Co-digestion of *Sargassum* sp. with glycerol and waste frying oil: optimization of the biomethane production using a design of experiments

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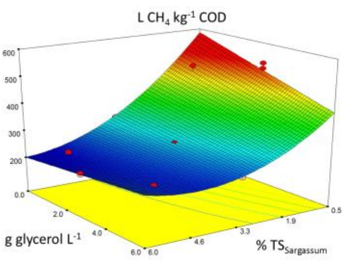

A response surface methodology was adopted to assess the optimal conditions for methane production from the macroalgae Sargassum sp. Three variables were tested: % total solids of algae (%TSsargassum); co-substrate concentration (gwaste L-1); and, co-substrate type (glycerol or waste frying oil (WFO)). The Biochemical Methane Potential (BMP) of Sargassum sp. was 300 ± 3 L CH4 kg-1 COD. The co-digestion with glycerol and WFO increased the BMP by 72% and 68% respectively. The methane production rate (k), showed similar behavior as the BMP, increasing 45% and 29% with glycerol and WFO, respectively. The higher BMP (517 ± 13 L CH4 kg-1 COD) and k (78± 4 d-1) was obtained in the assays with 0.5% TS and 3.0 g glycerol L-1. Co-digestion with glycerol or WFO is a promising process to enhance the BMP from the macroalgae *Sargassum* sp.

Introduction

After the sudden interest in the cultivation of highly productive algae due to the oil crisis in the 1970's, we are witnessing a rediscovery of algae potential. Seaweed (or macroalgae) can be used to produce bioenergy, namely bioethanol and biogas. This biomass has several advantages over terrestrial crops since it does not compete with land use and water consumption necessary for food crops production. Their fast growth rates and large yields make them even more attractive. *Sargassum* sp. is a brown macroalgae widely distributed in tropical and subtropical seas, and one of the most abundant seaweed in the Portuguese coast.

Macroalgae have great potential as energy crops because they contain easily hydrolysable sugars and low lignin content. The biochemical methane potential (BMP) of *Sargassum* sp. ranged between 119 and 380 L CH_4 kg^{-1} VS [1]. Therefore, by using *Sargassum* sp. as substrate in Anaerobic Digestion (AD) processes not only solves the problem of their disposal, but also provides a renewable source of energy.

Co-digestion is a process to enhance the anaerobic biodegradability of two or more complementary substrates due to synergetic effects. *Sargassum* sp. has high content in proteins, therefore its co-digestion with crude glycerol and/or waste frying oil (WFO) – two substrates with high carbon content and lack of nitrogen – may be a promising alternative to increase the BMP of *Sargassum* sp.

However, the efficiency of a co-digestion process depends on several variables, usually studied independently. A statistical analysis, using a design of experiments, is an efficient way to optimize the factors that are interrelated.

This work aimed at study the anaerobic biodegradability of *Sargassum* sp. and its co-digestion with glycerol and WFO. The effect on the BMP and methane production rate (*k*) of three operating conditions (*Sargassum* sp. and co-substrate concentrations, and type of co-substrate) was tested. A factorial design methodology was adopted to determine in a systematic way the statistical significance of each parameter and possible interactions.

Material and Methods

Inoculum: Anaerobic granular sludge from a brewery industry was used as inoculum in the biodegradability assays. The sludge samples contained 0.081±0.001 g $g^{-1}_{inoculum}$ of volatile solids (VS). The specific methanogenic activity (SMA) in the presence of acetate (30 mM) was 136±17 mL $CH_{4@STP}$ g^{-1} VS d^{-1}, and in the presence of H_2/CO_2 (80/20 v/v, 1 atm) was 592±65 mL $CH_{4@STP}$ g^{-1} VS d^{-1}. SMA was determined according to described in Costa et al. [2].

Substrate: *Sargassum* sp. was collected in the Portuguese coast (Póvoa de Varzim), and used as substrate in the anaerobic biodegradability assays. Crude glycerol, from vegetable oils, and WFO, from a kitchen restaurant in Braga (Portugal), were used as co-substrates. Each substrate was characterized in terms of solids, chemical oxygen demand (COD), nitrogen.

Experimental Design: A Factorial design of experiments was used to perform the anaerobic biodegradability assays. The effect of *Sargassum* sp. concentration (X_1), co-substrate concentration (X_2), and co-substrate type (X_3), on two response variables – biochemical methane production (Y_1) and methane productions rate (Y_2) – was studied using a response surface methodology. The limits selected for the anaerobic biodegradability assays are shown in Table 1. Furthermore, X_3 is a categorical factor, i.e. it was only 100% of glycerol or 100% of WFO. The experimental design consists in a full factorial experimental design with 18 runs. The experiments were randomly performed. The software package Design-Expert ® (Stat-Ease, Inc., Minneapolis) was used to determine the experiments matrix and their statistical analysis.

Biodegradability assays: The anaerobic biodegradability assays were performed according to the guidelines defined in Angelidaki et al. [3], with 50% (v/v) of inoculum, at 37 °C. All the assays were performed in duplicate, except the central point of factorial design and the blanks (without substrate) which were performed in triplicate. The blank was used to discount for the residual substrate present in the inoculum.

Analysis: Ammonium ($N\text{-}NH_4^+$), Total Kjeldahl nitrogen (TKN), Total Solids (TS) and VS were measured according to Standard Methods [4]. Total and soluble COD were determined using standard kits (Hach Lange, Düsseldorf, Germany). Protein content was determined based on the TKN measurement using the correction factor 6.25 [5]. The methane content of biogas was analyzed with a gas chromatograph (GC) (Chrompack 9000), as described in Costa et al. [6].

Results and discussion

The wastes characterization is shown in Table 2. Seaweeds were collected in their natural environment and without any pre-treatment. They contain several impurities, which could influence the anaerobic digestion process. The low value of VS and high concentration of nitrogen may limit their biodegradability. The co-digestion with glycerol and WFO can be a good alternative to bring the C:N ratio near to the optimum ratio for anaerobic digestion (around 20-30:1), since both co-substrates have high concentration of soluble COD and negligible content in nitrogen.

Table 1. Levels of factors selected for the response surface methodology.

Factors	Real values of coded levels				
	-α	**-1**	**0**	**+1**	**+α**
X_1 (% TS)	0.50	1.31	3.25	5.19	6.00
X_2 (g_{waste} L^{-1})	0.00	0.88	3.00	5.12	6.00

Table 2. Characterization of substrates used in the anaerobic biodegradability assays.

	Units	*Sargassum* sp.	Glycerol	WFO
TS	%	89.5±0.3	67.9±1.0	100
VS	% [a]	53.8±0.8	93.8±0.1	100
COD_T	$g\ g^{-1}_{waste}$	0.597±0.062	1.60±0.01	2.55±0.29
COD_S	$g\ g^{-1}_{waste}$	0.015±0.000	1.60±0.01	2.55±0.29
TKN	% [a]	2.08±0.04	n.d.	n.d.
Protein	% [a]	49.3±1.0	n.d.	n.d.

[a] Dry basis.
n.d. – Not detected

The response surface of the methane production from the co-digestion of *Sargassum* with glycerol and WFO is shown in Figure 1. The BMP varied significantly from 225 to 517 L CH_4 kg^{-1} COD for glycerol and from 243 to 505 L CH_4 kg^{-1} COD for WFO. The BMP of *Sargassum* sp. (without co-substrate) was 300 ± 3 L CH_4 kg^{-1} COD. The results suggest that the two parameters (concentrations of *Sargassum* sp. and co-substrate) had significant effects on the AD efficiency. Addition of glycerol and WFO showed similar results between them (Figure 1), although there are slightly better results with glycerol.

An increase in the concentration of *Sargassum* sp. leads to a decrease on the BMP (Figure 1). Habig and Ryther [7] refer that *Sargassum* sp. contains a fiber fraction difficult to biodegrade. For lowers concentrations of *Sargassum* sp., the BMP decrease with increasing concentrations of co-substrates. However, for concentration of *Sargassum* sp. > 4% TS, the addiction of different amounts of co-substrate did not influence significantly the BMP. In the assays with >4% TS of *Sargassum* sp. was observed an inhibitory effect possibly due to the accumulation of ammonia, i.e > 0.1 g NH_3-N L^{-1} [8].

The methane production can be described by the following model equation:

$$Y_1 = 271.1 - 80.5X_1 - 21.8X_2 - 6.06X_3 + 27.9X_1X_2 + 16.4X_1X_3 + 42.7X_1^2 \quad (1)$$

where all significant factors and interactions were considered. The significance was established at the $P < 0.05$ level.

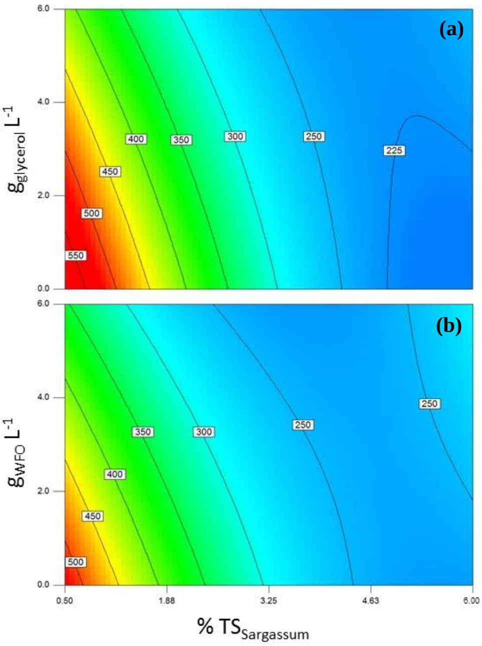

Figure 1. BMP (L CH_4 kg^{-1} COD) contour plot. Anaerobic co-digestion of *Sargassum* with glycerol **(a)** or WFO **(b)**. The lower (■) and higher (■) shading match to 201 and 585 L CH_4 kg^{-1} COD, respectively.

The methane production rate varied significantly between 29 to 74 d^{-1} for glycerol and 33 to 69 d^{-1} for WFO. The biodegradability of *Sargassum* sp. without co-substrate reached 54 ± 4 d^{-1}. As in the BMP, the concentration of *Sargassum* sp. and co-substrate had significant influences in the methane production rate. BMP and *k* assumed the same behavior.

Conclusions

The BMP of *Sargassum* sp. without co-substrate was 300 ± 3 L CH_4 kg^{-1} COD. The co-digestion caused an increase on the methane production up to 72% (with 0.5% TS of *Sargassum* sp. and 3.0 g glycerol L^{-1}), and 68% (with 1.31% TS of *Sargassum* sp. and 0.88 g WFO L^{-1}), compared with the digestion of *Sargassum* sp. without the addiction of co-substrate. Regarding the methane production rate, the same assays reached the highest values, increasing 45% and 29% for glycerol and WFO, respectively. The k of *Sargassum* sp. was 54 ± 4 d^{-1}.

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