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Short communication

Biomethane production using fresh and thermally pretreated *Chlorella vulgaris* biomass: A comparison of batch and semi-continuous feeding mode

Lara Mendez[a], Ahmed Mahdy[a,b], Mercedes Ballesteros[a,c], Cristina González-Fernández[a,*]

[a] IMDEA Energy, Avda. Ramón de la Sagra 3, Móstoles, Madrid 28935, Spain
[b] Department of Agricultural Microbiology, Zagazig University, Sharkia 44519, Egypt
[c] CIEMAT, Avda. Complutense, Madrid 28040, Spain

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ABSTRACT

The main challenge for an efficient anaerobic digestion using microalgal substrates is the optimization of cell wall disruption pretreatments. The objective of this work was to assess methane yield improvement using thermally pretreated *Chlorella vulgaris* in semicontinuous feeding operation. After thermal pretreatment, organic matter hydrolysis was confirmed by a 10-fold increase in soluble chemical oxygen demand (COD). Total COD removals ranged 36.5–49.7% for reactors fed with raw and thermally pretreated biomass, respectively. Despite the high nitrogen mineralization registered (52 and 78% for raw and thermally treated *C. vulgaris*), no ammonium/ammonia inhibition was detected. The reactor fed with thermally pretreated biomass resulted in 50% methane yield enhancement compared with the reactor fed with raw biomass. Even though no common inhibitions were detected, methane yield values attained were comparably lower than that obtained in batch mode digestion. This study highlighted the need of further testing promising pretreatments in semicontinuous mode anaerobic digestion before claiming their effectiveness.

1. Introduction

Photosynthetic microorganisms, such as microalgae, are nowadays studied as potential feedstock for next generation biofuels as an alternative to fossil fuels. Even though the upstream benefits of cultivating microalgae are clear (wastewater bioremediation, carbon dioxide mitigation and nutrients recovery, Ji et al., 2013), the problem encountered nowadays are related to the downstream biomass processing. Among biofuel production processes using this kind of biomass, biogas generation seems to be the least complex since all the organic macromolecules (proteins, carbohydrates and lipids) are used. Microalgae cell-wall provides resistance to bacterial degradation and thereby hinders or inhibits hydrolytic phase of the anaerobic digestion process (Mendez et al., 2013). The main challenge is the optimization of pretreatments intended for cell-wall disruption prior to anaerobic digestion (Mendez et al., 2013; González-Fernández et al., 2012; Cho et al., 2013). However, pretreatments can lead to by-products, such as those generated during the reactions of proteins and carbohydrates degradation (Mendez et al., 2013; Monlau et al., 2014) that can also reduce the methane yield. Previous studies have reported the use of several pretreatments, such as heat application on biomass, for an enhanced methane production in batch tests (Mendez et al., 2013; Passos and Ferrer, 2015; Mendez et al., 2014a). Data obtained in batch assays can provide guidance, but assessing the benefits of pretreatments in semicontinuously fed reactors (continuous stirred tank reactors, CSTR) is highly required in order to study in-depth the performance of anaerobic microorganisms fed with pretreated microalgae biomass. As a matter of fact, only few investigations have moved forward to CSTR (Passos and Ferrer, 2015; Schwede et al., 2013; González-Fernández et al., 2013). The performance of CSTRs fed with microalgae is still very limited and required to further confirm the beneficial effect of pretreatments. In this context, thermal pretreatment applied to *Chlorella vulgaris* has been shown beneficial in batch mode anaerobic digestions (Mendez et al., 2013, 2014a) by solubilizing polymeric cell structures (Mendez et al., 2014b), but confirming these in CSTR digestion mode is required. The aim of the present study was to compare the performance of

* Corresponding author.
E-mail address: cristina.gonzalez@imdea.org (C. González-Fernández).

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Table 1
Chemical chracterization of the different batches employed to feed the CSTRs.

Chemical parameter	Not treated biomass		Thermally pretreated biomass	
	Average	St D	Average	St D
TS ($g\,L^{-1}$)	59.7	5.1	65.2	0.4
VS ($g\,L^{-1}$)	55.1	4.3	60.2	0.8
tCOD ($g\,L^{-1}$)	103.4	9.4	99.1	11.6
sCOD ($g\,L^{-1}$)	1.9	0.5	19.9	3.5
TKN ($g\,L^{-1}$)	5.7	0.3	7.0	0.5
NH_4^+ ($g\,L^{-1}$)	0.4	0.3	0.3	0.5

two CSTRs digesting raw and thermally pretreated *C. vulgaris* in terms of methane production, organic matter degradation, nitrogen mineralization and potential inhibitors.

2. Materials and methods

2.1. Microorganisms

The selected microalga, namely *C. vulgaris*, was grown in a lab-scale photobioreactor under continuous artificial light (fluorescent, 6000 luxes) and agitation supplied by air bubbling. This microalga was selected as model microorganisms due to its easiness to cultivate outdoors and its robustness (hard cell wall). This biomass was cultivated on Modified Bold Basal Medium (Mendez et al., 2014a). Culture broth was periodically collected and microalgae were concentrated by centrifugation (Heraeus Thermo Scientific) at 5000 rpm for 10 min. Chemical characterization of the microalgae used as substrates can be seen in Table 1. On the other hand, the anaerobic sludge employed as inoculum for the CSTRs was collected at the wastewater treatment plant of Valladolid (Spain). Anaerobic biomass presented a total solids (TS) concentration of 21.1 $g\,L^{-1}$ and volatile solids (VS)/TS of around 60%.

Microalgae batches were diluted to a standard concentration of 65 $g\,TS\,L^{-1}$ prior to thermal pretreatment (Mendez et al., 2014a). Thermal pretreatment was carried out by autoclaving the biomass at 120 °C for 40 min (Mendez et al., 2014a).

2.2. Semi-continuous CSTRs

Anaerobic digestion was conducted in two CSTRs with a total volume of 1.5 L (1 L liquid volume and 0.5 L headspace volume). The reactors worked under mesophilic conditions (35 °C) using a water jacket connected to a water bath. Constant mixing of the digesters was accomplished with magnetic stirrers at 250 rpm. Biogas production was measured by water displacement. Methane content was analyzed by gas chromatography. Reactors were operated on semicontinuous mode, i.e. the same volume was withdrawn and fed in a daily basis using plastic syringes. To verify the enhancement of biogas production after pretreatment, one reactor was fed with raw *C. vulgaris* biomass (not-treated) and the other one was fed with thermally pretreated biomass. The organic loading rate (OLR) was set at 1.5 $g\,COD\,L^{-1}\,day^{-1}$ for both reactors. Previous studies performed in batch mode showed that methane productivity was almost negligible after 15 days of digestion (Passos and Ferrer, 2015; González-Fernández et al., 2013), thus a hydraulic retention time (HRT) of 15 days was selected for the present study. After three HRT, the system was considered at steady state.

2.3. Analytic procedures

Total solids (TS), volatile solids (VS) and total Kjeldahl nitrogen (TKN) were measured according to Standard Methods (Eaton et al., 2005). Proteins were calculated by multiplying TKN results by 5.95 (González López et al., 2010). Carbohydrates content was measured by phenol-sulphuric acid method (DuBois et al., 1965). Colorimetric methods were used for COD (Merck, ISO 15705) and ammonium (Merck, ISO 7150-1) determination. Soluble fractions were obtained after centrifugation at 14,600 rpm for 5 min (Minispin Eppendorf 5424).

Biogas composition was measured by gas chromatography (Agilent 7820A) equipped with 19095P-Q04 30 m column (HP-PLOT Q) connected to a flame ionization detector and thermal conductivity detector at 250 °C and H_2 at 4.5 $mL\,min^{-1}$ as carrier gas. Volatile fatty acids (VFAs) were analyzed in soluble phase by high-performance liquid chromatography in an Agilent 1260 chromatograph equipped with Aminex HPX-87H column and UV–vis detector.

3. Results and discussion

3.1. C. vulgaris *used as a substrate: characterization and hydrolysis after thermal pretreatment*

The characterization of fresh and thermally pretreated biomass is shown in Table 1. 92% of the TS were represented by VS and hence only 8% of the dry matter was inorganic material (ash). The freshness of the biomass can be observed by the low soluble COD in the non-pretreated biomass with regard to the total COD. The total COD/VS ratio ranged 1.70–1.88 for both biomass. This value is in good agreement with other microalgae biomass characterized previously (González-Fernández et al., 2013; Ramos-Suárez and Carreras, 2014). The total COD/TKN ratio was approximately 15. This value can change depending on the protein content of the microalgae which at the same time depends on microalgae growth conditions. Microalgae grown in wastewater or synthetic media provided similar values (Mendez et al., 2013; Passos and Ferrer, 2015), and therefore this value was considered to be in the conventional range. As a matter of fact, microalgae biomass composition studied herein exhibited 64.2 ± 3.4% w/w TS of proteins and 20.1 ± 3.2% w/w TS of carbohydrates. Taking these values into consideration together with the ash content, the remaining lipid fraction was estimated to be around 10%. This macromolecular profile is quite similar to other reported microalgae biomasses grown in non-stressed conditions (Mendez et al., 2013, 2014a).

In view of the promising results obtained by Mendez et al. (2013) in terms of organic matter solubilization and methane yield, *C. vulgaris* biomass was pretreated thermally at 120 °C for 40 min. With regard to that of raw biomass, thermal pretreatment of *C. vulgaris* led to an increase in the soluble phase of 4.5-fold for carbohydrates and 1.9-fold for proteins (Mendez et al., 2013). This solubilization of complex macromolecules was attributed to the cell-wall disruption taking place during thermal pretreatment. In the present study, the hydrolysis efficiency resulted in an enhancement of soluble COD of 10-fold. This value is similar to previous reports where 6-fold enhancement was registered when treating a mixture of 70% *Chlorella* sp. and *Scenedesmus* sp. at 120 °C for 30 min (Cho et al., 2013). Thus, the effectiveness of the thermal pretreatment was proven.

3.2. CSTRs semi-continuous anaerobic digestion

3.2.1. Organic matter fate

In order to investigate the digestion of *C. vulgaris* biomass in semicontinuous mode, two CSTRs were run in parallel (not treated and thermally treated biomass). Methane yield measured in the CSTR fed with raw *C. vulgaris* ranged 85 ± 15 $mL\,CH_4\,g\,COD\,in.^{-1}$ while the total COD and VS removal averaged 36.5 ± 6.0% and 31.9 ± 4.0%, respectively (Fig. 1). The values of soluble COD removed