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Thermal, alkali, and thermo-alkali pretreatments applied to monospecific microalgal biomass to improve anaerobic biogas production

G. Campo, V.A. Riggio*, A. Cerutti, B. Ruffino, D. Panepinto, M.C. Zanetti

DIATI, Politecnico di Torino, C.so Duca Degli Abruzzi, 24, 10129 Torino, Italy, emails: giuseppe.campo@polito.it (G. Campo), vincenzo.riggio@polito.it (V.A. Riggio), alberto.cerutti@polito.it (A. Cerutti), barbara.ruffino@polito.it (B. Ruffino), deborah.panepinto@polito.it (D. Panepinto), mariachiara.zanetti@polito.it (M.C. Zanetti)

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ABSTRACT

In this study, alkaline, thermal, and thermo-alkaline pretreatments of *Scenedesmus obliquus* microalgae were investigated with the goal of enhancing anaerobic biodegradability. In recent years microalgae, biomass has been used for several purposes; one is to employ this substrate to produce renewable energy. Biogas/methane production of untreated microalgae is limited by the complex cell-wall structure; neither the organic matter constituent of the cell wall nor the other matter inside the microalgae is available to microorganisms involved in anaerobic digestion (AD). Pretreatments could increase the biogas/methane production. High-temperature pretreatment was conducted at 120°C, and alkali pretreatments were performed using NaOH with a dosage range from 4% to 20% of the selected total solids matrix. Finally, hybrid pretreatment (thermo-alkali treatment) was conducted with the same dosage of NaOH and thermal conditions. All the described treatments had a duration of 1.5 h. Untreated and treated microalgae were subjected to AD, then biogas potential tests were performed in batch mode and mesophilic conditions (38°C). The thermo-alkali pretreatment proved to be the best technique in terms of methane production increase. The specific methane production after thermal and thermo-alkali treatments increased by 37% and 45%, respectively, compared with the raw digested microalgae sample. However, after a preliminary cost/revenue analysis the most promising hydrolysis technique applied to the microalgae tested was found to be the thermal treatment.

Keywords: Microalgae; *Scenedesmus obliquus*; Hydrolysis techniques; Disintegration rate; Anaerobic digestion; Renewable energy production

1. Introduction

The world community is currently seeking sources of renewable energy that are both economical and environmentally friendly. Biogas is one essential source of renewable energy that can be substituted for fossil fuels [1], and recently, microalgae cultivations have drawn attention from scientists and entrepreneurs as a new carrier for energy production. Several energy products can be obtained from the cultivation of microalgae, such as biodiesel, bioethanol, hydrocarbons, hydrogen, and methane. Methane contains carbon in its most reduced state, while carbon dioxide contains it in its most oxidized state. Methane production through anaerobic digestion (AD) is one of the most common diffused methods used for energy production from organic wastes and other biomass. AD involves the degradation of organic carbon into biogas, which consists mainly of methane. AD consists of four main steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The limiting step of AD of particulate matter is hydrolysis. During hydrolysis – an exo-bacteria phenomenon – exoenzymes convert complex matter and undissolved materials into less complex, dissolved compounds that can pass through cell walls and the membranes of fermentative bacteria. Several studies

* Corresponding author.

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have demonstrated the use of microalgae species for biogas production.

The structure of cell walls plays a crucial role in microalgae anaerobic biodegradability. According to Takade [2], the cell-wall structure of some microalgae, such as *Scenedesmus obliquus*, consists of glucose, mannose, and galactose: those compounds formed from cellulase and hemicellulose, which cause a high resistance to cell wall attacks on enzyme hydrolysis and limit their availability from AD.

Over the last three decades, in order to promote biogas production by different substrates, a number of pretreatment strategies have been developed in both the industrial and academic worlds, and some of these are currently used in fully operational processing plants. A complete review of the pretreatments studied was reported in Carrere et al. [3] and Carrere et al. [4]. The current lysis techniques can be divided into mechanical, thermal, chemical, biological, and hybrid treatments (hybrid treatments consist of a combination of two or more of these techniques). There are two types of thermal pretreatments: low and high. Low thermal pretreatments are conducted at temperatures lower than 100°C and at atmospheric pressure; high thermal lysis techniques, at temperatures higher than 100°C and at pressures generally between 1 and 8 bar. Currently, many of these pretreatment methods are being studied to see whether microalgae AD can be improved. A complete review of the pretreatments being studied, in order to better recover biogas production from microalgae, is reported in Passos et al. [5], Passos et al. [6], Jankowska et al. [7], and Córdova et al. [8]. The Italian Government provides incentives for the production of biomethane as a renewable fuel for the transport sector. Moreover, in "DM 2 Marzo 2018 Promozione dell'uso del biometano e degli altri biocarburanti avanzati nel settore dei trasporti" the biomethane generated after the upgrading of biogas produced from the AD of microalgae is denoted as advantageously combustible (DM 2 marzo 2018). A double incentive is given for the production of biomethane from the AD of microalgae.

The effectiveness of the pretreatments studied depends not only on the nature and structure of the microalgae chosen but also on the full applicability of the treatment and the cost/revenue ratio of the full cycle of microalgae, which includes cultivation, growth, harvest, treatment, digestion, and renewable energy production. The aim of this work was to evaluate the AD of *S. obliquus* biomass grown in a photobioreactor and in monospecific cultivation after chemical, thermal, and thermo-chemical pretreatments.

One of the best-known microalgae strains (*S. obliquus*) was selected for this study. The experiment was divided into two steps. During the first step *S. obliquus* samples were pretreated, and the effectiveness of each treatment tested was evaluated using the disintegration rate (DR) parameter. Two pretreatments evaluated during the first step were subjected to biochemical methane potential tests (BMPs) as second step of the experiment. The choice of which of the two lysis techniques to use was based on the price of the NaOH utilized and the projected profitability attributable to the increased methane production.

2. Materials and methods

2.1. Microalgae and inoculum used

The microalgal biomass used in this study was obtained by growing isolated *S. obliquus* (SAG 276–3a, *Acutodesmus obliquus*) inside an improvised flat-photobioreactor. The system assured high productivity of the mono-specific strain and could easily handle about 1,000 L of growing water. The system was placed in an outdoor greenhouse (Cascina Rapella, Chivasso – Turin, Italy) and the illumination of the strain followed the natural day-night cycle. The microalgae were batch fed with a BG11 medium and the proper quantity of CO_2 was dosed using electronic controls. In this way, the CO_2 consumption was drastically reduced and finely adjusted using intermittent injection. The CO_2, dissolved oxygen, temperature, and pressure were also monitored in real time, together with weather parameters from a nearby weather station. Biomass growth was monitored using optical density values scanned at 470 and 710 nm (Jenway 6051). The fresh microalgae biomass was syphoned off at the end of the growth stage (21 d), centrifuged with a Ruma separator (Model: MZ 35) operated at 4,460 rpm and then manually scraped from the interior bowl. The concentrated biomass was freeze-dried, and when needed, fed into the pretreatment reactor and then to the AD reactor at the respective volumetric organic loading rate. Tap water was used to resuspend the frozen biomass. The total and volatile solids of the microalgae biomass used are presented in Table 1.

The inoculum employed during the anaerobic batch digestion tests was collected from a semicontinuous pilot digester fed with waste-activated sludge. The biological sludge was generated in the largest Italian wastewater treatment plant (Castiglione Torinese WWTP), which is managed by the SMAT Company (the local water utility). The Castiglione Torinese Water Resource Recovery Facility (WRRF) treats municipal and industrial wastewater with a capacity of about 2,300,000 p.e. (serving about 1.5 million civil inhabitants, over 1,000 industrial discharges and tank truck wastewater). The pilot digester had an HRT equal to 20 d and a stated volume of 240 L, and the average organic loading rate was 1 kg VS/(m^3 d). The total and volatile solids of the inoculum used are presented in Table 1.

2.2. Analytical methods

All the analytical parameters: total solids (TS), volatile solids (VS), pH, electrical conductivity (EC), soluble chemical

Table 1
Content of total and volatile solids in *Scenedesmus obliquus* and inoculum used in the tests

Substrate	ST% (average)	SV% (average)	SV/ST% (average)
Scenedesmus obliquus	66.8	65.71	98.3
Inoculum	2.9	1.73	58.94

oxygen demand (sCOD), and ammonium (NH_4^+) were determined using standard methods [9]. Soluble COD is the fraction of COD separated after centrifugation at 4,000 rpm for 15 min and subsequent filtration through a 0.45-mm nylon membrane. The concentrations of volatile fatty acids (VFAs) and the total alkalinity were measured by means of a TitroLine® 5,000 xylem automatic titrator, and the values were expressed as FOS and TAC. FOS is an acronym for "Flüchtige Organische Säuren" which means VFA in German (expressed in mg Haceq/L); TAC is an acronym for "Totales Anorganisches Carbonat" which means total inorganic carbonate, also known as lime buffer, and it is expressed in mg $CaCO_3$/L.

2.3. Elemental analysis

The elemental analysis of *S. obliquus* microalgal was determined using a Flash 2000 Thermo-Fisher Scientific CHNS analyzer. Specific COD value O_2/VS (weight by weight ratio) was evaluated using the elemental composition of the microalgal as in van Lier et al. [10]. Using the formula (CaHbOcNd) of detected substrate, the total COD (COD_t) was calculated using the followed equation:

$$\mathrm{COD}_t = \frac{8\left(4a+b-2c-3d\right)}{\left(12a+b+16c+14d\right)} \left[\frac{w\,\mathrm{O}_2}{w\,\mathrm{C}_a\mathrm{H}_b\mathrm{O}_c\mathrm{N}_d}\right] \quad (1)$$

2.4. Thermal, alkali, and thermo-alkali pretreatments

The following hydrolysis techniques were used in this study: high thermal treatment (120°C), alkali pretreatment (NaOH) with different dosages (4%, 12%, and 20% of TS), and thermo-alkali pretreatment, a combination of the two processes. Alkaline and thermo-alkaline treatments were performed on mixed liquors containing algae biomass at 5% and 10% of TS by weight. In all the situations, the duration of pretreatments was fixed at 1.5 h. All the procedures were performed at the laboratory scale. The alkali, thermal, and thermo-alkali pretreatments were performed inside glass bottles (500 mL DURAN). The pretreatments were conducted on 250 mL of microalgae mixed liquor inserted into the glass bottles; moreover, when alkali and thermo-alkali pretreatments were applied the experimental solution consisted of 200 mL of raw microalgae biomass (5% and 10% TS) and 50 mL of alkaline solution. Alkali and thermo-alkali pretreatments were realized by dosing the correct concentration of alkaline solutions based on the microalgae TS quantity. Alkali solutions that contained the correct dose of NaOH were obtained starting from a 100 g/L alkali mother solution. The glass bottles were immersed in a pressurized thermo-static bath and the water inside was preheated to the temperature of 120°C. In the thermal and thermo-chemical pretreatments, at the end of the process, the bottles were cooled to room temperature using tap water.

Before and after pretreatment, the pH, EC, and sCOD were measured. At the end of the treatment, the biomass liquid phase was separated from the solid phase by means of centrifugation (4,000 rpm, 15 min) and subsequently filtered through 0.45 μm acetate-cellulose membranes. The series of tests performed are indicated in detail in Table 2. These were designed to investigate the effects of different parameters on the COD solubilization using the DR [11] parameter as an indicator and to compare the effectiveness of different substrate pretreatments. The formula used for the calculation of DR was the following:

$$\mathrm{DR} = \frac{\mathrm{sCOD}_i - \mathrm{sCOD}_0}{t\mathrm{COD} - \mathrm{sCOD}_0} \quad (2)$$

where the parameters tCOD, sCOD_0, and sCOD_i refer to the total COD of microalgae biomass and the soluble COD before and after pretreatments, respectively.

Table 2
Pretreatments, final values of considered parameters

	Pretreatment time, h	pH	Conductibility, mS/cm	Fos/Tac
S.O. 5%TS – 20°C	1.5	5.8	0.68	3.71
S.O. 5%TS – NaOH 4% – 20°C	1.5	11.8	3.62	0.33
S.O. 5%TS – NaOH 12% – 20°C	1.5	12.4	17.2	0.08
S.O. 5%TS – NaOH 20% – 20°C	1.5	12.4	27.7	0.05
S.O. 5%TS – 120°C	1.5	5.8	0.68	2.60
S.O. 5%TS – NaOH 4% – 120°C	1.5	10	2.8	1.00
S.O. 5%TS – NaOH 12% – 120°C	1.5	11.8	10.85	0.32
S.O. 5%TS – NaOH 20% – 120°C	1.5	11.5	19.39	0.46
S.O. 10%TS – 20°C	1.5	5.8	1.1	3.36
S.O. 10%TS – NaOH 4% – 20°C	1.5	11.8	6.3	0.34
S.O. 10%TS – NaOH 12% – 20°C	1.5	12.4	23.9	0.11
S.O. 10%TS – NaOH 20% – 20°C	1.5	12.6	43.2	0.28
S.O. 10%TS – 120°C	1.5	5.8	1.31	3.92
S.O. 10%TS – NaOH 4% – 120°C	1.5	10	4.6	1.08
S.O. 10%TS – NaOH 12% – 120°C	1.5	11.9	17.75	0.39
S.O. 10%TS – NaOH 20% – 120°C	1.5	12	30	2.05

S.O. – Scenedesmus obliquus.

2.5. Anaerobic digestion tests

AD tests, carried out in duplicate, were performed to investigate the effect of the selected pretreatment conditions on microalgae substrate. Due to the limited availability of laboratory-scale digesters, only two microalgae pretreatment methods were tested in terms of anaerobic degradability. The lysis techniques chosen for the AD tests were thermal (120°C) and thermo-alkali pretreatments (4 g NaOH/100 g TS 120°C). Eight digesters were used during the anaerobic tests. Two reactors were filled with only inoculum, two with untreated samples (the control), two with thermal-treated (120°C 1.5 h) microalgae only, and the last two with thermo-alkali treated (4 g NaOH/100g TS – 120°C – 1.5 h) algae.

Biomethane potential (BMPs) were performed in batch mode and in mesophilic conditions (38°C). The two lab-scale anaerobic digesters filled with only inoculum had a total volume of 2.5 L. The remaining six digesters used, each with a total volume of 6 L, were fed with untreated or treated microalgae. All the reactors were immersed in a controlled temperature water bath (38°C).

For each reactor, the produced biogas was collected in one 5-L Tedlar bag. The characterization and measurements of the produced biogas volume were carried out every day, throughout the duration of the test. Gas analysis was performed, as discussed in Ruffino et al. [12], and the volumetric composition of the biogas in terms of CH_4, CO_2, and O_2 was obtained by analyzing 500 mL of biogas with a biogas analyzer (Biogas Check, Geotechnical Instruments Ltd.). The residual volume of the biogas after the characterization was measured by replacing a measured volume of water with the residual gas. The temperature of the laboratory was measured daily, and the produced volume of biogas and methane were referred at normal conditions (0°C, 1 atm).

The substrate inoculum ratio *S* (substrate)/*I* (inoculum) was chosen as 0.11 g VS added/g VS inoculum. In order to prevent possible acidification inhibition, the ratio substrate inoculum chosen was lower, at the maximum (*S*/*I* equal to 0.5) suggested in Angelidaki et al. [13]. In our previous work [14], as visible in Table 3, we used a substrate inoculum *S*/*I* ratio of 0.76. In that experimentation, the digesters fed with thermal-treated (120°C – 1.5 h) microalgae and thermo-alkali treated microalgae showed typical acidification. Indeed, at that time during the first day of digestion the anaerobic bacteria, fed with pretreated algae, produced a biogas rich in CO_2 and poor in methane. The laboratory experience explained in this work differs from those previous experimentation results. In each digester, the inoculum occupied 67% of the total volume, the substrate (microalgae 7.7% VS) 1.7%, and the headspace the remaining 3%. The batch digesters were checked for any leakage and flushed with 100% pure nitrogen for approximately 3 min to ensure anaerobic conditions. The tests were considered concluded when the cumulative biogas curve reached an asymptotic trend as normed by Verein Deutscher Ingenieure [15].

3. Results and discussions

3.1. Microalgae analysis before pretreatment

An elemental analysis was performed on the *S. obliquus* biomass on a volatile dry basis. The results were: C 56.1%, H 8%, N 2.6%, and O 32.5% by weight. Assuming the chemical formula of $C_aH_bO_cN_d$ for volatile dry sludge, the values can be calculated as follows: a = 25.7; b = 43.4; c = 11.1; and d = 1. Consequentially the ratio between COD and VS was evaluated as 1.755.

3.2. Effect of pretreatment on DR and pH parameters

All the obtained results regarding the microalgae biomass treated with tested methods are reported in Fig. 1. It is evident that the DR value was much higher when the NaOH was used in combination with high temperature. Treated microalgae biomass with NaOH (0.2 g/g TS) at 120°C reached a DR value close to 40%. For the dosage of 0.2 g NaOH/g TS, the DR value was approximately 6.5 times higher than the situation with only thermal pretreatments without use of alkali solution. As already illustrated in the earlier paragraph this positive effect was enhanced when the dosage of NaOH inside the microalgae substrate was higher, but for economic and pH reasons the most interesting case for AD tests was the dosage of 0.04 g NaOH/g TS.

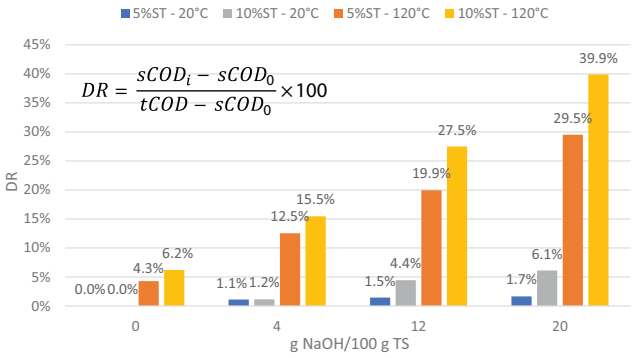

Fig. 1. Pretreatments versus disintegration rate.

Table 3
Anaerobic digestion, methane increases after pretreatment

Pretreatment	Pretreatment time, h	*S*/*I*	Methane increase, %	References
120°C	1.5	0.76	–9	[14]
120°C, 4 g NaOH/100 g VS	1.5	0.76	+42	[14]
120°C	1.5	0.11	+37	Current study
120°C, 4 g NaOH/100 g VS	1.5	0.11	+45	Current study

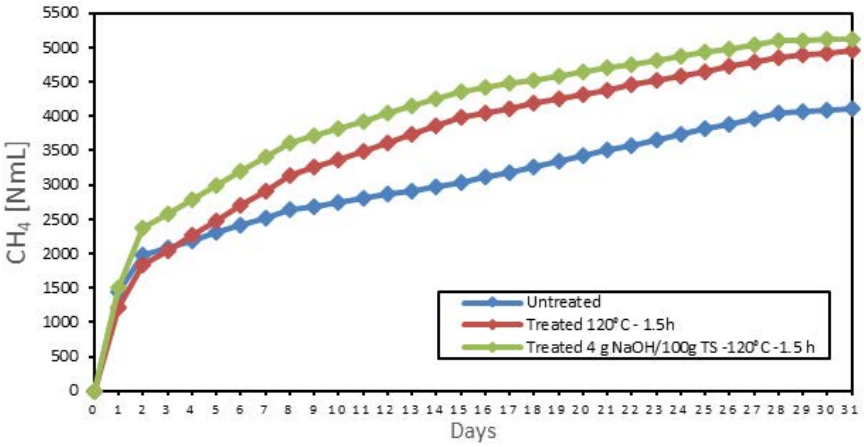

Fig. 2. Daily methane production. The reported values have not been subtracted from the methane production from the inoculum.

3.3. Anaerobic digestion test

Not all the tested pretreatments to assess the capability to release sCOD were evaluated in terms of effective biogas and methane potential increases. Indeed, only the cases considered more suitable for future applicability at a production scale were anaerobically tested at the laboratory scale. And only the results of the anaerobic tests considered the best in term of methane production, for each one of the conditions studied, were considered. Unfortunately, two of our reactors showed gas-seal problems during the digestion. The anaerobic batch tests of untreated algae showed a specific methane production of untreated microalgae of 0.30 Nm^3/kg SV. The high thermal pretreatment (120°C) was anaerobically tested and the results showed an increase in biogas yield production of 57% (37% methane) compared with the untreated microalgae substrate, as can be seen in Fig. 2. The pretreated substrate with 0.04 g NaOH/g TS, for 1.5 h at 120°C was the other tested condition. After thermo-alkaline treatment, the biogas production registered an increase of 59.5%, with respect to the control sample, while the overproduction of methane was 44.6%. Therefore, the specific methane production yields were 0.40 and 0.43 Nm^3/kg SV of algae after thermal and thermo-alkaline pretreatments, respectively. One of the possible keys for choosing the best treatment is cost/revenue analysis. This analysis was first done without considering the costs of the microalgae cultivation, the management and reuse of the digestate, the upgrading of biogas to methane and the possible codigestion of microalgae with other substrates. The thermo-alkaline treatment should be the best choice if the spread of the two SMPs (thermal and thermo-alkaline treatment of 0.03 Nm^3/kg SV) were able to produce an amount of electrical energy having an economic value at least equal to the price of the sodium hydroxide used. To evaluate the cost/benefit analysis some assessments were carried out. The price of sodium hydroxide was taken as 450 €/ton [12,16,17]. The electrical efficiency of the combined heat and power engine (CHP) was 35% and the economic value of electrical energy produced was 103.53 €/MWhe (https://www.gse.it/servizi-per-te/fotovoltaico/ritiro-dedicato/documenti 18/07/2018). Considering the previous hypothesis, the best pretreatment should be the thermal one. The extra methane producible after thermo-alkaline treatment, as far as it was possible to investigate in reality, was not sufficient to cover the NaOH price. Indeed, it would be necessary to spend 18 € per ton of TS (dry algae biomass) in addition to the sodium hydroxide, while the extra electrical energy producible would have an economic value of only 11 €/tons TS. Ometto et al. [18], using *Scenedesmus* and a thermal pretreatment like the one used in this study, obtained an increase in biogas production of 35% with respect to the control. Similar results of methane-specific production were obtained by Mendez et al. [19], who used *Scenedesmus* diluted at 13% (w/v) and treated at 120°C for 40 min. The biogas-specific production is comparable with data published by Ometto et al. [18], who used several types of pretreatment and obtained the best performance of biogas production using high temperature (165°C) and enzymatic hydrolysis. Specific methane production published by Mahdy et al. [20] using *Scenedesmus* biomass, pretreated with low temperature and thermo-alkaline pretreatment (at 0.05–2–5% of NaOH), showed very low enhancement compared with this study.

4. Conclusions

This work analyzed the effects of thermal, alkali, and thermo-alkali pretreatments of microalgae *S. obliquus* for the improvement of biogas and methane production. The BMPs conducted in this study show that thermal and thermo-alkali pretreatments (0.04 g NaOH/g TS) may improve the performance of the AD process. After thermal (120°C, 1.5 h) and thermo-alkali (120°C, 1.5 h 4 g NaOH/100 g VS) pretreatments the increases in methane were respectively equal to 37% (0.405 g CH_4/kg SV) and 44.6 % (0.428 g CH_4/kg SV). A methane-specific production of 0.429 Nm^3/kg SV for *S. obliquus* is a high value compared with which can be found in the literature. However, future anaerobic tests with the aim of validating these preliminary results mast be carried out before production-scale operation can be considered. In order to have better results, it will be necessary to repeat the experimentation using at list three replacements for each pretreatment methodology tested. Based on the achieved results it can be concluded that high thermal pretreatment

(120°C, 1 bar for 1.5 h) for *S. obliquus* biomass can efficiently enhance the AD process and can be used as a starting point for further development.

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