Fuel xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Fuel

journal homepage: www.elsevier.com/locate/fuel

Protease pretreated *Chlorella vulgaris* biomass bioconversion to methane via semi-continuous anaerobic digestion

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HIGHLIGHTS

- Performance of CSTR fed with protease pretreated *C. vulgaris* biomass.
- Protease treatment enhanced organic matter solubilization and methane production.
- Protease treatment resulted in high nitrogen mineralization.
- High effluent ammonium concentration mediated an unbalanced microbial activity.

ARTICLE INFO

Article history:
Received 10 January 2015
Received in revised form 25 February 2015
Accepted 21 April 2015
Available online xxxx

Keywords:
Anaerobic digestion
Batch
Semi-continuous
Chlorella vulgaris
Methane production
Pretreatment

ABSTRACT

This study evaluated the enhancement of biogas production in semi-continuous anaerobic digestion fed with enzymatically pretreated *Chlorella vulgaris*. Organic matter in soluble phase increased from 2.5% to 45% after pretreatment with proteases. The soluble COD was easily biodegradable and almost all organic matter available in soluble phase was removed (94.4%) in the anaerobic reactor (CSTR). Methane yield increased 2.6-fold when compared to the CSTR fed with raw biomass. With regard to the nitrogen fate, 77% of the organic nitrogen was mineralized during anaerobic digestion. Slight ammonium inhibition was detected due to the high nitrogen mineralization registered. pHs remained close to neutrality throughout the experimental time. VFAs were accumulated in the last retention time as a consequence of the slight anaerobic digestion inhibition, which revealed an unbalanced equilibrium among the anaerobic microbial population. This fact was corroborated in batch digestion assays. The anaerobic sludge collected from the CSTR exhibited a different profile in terms of methane productivity when compared to the inoculum from WWTP normally used. Further studies are nowadays undertaken to overcome the inhibition and thus increase the methane yield.

1. Introduction

Energy supply and cost are key issues for most countries. The production of bioenergy using different biomasses is considered as one of the most important future renewable energy sources since it releases pressure on finite natural resources. Biomass is regarded as a sustainable feedstock for energy purposes [1]. This feedstock is synthesized by photosynthetic capture of solar energy and stored as chemical energy. Microalgae, aquatic biomass, seems to present several advantages over land crops since the former exhibits higher biomass productivity and do not compete with arable land. Three main conversion processes; namely, fermentation, oil extraction, and anaerobic digestion; have been investigated lately to convert biomass into energy. The criterion for the selection of the most appropriate conversion process is biomass composition. Since microalgae are not exhibiting high fermentable sugars or transesterificable triglycerides content under non-restrictive cultivation conditions, bioethanol and biodiesel production do not seem the most suitable energy form to be produced using this substrate. On the other hand, biogas production can be achieved by anaerobic digestion using all macromolecular fractions, i.e. carbohydrates, proteins and lipids, from cyanobacteria and microalgae biomass [2,3].

Although anaerobic microorganisms can use almost all types of organic matter for bio-methane conversion, two serious hurdles have been encountered when using micro-algal biomass as substrates: (i) the rigid cell wall of some microalgae strains that hinders an efficient bioconversion by hampering the hydrolytic stage of the digestion [4], and (ii) the low carbon to nitrogen (C/N) ratio

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http://dx.doi.org/10.1016/j.fuel.2015.04.052
0016-2361/

Nomenclature

CSTR	continuously stirred tank reactor	VFAs	volatile fatty acids
BMP	biochemical methane potential	HRT	hydraulic retention time
tCOD	total chemical oxygen demand	OLR	organic loading rate
sCOD	soluble chemical oxygen demand	WWTP	wastewater treatment plant
TS	total solids		
VS	volatile solids		
TKN	total Kjeldahl nitrogen		

characteristic of this biomass [5] that can result in anaerobic microorganisms inhibition by the high NH_4^+/NH_3 concentration taking place during digestion [6].

Thereby, cell wall hydrolysis/disruption prior to anaerobic digestion is a must to improve the anaerobic biodegradability of microalgae biomass. In this sense, numerous pretreatment strategies such as thermal [7,8], thermochemical [9], microwave [10], ultrasound [11], chemical [12] and enzymes addition [13,14] have been recently studied to solubilize organic matter and enhance the hydrolytic limiting stage of anaerobic digestion. Among these methods, enzymatic pretreatment involves mild conditions and minimal formation of toxic byproducts. Protease addition has been shown to provide high organic matter solubilization and increase the inherent low biodegradability of microalgae biomasses such as *Chlorella* sp. and *Scenedesmus* sp. [15]. Enzymatic pretreatment of *Chlorella vulgaris* with proteases evidenced an organic matter solubilization of 50% in comparison to 1–5% available in the non-treated biomass [16]. As a result, the anaerobic biodegradability was enhanced from 40% registered for the raw material to 70% for the enzymatically pretreated biomass. Despite of the high protein content (63.8%) and the use of a proteolytic enzymatic cocktail, no ammonia inhibition was detected in the batch anaerobic digestion assays. At this point it should be stressed out that anaerobic digestion using pretreated microalgae biomass has been mostly carried out in batch mode. Methane potential batch assays are used to determine the amount of organic carbon in a given material that can be anaerobically converted to methane. Data from batch assays can provide guidance, but assessing the benefits of a pretreatment in a continuously fed reactor (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, the follow up of CSTRs can result in lower methane yields than observed on batch assays. Batch assays results could be extrapolated to CSTRs only when no inhibition occurs.

The present study was designed to evaluate the performance of a CSTR fed semi-continuously with enzymatically pretreated *C. vulgaris* biomass. The pretreatment was conducted in accordance to previous results obtained during the optimization of *C. vulgaris* hydrolysis by protease addition [13,16]. Organic matter removal, biogas quality, methane yield, and nitrogen mineralization achieved during anaerobic digestion of this substrate was assessed. Additionally, ammonium and volatile fatty acids concentration of the effluents were monitored in order to discard the most common inhibitions related to protein rich substrates and organic overloading, respectively.

2. Material and methods

2.1. Substrate and inoculum

C. vulgaris cultivation media, operational growing conditions and harvesting method employed can be found elsewhere [16]. The CSTRs were fed with raw and pretreated *C. vulgaris*. Microalgae biomass was concentrated at 65 g TS L^{-1} using a centrifuge, and subjected to enzymatic hydrolysis (as described in Section 2.2.).

The inoculum employed in the semi-continuous fed CSTRs was anaerobic sludge (kindly provided by the wastewater treatment plant of Valladolid, Spain). Anaerobic sludge presented TS concentration of 10 g L^{-1} and VS/TS of around 60%. In the case of the batch mode assay, the anaerobic sludge was collected from the CSTR fed with the enzymatically pretreated biomass.

2.2. Microalgal biomass pretreatment

Enzymatic pretreatment conditions were applied to hydrolyze microalgae cell wall and solubilize organic matter. The biological catalyst selected was a commercial protease "Alcalase 2.5L" supplied by Novozymes (Denmark). This enzyme was selected in accordance to previous results on *C. vulgaris* cell wall hydrolysis [13]. The enzyme activity was 2.4 AU-A g^{-1} of endopeptidase activity according to the supplier information. The enzymatic dosage (0.585 AU) and biomass load of substrate (65 g L^{-1}) were selected in accordance to previous results [16]. The hydrolysis efficiency was defined according to the following equation:

$$\%\text{Hydrolysis efficiency} = \frac{\text{sCOD concentration after treatment} - \text{sCOD concentration in raw biomass}}{\text{tCOD concentration} - \text{sCOD concentration in raw material}} * 100 \quad (1)$$

2.3. Anaerobic digestion

2.3.1. Continuous stirred tank reactors

Two CSTRs with a working volume of 1 L were used as digesters. In order to prevent any photosynthetic activity, the digesters were covered with aluminum foil. The digesters were maintained at mesophilic temperature (35 °C) by circulating water through a water jacket. Constant mixing was provided by a magnetic stirrer.

Please cite this article in press as: Mahdy A et al. Protease pretreated *Chlorella vulgaris* biomass bioconversion to methane via semi-continuous anaerobic digestion. Fuel (2015), http://dx.doi.org/10.1016/j.fuel.2015.04.052