Chemical Engineering Journal 242 (2014) 86–95

Contents lists available at ScienceDirect

Chemical Engineering Journal

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

Use of microalgae residues for biogas production

Juan L. Ramos-Suárez *, Nely Carreras

Environment Department, Ciemat. Avda. Complutense, 40, 28040 Madrid, Spain

HIGHLIGHTS

- *Scenedesmus* residues were successfully converted to methane.
- Extraction methods enhanced biodegradability and methane production.
- Highest methane production was obtained from amino acid-extracted biomass.
- Kinetics of the process was improved by co-digestion.

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 9 December 2013
Accepted 19 December 2013
Available online 30 December 2013

Keywords:
Microalgae residues
Anaerobic digestion
Biogas
Co-digestion
Scenedesmus

ABSTRACT

In biorefineries, the extraction of metabolites from microalgae would produce great amount of organic residues that would need to be treated. In this work, *Scenedesmus* residues were evaluated as substrates for biogas production and compared to raw biomass (SB). Microalgae residues were generated after the extraction of amino acids (SRA) and lipids (SRL). The influence of the processes applied on physicochemical properties and anaerobic biodegradability of microalgal biomass was studied in batch digestion tests. Co-digestion of microalgae residues with carbon rich substrates was also assessed by studying synergisms and kinetics of the discontinuous process. Methane yields of SRA and SRL in mono-digestion were $272.8 \pm 7.3\ L_{CH4}\ kgVS^{-1}$ and $212.3 \pm 5.6\ L_{CH4}\ kgVS^{-1}$, respectively, increasing that of SB ($140.3 \pm 29.4\ L_{CH4}\ kgVS^{-1}$). Kinetics of the process was also improved after the extraction of amino acids and lipids. Improvements were attributed to the disruption of microalgae cell walls and the increase in the solubilization of the organic matter. The amino acid extraction process improved the digestion process in a higher extent than lipid extraction because of its higher hydrolytic effect on biomass. Co-digestion influence on methane yield depended on the co-substrates used. However, co-digestion improved kinetics of the process.

1. Introduction

Extraction on a large scale of high value compounds from microalgae would produce great amount of organic residues that would require appropriate treatment. In most microalgae species cultured without nutrient limitation protein is the main organic component [1]. These proteins can be used for human and animal nutrition. Moreover, amino acid hydrolysates can be useful for the production of bacteria and yeast in the fermentation industry, as antioxidants, as an energy source or as biofertilizer [2]. When proteins are extracted from microalgae, sugars and lipids remain in the residual biomass. Therefore, the potential for energy production from these residues is very high. Anaerobic digestion is a well-known process used for the treatment of organic residues reducing their organic load and, at the same time, producing biogas and stabilized organic matter where most of the nutrients have been mineralized. The treatment of protein-extracted microalgae residues, rich in lipids and carbohydrates, through anaerobic digestion would yield high biogas.

Microalgae are also being studied as an energy crop for biodiesel production due to their high biomass productivities and high lipid accumulation [1]. If lipids are extracted from microalgae for biodiesel production, proteins and carbohydrates would remain in the residual biomass, with the subsequent opportunity to convert these organic components into biogas by anaerobic digestion. In fact, this option has already been pointed out as crucial in order to make sustainable microalgal biodiesel [3].

However, the anaerobic digestion of microalgae has shown two main problems. Some microalgae have shown low biodegradability [4–7]. Cell walls of some microalgae species are composed of complex carbohydrates that are hardly biodegradable by bacteria [5,7]. These cell walls act as a protection of the intracellular organic macromolecules from bacterial attack, reducing biodegradability of microalgal biomass. Another drawback to the anaerobic degradation of microalgae biomass is its high nitrogen content and low

* Corresponding author. Tel.: +34 91 496 2586; fax: +34 91 346 6269.
E-mail address: juanluis.ramos@ciemat.es (J.L. Ramos-Suárez).

1385-8947/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cej.2013.12.053

C/N ratio, consequence of the high protein fraction. Feedstocks of low C/N ratio can produce excessive ammonia inhibiting the growth of microorganisms and consequently spoiling or even stopping the digestion process [8].

Solutions for both drawbacks have been studied. Some pretreatments are able to break microalgae cell walls. Intracellular organic molecules are released and their solubilization is increased, being available for bacterial biodegradation. Consequently, biomass biodegradability and methane yields are increased [7,9–11]. On the other hand, the low C/N ratio of microalgal biomass can be balanced by the addition of high carbon content substrates [12–15], avoiding ammonia inhibition. Results on co-digestion of microalgae show, in most cases, that it improves the digestion process through the synergistic effects produced, such as the balance of nutrients, the increase buffer capacity or the increase of enzyme activity [12–15]. Conversely, in other studies co-digestion of microalgae with carbon-rich substrates produced no synergistic effects and a decrease in the methane yield [16].

Furthermore, the extraction of intracellular metabolites, such as lipids, also increases biodegradability and methane yields, as a consequence of the disruption of the cell wall [9,10]. These extraction methods have been claimed as similar to other types of pretreatments applied to microalgae that seek to enhance the biodegradability and the methane yield [11]. Therefore, either by applying pretreatments or by the extraction of metabolites the biodegradability of intracellular organic molecules of microalgae is increased leading to a higher accumulation of ammonia from degraded proteins inside digesters. In fact, Schwede et al. [11] observed inhibition caused by ammonia and salts accumulation during the anaerobic degradation of thermally pretreated *Nannochloropsis salina* in semi-continuously fed reactors. The combination of pretreatments or metabolite-extraction processes and co-digestion could be the solution to increase the anaerobic biodegradability of microalgal biomass avoiding at the same time ammonia toxicity.

The main goal of this study was to assess *Scenedesmus* residues as substrates for anaerobic digestion and to compare the digestion process of these residues and the digestion process of raw *Scenedesmus* biomass. Moreover, co-digestion assays were performed to study the effect of adding carbon-rich substrates to microalgae residues. Microalgae residues were generated after two different processes of amino acid and lipid extraction. Lipid-extracted microalgae residues have been assessed as substrate for biogas production several times. However, to the authors' knowledge, this is the first time that amino acid-extracted microalgae residues are evaluated for biogas production.

2. Materials and methods

2.1. Substrates and anaerobic biomass

Scenedesmus biomass (SB) and residues were kindly provided by *Fundación Cajamar* and University of Almería (Spain).

In order to extract amino acids, *Scenedesmus* biomass underwent an enzymatic hydrolysis process described in detail in Romero García et al. [2]. This process included a pretreatment to break microalgae cell walls by mechanical means (horizontal-bed ball-mill). After this pretreatment the enzyme Viscozyme® was added in order to reduce the viscosity of the solution to increase the yield of the enzymatic hydrolysis process. Viscozyme® is an enzyme with activity betaglucanase-cellulase-xylanase thus breaking carbohydrates and reducing their influence in the enhancement of the viscosity of the medium. After 30 min, the enzyme Alcalase® 2.5 L was added at pH 8.0 (kept constant by the addition of NaOH 1 M during 120 min). Then, pH was decreased by the addition of H_2SO_4 1 M and the enzyme Flavourzyme® 1000 L was added (pH was kept constant at 7.0 by H_2SO_4 1 M during 60 min). All these three steps were performed at 50 °C. Overall, biomass was subjected to 50 °C during 3.5 h (0.5 h during the viscosity reduction process and 3 h during the enzymatic hydrolysis). Finally, in order to deactivate enzymes, biomass was heated up to 75 °C during 15 min. After the separation of amino acid hydrolysate, residual biomass (from now on referred as SRA) was freeze-dried to make easier its transport, manipulation and conservation.

The extraction of lipids from *Scenedesmus* biomass was performed using hexane as solvent in a standard Soxhlet apparatus. Lipid-extracted residual biomass (from now on referred as SRL) was also freeze-dried.

SRA was co-digested in batch mode with paper sludge (PS) and *Opuntia maxima* cladodes (OM) in order to increase the C/N. PS is an abundant residue in the recycling paper industry with costs associated to its treatment or disposal. Due to its high carbon content, in principle it is a suitable co-substrate for anaerobic co-digestion of high nitrogen content feedstocks. PS was composed of sludge of the deinking process and of biological sludge from a wastewater treatment plant. Both came from the same factory in Madrid. Sludge produced in the deinking process was mainly composed of cellulose, ink and $CaCO_3$ whereas biological sludge, produced in the wastewater treatment plant of the factory, was around 6% in weight of the final sludge. PS was dried and finely milled to produce particles as small as possible in order to increase the surface contact with anaerobic biomass. *O. maxima* is rich in carbon and yield high biogas. It has been proposed in Mediterranean countries as an energy crop [17], since it can grow with high biomass yields with low water and fertilizer inputs [18]. OM was harvested in Madrid, where it was growing without fertilizers nor irrigation. Young cladodes (one or two years) were the only part of the plant harvested, since old cladodes increased their fraction in lignocellulosic material [19], hardly biodegradable. The cladodes were grinded and homogenized before their use; however the sample still contained some lumps because of its high mucilage content.

SRL was co-digested with residual glycerin (GLY) obtained during a biodiesel production process from waste cooking oil. GLY is rich in carbon and has been proved to be beneficial to the digestion process when added in low amounts to lipid-extracted microalgae residues [14,20]. Glycerol residue used in this study was not liquid and had to be homogenized by an intensive blending. However, samples still contained lumps and showed color gradients.

Different inoculums were used in the batch assays performed. In the first BMP assay (SRA–PS), the anaerobic biomass was anaerobic sludge adapted to the co-digestion of *Scenedesmus* biomass and *O. maxima* in laboratory reactors. Total and volatiles solids (TS and VS) concentration were 39.4 g L^{-1} and 23.3 g L^{-1}, respectively. Total Kjeldahl nitrogen (TKN) concentration was 2.2 g L^{-1}. Total and soluble chemical oxygen demand (COD_t and COD_s) were 33,333 and 2149 mgO_2 L^{-1}, respectively, and it showed a high partial alkalinity (PA) (3868 mg$CaCO_3$ L^{-1}) and a low intermediate alkalinity (IA) (950 mg$CaCO_3$ L^{-1}), consequently a low IA/PA ratio (0.25). pH was 7.8.

In the second BMP assay (SRA–OM) the anaerobic biomass was obtained during the digestion process of SRA in continuous mode in lab reactors. TS and VS concentration was 47.7 gTS L^{-1} and 27.8 gVS L^{-1}. TKN concentration was 4.0 g L^{-1}. COD_t and COD_s were 51,830 and 7877 mgO_2 L^{-1}, respectively, and it showed a high PA (6634 mg$CaCO_3$ L^{-1}) and a low IA (1398 mg$CaCO_3$ L^{-1}), with a consequent low IA/PA ratio (0.21) pH was 7.9.

In the third BMP assay (SRL–GLY) the anaerobic biomass was obtained during the digestion process in continuous mode of diluted SRA. TS and VS concentration were 58.3 g L^{-1} and 32.4 g L^{-1}, respectively. TKN concentration was 4.8 g L^{-1}. COD_t and COD_s were 61,867 mgO_2 L^{-1} and 8003 mgO_2 L^{-1}, respectively,