PRETREATMENTS APPLIED TO MICROALGAE RESIDUES TO ENHANCE ANAEROBIC DIGESTION

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ABSTRACT
Biomass of microalga *Chlorella protothecoides*, grown under autotrophic and heterotrophic conditions and subjected to pretreatments, were energetically valorized through anaerobic digestion process according to the substrates: autotrophic algae (A), heterotrophic algae (H), heterotrophic algae extracted (HE), autoclave pretreated heterotrophic algae (HPA), enzyme pretreated heterotrophic algae (HPE), ultrasound pretreated heterotrophic algae (HPU), and inoculum (I). Despite the application of pretreatments, the highest methane production was obtained in the algae extracted digestion with 172 mL CH_4, against 153, 126 and 142 mL obtained in HPA, HPE and HPU, respectively.
The COD removal capacity was higher in the HPA sample while the TS and VS removal reached higher values in the autotrophic alga.

Keywords: Anaerobic digestion, microalgae, *Chlorella protothecoides*, biofuel, bioenergy.

INTRODUCTION
The fossil fuels are non-renewable resources limited to the existence of underground reserves, which are responsible for a big impact on global warming and the destruction of sensitive and fundamental ecosystems to the human population, with their exploration, extraction and refining. Human awareness has led the world leaders of 195 countries to a commitment by signing the Paris Agreement, for which was necessary to intensify the research into renewable energy sources, like biogas. With the research development, a new perspective was developed using microalgae due to its advantages: they grow faster and consume less water than other terrestrial crops [1], their cultivation consists in a conversion of solar energy into biomass and that can be used to anaerobic digestion processes of energetic valorization. The anaerobic process leads to the production of organic acids and biogas, a renewable energy source used for electricity, biofuel production or natural gas grid injection [1, 2].
The microalgae cell wall is mostly composed of organic components with low degradability (such as cellulose) which leads to a reduction in the production of methane since the organic matter is in the cytoplasm, which is not accessible to the bacteria of the anaerobic digestion.
Given this problem, it is necessary to apply pretreatments that allow to degrade the cell wall of the microalgae, improving the anaerobic digestion and, consequently, the production of biogas.
In this study, biogas production and removal capacity of autotrophic and heterotrophic microalgae were compared, as well as the impact of different pretreatments.

MATERIAL AND METHODS

Pretreatments methods
Microalgae were pretreated using different methods, under laboratory conditions: (i) Lipid extraction with hexane for 10h; (ii) enzymatic treatment with lysozyme 100mg/ml, incubating at 37ºC and 100 rpm for 16h [3]; (iii) autoclaving at 121ºC and 2 bar for 30 min; (iv) ultrasound treatment realized in 3 times for 5 min at 20 kHz output, on ice.

Microalgae and anaerobic digestion assay
The microalgae used in the trial was *Chlorella protothecoides.* The microalga biomass was previously cultivated at Bioenergy Unit at LNEG using two different liquid media: autotrophically (A) in inorganic culture medium at room temperature (22ºC) under continuous illumination and heterotrophically (H) in organic culture medium at 28ºC [4].
The anaerobic digestion assays were performed in batch mode, in triplicate and under mesophilic conditions (37ºC). Glass units of 40 mL of working volume were used for 47 days. The assay was carried out comparing the material without any physically disrupted cell to non-treated microalgae, the positive control. The negative control assay has the same inoculum concentration but without microalgae biomass. Biological solids, collected from an anaerobic digester Wastewater Treatment plant (ETAR Quinta do Conde, Portugal), were used as inoculum (I).

Analytical and chromatograph methods
Total and volatile solids (TS and VS) and chemical oxygen demand (COD) were determined according to Standard Methods [5]. Biogas production was daily monitored, and gas composition was analysed by gas chromatographic techniques according to ASTM Standard Method [6]. All gas volumes were adjusted to STP conditions (1 bar, 0°C).

RESULTS AND DISCUSSION
Biogas production was recorded in all assayed units during the experimental time of 47 days (Fig. 1). No lag phase was observed, and all the substrates provided higher biogas volumes than the inoculum (I), indicating that each process was able to convert suitably the substrates tested into gas. The higher biogas volume (172 mL) was obtained in the unit containing the extracted microalgae (HE) followed by the autoclaved microalgae (HPA, 153 mL), showing better results compared to substrates where pretreatments were applied. This agrees to Jankowska et al. [7], where thermal pretreatment is the most efficient in cell wall degradation among the pretreatments and consequently gives the best results in biogas production. In contrast, the non-pretreated heterotrophic microalgae (H), which represents the control of the assay, produced the lowest value of biogas (118 mL). Comparing autotrophic and heterotrophic algae (A and H), better results were obtained from the A digestion than from H. Around 20 days were sufficient for A, attaining a biogas production of 110 mL while H requires more than 35 days to reach an equivalent biogas volume.
As it can be observed (Table 1), HE was more efficient than the other heterotrophic algae units. COD removals of 23-26% were recorded in heterotrophic algae units with the exception of HPE which had the lowest value (14%) according to the low gas production.
The low conversion capacity of the substrates tested may be related to the appearance of a purple pigmentation in each of the digester units, and this could be attributed to purple non-sulfur bacterium (PNSB) [8].

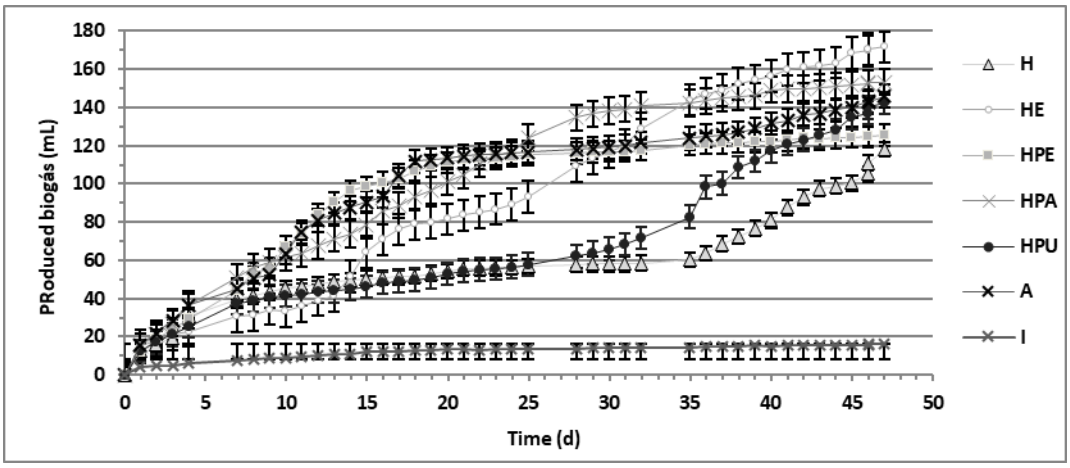

Fig. 10. Produced gas (SPT conditions: mean values and standard deviation of triplicates)

Table 11. Anaerobic digestion characterization and removal capacity

Pretreatments	Biogas	Initial parameters			Removal capacity		
	Final (mL)	COD (g L^{-1})	TS (g L^{-1})	VS (g L^{-1})	COD (%)	TS (%)	VS (%)
HE	171.6	21.3	11.6	10.1	25	45.7	52.5
HPA	153.3	27.0	12.1	10.5	26	29.8	34.3
A	145.9	18.5	11.6	9.3	18	63.8	66.7
HPU	142.3	26.3	11.7	10.2	25	33.3	39.2
HPE	125.5	29.1	10.0	8.6	14	49.0	52.3
H	118.0	32.7	11.6	10.2	23	21.5	39.2
I	16.2	11.4	5.1	1.1	43	13.7	0.0

All the digester units presented high quality in methane composition (Fig. 2), where the highest value was recorded in the inoculum with 82.3 % (V/V) and the lower value in unit H, with 69.6 % (V/V).

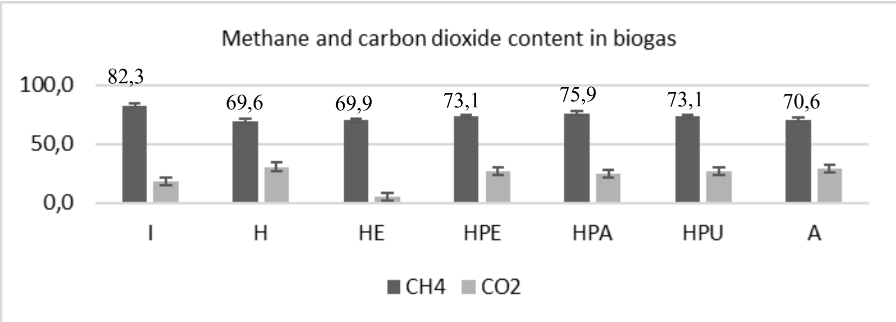

Fig. 11. Methane and Carbon Dioxide content (%) in biogas on 23rd day.

CONCLUSIONS

All pretreatments were successfully applied and digested in anaerobic conditions. The highest value of biogas obtained was 172 mL, for the extracted microalgae (HE) with good quality in methane composition, as well as in the removal capacity of TS and VS in heterotrophic microalgae samples, showing great efficiency and that should be taken into account in future studies.

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