Evaluation of different strategies to maximize biogas production from algae

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Abstract

Seaweeds (macroalgae) are a promising substrate for biogas production due to the high percentage of carbohydrates and high growth rate. Therefore, the biogas produced from the anaerobic digestion of seaweeds is a sustainable and renewable alternative source of bioenergy. Seaweeds are available in coastal areas and may also be produced in aquacultures. This work presents results of the biochemical methane potential (BMP) of the wild seaweed, *Gracilaria vermiculophylla*, as well as the effect of physical and thermochemical pre-treatments on their biodegradability. The co-digestion with glycerol and sewage sludge was also studied. The BMP of *G. vermiculophylla* after a physical pre-treatment (washing and maceration) reached 481±9 L CH_4 kg^{-1} VS, corresponding to a methane yield of 79±2%. Regarding the thermochemical pre-treatments, it was found that the increase of temperature (from 20 to 90ºC), NaOH concentration (from 0.1 to 0.5 g NaOH per g TS) and pressure (from 1 to 6 bar) caused an increase in the seaweeds solubilisation up to 44%. However, the subsequent methane production was not increased as expected, although a faster methane production rate was observed. The co-digestion of *G. vermiculophylla* with glycerol or sewage sludge has proved to be quite effective for increasing the methane production. Addition of 2% glycerol (w:w) increased the methane production by 18% (599±16 L CH_4 kg^{-1} VS) and methane yield by 22%, achieving almost complete substrate methanation. Moreover, the co-digestion of seaweed and secondary sludge (15:85%, TS/TS) caused an increase of 25% in the BMP (605 ± 4 L CH_4 kg^{-1} VS), relatively to the individual digestion of algae. The addition of glycerol in this assay did not cause significant improvements.

Keywords

Algae; Anaerobic Digestion; *Gracilaria* sp.; Glycerol; Pre-treatment; Sewage sludge

INTRODUCTION

Raising concerns on climate change effects and the scarcity of fossil fuels increased the demand for alternative energy sources. Algae should be seen as a promising source for bioenergy production in the future, since it has several advantages over other energy crops, including high yields and growth rates. *Ulva* spp. and *Gracilaria* spp. had similar Biochemical Methane Potential (BMP), 196±9 and 182±23 L CH_4 kg^{-1} VS, respectively, without any pre-treatment (Costa et al. 2012). However, in most cases the methane yield is less than 50%, suggesting the need of pre-treatments to improve the substrate solubilisation and subsequent methane production. Co-digestion is another process that can increase the methane production from macroalgae. The co-digestion of *Ulva* sp. (15%) with sewage sludge (85%) is feasible at a rate of methane production 26% higher than sewage sludge alone, without decreasing the overall biodegradability of the substrate (42–45% methane yield) (Costa et al. 2012). The aim of this study was to evaluate the effect on the *G. vermiculophylla* methane yield and methane production rate, after physical and thermochemical pre-treatments. Macroalgae co-digestion with glycerol and sewage sludge was also assessed.

MATERIAL AND METHODS

Inoculum and Substrate. Anaerobic granular sludge from a brewery industry was used as inoculum in all biodegradability assays. The sludge samples contained 0.06±0.01 g VS g^{-1} inoculum. The specific methanogenic activity in the presence of acetate (30 mM) was 214±17 mL $CH_{4\,@STP}$ g^{-1} VS

d^{-1}, and in the presence of H_2/CO_2 (80/20 v/v, 1atm) was 389±16 mL $CH_{4\ @STP}$ g^{-1} VS d^{-1}.

G. vermiculophylla was collected in Ria de Aveiro, in March 2012. Glycerol was produced in December 2010 from vegetable oils and stored at 4°C since then. Sewage sludge was collected in June 2012 from the secondary treatment of the Frossos WWTP.

Experimental procedure. Evaluation of the methane production potential from *G. vermiculophylla* was performed according to the scheme shown in Figure 1. The biodegradability assays were performed according to the guidelines defined in Angelidaki et al. (2009), with 50% (v/v) of inoculum, at 37 °C and with an inoculum to substrate ratio of 4 g $VS_{inoculum}$ g^{-1} $VS_{substrate}$. All the assays were performed in triplicate and a blank (without substrate) was used to discount for the residual substrate present in the inoculum. The algae was washed in a water bath during 30 minutes to remove impurities (W) and washed and dried at 37 ºC (WD). During maceration the algae were cut to less than 0.5 cm and then crushed with a mortar.

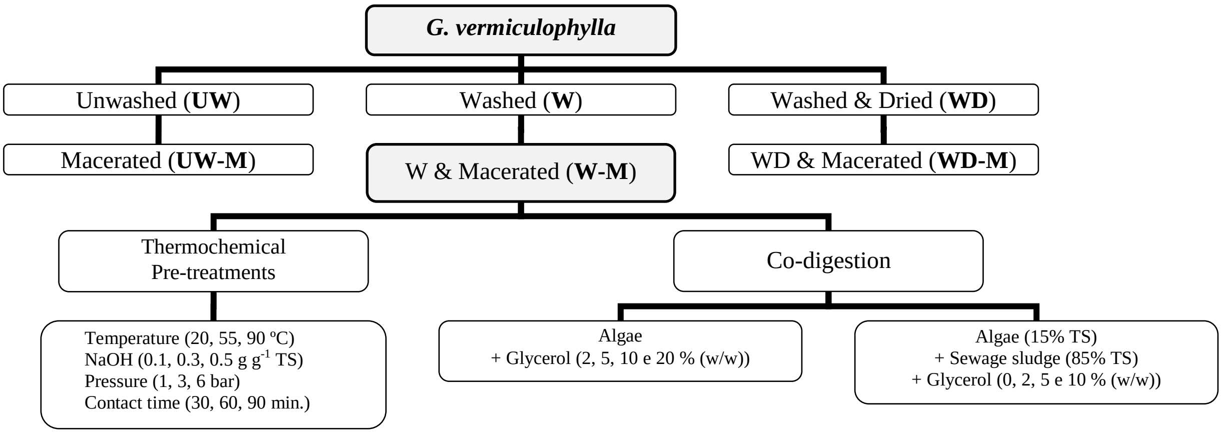

Figure 1. Sequential degradation tests performed with *G. vermiculophylla*, glycerol and sewage sludge.

Analytical Methods. Total kjeldahl nitrogen (TKN), ammonium ($N\text{-}NH_4^+$), Total (TS) and volatile (VS) solids were measured according to standard methods (APHA, 1998). Total (COD_T) and soluble (CODs) chemical oxygen demand were determined using standard kits (Hach Lange, Germany). Volatile Fatty Acids (VFA) were determined by HPLC (Costa et al. 2012). Long Chain Fatty Acids (LCFA) were analysed according to the method describe in Neves et al. (2009). Methane (CH_4) content was analysed in a gas chromatograph (Chrompack 9000) equipped with a FID detector and Carbowax 20M (80-120 mesh) column. Nitrogen was used as carrier gas (30 mL min^{-1}). The column, injector, and detector temperatures were 35, 110, and 220 ºC, respectively.

RESULTS AND DISCUSSION

Substrates characterization

The substrates characterisation is shown in Table 1. Since algae were captured directly in their environment, the presence of impurities and potentially toxic compounds to AD, such as high salinity of sea water, sand, small shellfish, etc., influence their characterisation. For example, sand and small molluscs shells have a strong influence on the solids content of the algae as observed by the VS of the unwashed (UW) sample. As expected, the washed and dried sample (WD) present higher TS and COD concentration. The small CODs concentration, only 3 to 10% of COD_T, confirms the need of a pre-treatment to increase the organic matter solubilisation.

Table 1. Characterisation of the different samples of *G. vermiculophylla*, glycerol, and sewage sludge used in the anaerobic biodegradability assays.

Parameter	***G. vermiculophylla***			***Glycerol***	***Sewage Sludge***
	UW	**W**	**WD**		

TS	%	21.5 ± 1,9	13.6 ± 0.6	91.7 ± 1.7	71.5 ± 2.8	16.7 ± 0.2
VS	% (a)	47.7 ± 6,5	69.4 ± 4.3	70.0 ± 2.8	94.7 ± 3.9	83.8 ± 0.8
COD_T	$g\ g^{-1}_{substrate}$	0.16 ± 0,01	0.16 ± 0.01	0.82 ± 0.04	1.44 ± 0.02	0.29 ± 0.01
CODs	$g\ g^{-1}_{substrate}$	0.011 ± 0,000	0.005 ± 0.000	0.086 ± 0.001	1.44 ± 0.02	0.052 ± 0.000
TKN	% (a)	2.51 ± 0,03	4.75 ± 0.92	3.95 ± 0.09	*nd*	*nd*

(a) dry basis; *nd:* not determined; UW – unwashed; W – washed; WD – washed and dried at 37ºC

Physical and thermochemical pre-treatments

The cumulative methane production from *G. vermiculophylla* after different physical pre-treatments is shown in Figure 2a, while after thermochemical pre-treatments is shown in Figure 2b. The macerated samples achieved higher methane yields compared with the respective unmacerated samples, approximately 10% for W and UW samples and 5% for WD. The maceration does not change the substrate theoretical methane production, but causes differences in the AD process. The increased surface area makes the substrate more accessible to the inoculum, facilitating a fast hydrolyses and providing higher biodegradation rates. Increases of 25-40% in the methane production rates were observed in the macerated samples. The higher BMP was achieved with the washed and macerated (W-M) sample, i.e. 481±9 L CH_4 kg^{-1} VS.

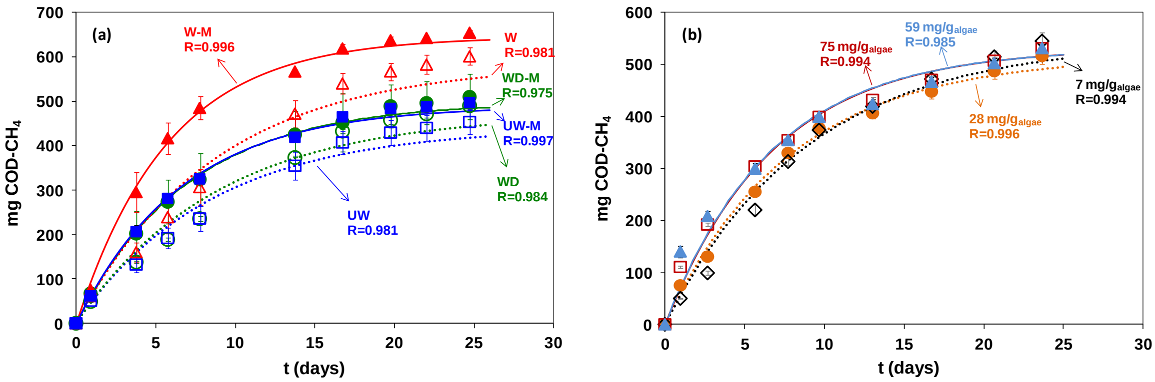

Figure 2. Cumulative methane production during biodegradability assays: **(a)** after physical pre-treatments, UW (□), UW-M (■), W (△), W-M (▲), WD (○) and WD-M (●); and **(b)** after thermochemical pre-treatments, 75 (□), 59 (▲), 28 (●) and 7 (◇) mg CODs g^{-1} macroalgae. The lines represent the predicted data by the exponential model, $M(t)=P[1-exp(-kt)]$, where $M(t)$ is the methane cumulative production (mg COD-CH4), P is the maximum methane production (mg COD-CH4), k is the methane production rate constant (day^{-1}), and t is the time (days).

The macroalgae solubilisation percentage ranged from 1.1% (30 min, 0.1 g NaOH g^{-1} TS, 20 °C and 1 bar) to 44.3% (30 min, 0.5 g NaOH g^{-1} TS, 90 °C and 6 bar). The results demonstrated a strong influence of temperature, alkali concentration and pressure, and little influence of contact time on the algae solubilisation (data not shown). Afterwards, four samples, with different CODs concentration (75, 59, 28 e 7 mg CODs g^{-1} algae), were added to vials and the methane production was determined. No significant effects were observed in the biodegradability results; BMP ranged from 353–380 L CH_4 kg^{-1} VS, the methanation percentage from 58–63%, and the final solubilisation percentage (pre-treatment + AD) from 82–87%. However it should be noted the highest biodegradability rate of the assay with high initial CODs (data not shown). Although the washing step seems to decrease the inhibitory effect caused by the high salinity, further studies in continuous and long-term operations should be performed to properly evaluate the potential inhibitions caused by the salinity.

Co-digestion

The cumulative methane production obtained in the co-digestion assays with *G. vermiculophylla* and with varying percentages of glycerol (w/w) is presented in Figure 3a, while the co-digestion of algae (15% TS), sewage sludge (85% TS), and glycerol is shown in Figure 3b. The absolute methane production increased with increasing percentages of glycerol, except in the assay with 20%

glycerol, which was inhibited. The sample with 10% glycerol presented the highest methane production, but a latent stage between days 10 and 25 was observed. In the assays with high glycerol concentrations (10 and 20%), the accumulation of VFA and LCFA caused the methanogenesis inhibition. Additionaly, in the assays with 5 and 10%, high concentrations of ammonia were detected (>0.11 g NH_3-N L^{-1}). The highest BMP was obtained in the assay with 2% glycerol (599±16 L CH_4 kg^{-1} VS). Thus, special attention should be considered when adding glycerol as co-substrate because it can easily inhibit the AD process.

In the tests with sewage sludge was observed that the assays with 0, 2 and 5% glycerol exhibit similar results. The BMP varied between 605–611 L CH_4 kg^{-1} VS. However, the assay with 10% glycerol was inhibited by the high concentration of VFA, LCFA and ammonia. Therefore no significant effects are found by adding glycerol to the co-digestion.

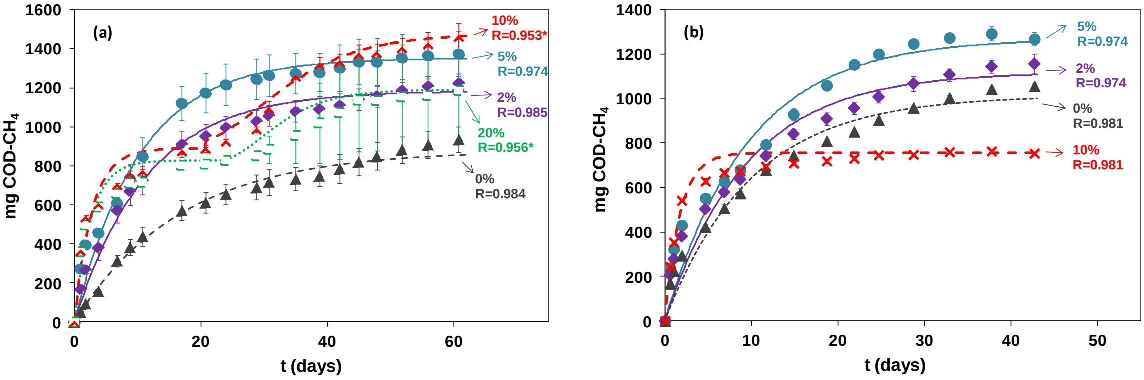

Figure 3. Cumulative methane production during co-digestion of *G. vermiculophylla* and glycerol **(a)**: 0% (▲), 2% (◆), 5% (●), 10% (x) and 20% (☐) of glycerol; and *G. Vermiculophylla*, sewage sludge and glycerol **(b):** 0% (▲), 2% (◆), 5% (●) and 10% (x) of glycerol. The lines represent the predicted data by the exponential model (see figure 2).

CONCLUSIONS

The prior washing and maceration (W-M) of *G. vermiculophylla* allow the highest specific methane production (481±9 L CH_4 kg^{-1} VS) due to the removal of impurities and/or potential toxics to AD and increased surface area of the substrate. The application of thermochemical pre-treatment had no positive effect on methane production, although the substrate solubilisation increased up to 44%. The co-digestion of *G. vermiculophylla* with glycerol proved to be a promising alternative. The addition of only 2% glycerol increased the methane production by 25% (599±25 L CH_4 kg^{-1} VS) compared with the digestion of W-M algae alone. The results indicate a synergetic effect of the co-substrates, with glycerol concentrations between 2 to 5%, being inhibited above this value. The co-digestion with sewage sludge (15:85, w/w) caused an increase in methane production (605±4 L CH_4 kg^{-1} VS). However, in this case the addition of glycerol had no significant effect.

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