Enhancement of microalgae anaerobic digestion by thermo-alkaline pretreatment with lime (CaO)

Maria Solé-Bundó[1,2], Hélène Carrère[2], Marianna Garfí[1], Ivet Ferrer[1,*]

[1] GEMMA - Group of Environmental Engineering and Microbiology, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya·BarcelonaTech, c/ Jordi Girona 1-3, Building D1, E-08034, Barcelona, Spain

[2] INRA, UR0050 Laboratoire de Biotechnologie de l'Environnement, Avenue des Etangs, F-11100 Narbonne, France

[*] Corresponding author

Tel.: +34 93 401 64 63

E-mail address: ivet.ferrer@upc.edu

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Highlights

- The effect of thermo-alkaline pretreatment on microalgae anaerobic digestion was evaluated.
- Different lime doses and temperatures were tested to determine the best pretreatment condition.
- All pretreatment conditions improved process kinetics as compared to untreated microalgae.
- The highest methane yield increase was achieved by adding 10% CaO at 72°C.

Abstract

The aim of this study was to evaluate for the first time the effect of a thermo-alkaline pretreatment with lime (CaO) on microalgae anaerobic digestion. The pretreatment was carried out by adding different CaO doses (4 and 10%) at different temperatures (room temperature (25ºC), 55 and 72ºC). The exposure time was 4 days for pretreatments at 25ºC, and 24h for pretreatments at 55 and 72ºC. Following, a biochemical methane potential test was conducted with pretreated and untreated microalgae. According to the results, the pretreatment enhanced proteins solubilisation by 32.4% and carbohydrates solubilisation by 31.4% with the highest lime dose and temperature (10% CaO and 72°C). Furthermore, anaerobic digestion kinetics were improved in all cases (from 0.08 to 0.14 day^{-1} for untreated and pretreated microalgae, respectively). The maximum biochemical methane potential increase (25%) was achieved with 10% CaO at 72°C, in accordance with the highest biomass solubilisation. Thus, lime pretreatment appears as a potential strategy to improve microalgae anaerobic digestion.

Keywords

Algae; Anaerobic digestion; Biogas; Biomass solubilisation; Chemical Pretreatment

1. Introduction

Microalgae-based wastewater treatment systems are promising solutions to shift the paradigm from wastewater treatment to energy and resources recovery. In these systems, microalgae assimilate nutrients and produce oxygen which is used by bacteria to biodegrade organic matter improving water quality. Moreover, microalgae biomass can be harvested and reused to produce biofuels or other non-food bioproducts [1,2]. In this context, anaerobic digestion is one of the most consolidated and well-known technologies to convert organic waste generated in a wastewater treatment plant into bioenergy [3].

Over the last decades, the feasibility to obtain biogas from microalgae has been proved. However, some microalgae species can present a low biodegradability due to the complex structure of their cell walls. This fact may hamper the hydrolysis step [4]. For that reason, some pretreatment techniques have been evaluated to improve both the microalgae anaerobic biodegradability and the kinetics of the process [4,5]. The most studied methods have been mechanical and thermal pretreatments, which may increase the biomass solubilisation, methane yield and methane production rate. Nevertheless, energy balances are not always positive, since some of these pretreatments have a high energy demand [5]. Thus, pretreatments which require minimal energy input, such as low-temperature, biological and chemical methods, have recently been gaining interest [6,7].

Chemical pretreatments consist of adding acids (acid pretreatment) or bases (alkaline pretreatment) under different conditions (e.g. different temperatures and exposure times). First applications of alkaline pretreatments were found to improve the biodegradability of lignocellulosic biomass due to their effectiveness at breaking ester bonds between lignin and polysaccharides [8] and partially solubilising hemicelluloses and celluloses to a lower extent [9]. Although microalgae do not contain lignin, some benefits have also been reported in the application of an alkaline pretreatment to microalgae. Indeed, Mahdy et *al*. [10] reported that both organic matter solubilisation and methane yield increased by applying an alkaline pretreatment. In addition, while an acid pretreatment of microlagae only increased carbohydrate solubilisation, an alkaline pretreatment enhanced the solubilisation of both proteins and carbohydrates [11]. Moreover, the combination of thermal and alkaline pretreatments applied to different microalgae species was more effective than alkaline or thermal pretreatments applied separately [12]. The combination of temperature and alkali pretreatments has been tested at low (<100 °C) and high (>100 °C) temperatures. However, it has been demonstrated that high temperatures may lead to the production of refractory organic compounds or inhibitory intermediates generated through intramolecular reactions (i.e. Maillard reactions) [13]. Therefore, the use of lower temperatures might be more appropriate.

To date, the most used alkali for microalgae pretreatment is NaOH, although a recent study also analysed the effect of KOH, Na_2CO_3 and NH_4OH [14]. However, some environmental and economic drawbacks should be considered when applying these chemicals. In particular, NaOH increases the concentration of Na^+ in digestates, which is known to be inhibitory to methanogens [15] and could be harmful for soil upon digestate agriculture reuse [16]. On the other hand, NH_4OH may not be recommended for microalgae, as their high nitrogen content combined with the addition of NH_4OH could inhibit anaerobic digestion [17]. Concerning KOH, it is more expensive than other alkalis. Conversely, lime ($Ca(OH)_2$ or CaO) is more environmentally friendly and cheaper [18]. In particular, lime is around 1.5 and 4-fold less expensive than NaOH and KOH, respectively. Lime pretreatment has already been tested on lignocellulosic biomass (i.e. wheat straw or sunflower stalks), showing a significant increase in biomass solubilisation and methane yield [8,9]. To the best of our knowledge, no studies have assessed the effect of lime pretreatment on microalgae anaerobic digestion.

The aim of this study is to evaluate and determine the best pretreatment conditions (alkali dose and temperature) for a thermo-alkaline pretreatment of microalgae with lime (CaO) by means of biomass solubilisation and methane production analysis.

2. Material and Methods

2.1 Microalgal biomass

Microalgae used in this study were harvested from a pilot raceway pond (17 m^3) located at the INRA-LBE facilities (Narbonne, France), which treated synthetic wastewater based on the composition tested by Bracklow et al. (2007) [19]. A detailed description of the system can be found in Hreiz et al. (2014) [20]. Microalgal biomass, which consisted of a mixed culture of microalgae and bacteria, was harvested by membrane concentration followed by gravity settling (24h at 4 ºC). Microalgae species were identified by optical microscopy (Olympus BX53).

2.2 Microalgae pretreatment

Thermal and thermo-alkaline pretreatments of microalgal biomass were carried out in glass bottles of 160 mL containing 27.62 g of microalgal biomass with a concentration of 14.5 g VS L^{-1}. In order to assess the best pretreatment condition, two lime (Akdolit® Q90; purity ≥ 92%) doses were tested: 4 and 10% CaO on a TS basis, based on the common doses used when applying this pretreatment [21]. According to the literature, lime pretreatment requires long exposure times, ranging from several days to weeks, which can be reduced by increasing temperature [18]. For this reason, the following combinations of temperature and exposure time were tested: 4 days at room temperature (25°C) and 24 h at 55 and 72°C. After adding lime, bottles were closed and incubated with constant agitation. All conditions were compared with control trials (without lime): microalgae stored for 4 days at 4°C, and microalgae exposed to 25°C for 4 days and 55 and 72°C for 24h.

Each pretreatment condition was performed in five different bottles. Later, three of them were used in the biochemical methane potential (BMP) test (triplicates) (Section 2.3) and the rest were devoted to all analysis (Section 2.4). As far as the pretreatment at room temperature is concerned, 4 extra bottles were used in order to monitor the pH (duplicates), and the gas pressure and composition inside the bottles (duplicates).

2.3 Biochemical methane potential tests

Methane potentials of untreated and pretreated microalgae were tested by means of BMP tests. Each condition was performed in triplicate. The inoculum was granular sludge from a mesophilic digester which treated the effluent of a sugar factory. The sludge was diluted with distilled water to reach a concentration of 60 g TS L^{-1} and 47.6 g VS L^{-1}. Then, it was kept under anaerobic conditions at 35°C with continuous stirring until use.

In order to avoid biomass loss during the experimental process, the test was carried out using the same glass bottles as the pretreatment. As already mentioned, each bottle contained 4 g VS L^{-1} of microalgae. The substrate to inoculum ratio (S/I) was 1 g VS substrate / g VS inoculum. Macronutrients, oligoelements and buffer solutions were added providing 360 mg $N\text{-}NH_4 \cdot L^{-1}$, 118 mg $P\text{-}PO_4 \cdot L^{-1}$, 37.1 mg Mg $\cdot L^{-1}$, 42.3 mg Ca $\cdot L^{-1}$, 5.6 mg Fe $\cdot L^{-1}$, 1.24 mg Co $\cdot L^{-1}$, 0.28 mg Mn $\cdot L^{-1}$, 0.25 mg Ni $\cdot L^{-1}$, 0.24 mg Zn $\cdot L^{-1}$, 0.09 mg B $\cdot L^{-1}$, 0.23 mg Se $\cdot L^{-1}$, 0.15 mg Cu $\cdot L^{-1}$, 0.04 mg Mo$\cdot L^{-1}$and 2.6 g $NaHCO_3 \cdot L^{-1}$. Bottles were filled with distilled water up to 100 mL, flushed with nitrogen gas, sealed with butyl rubber stoppers and incubated at 35 ºC until biogas production ceased.

Accumulated biogas production was measured with a manometer (LEO 2, Keller) while biogas composition (CH_4, CO_2, N_2, O_2, H_2) was analysed by means of a gas chromatograph (Clarus 580, PerkinElmer) equipped with RtQBond and RtMolsieve columns coupled to a thermal conductivity detector (TCD). The carrier gas was argon, and the temperatures of the injector, detector and oven were 250, 150 and 60°C, respectively.

A blank treatment was used to quantify the amount of methane produced by the inoculum. The net biogas production was calculated by subtracting the blank results to each trial.

2.4 Analytical methods

Microalgal biomass was characterised by the concentration of TS, VS and total chemical oxygen demand (COD), following APHA Standard Methods [22]. Biomass macromolecular composition was expressed in terms of percentage of proteins, carbohydrates and lipids over the VS content.

Proteins were calculated by multiplying the total Kjeldahl nitrogen (TKN) by 5.95 [23], and TKN was titrated using a Buchi 370-K after mineralisation of samples. The total carbohydrate content (CH) was analysed by the phenol-sulphuric method [24] after acid hydrolysis. The lipid content was determined after heptane extraction (ASE®200, DIONEX).

The liquid fraction from each pretreatment was analysed for soluble COD (CODs), TKN (TKNs) and CH (CHs) as described before. Soluble sugars were also quantified by High Performance Liquid Chromatography (HPLC) coupled to refractometric detection (Waters R410) after mild acid hydrolysis [25]. Chemicals were separated by an Aminex HPX-87H column (300 x 7.8mm, Biorad) equipped with a protective precolumn (Microguard cation H refill catbridges, Biorad). The eluting solution was 2 mM H_2SO_4, the flow rate was 0.3 $ml \cdot min^{-1}$, the column temperature was 45°C and the refractive index detector (Waters 2414) worked at 45°C to quantify sugars. All physico–chemical analyses were performed in triplicate.

2.5 Solubilisation rates and biomass loss calculation

Biomass solubilisation was evaluated by the soluble to total COD, CH and TKN ratios using the following equations (Eq. 1-3):

$$COD\ solubilised\ (\%) = \frac{(COD_s)_p}{(COD)_0} \cdot 100 \qquad \text{[Eq. 1]}$$

$$CH\ solubilised\ (\%) = \frac{(CH_s)_p}{(CH)_0} \cdot 100 \qquad \text{[Eq. 2]}$$

$$TNK\ solubilised\ (\%) = \frac{(TNK_s)_p}{(TNK)_0} \cdot 100 \qquad \text{[Eq. 3]}$$

where sub-indexes refer to pretreated (p) and untreated (0) biomass.

The biomass loss after pretreatment was calculated in terms of COD loss according to Eq. 4, where $(COD)_p$ is the total COD concentration of pretreated samples and $(COD)_0$ is the total COD concentration of untreated microalgae (control).

$$COD\ losses\ (\%) = \frac{(COD)_p - (COD)_0}{(COD)_0} \cdot 100 \qquad \text{[Eq. 4]}$$

2.6 Kinetic data analysis

In order to evaluate the kinetics of the process, experimental data from BMP tests was adjusted to a first-order kinetic model [Eq.5] by the least square method.

$$B = B_0 \cdot \{1 - exp[-k \cdot (t - \lambda)]\} \qquad \text{[Eq.5]}$$

where, B_0 stands for the methane production potential (ml CH_4·gVS^{-1}), k is the first order kinetic rate constant (day^{-1}), B is the accumulated methane production at time t (ml CH_4·gVS^{-1}), t is time (day) and λ represents the lag phase (day).

The error variance (s^2) was estimated by the following equation:

$$s^2 = \frac{\sum_1^i (y_i - \hat{y}_i)^2}{N-K} \qquad \text{[Eq.6]}$$

where y_i is the experimental value, $\hat{y}_i$ is the value estimated by the model, N is the number of samples and K is the number of model parameters.

2.7 Statistical analyses

Linear regressions were fit to find the relationship between solubilisation and explanatory variables (i.e lime dose, temperature). Differences among experimental conditions for the methane yield were determined by the ANOVA and Tukey tests. Differences were considered significant at p values below 0.05. All statistical analyses were performed using R 3.0.2 software.

3. Results and discussion

3.1 Microalgae biomass characteristics

Microscope examination showed that the predominant microalgae were *Chlorella* sp. and *Scenedesmus* sp. (Fig. 1). Both genus are characterised by a resistant cell wall which hampers their biodegradability, especially in the case *Scenedesmus* which has a complex multilayer cell wall [26].

Figure 1. Microscopic image of microalgal biomass mainly composed of *Chlorella* sp. and *Scenedesmus* sp.

Biochemical analysis indicated that microalgae biomass was mainly composed of proteins (52%), followed by carbohydrates (16%) and lipids (9%) (Table 1). These results are in accordance with the literature [27]. Carbohydrates were mainly constituted by glucose and xylose (48 and 39% of the total carbohydrates, respectively). This is in agreement with previous studies which found a similar carbohydrate composition in *Chlorella sorokiniana* and *Scenedesmus almeriensis* [28].

Table 1. Biochemical composition of microalgal biomass (mean ± standard deviation).

Parameter	**Value**
TS ($g \cdot L^{-1}$)	17.8 ± 0.1
VS ($g \cdot L^{-1}$)	14.5 ± 0.1
COD ($g\ O_2 \cdot L^{-1}$)	23.5 ± 0.2
Carbohydrates (% VS)	16.3 ± 0.5
Proteins (% VS)	52.0 ± 0.5
Lipids (% VS)	8.8 ± 0.0
Ash (%)	18.4 ± 0.9

3.2 pH monitoring over lime pretreatment

pH is an important parameter in alkaline pretreatments, as alkaline conditions must be ensured during the whole pretreatment process. For that reason, pH was measured before and after applying the pretreatment with lime. While untreated microalgae showed a pH of 8.1, this value increased to 11.9 and 12.4 when 4 and 10% CaO was added, respectively. However, the final pH decreased after 4 days of alkaline pretreatment at room temperature and after 24h of thermal and thermo-alkaline pretreatment (Table 2).

Concerning the alkaline pretreatment, pH values achieved at the end of the pretreatment were very low (7.6 and 8.1 with 4 and 10% CaO, respectively). These results were unexpected, since lime was applied to induce alkaline conditions during the whole pretreatment. To further investigate the pH drop, the lime pretreatment at room temperature was repeated mesuring the pH and gas content in the bottles over time (Fig. 2). As can be observed in Fig. 2, after the first 20-30 hours the pH decreased and then it stabilised at similar values as those obtained during the thermal pretreatment without lime (pH = 7.3 ± 0.3). The same graph also shows that the CO_2 content increased over time. This can be explained by the presence of heterotrofic bacteria in the microalgal biomass, which release CO_2 as a result of organic matter biodegradation. The higher the dose of lime, the lower the CO_2 concentration in the gas phase, especially at the beginning of the pretreatment when CO_2 increase was moderate (even null for 10% CaO). This fact suggests that CO_2 was dissolved, decreasing the pH. Hence, the alkaline pretreatment of this type of biomass at room temperature only makes sense with contact times below 24 h.

Regarding the thermo-alkaline pretreatment at 55 and 72ºC, higher final pH values were achieved as compared to the alkaline one (8.8 for 4% CaO and 11.9 for 10% CaO) (Table 2), even though they showed a pH decrease at the end of the pretreatment. On the other hand, thermally pretreated samples presented a slight pH decrease with respect to untreated microalgae (7.71 and 7.78 at 55 and 72ºC, respectively). In this case, the decrease could be attributed to a certain acidification caused by organic matter biodegradation. The same evidence was detected after pretreating the macroalga *Palmaria palmata* with 4% NaOH, when the pH decreased from 11.3 to 9.3 and 9.9 after 24 h at 70 and 85°C, respectively [29]. Nonetheless, in comparison with the alkaline pretreatment at room temperature, mild temperatures enhanced alkaline conditions during the pretreatment.

Table 2. Pretreatment conditions and final pH achieved after the pretreatment.

Trial	Pretreatment conditions			Final pH
	Temperature (°C)	Contact time (h)	CaO dose (% TS)	
Untreated microalgae	-	-	-	8.06
Room temperature	25	96	0	8.12
Room temperature + 4% CaO	25	96	4	7.55
Room temperature + 10% CaO	25	96	10	8.09
55 ºC	55	24	0	7.71
55 ºC + 4% CaO	55	24	4	8.85
55 ºC + 10% CaO	55	24	10	11.92
72 ºC	72	24	0	7.78
72 ºC + 4% CaO	72	24	4	8.82
72 ºC + 10% CaO	72	24	10	11.91

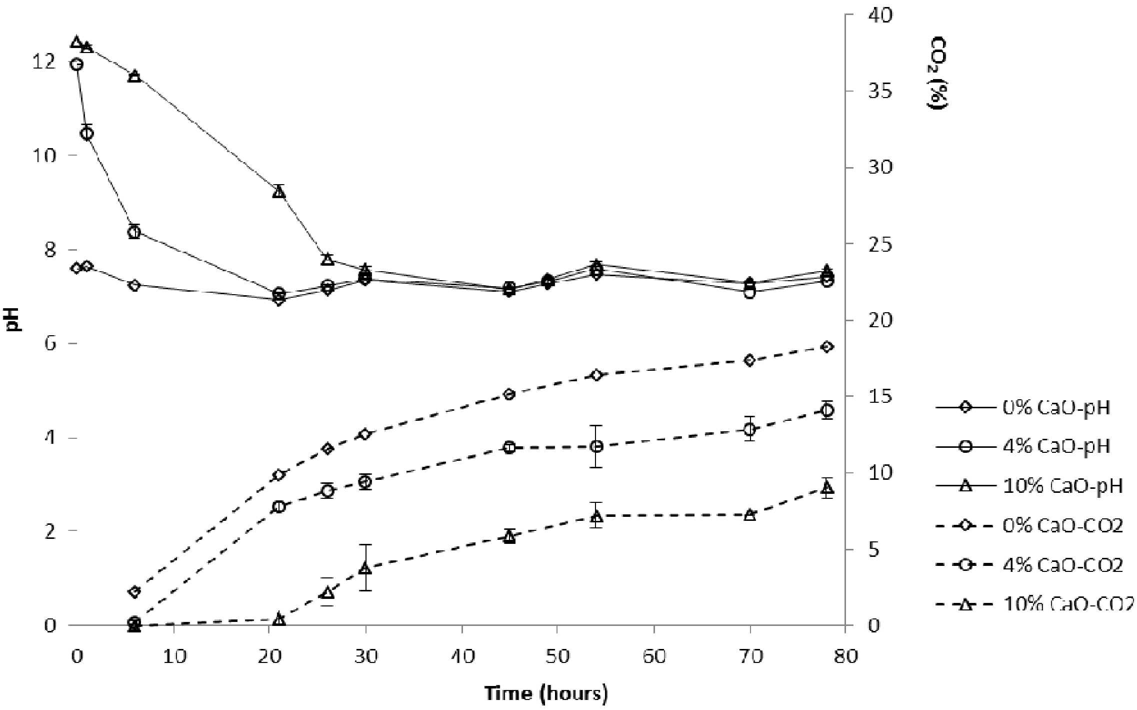


Figure 2. pH and CO_2 measured in the bottles after addition of 0, 4 and 10% CaO at room temperature.

3.3 Effect of the pretreatment on microalgal biomass solubilisation and biomass loss

3.3.1. Organic matter solubilisation

Thermal and thermo-alkaline pretreatments enhanced organic matter solubilisation under all pretreatment conditions (Fig. 3). Indeed, the soluble to total COD ratio increased by 10-25%, depending on the pretreatment condition. Moreover, the addition of lime enhanced biomass solubilisation under all temperatures assayed. The highest soluble COD values were observed for the thermo-alkaline pretreatment with 10% CaO at 55 and 72°C (20 and 25% CODs, respectively).

Similar results were observed in a previous study that analysed COD solubilisation after applying NaOH at mild temperature (50°C) to different microalgae species [10]. They obtained values of 16-20% of COD solubilised when pretreating *Chlorella* sp. and 4-18% for *Scenedesmus* sp. The authors attributed such a low COD solubilisation to the fact that the tested pretreatments were unable to break down microalgae cell walls. Hence, soluble COD increase seemed to be caused by exopolymers release rather than intracellular material. Higher COD solubilisation was observed by applying NaOH to *Chlorella* sp. and autoclaving at 120°C, achieving up to 81% CODs [12]. This shows how higher solubilisation can be achieved by combining alkaline pretreatment with high temperatures as compared to mild temperatures.

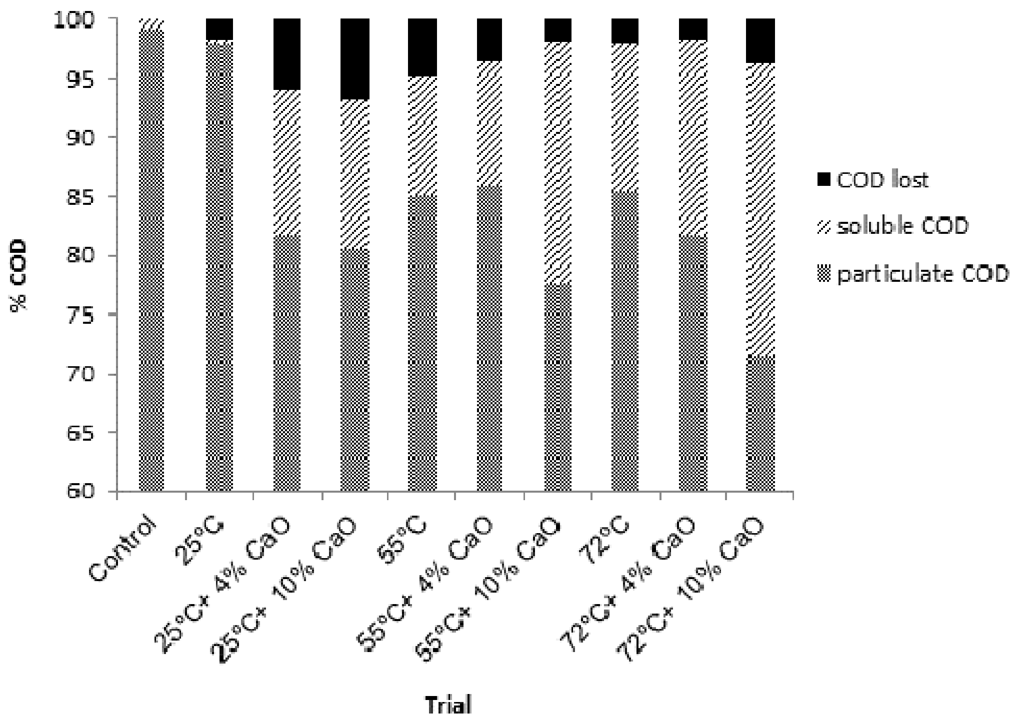


Figure 3. COD fractions after thermo-alkaline pretreatment, expressed as % of the total initial COD of untreated microalgae. Soluble fractions were calculated according to Eq. 1; particulate fractions were calculated as the difference between total COD and soluble COD; and removed COD fractions were calculated according to Eq. 4. Mean values (relative error < 2%).

3.3.2. Biomass loss during the pretreatment

During the pretreatment step biomass loss should be minimised not to reduce the methane potential. In this study, biomass loss was expressed as the total COD removed during the pretreatment (Eq. 4) and the values were low (< 7%). As can be observed in Fig. 3, organic matter loss was the highest (between 6-7%) after alkaline pretreatment at room temperature. This was due to the fact that alkaline conditions were not preserved during the whole pretreatment (Table 2). Thus, biomass solubilisation by the pretreatment enhanced the consumption of readily biodegradable organic matter by heterotrophic bacteria. On the contrary, in the pretreatments at mild temperatures (55, 72 ºC), lime addition contributed to avoid organic matter biodegradation (except for the sample pretreated at 72°C with 10% CaO). In that case, thermal effects prevailed over biological ones.

3.3.3. Carbohydrate and protein solubilisation

CH and proteins are the main macromolecules of microalgae biomass (Table 1). In addition, CH are the main constituents of microalgae cell wall, which hampers microalgae hydrolysis. In order to evaluate the effect of the pretreatment on both macromolecules, CH and TKN (which is directly related to proteins) contents in the liquid phase were analysed after each pretreatment (Fig. 4 and 5).

According to the results, CH solubilisation increased with temperature and lime dose (from 5% of solubilised CH for samples pretreated at room temperature with 4% CaO to 31% for samples pretreated at 72°C with 10% CaO). In fact, the combination of alkali and temperature could induce cellulose swelling, increasing the internal surface area and reducing the degree of crystallinity and polymerization [30]. Moreover, the hydrolysis of CH may occur through a variety of reactions induced by lime, including the disruption of H-bonds and saponification of intermolecular ester bonds in cellulose and hemicelluloses and crosslinking hemicellulose with other polymeric components [18]. Indeed, carbohydrate release after thermo-chemical pretreatment of microalgae has already been reported [10,28]. However, the comparison of alkali and acid pretreatments showed how alkaline hydrolysis cleaved intermolecular linkages between complex polysaccharides and fibbers and other polymeric compounds, but only acid hydrolysis was able to break down complex carbohydrates into simple sugars [28].

a)

b)

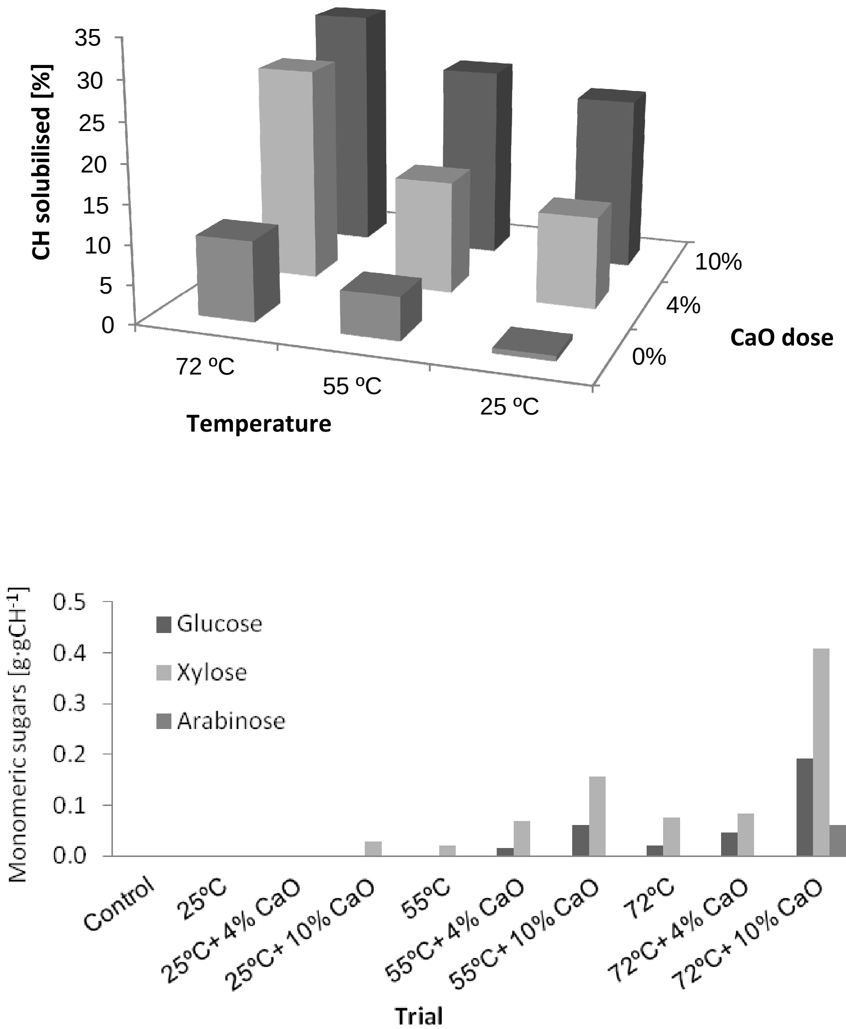


Figure 4. Carbohydrates solubilised (CHs) expressed as percentage over the total carbohydrates (CH) (Eq. 2) (a) and main sugar monomers solubilised (b) after each pretreatment. Mean values (relative error < 2%).

Opposite to [10], who observed low COD solubilisation (4-20%) attributed to exopolymers release, in the current study, the high COD and CH solubilisation (> 30%) observed with the highest lime dose and temperature (10% CaO and 72ºC) could not only be attributed to exopolymers release but also other structural macromolecules. Indeed, the soluble fraction of different structural sugar monomers (i.e. glucose, xylose and arabinose) was also analysed (Fig. 4b). The goal was to verify if carbohydrates released during the pretreatment came not only from intracellular material but also from structural carbohydrates from the cell wall. The results showed a substantial increase in glucose and xylose after the pretreatment at the highest temperature and lime dose (72ºC and 10% CaO). Moreover, arabinose release was only detected in that case. Such a significant sugar release could be attributed to the cell wall damage, since the cell wall of the studied microalgae species is constituted by these monomeric sugars [31,32].

Regarding proteins, there was no direct correlation between their solubilisation and the lime dose (Fig. 5). For the pretreatment at room temperature, the percentage of solubilised TKN was the highest with the lowest lime dose (17.2 and 12.9% with 4 and 10% CaO, respectively). Taking into account that the pH decreased after lime addition at room temperature (Table 2), it seems that the biological degradation of proteins prevailed over the chemical one. Thus, at room temperature the lowest lime dose favoured the biological degradation of organic matter and consequently its solubilisation. A different behaviour was observed at 55 and 72ºC (Fig. 5), at which thermo-chemical effects prevailed over biological ones. Nevertheless, the highest soluble TKN fraction (32%) was reached with the most severe pretreatment condition (10% CaO and 72ºC).

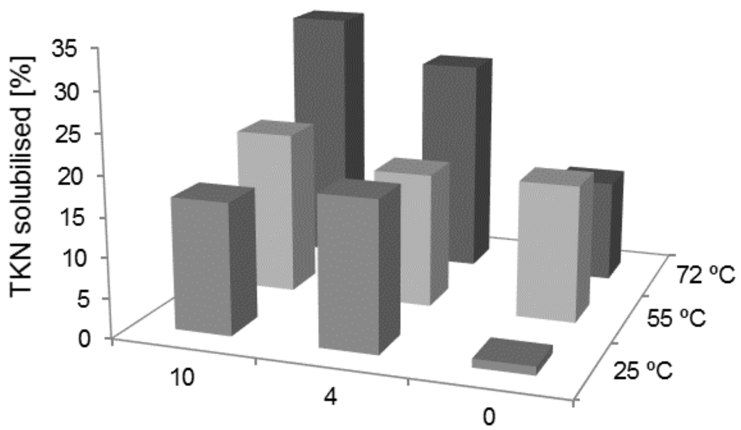


Figure 5. Soluble TKN (TKNs) after each pretreatment expressed as percentage over the TKN (Eq. 3). Mean values (relative error < 2%).

In conclusion, the use of alkali mainly enhanced protein solubilisation, while the combination of alkali and temperature was required to solubilise carbohydrates. This is in accordance with the literature. For instance, Mendez et *al*. (2013) found that proteins prevailed over carbohydrates solubilisation when *Chlorella* was subjected to alkaline conditions [11]. Similarly, Yang et *al*. (2011) concluded that protein solubilisation of lipid-extracted microalgal biomass was influenced by NaOH addition while carbohydrate solubilisation was not [33].

3.4 Effect of the pretreatment on the methane production

To evaluate the effect of pretreatments on the methane production, both methane production rate and extent were evaluated in BMP tests.

3.4.1. Biochemical methane potential increase with the pretreatment

Fig. 6 shows the cumulative methane yield obtained after 105 days of assay, while Table 3 reports the final methane potential achieved for each pretreatment condition. It should be notice that the methane yield is referred to the initial VS of untreated microalgae. In Table 3, the methane yield increase is compared to the methane yield increase considering methane potential losses resulting from organic matter losses during the pretreatment step. To do so, COD losses (Eq. 4) were converted into methane losses.

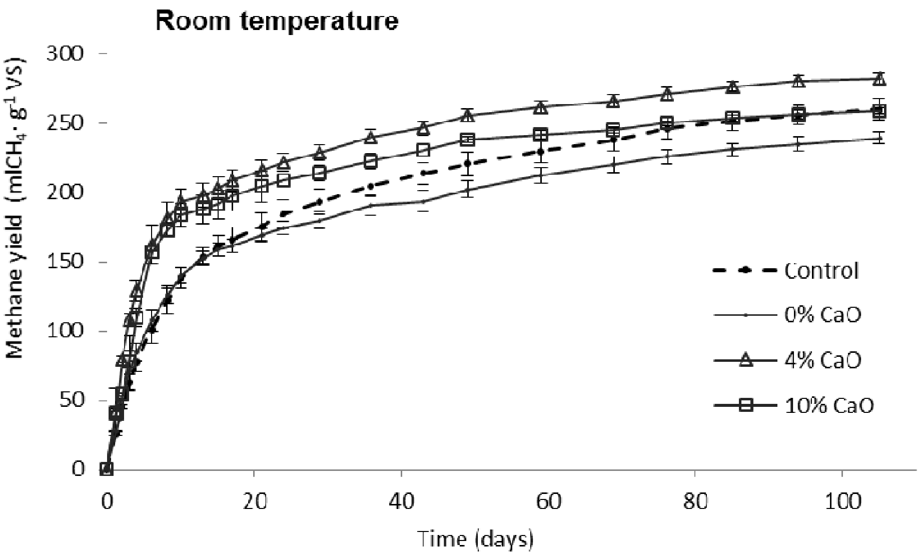
The results show how untreated microalgae produced 260 mL $CH_4 \cdot gVS^{-1}$, which is in accordance with reported methane yields for *Chlorella* sp. (189-403 mL $CH_4 \cdot gVS^{-1}$) and *Scenedesmus* sp. (240-287 mL $CH_4 \cdot gVS^{-1}$) [3]. Some samples presented a similar methane yield after the pretreatment (i.e. 10% CaO at 25ºC; 0% and 4% CaO at 55ºC), while in others the methane yield increased by 10% (i.e. 4% CaO at 25 and 72ºC; 10% CaO at 55ºC). The most significant methane yield increase (25%) was achieved by the pretreatment with 10% CaO at 72ºC (325 mL $CH_4 \cdot gVS^{-1}$). This methane yield increase is even higher (> 33% increase) if the biomass loss during the pretreatment step is taken into account. The highest methane production can be attributed to the highest solubilisation of both carbohydrates and proteins after the thermo-chemical pretreatment

(Fig. 4 and 5), and to the release of sugar from the cell wall, namely glucose, xylose and arabinose (Fig. 4b). Accordingly, the methane production increase may have resulted from the cell wall damage after the pretreatment with 10% CaO at 72ºC. Similar results were obtained by pretreating *Chlorella* sp. and *Scenedesmus* sp. with 5% NaOH at 50°C increasing the methane yield by 17 and 20%, respectively [10]. Comparing the lime pretreatment with others, similar methane yield increase (29%) was achieved by applying a thermal pretreatment at 120 °C on Chlorella sp. and Scenedesmus sp. culture [34] and a low-temperature pretreatment at 80°C on *Chlorella vulgaris* (11–24%) [35]. Regarding mechanical pretreatments, lower values were obtained by applying ultrasounds (6-15%) [34] but higher improvements were found with other mechanical pretreatments (i.e. milling) on *Acutodesmus obliquus* (51%) [36].

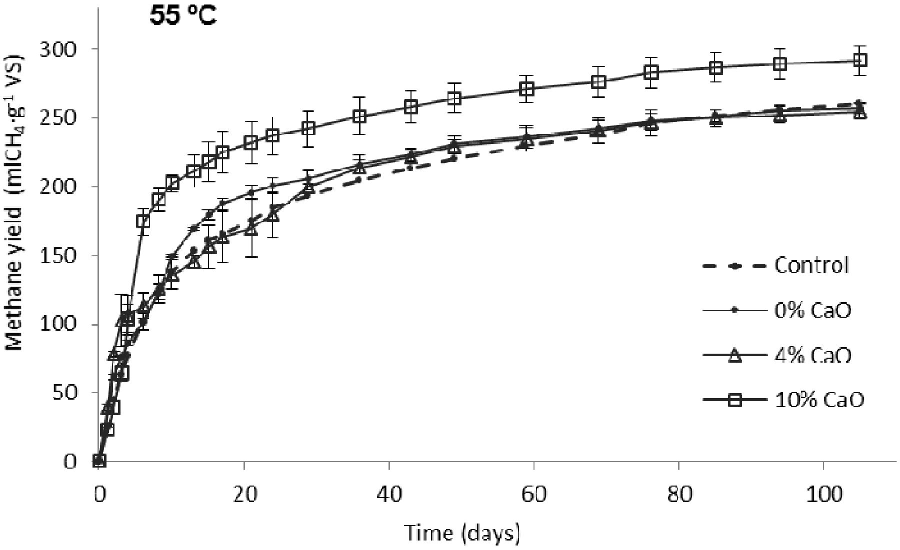
Table 3. Final methane yield and methane content obtained in BMP tests for each pretreatment condition (mean ± standard deviation).

Trial	Methane yield (mL CH_4·g VS^{-1} untreated microalgae)	Methane content (%)	Methane yield increase (%)	Methane loss (mL CH_4·gVS^{-1})	Methane yield increase considering methane loss (%)
Untreated microalgae	260 ± 8	67.2 ± 0.6	-	-	-
Room temperature	239 ± 5	67.5 ± 0.5	-8.0	10.3	-4.0
Room temperature + 4% CaO	282 ± 4	70.0 ± 1.0	8.4	29.7	19.8
Room temperature + 10% CaO	259 ± 2	75.5 ± 2.8	-0.5	39.9	14.9
55 ºC	257 ± 4	69.8 ± 0.7	-1.0	28.1	9.8
55 ºC + 4% CaO	255 ± 6	69.7 ± 0.3	-2.1	21.5	6.2
55 ºC + 10% CaO	292 ± 11	77.3 ± 1.8	12.2	11.2	16.5
72 ºC	230 ± 7	71.4 ± 0.5	-11.6	12.3	-6.8
72 ºC + 4% CaO	287 ± 4	74.3 ± 0.5	10.3	10.6	14.3
72 ºC + 10% CaO	325 ± 12	77.9 ± 0.6	25.0	22.1	33.5

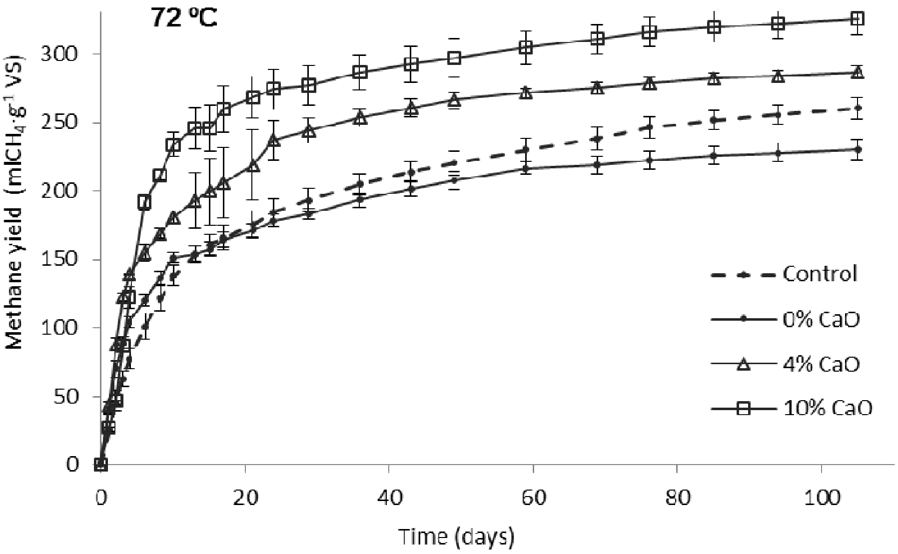
Comparing the effect of lime for each tested temperature, two different trends were observed. For thermally pretreated samples, the higher the dose of lime, the higher the methane yield (increasing from 257 to 292 ml CH_4 $g^{-1}VS$ at 55°C and from 230 to 325 ml CH_4 $g^{-1}VS$ at 72°C). Conversely, the pretreatment at room temperature presented the highest methane yield with 4% CaO (282 ml CH_4 gVS^{-1}). These results are consistent with the higher protein solubilisation obtained with 4% CaO compared to 10% CaO, and also with the higher biomass loss of the pretreatment with 10% CaO. According to the results, the thermo-alkaline pretreatment had more effect in terms of biomass solubilisation than methane production. Indeed, it has been shown that organic matter solubilisation can increase significantly more than the methane yield of several microalgae species [12,34]. Nevertheless, with the most severe condition (10% CaO at 72ºC) not only biomass solubilisation but also the final methane yield was improved.



a)



b)



c)

Figure 6. Cumulative methane yield of chemically pretreated microalgae at room temperature (a) and thermo- chemically pretreated microalgae at 55°C (b) and 72°C (c) with 0, 4 and 10% CaO.

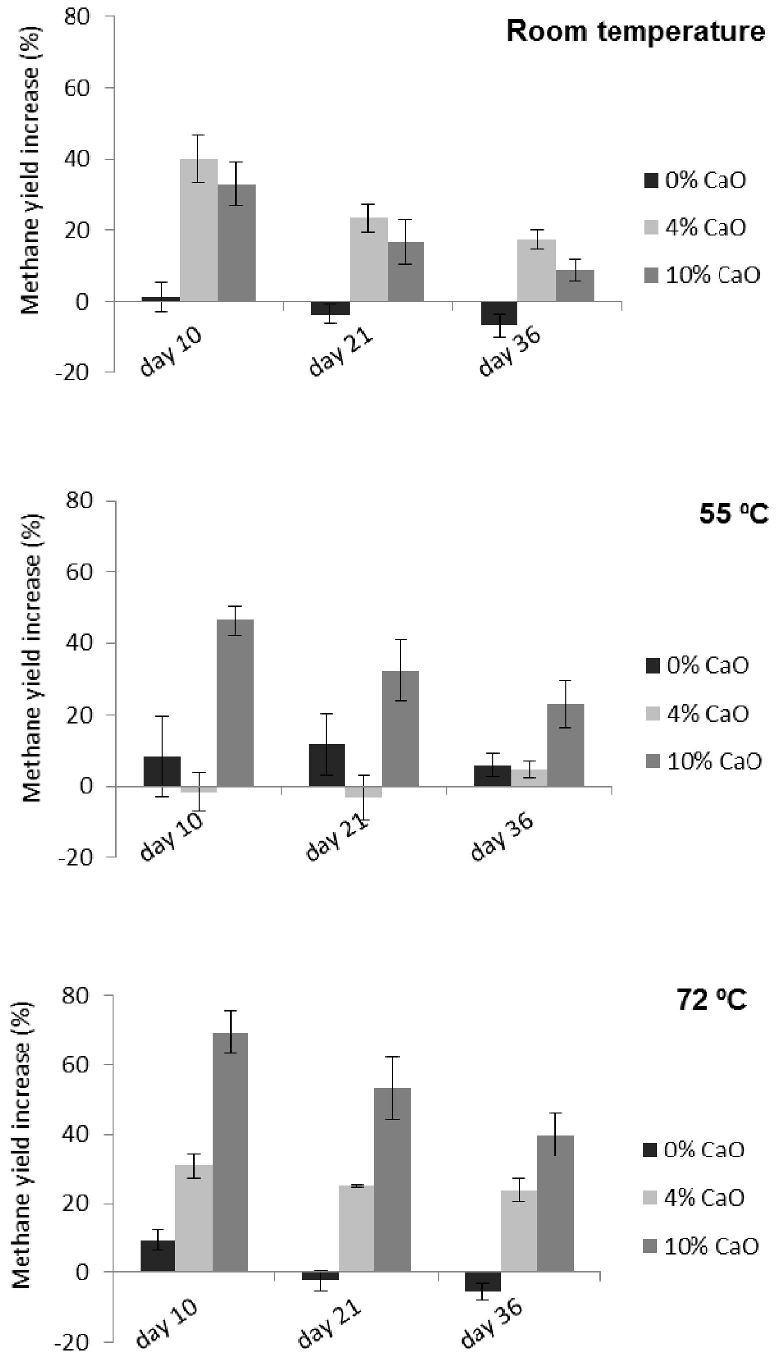
3.4.2. Kinetics improvement with the pretreatment

All the pretreatments improved the kinetics of the process as shown by the first order kinetic constant (*k*) (Table 4). While untreated microalgae showed the lowest *k* (0.08 day^{-1}), *k* values increased to 0.09-0.14 day^{-1} when biomass was pretreated. In general, the higher the lime dose, the higher the *k*. This kinetics enhancement was attributed to organic matter solubilisation after the pretreatment. Altogether, no correlation between the percentage of COD solubilised and the kinetic rate constant was found (R^2=0.136). However, since alkaline and thermo-alkaline pretreatments presented different behaviours in terms of macromolecules solubilisation and methane production, the correlation was analysed separately. By doing so, higher correlation coefficients were found (R^2=0.985 and R^2=0.779 for the alkaline and thermo-alkaline pretreatments, respectively).

Table 4. Kinetic parameters obtained from Eq.5. Estimated error variance (S^2) of each fitting calculated from Eq. 6.

Trial	λ (day)	Bo (ml CH_4 gVS^{-1})	k (day^{-1})	S^2
Untreated microalgae	0.00	238	0.08	173
Room temperature	0.00	214	0.10	209
Room temperature + 4% CaO	0.00	255	0.14	325
Room temperature + 10% CaO	0.00	237	0.14	201
55 ºC	0.00	240	0.09	132
55 ºC + 4% CaO	0.00	236	0.09	456
55 ºC + 10% CaO	1.17	271	0.12	261
72 ºC	0.00	209	0.12	274
72 ºC + 4% CaO	0.00	265	0.12	398
72 ºC + 10% CaO	1.17	305	0.13	223

The kinetics improvement could be responsible for the higher methane production rate during the first days of the BMP test (Fig. 6). To ease comprehension, the methane yield increase for each pretreatment condition with respect to untreated microalgae at days 10, 21 and 36 was compared (Fig. 7). As can be observed in Fig. 7, alkaline and thermo-alkaline pretreatments presented different behaviors. Once again, higher values were obtained with 4% CaO for the alkaline pretreatment at room temperature and 10% CaO for all thermo-alkaline pretreatments.



a)

b)

c)

Figure 7. Methane yield increase of pretreated samples at room temperature (a), 55 ºC (b) and 72 ºC (c) with respect to untreated microalgae (control) after 10, 21 and 36 days of BMP assay.

4. Conclusions

This study evaluated the effect of a thermo-alkaline pretreatment with lime on microalgal biomass anaerobic digestion. The pretreatment increased proteins and carbohydrates solubilisation up to 32.4% and 31.4%, respectively. Consequently, anaerobic digestion kinetics were also improved (the first order kinetic rate constant increased from 0.08 to 0.14 day^{-1}). The pretreatment with the highest lime dose (10% CaO) and temperature (72°C) showed both the highest macromolecules solubilisation (31-32%) and the highest biochemical methane potential increase (25%). Bearing in mind that lime is not toxic and that it is less expensive than other chemicals (e.g. NaOH), the use of lime could also contribute to reducing pretreatment costs and potential environmental impacts. Nevertheless, the application of the best pretreatment condition should be further investigated in continuous reactors to estimate the energy balance and economic cost of the process.

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Declaration of contributions

Maria Solé-Bundó (maria.sole-bundo@upc.edu), Hélène Carrère (helene.carrere@supagro.inra.fr), Marianna Garfí (marianna.garfi@upc.edu) and Ivet Ferrer (ivet.ferrer@upc.edu) take responsibility for the integrity of the work as a whole, from inception to finished article.

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