

BOOK  
OF  
PROCEEDINGS

# WASTES

solutions treatments opportunities

5<sup>th</sup> Costa da Caparica  
4 > 6  
september

2019 International  
Conference

CVR - Centro para a Valorização de Resíduos  
Campus de Azurém da Universidade do Minho  
4800-058 Guimarães  
PORTUGAL

Telef.: +351 253 510 020  
Fax: +351 253 510 029  
<http://www.cvresiduos.pt>  
e-mail: [geral@cvresiduos.pt](mailto:geral@cvresiduos.pt)



***Book of proceedings - 5<sup>th</sup> International Conference  
WASTES: Solutions, Treatments and Opportunities***

EDITION

CVR - Centro para a Valorização de Resíduos

EDITORS

Fernando Castro, Cândida Vilarinho,  
Joana Carvalho

COVER DESIGN

Rui Ferreira

ISSN

2183-0568

september 2019

## PRETREATMENTS APPLIED TO MICROALGAE RESIDUES TO ENHANCE ANAEROBIC DIGESTION

M. P. Rodrigues<sup>1</sup>, A. Eusébio<sup>2</sup>, C.A. Santos<sup>1</sup> and I.P. Marques<sup>2</sup>

<sup>1</sup> Instituto Politécnico de Setúbal, Escola Superior de Tecnologia do Barreiro, 2839-001 Lavradio, Portugal; carla.santos@estbarreiro.ips.pt

<sup>2</sup> LNEG - Laboratório Nacional de Energia e Geologia, Unidade de Bioenergia, Lisboa, Portugal; isabel.paula@lneg.pt

### 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<sub>4</sub>, 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 37°C and 100 rpm for 16h [3]; (iii) autoclaving at 121°C 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 (22°C) under continuous illumination and heterotrophically (H) in organic culture medium at 28°C [4].

The anaerobic digestion assays were performed in batch mode, in triplicate and under mesophilic conditions (37°C). 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].

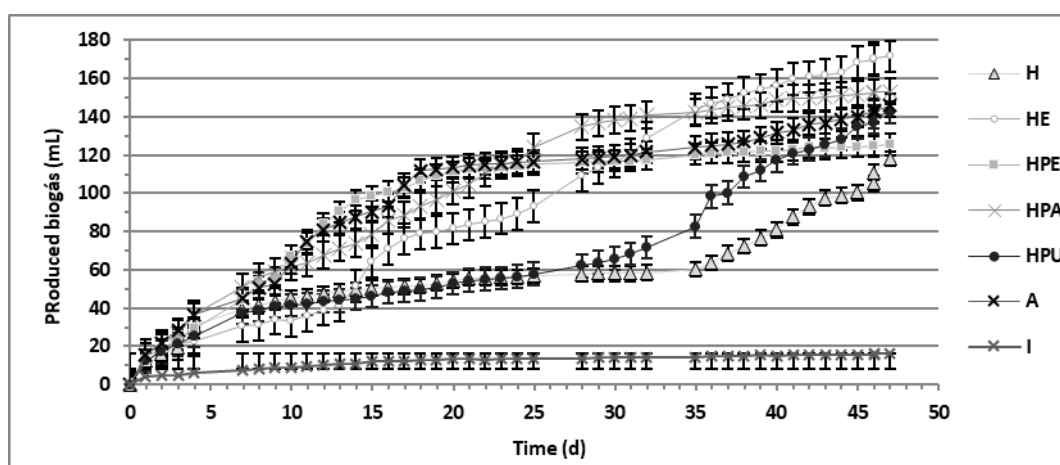


Fig. 10. Produced gas (STP conditions: mean values and standard deviation of triplicates)

Table 11. Anaerobic digestion characterization and removal capacity

Pretreatments	Biogas Final (mL)	Initial parameters			Removal capacity		
		COD (g L <sup>-1</sup> )	TS (g L <sup>-1</sup> )	VS (g L <sup>-1</sup> )	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).

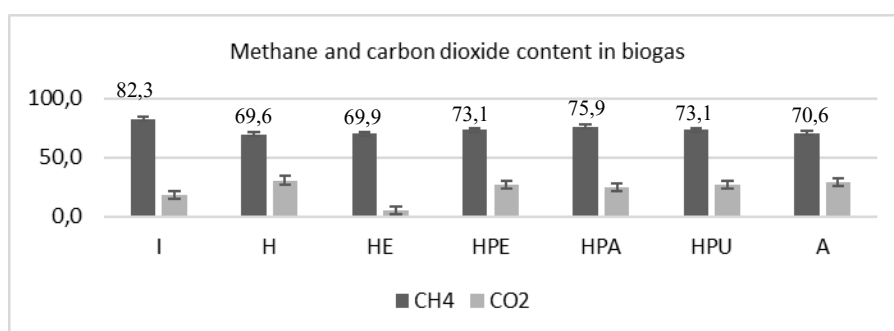


Fig. 11. Methane and Carbon Dioxide content (%) in biogas on 23rd day.

## 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.

## References

- [1] Passos F., Solé M., Carrère H., Ferrer I., Pretreatment of microalgae to improve biogas production: a review. *Bioresour Technol.*2014, 172, 403-412.
- [2] Chernicharo, Carlos. *Anaerobic Reactors*. Biological Wastewater Treatment Series, four ed.,2007
- [3] Al-Zuhair S, Ashraf S, Hisaindee S. Enzymatic pre-treatment of microalgae cells for enhanced extraction of proteins. *Engineering in Life Sciences.*2017,17,175-185.
- [4] Santos CA, Nobre BP, Lopes da Silva T, Pinheiro HM and Reis A (2014). Dual-mode cultivation of *Chlorella protothecoides* applying inter-reactors gas transfer improves microalgae biodiesel production. *Journal of Biotechnology* 184:70-83. doi: 10.1016/j.jbiotec.2014.05.012
- [5] APHA-American Public Health Association. 2012. *Standard Methods for examination of water and wastewater*, Washington DC.
- [6] *Standard Practice for Analysis of Reformed Gas by Gas Chromatography*, ASTM D1946-90, ASTM International, West Conshohocken, PA (2000).
- [7] Jankowska E., Sahub A.K., Oleskowicz-Popiel P., 2017, Biogas from microalgae: Review on microalgae's cultivation, harvesting and pretreatment for anaerobic digestion, *Renewable and Sustainable Energy Reviews* 75.2017., 692–709
- [8] Socorro M., Mehid J., Ladion W., Teves F., Purple Nonsulfur Bacteria (PNSB) Isolated from aquatic sediments and rice paddy in Iligan City. *Multidisciplinary Studies*. 1<sup>o</sup> ed.2013