55 ± 1	18
B2	29	59	257	266 ± 2	5	54 ± 0	17
T1	38	34	190	413 ± 5	62	76 ± 1	62
T2	39	40	263	382 ± 4	50	71 ± 1	52
T3	40	43	399	359 ± 3	41	68 ± 1	46

Biodeg: Biodegradability.

affecting the methane productivity. The fact that CH_4 productivity for B2 was lower than that recorded for B1 in microalgae A, suggest that aerobic decomposition was greater the longer the duration of the pretreatment. Unlike microalgae A, microalgae B and C were microalgal-bacterial consortia. The slight increase in CH_4 productivity recorded for microalgae C compared to the reduction observed for microalgae B suggest the presence of a larger population of bacteria capable of excreting hydrolytic enzymes in microalgae C.

At the end of the anaerobic digestion assays the total NH_4^+ concentrations corresponded to the soluble TN regardless of the microalgae pretreated (data not shown), which suggests that all the soluble organic nitrogen was ammonified. Moreover, similarly to the ratio and concentration tests, the ammonium released per

gram of VS added or per gram of VS eliminated was not correlated neither with the pretreatment applied nor with the enhancements in CH_4 productivity or biodegradability.

4. Conclusions

The anaerobic digestion of microalgae should be conducted at a S/I ratio of 0.5 to avoid process imbalance due to VFA accumulation and at concentrations of 10 gTS/kg to obtain the highest biodegradabilities and methane productivities. The results obtained confirm that the BMP depended on the microalgae species. Likewise, the NH_4^+ released was independent of the biomass concentration and the S/I ratio. Thermal hydrolysis was the most effective

pretreatment, supporting productivity and biodegradability increases over 60%. The optimum temperature of this pretreatment depended on the microalgae species as recalcitrant compounds could be generated at such a high temperatures.

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