Thermal, Alkali And Thermo-Alkali Pretreatments Applied On Monospecific Microalgal Biomass To Improve Anaerobic Biogas Production.

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Abstract

In last years microalgae biomass has been used for several scopes. One of them is to investigate their use as energy products. In this work microalgae were studied for anaerobic digestion use, but, to do so with a positive cost/revenue ratio, the cell walls must be efficiently removed before that the anaerobic digestion process takes place. This is the only way to make available the organic matter for the microbial activity and consequently enhance quantity and quality of produced biogas. In this study were used *Scendesmus obliquus* strain treated with thermal, alkali and alkali-thermal pre-treatments. Samples of microalgae biomass were collected and subjected to a first pre-treatments test at 120°C (autoclave) for one hour and half. The second test was performed with an alkali pre-treatments using NaOH with a dosage range from 4 to 20% of the selected TS matrix (2, 5, 10% by weight). The last test was conducted using a hybrid pre-treatment (alkali – thermal), with the same dosage of NaOH and thermal exposition described above. Both tests with the use of NaOH showed a better efficiency in releasing intracellular organic matter and breaking polymeric bridges. Then biogas potential tests were performed under batch mesophilic conditions after each pre-treatment to evaluate possible enhancement in biogas production and quality.

Keywords: Microalgae, Scenedesmus obliquus, Anaerobic Digestion, alkali pre-treatments, thermal pre-treatments, thermos-alkali pretreatments, renewable energy production

1. Introduction

Nowadays microalgae cultivations have drawn attention within scientists and entrepreneur as a new carrier for energy production. Several energy products can be obtained from the cultivation of microalgae as biodiesel, bioethanol, hydrocarbons, hydrogen and methane. Methane production through anaerobic digestion is one of the most diffused methods which have been used for energy production from organic wastes to date. However, to be economically sustainable, microalgae biomasses should be grown with highly productive and integrated systems, and then efficiently treated. In the past anaerobic digestion tests noticed that after more than 40 days the number of microalgae cell still intact was very high, a situation which leads to poor production performances. To enhance cost/revenue ratio the cell walls must be removed before that the anaerobic digestion process takes place, in this way the organic matter inside the reactor can be easily available for the microbial activity and consequently the biogas production can be increased in quantity and quality. One of the most known microalgae strain was selected for the realization of this study. This work presents the results of a technical assessment of thermal, alkali–and alkali-thermal pre-treatments. Samples of microalgae biomass were collected and subjected to a preliminary pre-treatments test at 120°C (autoclave) for one hour and half. The second test was performed with an alkali pre-treatments using NaOH with a dosage range from 4 to 20% of the selected TS matrix (5 and 10% by weight). The last test was conducted using a hybrid pre-treatment (thermo - alkali), with the same dosage of NaOH and thermal exposition described above. Both tests with the use of NaOH showed a better efficiency in releasing intracellular organic matter and breaking polymeric bridges. The combined effect of the chemicals and temperature at 120°C made possible to obtain disintegration drate (DR) value increases about to 40%. Biogas potential tests were performed under batch mesophilic conditions after each pre-treatment to evaluate possible enhancement in biogas production and quality.

2. Materials and methods

2.1 Microalgae and inoculum used

The algal biomass used in this study was obtained by growing isolated *Scenedesmus obliquus* (SAG 276-3a, *Acutodesmus obliquus*) inside an own made flat-photobioreactor. The system assures high productivity of mono specific strain and can easily handle about 1000 L of growing water. The system was placed in a greenhouse outdoors (Cascina Rapella, Chivasso – Turin, ITALY) and the illumination of the strain followed the natural day-night cycle. The microalgae were batch feed with BG11 medium and the right quantity of CO_2 was dosed using electronic control. 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 the weather station parameters. Biomass growth was monitored using optical density values scanned between 470 and 710 nm (Jenway 6051). The fresh microalgae biomass was syphoned off at the end of the growth stage (21 days), 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 anaerobic digestion reactor at the respective volumetric organic loading rate. Tap water was used to re-suspend the frozen biomass. The total and volatile solids of used microalgae biomass are presented in Table 1. The inoculum employed during the anaerobic batch digestion tests was collected from a semi-continuous pilot digester fed with waste activated sludge. The biological sludge is generated into the biggest Italian wastewater treatment plant (Castiglione Torinese WWTP), that is managed by the SMAT Company (Local Water Utility). The Castiglione Torinese Water Resource Recovery Facility (WRRF) treats municipal and industrial wastewater with a capacity of about 2,300,000 e.i. (about 1.5 million of civil inhabitants, over 1000 industrial discharges and also tank truck wastewater). The pilot digester has a HRT equal to 20 days, a worded volume of 240 L and an average organic loading rate is equal to 1 kg VS/(m^3d). The total and volatile solids of used inoculum are presented in Table 1.

2.2 Thermal, alkali and thermo-alkali pretreatment.

The following hydrolysis techniques were used in this study: high thermal treatment (120°C), alkali pretreatment (NaOH) with different dosage in a range between 0.04 and 0.2 g hydroxide/g TS and thermo-alkali pre-treatment, a combination of the above-mentioned processes. In all the situations, the duration of pretreatments was fixed at 1.5 hours. All the procedures were performed at laboratory scale. The alkali, thermal and thermo-alkali pretreatments have been performed in glass bottles (500 mL DURAN). The pretreatments have been conducted on 250 mL of microalgae inserted into the glass bottles; moreover, they consisted of 200 mL of raw microalgae biomass (5 and 10 % TS) and 50 mL of alkaline solutions. Alkali and thermos-alkali pre-treatments were realized dosing the correct quantity 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 thermos-static bath, the water inside was preheated at the temperature of 120°C. In the case of thermal and thermo-chemical pretreatment at the end of the process the bottles were cooled to room temperature using tap water. Before and after pretreatment the measurement of pH, EC and sCOD was performed. At the end of the treatment, the biomass liquid phase was separated from the solid phase by means of centrifugation (4000rpm, 15min) and subsequently filtered on 0.45 µm acetate-cellulose membranes. The series of test performed are indicated in details in Table 2. They were designed to investigate the effect of different parameters on the COD solubilization. In this way was possible to use the Disintegration Rate (DR) parameter as an indicator to compare the effectiveness of different substrates pre-treatments (Dohanyos et al., 1997). The formula used for the calculation of DR was the following:

$$DR = \frac{sCOD_i - sCOD_0}{tCOD - sCOD_0}$$

Where the parameters tCOD, $sCOD_0$ and $sCOD_i$ are referred to the total COD of microalgae biomass and the soluble COD before and after pretreatments respectively.

2.3 Anaerobic digestion test

Anaerobic digestion 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 lab-scale digesters, only two microalgae pre-treatment methods were tested in terms of anaerobic degradability. The treated methods were chosen based on the most interest results obtained during the previous lysis tests. Eight digesters were used during the anaerobic tests. Two reactor were filled with only inoculum, two with untreated samples (control), one pair of digesters with microalgae only thermal treated (120 °C 1,5 h) and the last two reactors with algae thermo-alkali treated (4 gNaOH/ 100gTS -120°C-1,5 h). Biogas production tests (BPTs) were performed at batch mode and in mesophilic conditions (38°C). The lab-scale anaerobic digesters filled with inoculum had a total volume of 2,5 L. The remaining six digesters used, each one 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. For each reactor, the produced biogas was collected in one 5 L tedlar bag. The characterization and measurements of the produced biogas volume was carried every day, throughout the whole duration of the test. The gas analysis, which is the volumetric composition of the biogas in terms of CH_4, CO_2, 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 volumes 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 condition (0°C, 1 atm). The substrate inoculum ratio S (substrate)/I (inoculum) has been chosen at 0.11 g VS added/g VS inoculum. In our previous work (Riggio et al. 2016) it was used a substrate inoculum S/I ratio equal to 0.76. In that experimentation, the digesters fed to microalgae thermal (120 °C-1.5h) and thermo-alkali treated showed a typical acidification phenomena. Indeed, during the first days of digestion the anaerobic bacteria, fed with pre-treated algae, produced a biogas rich of CO_2 and poor of methane. The laboratory experience explained in this paper shows the difference with the previous experimentation results. In each digester, the inoculum occupied the 67 % of total volume, the substrate (microalgae 7,7 % VS) occupied the 1,7 % of total volume and the head space occupied the remain 31%. The batch digesters were checked for any leakage and flushed with 100% pure nitrogen for

approximately 3 minutes to ensure anaerobic conditions. The tests were considered concluded when the cumulative biogas curve reached an asymptotic trend as normed by Verein Deutscher Ingenieure (VDI, 2006).

3. Results and discussions

3.1 Microalgae analysis before pre-treatments

An elemental composition analysis was performed on the *Scenedesums obliquus* biomass based on volatile dry basis. The results were equal to: C 56.1%, H 8%, N 2.6%, 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 follow: a = 25.7; b = 43.4; c = 11.1; d = 1. Consequentially the ratio between COD and VS has been evaluated equal to 1.755.

3.2 Effect of pretreatment on DR and pH parameters

All the obtained results regarding the microalgae biomass treated with tested methods were reported in Table 2. It is evident that the DR value is much higher when the NaOH is used in combination of high temperature treatment. Treated microalgae biomass with NaOH (0.2 g/g TS) at 120°C reached an increase of DR value close to 40%. For the dosage of 0.2 g NaOH/g TS, 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 above paragraph this positive effect is more enhanced when the dosage of NaOH inside the microalgae substrate is higher, but for economic and pH reasons the most interesting case for anaerobic digestion tests is the dosage equal to 0.04 g NaOH/g TS.

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 the full scale were anaerobically tested at laboratory scale. Only the results of the anaerobic tests considered the best in term of methane production, for each one of the conditions studied, were taken into account. Unfortunately, two of our reactors showed same problem of gas seal during the digestion. The high thermal pretreatments (120°C) was anaerobically tested. The result shows an increase in biogas yield production equal to 57% (37% methane) compared to the not treated microalgae substrate, as visible in Figure 1. Ometto et Al. (2014) using *Scenedesmus* and a thermal pretreatment like the one used in this study obtained an increase of biogas production of 35% to respect of control. Similar results of methane specific production were obtained by Mendez et al. (2014) that used *Scenedsmus* diluted at 13% (w/v) and treated at 120°C for 40 minutes. The pretreated substrate with 0.04 g NaOH/g TS, for 1.5h and at 120°C was the other tested condition. The biogas production registered an increase of 59,5%, respect to control sample, moreover the results in overproduction of methane are equal to 44,6%. The biogas specific production is comparable with the data published by Ometto et al. (2014) that 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. (2014) of *Scenedesmus* biomass, pretreated with low temperature and thermos-alkaline pretreatment (at 0.05 – 2 – 5% of NaOH), showed very low enhancement compared to this study. The CO_2/CH_4 ratio is visible in Figure 4, where it can be notice that the pretreated substrates produced a higher quantity of CO_2 in the first 3 days of test, respect to control sample

4. Conclusions

This work analyzed the effects of thermal, alkali and thermo-alkali pretreatments of microalgae *Scenedesmus obliquus* for the improvement of biogas and methane production. The BPTs conducted in this study shown that, thermal and thermo-alkali pretreatment (0.04 g NaOH/g TS) may improve the performance of the AD process. The anaerobic batch tests show that the specific methane production of untreated microalgae is equal to 0.296 Nm^3/Kg SV. After, thermal (120°C, 1.5h) and thermo-alkali (120°C, 1.5h 4g NaOH/100g VS) pretreatments the increase of methane was 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 equal to 0.429 Nm^3/Kg SV for *Scenedesmus obliquus*un is a high value compared to what can be found in literature. However, other future anaerobic tests with the aim to validate this preliminary results mast be required before real scale consideration can be done. In order to have better results it will be necessary to repeat the experimentation using at list three replaces for each one pre-treatment methodology tested. Based on the achieved results it can be concluded that thermo-alkali pretreatment for *Scendesmus obliquus* biomass can efficiently enhanced the anaerobic digestion process and can be used as a starting point for further development.

Table 1. Contenent 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

Table 2. Pre-treatment starting conditions and final values of considered parameters.

	Pretreatment time [h]	pH	Conducibility [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

Table 3. Anaerobic digestion, methane increase after pretreatment

Pre treatment	Pretreatment time [h]	S/I	Methane increase [%]	Reference
120 °C	1.5	0.76	-9	Riggo et al. (2016)
120°C, 4 gNaOH/ 100gVS	1.5	0.76	+42	Riggo et al. (2016)
120 °C	1.5	0.11	+37	Riggo et al. (2017)
120°C, 4 gNaOH/ 100gVS	1.5	0.11	+45	Riggo et al. (2017)

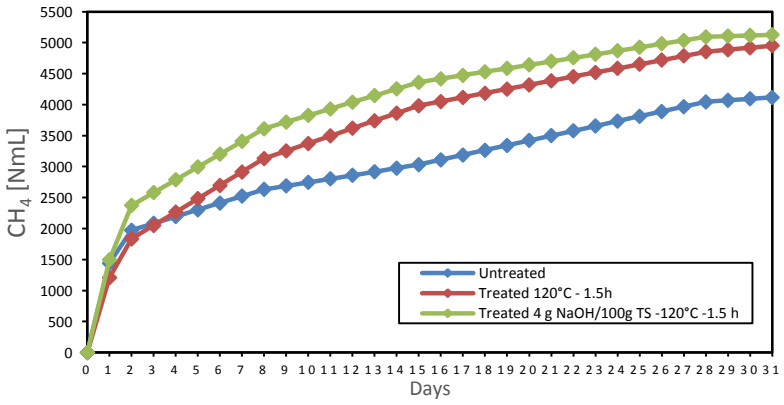

Figure 1. Daily methane production. The reported values have not been subtracted from the methane production from the inoculum

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