Bioresource Technology 257 (2018) 30–38

Contents lists available at ScienceDirect

Bioresource Technology

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

Effect of pretreatments on biogas production from microalgae biomass grown in pig manure treatment plants

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ARTICLE INFO

Keywords:
Anaerobic digestion
Fertilizer
Inhibition
Kinetic model
Methane

ABSTRACT

Methane production from pretreated and raw mixed microalgae biomass grown in pig manure was evaluated. Acid and basic pretreatments provided the highest volatile solids solubilisation (up to 81%) followed by alkaline-peroxide and ultrasounds (23%). Bead milling and steam explosion remarkably increased the methane production rate, although the highest yield (377 mL CH_4/g SV) was achieved by alkali pretreatment. Nevertheless, some pretreatments inhibited biogas production and resulted in lag phases of 7–9 days. Hence, experiments using only the pretreated solid phase were performed, which resulted in a decrease in the lag phase to 2–3 days for the alkali pretreatment and slightly increased biomass biodegradability of few samples. The limiting step during the BMP test (hydrolysis or microbial inhibition) for each pretreatment was elucidated using the goodness of fitting to a first order or a Gompertz model. Finally, the use of digestate as biofertilizer was evaluated applying a biorefinery concept.

1. Introduction

Over the past decades, the concurrent developments in society, science, and technology have resulted in a higher demand for energy. One of the principal challenges in today's society is to provide a reliable energy supply for the future, which is hindered by the increasing prices of oil and gas (Kavitha et al., 2017a). Multiple eco-friendly alternatives, such as the production of bioethanol, biodiesel or biogas from wastes, have been considered and developed to make processes more environmentally friendly and feasible. The conversion of residual biomass into biogas via anaerobic digestion is considered the simplest and most straightforward way, since it requires mild pretreatments and low-cost equipment (Kavitha et al., 2017b).

Biomass grown in wastewater treatment plants is a suitable substrate for biogas production. Among the possible biological wastewater treatment alternatives, the use of microalgae is an emerging challenge, especially for effluents such as pig manure with a high nutrient concentration. Microalgae are able to grow in these wastewaters assimilating organic matter, N and P. Although wastewater treatment coupled to the anaerobic digestion of the microalgae biomass produced is a sustainable and interesting alternative, most studies on biogas production from microalgae have focused on single species (Mussgnug et al., 2010).

The type of microalgae and the cultivation conditions are essential parameters affecting its macromolecular composition and the cell wall resistance, and hence its potential biogas production (Klassen et al., 2016). Murphy et al. (2015) reported different theoretical methane yields from each organic fractions of the biomass (1.390 L/g VS from lipids, 0.851 L/g VS from proteins, and 0.746 L/g VS from carbohydrates). Additionally, biomass grown in microalgae-based treatment plants contains resistant microalgae species and a huge number of bacteria. To evaluate the feasibility of the combined process of wastewater treatment and biomass valorisation, the study of biogas production from this type of mixed microalgae biomass is required (Jankowska et al., 2017).

The application of pretreatments to disrupt the cell wall represents a promising alternative to increase the biodegradability of mixed microalgae biomass composed of recalcitrant microalgae species. Most of the information reported in literature refers to microalgae grown in domestic wastewater. Passos et al., (2015) carried out different pretreatments such as ultrasound and hydrothermal pretreatments in a mixed microalgae biomass cultivated in domestic wastewater (*Stigeoclonium* sp. and *Monoraphidium* sp. and diatoms *Nitzschia* sp. and *Navicula* sp.). Hydrothermal pretreatment (130 °C) increased the methane yield (135 mL CH_4/g VS) compared to the untreated control (106 mL CH_4/g VS). However, in this case, ultrasound pretreatment (26700 J/g

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https://doi.org/10.1016/j.biortech.2018.02.063
Received 25 December 2017; Received in revised form 12 February 2018; Accepted 13 February 2018

TS) did not significantly improve methane production. In another study, Passos et al., (2016a) studied the effect of two thermochemical pretreatments (KOH and HCl) on biogas production from microalgal biomass. They reported an increase in methane production up to 82% and 86% compared to the untreated biomass (78 mL CH_4/g VS) for alkaline and acid pretreatments, respectively.

Nevertheless, Passos et al. (2016a) also observed an inhibitory effect under severe pretreatment conditions. Most of the reported degradation compounds generated by pretreatments in algae (Martín Juárez et al., 2016) or other types of biomasses were soluble and released to the liquid phase (Toquero and Bolado, 2014, Bolado-Rodríguez et al., 2016). Therefore, the systematic comparison of biogas production using both fractions (solid and liquid fractions) or only the solid fraction of pretreated samples will provide a valuable information about the effect of the pretreatment technology on the biodegradability of biomass and generation of inhibitory compounds.

Following the valorisation as biogas of the organic matter present in microalgae, a significant load of nutrient is expected in the digestates, especially from biomass grown in wastewater with high N and P content. The use of the residual effluent from microalgae anaerobic digestion as fertilizer would lead the integral valorisation of the mixed microalgae biomass (Acién et al., 2014).

This study aimed at investigating the production of biogas by anaerobic digestion of mixed algal biomass grown in pig manure treatment plants. This work evaluated first the efficiency of different pretreatments (bead mill, alkaline, steam explosion, alkali-peroxide, ultrasound, and acid pretreatments) under two extreme operating conditions on CH_4 productivity. Furthermore, the methane productions from the whole suspension and the only solid fraction from pretreatment were compared in terms of the methane production yield to evaluate the generation of any potential inhibition induced by the pretreatments, kinetic modelling being used to identify the limiting step of the anaerobic digestion of the pretreated biomass. Finally, the composition of the digestates was analysed and their potential use as bio-fertilizers was evaluated to recover the high nutrients load of pig manure using a bio-refinery approach.

2. Materials and methods

2.1. Microalgae biomass

Fresh mixed microalgae biomasses were cultivated in a thin-layer photobioreactor with a volume of 1200 L fed with pig manure diluted at 10% at two different times of the year: February and March. The composition during February was 23.67% carbohydrates, 43.31% proteins, 16.74% lipids, 83.17% volatile solids, and 987 mg O_2/kg of COD, all of them in a dry basis. The microalgae species were *Tetradesmus obliquus* (29%), *Tetradesmus lagerheimii* (26%), *Desmodesmus opoliensis* (16%), *Aphanothece saxicola* (11%), *Chlorella vulgaris* (5%), *Scenedesmus magnus* (4%), *Parachlorella kessleri* (3%), and others in lesser amounts. The composition during March was 38.11% carbohydrates, 24.83% proteins, 12.51% lipids, 74.5% % volatile solids and 1150 mg O_2/kg in a dry basis. The microalgae species were *Desmosdesmus opoliensis* (47%), *Navicula reichardtiana* (27%), *Tetradesmus obliquus* (12%), *Scenedesmus* sp. (9%), and *Scenedesmus acuminatus* (5%). The biomass was supplied by the Cajamar Foundation (Almeria, Spain) and centrifuged at 78.75% (February) and 77.91% (March) of moisture and refrigerated at 4 °C prior to use.

2.2. Pretreatments

The pretreatments performed for the biomass from February were bead mill, alkaline (NaOH), steam explosion, and alkaline-peroxide (H_2O_2) pretreatments, all of them at 5% (w/w) dry weight. Two levels of bead mill pretreatments (Postma et al., 2017) were carried out: A (small beads 1.25 mm and 5 min) and B (big beads 2.50 mm and 60 min), using distilled water in the mill (Pascal Engineering Co. Ltd) until 200 mL of total volume. The alkaline pretreatment was carried out in 1 L borosilicate bottles with NaOH 0.5 M (C) and 2 M (D). Adequate volumes of NaOH solutions (of the selected concentrations) were added to the known mass of microalgae to obtain 200 mL volume, and, then, suspensions were autoclaved at 121 °C for 60 min (Bolado-Rodríguez et al., 2016). The steam explosion pretreatment was carried out using saturated steam at 130 °C during 5 min (E) and at 170 °C during 20 min (F) in a 5 L stainless steel reactor filled with 800 mL of suspension (Alzate et al., 2012). After the selected operation time, the steam was flashed and the biomass was cooled down in another vessel (Marcos et al., 2013). For the alkaline-peroxide pretreatment, known mass of microalgae were placed in 1 L bottles and adequate volumes of H_2O_2 solutions of the selected concentrations 0.5% (w/w) (G) and 7.5% (w/w) (H) were added to obtain 200 mL of total volume (Martín Juárez et al., 2016). Then, the pH was adjusted to 11.5 with 2 M NaOH, a few drops of antifoam were added, and the systems were incubated in a rotatory shaker at 50 °C and 120 rpm for 60 min.

Ultrasound and acid (HCl) pretreatments at 5% (w/w) dry weight were performed on the biomass from March. The ultrasound pretreatment was carried to a total volume of 400 mL of microalgae biomass diluted with distilled water in Ultrasound Technology (Hielscher UIP1000hd), during 5 (I) and 21 min (J), (Alzate et al., 2012). Power was calculated to expend identical amount of energy (7186 J/g TS) for the two operation conditions, according to Eq. (1). This consumption of energy, considered a limit value, was calculated as the difference between energy from the maximum theoretical potential of biogas production and the experimental biogas production from the raw biomass.

$$Energy = \frac{P \cdot t}{V \cdot TS} \tag{1}$$

where P is the average ultrasonic power (watts), t is the ultrasonic time (seconds), V is the sample volume (liters), and TS is the initial total solid concentration (g TS/L).

The acid pretreatment was carried out in borosilicate bottles with HCl 0.5 (K) and 2 M (L) (Bolado-Rodríguez et al., 2016). The known mass values of microalgae were placed in 1 L bottles, adequate volumes of HCl solutions (of the selected concentrations) were added to obtain a volume of 200 mL, and suspensions were autoclaved at 121 °C for 60 min. All the pretreatments were conducted in duplicate.

After the pretreatments, the resulting suspensions were centrifuged at 10000 rpm, for 10 min. The solid and liquid fractions were weighed. Next, the total and the volatile solids were analyzed in the solid and liquid fractions and in the pretreated whole suspensions. Samples of whole pretreated suspensions (named 1) and only solid fractions (named 2) were stored at 4 °C for biogas production experiments. The following parameter was defined to calculate the percentage of volatile solids retained:

$$\text{Retained volatile solids} = \frac{\%SV_{solid\ fraction} \cdot g\ of\ dried\ solid\ fraction}{\%SV_{raw\ material} \cdot g\ of\ dried\ raw\ material} \cdot 100 \tag{2}$$

2.3. Biogas production

Biochemical methane potential (BMP) tests were carried out to study the biodegradability of the microalgae biomass in triplicate following the protocol of Angelidaki et al. (2009). Batch mode assays were performed under mesophilic conditions in 300 mL borosilicate glass bottles with a working volume of 100 mL. The effluent from a pilot scale mesophilic anaerobic digester processed mixed sludge from a municipal wastewater treatment plant, with a volatile solids (VS) concentration of 9.1 ± 0.08 g VS/kg was used as inoculum for the tests. Two series of experiments were performed to determine the influence of the pretreatment and the inhibitory effect of the compounds present in the liquid phase: (1) using the whole pretreated suspension; and (2)