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Anaerobic Digestion of Microalgal Residues to Enhance the Energetic Profit of Biocrude Production

Elia Armandina Ramos Tercero*[a], Luca Alibardi[b], Raffaello Cossu[a], Alberto Bertucco[a]

[a]Department of Industrial Engineering, University of Padova, Via Marzolo 9, 35131 Padova, Italy
[b]Department of Civil, Environmental and Architectural Engineering, University of Padova, Via Marzolo 9, 35131 Padova, Italy.
eliaarmandina.ramostercero@studenti.unipd.it

Microalgae as source of energy have generated an enormous interest in the last decades. Microalgae seem to be the most feasible option to obtain renewable liquid fuels due to high growth rates, CO_2 fixation capability and large accumulation of oil compared to other crop plants. The bottleneck of this technology is anyway represented by the costs of the process, both from the economic and energetic points of view.
In order to reduce the energetic costs and to make microalgae cultivation more attractive, the possibility of exploiting the energetic content of microalgal biomass residues after oil extraction by means of anaerobic digestion to produce biogas was studied. Two microalgal species, *Scenedesmus obliquus* and *Chlorella protothecoides,* selected for their high oil contents and fast growth rates, were tested for biogas production, before and after the oil extraction. Oil extraction was carried out by Soxhlet method, using a mixture of methanol and chloroform as the solvent. Biochemical Methane Potential (BMP) tests were carried out to evaluate biogas production capacity from microalgae and degradability rates. Two different kinds of inocula were used to compare the specific hydrolytic capacities and to assess the most suitable one to maximize the biogas conversion of microalgae. The digestion tests were performed at controlled temperature of 37 °C, in batch reactors. Production of biogas and the proportion of CO_2 and CH_4 content were measured. The results are discussed in view of feasible industrial application.

1. Introduction

In recent years the interest in liquid biofuels has increased because of the declining in petroleum reservoirs and growing demand of energy. Microalgae seem to be a promising energy source, due to their advantages i.e. higher productivity compared with energy crops. In view of future exploitation of microalgae for biofuels production, the energetic profitability must be maximized by means of bioprocesses and technological chains that increase the energy return on energy investment (EROEI) (Ramos Tercero et al., 2013). To this regard the reuse of biomass residues obtained after oil extraction plays an important role on increasing EROEI for biodiesel production from microalgae. The energetic utilization of residues after biodiesel production provides benefits also from an economic point of view. The production of biocrude is in fact not sustainable yet (Campbell et al., 2011) as the costs of biocrude and biodiesel are still high and not competitive with commercial products (Davis et al., 2011). Methane production by means of anaerobic digestion (AD) from biomass residues could be a practical and competitive alternative to enhance energy return and to reduce costs of biodiesel production from microalgae. Current investigations in which AD is used for microalgal biomass to produce biogas are addressed with different purposes, e.i. integrate AD in biorefinery facility (Mussgnug et al., 2010). Methane potential productions from microalgae are species-specific and vary from about 200 to 400 mL CH_4/g VS (Frigon et al., 2013). Different digestion conditions in terms of temperature, biomass concentration, carbon to nitrogen ratio and retention time for biogas production from *Chlorella* biomass residues after biodiesel production with transesterification process, were evaluated by Ehimen et al., (2011). Methane potential production can be also influenced by microalgae growth conditions i.e. photoautotrophic, using wastewater as resource of nutrients (Alcántara

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et al., 2013), or mixotrophic growth (Singh et al., 2011). Substrate/inoculum ratio was also investigated (Alzate et al., 2012).
In this study the biochemical methane potential (BMP) of *S. obliquus* and *C. protothecoides* were investigated, *S. obliquus* characterized by its high lipid content (Sforza et al., 2013) can be used for biodiesel production (Mata et al., 2013) while *C. protothecoides* has been resulted an efficient species for depuration of urban wastewaters (Ramos Tercero et al., 2014). Two kinds of inocula were tested in order to determine their applicability with microalgae as substrate and to compare their specific hydrolytic capacities. Hydrolysis in fact represents the limiting factor in anaerobic digestion of complex organics (Vavilin et al., 2008) and a rapid and efficient hydrolysis can improve the overall biogas conversion of microalgae. Kinetic constants and production rates of biogas and methane were determined. Furthermore, *S. obliquus* biomass after lipid extraction was tested as a substrate for anaerobic digestion, obtaining interesting results regarding the extraction method and in particular on the type of solvent mixture used.

2. Materials and Methods

2.1 Microalgae strains and cultivation

Two species of microalgae were investigated: *Scenedesmus obliquus* 276-7 and *Chlorella protothecoides* 33.80 strains were obtained from SAG-Goettingen and were cultured in freshwater media (BG11) (Rippka et al., 1979). Maintenance and propagation of cultures were performed using the same medium added with 10 g L^{-1} of Plant Agar (Duchefa Biochemie). Temperature was kept at 23 ± 1°C in a growth chamber. *S. obliquus* was grown in a batch flat panel reactor of 50 L bubbled with a flow of air enriched with CO_2 to maintain the pH between 6 to 7 and provide a non-limiting concentration of carbon source. Light irradiance provided was 200 µE m^{-2} s^{-1}, measured by a photoradiometer (Model LI-189, LI-COR, USA). *C. protothecoides* was grown in continuous flat panel reactor of 2 L, under CO_2-air bubbling (5%v/v), and irradiated by fluorescent tubes at the intensity of 237 µE m^{-2} s^{-1}.

2.2 Anaerobic inoculum

Biogas production experiments were carried out using two different types of inocula. The first one (hereafter named G) was an anaerobic granular sludge, collected from a real scale Upflow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy. Inoculum G was composed by a Total Solids (TS) concentration of 11 % and a Volatile Solids (VS) concentration of 70 % referred to dry weight. The second inoculum (hereafter named CN) was an anaerobic sludge collected from an anaerobic digester of sewage sludge from a municipal wastewater treatment plant located in Padova, Italy. Inoculum CN was characterized by a TS concentration of 5 % and a VS concentration of 55 % referred to dry weight.

2.3 Experimental set up

Biochemical Methane Potential (BMP) tests were carried out in batch conditions, in reactors of 0.5 L, hermetically closed by means of silicon plug enabling sampling of the gas produced during the fermentation. The working volume of each reactor was 0.3 L. After preparation the reactors were flushed with N_2 gas and incubated without stirring in a thermostatic chamber at 35 ± 2 °C. The incubation time was approximately 45 days. Blank tests using the inoculum alone were also prepared to measure the quantity of biogas produced only by the biomass used as inoculum. The biogas volume was measured adopting the dislocation method. By this method the excessive pressure produced in the reactor by biogas production process moves an equal quantity of liquid to a second bottle. The volume of the liquid moved, and, accordingly, the volume of biogas produced, is measured with a graduated cylinder. The liquid used in measurements was an acidified (pH<3) and saline (NaCl 25 %) solution in order to avoid the dissolution of methane and carbon dioxide into the liquid. Conversely, the quality of the biogas produced in percentage terms of carbon dioxide and methane was measured using a portable gas analyzer (LFG 20, Eco-Control).
Methane and carbon dioxide volumes produced in the time interval between two subsequent measurements, were calculated using a model taking into consideration the gas concentration at time t and time t-1, together with the total volume of biogas produced at time t, the concentration of the specific gas (methane or carbon dioxide) at times t and t-1, and the volume of the reactors' head space (Ginkel et al., 2005). The following equation was applied:

$$V_{C,t} = C_{C,t} * V_{G,t} + V_H * (C_{C,t} - C_{C,t-1}) \quad (1)$$

where: $V_{C,t}$ is the volume of generic biogas (CH_4 or CO_2) produced in the interval between t(d) and t-1(d); $C_{C,t}$ and $C_{C,t-1}$ represent the gas (CH_4 or CO_2) concentrations measured at times t(d) and t-1(d); $V_{G,t}$ is the volume of biogas produced between time t(d) and t-1(d); V_H is the volume of the reactors' headspace.
Batch tests were carried out in triplicates for each sample and three control test for each condition.

To compare results obtained from the batch tests, data were interpolated using a Gompertz equation (Favaro et al., 2013). The Gompertz equation used is as follows:

$$B(t) = B_0 * exp\left\{-exp\left[k * e\frac{(\lambda - t)}{B_0}\right] + 1\right\} \quad (2)$$

where: B(t) is the cumulative biogas or methane production at time t (d) (mL/g VS); B_0 is the maximum biogas or methane production (mL/g VS); k is the biogas/methane production rate (mL/d g VS); λ is the latency phase (d); e is Euler's number. The quantities B_0, λ, and k constants have been obtained based on experimental data the for biogas and for methane, with a non-linear regression.

2.4 Analytical methods

Total lipids of microalgal biomass were extracted overnight from dried cells using chloroform:methanol (1:2 v/v) in accordance with Bligh & Dyer method, in a Soxhlet apparatus. The lipid mass was measured gravimetrically after solvent removal using rotary evaporator. Chemical and physical characterization parameters were measured in accordance to standard methods (APHA and AWWA, 1999). Data on biogas and methane productions are reported in terms of 1 atm of pressure and 0 °C of temperature.

3. Results and discussion

3.1 Anaerobic digestion of fresh microalgal biomass

Biomethane potential (BMP) of *S. obliquus* was investigated with two Food/Microorganism ratios (F/M ratio as g VS substrate/g VS inoculum). The F/M ratios applied were 0.5 and 0.1, using both inocula reported in section 2.2. The best yield was obtained with inoculum CN and 0.5 of F/M ratio, resulting in a biogas production of 420 mL gVS^{-1} composed by 55 % of methane. The suitability of fresh *S. obliquus* biomass for the production of biogas is show in Figure 1, where production as a function of time is shown, comparing the performance of both inocula, with F/M ratio of 0.5 (Figure 1A) and 0.1 (Figure 1B). It can be easily noted that maximum biogas production is achieved with the flocculent sludge (CN) in a shorter period. With both organic loads, methane production started almost immediately. On the other hand, by using the granular sludge (G), an initial adaptation time of about a week was observed at higher F/M ratio. With the lower F/M value this lag phase is almost depreciable. This is in agreement with study on hydrolysis rates indicating that at low F/M ratios, the higher amount of bacteria leads to a higher enzyme availability and a faster substrate hydrolysis and consumption (Trzcinski and Stuckey, 2012). In addition, it is possible to observe that the slope of the curve, i.e. the production rate, varied considerably between granular sludge and flocculent sludge when the F/M is higher, whereas if the F/M ratio is lower with both sludge the difference is minimal. The same behavior is observed in the cumulate production of biogas, the difference in production is more marked when the ratio is higher, although in all cases the net methane production is good. Biogas production and composition of CO_2 and CH_4 were also investigated with the specie *C. protothecoides* in order to compare the performance and to investigate if production could be influenced by the microalgae species. In this case, a F/M ratio of 0.5 was used, using both inocula described previously. Results are shown in Figure 2. It can be observed that, with both inocula, the trend of biogas production was very similar to that obtained with the species *S. obliquus* at the same F/M ratio, highlighting a lag phase with G inoculum and immediate production with CN sludge. Although the final production of biogas was relatively lower with *C. protothecoides* with both inocula, a major difference was observed with CN, this is probably due to the difference in biochemical composition of microalgae species, causing the variance in BMP (Sialve et al., 2009). Values of biogas production and composition are reported in Table 1.

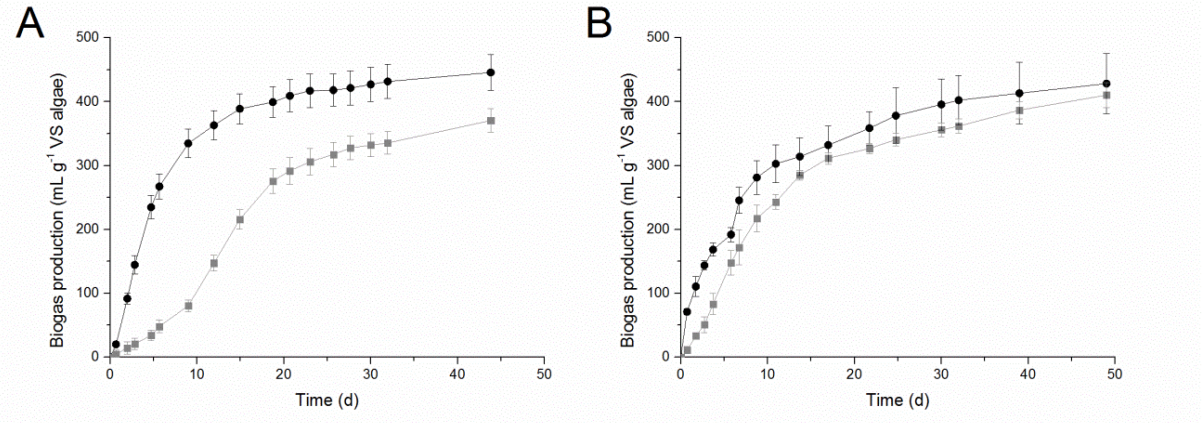

Figure 1. S. obliquus biogas production curves in mL/gVS of microalgae biomass, over time. A) F/M ratio of 0.5, B) F/M ratio of 0.1. Black circles represent inoculum CN, grey squares inoculum G.

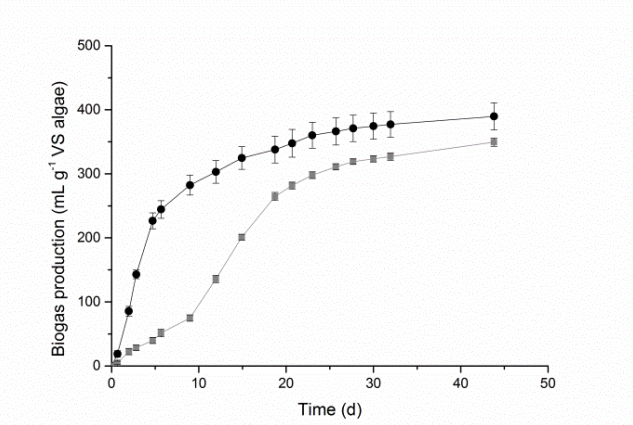

Figure 2. C. protothecoides biogas production curves in mL/gVS of microalgae biomass, over time. Testing the different inocula, CN is represented by black circles, and G by grey squares, both experiments with F/M ratio of 0.5.

Table 1: Biogas and methane production from fresh microalgae at day 30 of the curve (n=3; ±SD).

Species	Inoculum	F/M (gVS/gVS)	Biogas (mL/g VS)	Methane (mL/g VS)	% Methane
S. obliquus	CN	0.1	395 ± 40	215 ± 4.3	55
		0.5	420 ± 26	230 ± 3.8	55
	G	0.1	350 ± 10	190 ± 7.2	55
		0.5	331 ± 17	176 ± 8.4	53
C. protothecoides	CN	0.5	371 ± 20	206 ± 8.9	56
	G	0.5	319 ± 4.9	166 ± 4.9	52

3.2 Production rates and kinetic parameters

Results of kinetic analysis of biogas and methane productions are reported in Table 2. Biogas production rate with inoculum CN resulted higher than those from tests with inoculum G, even in the cases with lower F/M ratio. This can be explained by the fact that inoculum CN is a flocculent type of anaerobic biomass. Therefore the distribution of inoculum in the reactor is more homogenous allowing a higher contact between bacteria and microalgae. Inoculum G is characterized by fast settleability and bacteria are grouped in complete communities only in the granule. The contact between the substrate and inoculum is more limited and distribution of organics to be degrades is mainly guided by diffusion effects without constant mixing of the reactors. This effect influenced mainly the first phases of the anaerobic degradation, the hydrolysis of organics, resulting in lower biogas production rates in the first 10 days of degradation. After this initial phase, biogas and methane productions reached comparable results with CN inoculum (at day 30 of the curve).

Table 2: Kinetic constants and production rates of biogas and methane obtained from data interpolation.

Species	Inoculum	F/M	Biogas				Methane			
			Bo (mL/g VS)	k (mL/d g VS)	λ (d)	R^2	Bo (mL/g VS)	k (mL/d g VS)	λ (d)	R^2
S. obliquus	CN	0.1	394.60	34.93	0.00	0.988	213.88	20.88	0.00	0.986
		0.5	415.07	48.92	0.18	0.997	228.89	29.61	0.31	0.998
	G	0.1	386.64	23.50	0.27	0.994	208.79	13.14	-0.08	0.995
		0.5	362.79	19.58	4.08	0.998	188.01	12.05	5.15	0.998
C. protothecoides	CN	0.5	360.60	47.12	0.19	0.994	198.79	28.49	0.29	0.996
	G	0.5	354.07	17.43	3.11	0.997	178.80	12.50	6.27	0.998

As can be observed in Table 2 (columns refer k), the production rates of biogas and methane (k) are proportionally correlated in all cases investigated, the higher the biogas production rate the faster the methane production. Higher speeds were observed with *S. obliquus* with CN and F/M of 0.5, being 48.92 and 29.61 (mL/d g VS) in biogas production and methane respectively, followed by 47.12 and 28.49 (mL/d g VS) achieved with *C. protothecoides* with CN and F/M of 0.5. In these cases little influence was

observed with respect of the microalgae species. The same behavior can be noticed for the biogas production curves, having rates very similar in both species with inoculum G, 17.43 (mL/d g VS) with *C. protothecoides* and 19.58 (mL/d g VS) with *S. obliquus*, while methane around 12 (mL/d g VS) for both. In this case the rate was less than a half then comparing G with CN. The results indicate that inoculum CN is characterized by faster hydrolytic capacity for the specific type of substrate used. This fact could explain the lag phase with inoculum G of about 3 to 4 days for biogas and 5 to 6 days for methane. When substrate concentration increased in the reactors, without constant mixing, the methanogenic bacteria only degrade the substrate that is in direct contact with them. On the contrary, this behavior is not observed when the F/M ratio was lower because a higher concentration of microorganisms was present and a higher contact area with the substrate was possible.

3.3 Biogas from de-oiled biomass

Previous work in energetic analysis of biocrude production indicates that the process can be energetically self-sufficient (Ramos Tercero et al., 2013) as long as the energy in the residual biomass is exploited. For this reason the BMP of de-oiled biomass was experimented. The mixture chloroform/methanol is considered as an excellent solvent for extracting lipids from biomass of microalgae (Lam and Lee, 2012). However BMP tests showed evidence of strong inhibition of methanogenic activity during tests within microalgal residues after oil extraction. Test was carried out using de-oiled *S. obliquus* as substrate, under F/M ratio of 0.5 (Figure 3A) and 0.3 (Figure 3B) with CN inoculum. As reported in Figure 3, methane was not detectable in biogas and only CO_2 was produced. From these results it can be hypothesized that the chloroform/methanol mixture, even if particularly volatile and probably it was largely removed from the biomass before digestion, can still produce inhibitory effects for methanogenic bacteria even at a lower concentration of substrate with the same concentration of bacteria (F/M 0.3). The same behavior was observed by Zhao et al., (2012). Biogas analysis showed also concentration of H_2S, higher than 1,000 ppm (data not shown). Apparently the methanogenic inhibition allowed sulfur-reducing bacteria to predominate the final digestion phases thanks to their lower sensibility to inhibition or unfordable digestion conditions if compared to methanogens (Deublein and Steinhauser, 2008).

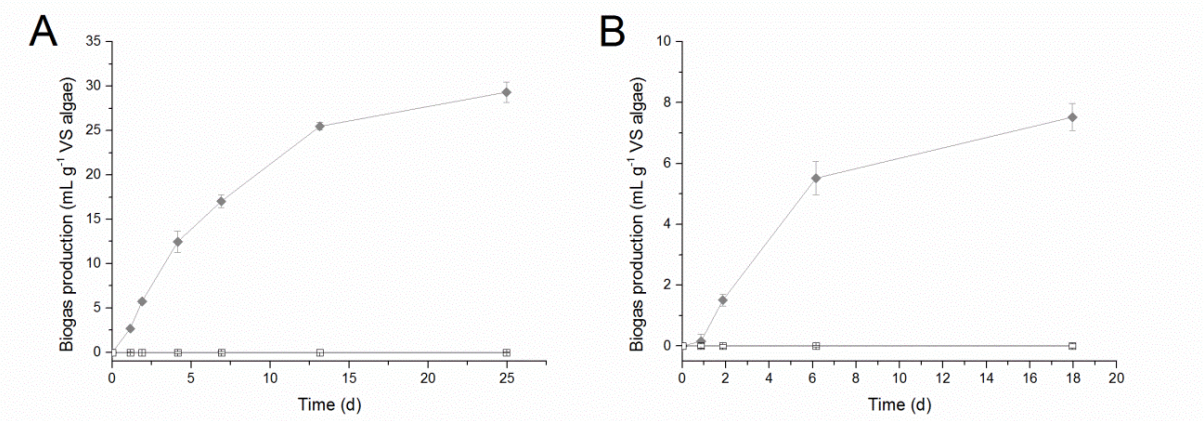

Figure 3. Cumulative CO_2 (grey rhombus) and CH_4 (squares) production in mL/g VS of de-oiled microalgae biomass, over time, A) F/M = 0.5, B) F/M = 0.3.

4. Conclusions

In this work, anaerobic digestion of microalgal biomass was investigated, resulting in a feasible operation with degradation kinetics comparable to those of conventional biomass used for biogas production. Reuse of biomass residues after oil extraction could improve the energy profits of the process but it is important to highlight the essential role of the extraction method, in particular of the solvents. The mixture chloroform/methanol must be object of more studies to propose an alternative method to totally eliminate the solvent content in the biomass. In addition different solvent mixtures for extraction must be investigated, if the aim is to produce biomethane with de-oiled biomass. Anaerobic digestion can play a central role in the biorefinery concept for microalgal energy exploitation, as it is a well establish technology and fresh microalgae represent suitable substrates for biogas production. In microalgae production process, combination of bioprocesses with chemical processes anyway must be assessed from a global point of view in order to define the optimal process schemes that allow the highest energy conversion rates maintaining the best process condition for the biological degradation.

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