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Benefits of combining anaerobic digestion and amino acid extraction from microalgae

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HIGHLIGHTS

- High biogas yields were obtained from amino acid-extracted microalgae.
- Ammonia inhibition could be avoided by dilution of the substrate.
- The acclimation of microorganisms improved the efficiency of the process.
- Coupling anaerobic digestion to microalgae biorefineries could save costs in fertilizers and CO_2.

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ABSTRACT

The anaerobic digestion of *Scenedesmus* residues generated after a process of amino acid extraction with an extraction efficiency of 59% was thoroughly studied in 3 L working volume semi-continuous reactors. Anaerobic digestion of high concentrated *Scenedesmus* residues (17.6%TS) was inhibited due to ammonia and volatile acids accumulation. Dilution of the substrate to 10.5%TS avoided the inhibition of the process. The acclimation of microorganisms to the digestion of the substrate caused a further improvement in the process performance. High biogas and methane yields (409.3 and 291.5 L kg VS^{-1}, respectively) were achieved at an organic loading rate of 3.85 g VS L^{-1} d^{-1} with an hydraulic retention time of 20 days. Electrical and thermal energy (0.525 kWh and 2305.9 kJ per $kg^{-1}_{dry\ cultivated\ biomass}$) generated by the combustion of methane could be used for the different steps within the culturing of microalgae and the amino acid extraction processes. Moreover, a mass balance suggested that nitrogen and carbon dioxide needs for growing microalgae could be cut to a maximum of 30% and 25%, respectively, thanks to the coupling of anaerobic digestion to the extraction of amino acids from microalgae.

1. Introduction

Microalgae accumulate different kind of metabolites. Pigments, ω-3 fatty acids, proteins, nutritional supplements for humans and natural food colorants can be obtained from microalgal biomass [1]. In recent years, microalgae have also raised great expectations due to their capacity to accumulate a high fraction of lipids that can be extracted to produce biodiesel. However, the major fraction of biomass cultured without nutrient limitation is proteins [2], which could be up to 52% [3]. Whatever the biochemical composition of the biomass, to achieve profitable processes it is necessary to use completely the biomass according to a biorefinery concept, minimizing the production of residues. There is a need to find technologies able to treat the organic residues that would be produced after the extraction of valuable compounds from microalgae [3]. Anaerobic digestion is a suitable technology for the treatment of organic residues generated in industrial processes. In fact, anaerobic digestion of microalgae biomass has been studied since the late 50's [4]. The success of the process in the treatment of microalgal biomass is specie-specific [5]. Two main drawbacks for the proper degradation of microalgae have been described by several authors. The first one is the low biodegradability of microalgal cells [4–6] consequence of the composition of the cell wall of certain species, such as *Scenedesmus* and *Chlorella*, which are composed of complex carbohydrates [7]. The second drawback is the low *C/N* ratio of microalgal biomass [8–10] due to its high protein content [11]. The degradation of proteins leads to the production of ammonia which can be inhibitory to microorganisms [12].

Proteins from microalgae can be extracted to be used in human and animal nutrition [1]. Additionally, amino acid hydrolysates are useful for the production of bacteria and yeast in the fermentation

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industry, as antioxidants, as energy source and as biofertilizer [13]. After the extraction of proteins, the residual biomass could be converted into biogas. Furthermore, the digestion process is improved due to the disruption of the cell wall prior to protein extraction and the increase in the *C/N* ratio [14]. The coupling of anaerobic digestion to the extraction of proteins from microalgae could improve the economics of the process by the generation of renewable energy and the recycling of the digestate as growth medium [15].

In this work, valuable amino acids were extracted from *Scenedesmus* biomass by an enzymatic hydrolysis method described in Romero García et al. [13]. This method included a pretreatment that broke microalgal cell walls, followed by an enzymatic hydrolysis and a final centrifugation that separated L-amino acid concentrate from residual biomass. Residual *Scenedesmus* biomass (SR) was used as substrate for biogas production in semi-continuous reactors. This is the first time that microalgae residues generated after the extraction of amino acids are evaluated as source for biogas production in continuously stirred tank reactors (CSTR). The goals of the study were to evaluate the biodegradability and biogas production of SR in semi-continuous digestion, as well as to optimize the digestion process and to study possible inhibition mechanisms. Moreover, a mass balance was performed in order to estimate the nutrient and energy recovery possibilities.

2. Materials and methods

2.1. Substrates and anaerobic biomass

SR was provided by Cajamar Foundation and University of Almería (Spain). *Scenedesmus* biomass underwent an enzymatic hydrolysis described in detail by Romero García et al. [13], from which L-amino-acid concentrates were obtained. This process included a high pressure homogenization (1000 bar) performed on wet biomass that caused the disruption of the cell walls before the enzymatic hydrolysis. 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. 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 for 3.5 h (0.5 h during the viscosity reduction process and 3 h during the enzymatic hydrolysis). Finally, in order to deactivate the enzymes, biomass was heated up to 75 °C for 15 min. During the whole process a Rushton turbine provided constant agitation to the microalgal broth and the enzymes. The supernatant (free amino acids concentrate) was separated from the waste biomass (SR) using a discontinuous centrifuge at 4500 rpm (Rina, Barcelona, Spain). Finally, SR was frozen in order to ease transport and conservation. Freezing can disrupt the cell wall of microalgae [16,17] and therefore could constitute a pretreatment with a positive impact on the anaerobic digestion of microalgae biomass [18]. In the case of SR, the intensive disrupting processes that suffered fresh microalgae biomass during pretreatment and amino acid extraction were enough to disrupt microalgae cell walls minimizing the possible effect that the subsequent freezing process may have on them.

SR showed a TS concentration of 230 g L^{-1} after the final centrifugation. However, SR finished the amino acid extraction process with 170 g TS L^{-1}. Therefore, it was thawed and diluted to the desired concentration before being fed into the digesters. SR was produced in different batches. Although the same method was applied for the extraction of amino acids, composition changed from one batch to another. Average composition of different batches of SR is shown in Table 1. Table 2 shows the physical and chemical characteristics of diluted SR that was finally used to feed the digesters.

Different inocula were used for the two assays performed. In the first semi-continuous assay (17.6%TS feedstock concentration), anaerobic biomass used was anaerobic sludge adapted to the co-digestion of raw *Scenedesmus* biomass and *Opuntia maxima* cladodes in laboratory reactors. In the second semi-continuous assay (10%TS feedstock concentration), inoculum was collected from a wastewater treatment plant working at 37 °C in Madrid (Spain). Composition of the inocula is shown in Table 2 and it is further discussed in Section 3.1.

2.2. Semi-continuous assay

The assay in semi-continuous mode was performed in duplicate in CSTR. The working volume of reactors was 3 L. Reactors were kept at constant temperature in the mesophilic range (37 °C) by water heated by thermostatic baths that was pumped through their double wall glass. The content of reactors was continuously stirred at 35 rpm by mechanical means.

In the first semi-continuous assay, SR was diluted with tap water to 176 g TS L^{-1}. Different OLR were tried (see Table 3). OLR levels were increased when constant methane production was achieved during long periods, as suggested in VDI-4630 guidelines for fermentation of organic substrates [19]. Feed and discharge of reactors was done manually once a day. The experiment was stopped when evidences of inhibition were observed. In the second semi-continuous assay SR was diluted to 10.5%TS with tap water. The feed and discharge of reactors was done automatically by

Table 1
Composition of SR. Elemental analysis expressed based on dry matter.

	SR
TS (%)	23.0 ± 2.5
VS (%TS)	72.9 ± 4.2
VS (%)	16.7 ± 1.3
pH[a]	6.3–6.7
COD_t (gO_2 L^{-1})	325.0 ± 44.4
COD_s (gO_2 L^{-1})	107.1 ± 8.2
TKN (gN L^{-1})	14.5 ± 2.9
TAN (gNH_4^+ L^{-1})	1.8 ± 0.7
C (%)	42.0
H (%)	6.0
N (%)	6.3
S (%)	0.7
C/N	7.3
Ca (%)	4.1
P (%)	3.1
K (%)	0.7
Na (%)	0.7
Mg (%)	1.0
Al (ppm)	98
Fe (ppm)	838
Cu (ppm)	129
Mn (ppm)	1300
Zn (ppm)	266
Sr (ppm)	1200
Ba (ppm)	204
Ti (ppm)	6.5
Pb (ppm)	25
Be (ppm)	<6
Bi (ppm)	<6
Cd (ppm)	<6
Co (ppm)	<6
Cr (ppm)	<6
Mo (ppm)	<6
Ni (ppm)	<6
V (ppm)	<6
Hg (ppb)	18.8

[a] Range in which the pH of different batches oscillated.