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Anaerobic Digestion of pre-treated Microalgae Biomass

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Chlorella vulgaris microalgae biomass was cultivated in brewery secondary effluents and used as a recalcitrant effluent to be valorised energetically by anaerobic digestion process. All previous techniques applied for cellular disruption – autoclave, freeze/heating, ultrasound, microwave - provided either high absorption values and release of reducing sugars in the medium or membrane cells damage, compared to the untreated sample, indicating that the pre-treatment action was effective.
The highest methane production was attained by the autoclave and untreated microalgae assays (samples with less permeabilized cells) while the lowest was provide by the microwaves biomasses pre-treatment: 163-178 mL versus 67 mL CH_4. COD removal of 27-29 % and 16 % and TS removal of 28-32 % and 17 % were obtained, respectively. The corresponding methane yield achieved values of 0.04 and 0.030 L g^{-1} COD and 0.205-0.235 L g^{-1} TS related to concentrations determined in the influent.

1. Introduction

The use of fossil fuels raises serious environmental concerns, such as greenhouse gas (GHG) emissions that contribute to global warming and cause adverse effects on mankind. A global movement towards the generation of renewable energy is therefore under way, namely regarding the recent Paris Agreement on Climate Change, signed by 195 countries, to limit global warming to well below 2 °C. For this reason, the development of alternative renewable energy sources is necessary in order to simultaneously reduce dependence on oil and mitigate climate change (Uggetti et al. 2014).
Anaerobic digestion is an appealing technology for the organic effluents valorisation as it adds value by providing biogas/methane, a local renewable energy source, several bioactive compounds for further industrial applications and nutrient-rich streams for local landscape proposals.
In recent years, there is a growing interest in the use of microalgae for the production of biofuels because algal biomass offers several potential advantages compared with other feedstocks, including a higher real biomass productivity, high lipid content and higher value products (Batista et al. 2017, Frigon et al. 2013). The use of the whole microalgae cells for methane production as a biofuel has been suggested and verified under a life cycle analysis (Collet et al. 2017). However, the microalgae cell wall is mostly composed of organic compounds with slow biodegradability and/or bioavailability, such as cellulose and hemicellulose that hinders the methane production, since organic matter retained in the cytoplasm is not easily accessible to anaerobic bacteria (Passos et al. 2014). For this reason, pre-treatment techniques have been proposed to release carbohydrates, lipids and proteins from the microalgae cell inner.
The present work study the impact of the different biomass pre-treatments on algae cells to promote cell rupture and intracellular compounds release and, afterwards, the pre-treatments effects on anaerobic digestion process for biogas production.

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2. Material and methods

2.1 Microalgae biomass

Microalgae biomass was obtained from a culture of *Chlorella vulgaris* previously grown in brewery wastewater. The biomass of cultured cells was harvested by centrifugation to wet matter content of 35 % (w/v), and stored at 4 ºC for further use.

2.2 Analytical methods

The effect of the pre-treatment methods on cell disruption was analysed under absorbance spectroscopy of centrifuged samples (1 min at 12800 g; dilution 1/10) at 280 nm (maximal absorption of the three aromatic amino acids phenylalanine, tyrosine and tryptophan) to monitor the released protein. The release of organic compounds such as protein, carbohydrates and DNA in the supernatant was also analysed by the absorbance in spectrograms with wavelength between 190 and 290 nm. Reducing sugars were estimated by the dinitrosalycilic acid method (Miller, 1959). Flow cytometry (BD Biosciences) was used to evaluate the percentage of cell disruption and to monitor cytoplasmic membrane integrity when coupled with propidium iodide.

The substrates liquid phase were characterized in terms of total solids (TS), volatile solids (VS), chemical oxygen demand (COD), volatile fatty acids (VFA) and pH, at the beginning and in the end of the anaerobic batch assays according Standard Methods (Rice et al., 2012). VFA analysis was performed using a Hewlett Packard 5890 gas chromatograph with flame ionization detector and the values expressed in terms of acetic acid (HAc).

Biogas production was monitored daily with a pressure transducer and gas composition, in terms of methane, was analysed weekly, according to Standard Method [D1946–90, 2000] and using a VARIAN 430 GC gas chromatograph with a thermal conductivity detector and a column Select Permanent Gases/CO2 HR Molsieve 5A Parabond Q tandem. All gas volumes were corrected to STP conditions (Standard Temperature and Pressure: 0 °C, 1 atm).

2.3 Pre-treatment methods

Microalgae biomass at 35 % (w/v) was pre-treated by using four different methods of cell disruption, under aerobic conditions: (1) autoclaving at 120 ºC for 30 min, under 1.2 bar; (2) freezing overnight at -20 °C, followed by heating for 3 h at 100 °C in a dry bath (Model D1100, Labnet); (3) ultrasound treatment (three times for 5 min at 30 kHz output, on ice; vibra-cell VC505, Sonics) and (4) microwave heating (1 min until boiling at 550 W, followed by 4 min at 750 W and 2450 MHz; Whirlpool).

2.4 Anaerobic digestion assays

Anaerobic digestion of physically disrupted cell material was carried out in comparison to untreated microalgae biomass (positive control assay). A negative control assay was also provided using the same inoculum concentration, but without substrate (microalgae biomass) addition. Anaerobic digestion assays were conducted in batch mode, in triplicate and under mesophilic conditions (37 °C). Glass vials of 71.5 mL of total volume and 40 mL of working volume were used for 32 days, until methane production plateau was reached. Sludge from an anaerobic digester plant was obtained in the region of Leiria (Portugal) and it was used as inoculum of the experiment. The inoculum was characterized by a pH of 7.4, a content of solids of 15.0 ± 1.3 g L^{-1} (TS) and 5.4 ± 0.2 g L^{-1} (VS), and a COD concentration of 18.2 ± 1.6 gO_2 L^{-1}.

3. Results and discussion

3.1 Pre-treatments and cell disruption

All disruption techniques were successfully applied, as shown in Table 1 and Figure 1. They were able to release reducing sugars, providing higher absorptions values and damaged membrane cells, compared to the untreated sample (control).

Photometrical measurement due to released cytosolic protein, by cell wall disruption, was highest in ultrasound treated samples. Comparatively, decreasing amounts were detected in samples submitted to autoclave. Methods of cell disruption using microwave and freeze/heating were less successful. Similar results were obtained with the analysis of reducing sugars (Table 1). According to the flow cytometry results (Table 1), values higher than 50 % of damage were registered indicating that all pre-treatments affected cell membranes. Many studies have been reviewed (Günerken et al. 2015, Jankowska et al. 2017) for the application of pre-treatment methods to increase microalgal biomass cell wall digestibility. Overall, the best results in microalgae pre-treatment for the anaerobic biodegradability have so far been achieved with thermal

and ultrasound methods (Passos et al. 2013, Jankowska et al. 2017) indicating that the results shown in this study are in accordance with literature.

Table 1: Microalgae cell disruption: absorbance at 280 nm and reducing sugars of treated vs. untreated (control) sample, and measurement of permeabilized cells by flow cytometry. All results were mean of duplicates.

Pre-treatments	Reducing sugars (mg ml^{-1})	A_{280nm}	Cells with damaged membrane (%)
Control	0.076 ± 0.001	0.068 ± 0.002	3
Microwave	0.101 ± 0.004	0.200 ± 0.001	99
Freeze-heating	0.146 ± 0.004	0.514 ± 0.009	91
Autoclaving	0.157 ± 0.003	0.689 ± 0.012	55
Ultrasound	0.408 ± 0.006	1.000 ± 0.000	84

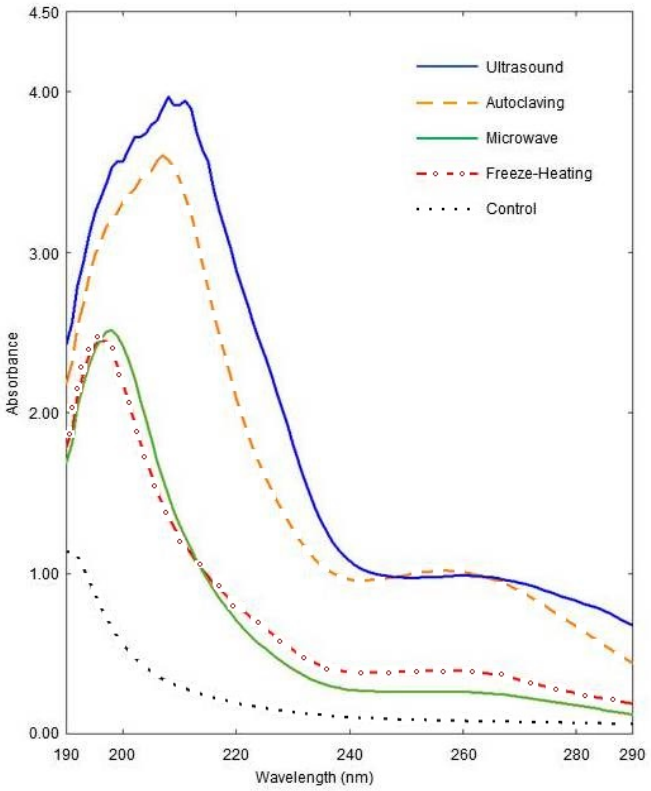

Figure 1: Spectrograms of treated vs. untreated (control) sample obtained with wavelength range 190-290 nm

3.2 Anaerobic digestion of pre-treated substrates

Highest methane volume was recorded in the units digesting the autoclaved microalgae (Figure 2) followed unexpectedly by the control untreated biomass (178 and 162 mL CH_4, respectively). Indeed, comparatively, control units exhibited a very interesting behaviour suggesting that the anaerobic digestion of the raw microalgal substrate should be taken into account in future studies, in order to evaluate the advantage and drawbacks of including pre-treatment or digesting directly *Chlorella* of brewery effluents. In opposition, the microwave pre-treatment, given only a volume of 67 mL CH_4, provided the lowest methane production of the assay.

By ordering the gas productions recorded in anaerobic digestions of each pre-treated substrates in a descending order and considering the organic load involved, as shown in Table 2, it is notorious the relationship between the methane volume and the organic matter (COD and solids contents) disposable for anaerobic process. This relationship, despite being obvious and expected, shows that the difference among

the gas productions of the diverse pre-treated substrates results from the variation of the respective load accessible for conversion and not due to any inhibition process in terms of anaerobic digestions development. In other words, the feature of relevant importance regarding the application of a defined pre-treatment is related to the pre-treatment ability of providing material for the anaerobic digestion, but also with the quantities of organic matter that it can make available to be converted in biogas/methane.

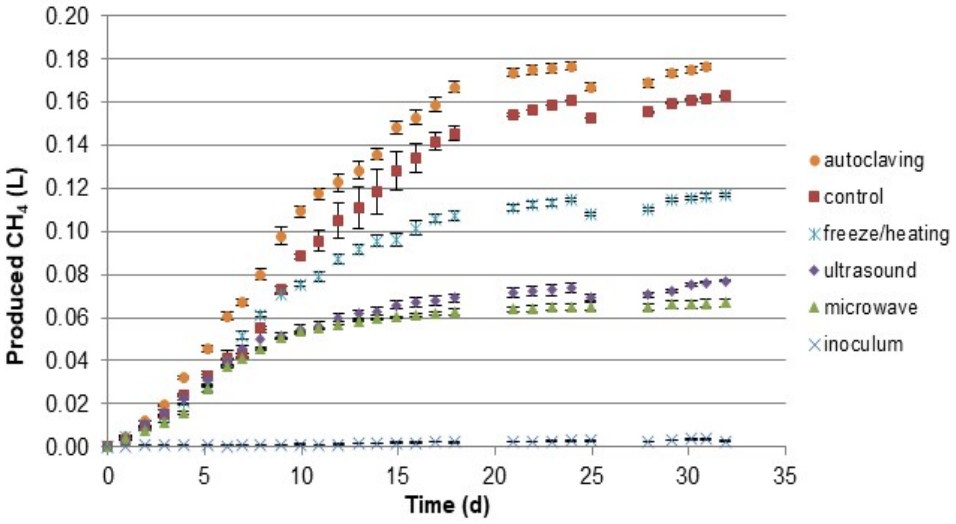

Figure 2. Accumulated methane (CH4) production (STP conditions; mean values and standard deviation of triplicates)

Accordingly, the digestion of the most concentrated substrates – autoclave and control assays, with COD values of 101-109 g L^{-1} and solids of 19-20 g L^{-1} (TS) and 15 g L^{-1} (VS) – provided the highest gas proportions (250-265 mL of biogas and 162-178 mL of methane) and the greatest removal amounts. Values of COD removal of 27-29 %, TS removal of 28-32 % and VS removal of 35-41 % were registered (Table 2 and Table 3).

Table 2: Anaerobic digestion characterization

Pre-treatments	Methane	Anaerobic digestion influent		
	(mL)	COD (g L^{-1})	TS (g L^{-1})	VS (g L^{-1})
autoclave	178.1	101.4	18.9	14.9
control	162.5	108.5	19.8	15.4
freeze/heating	117.1	90.3	16.8	12.9
ultrasound	76.9	69.4	12.6	9.0
microwave	66.7	55.8	12.0	8.4
inoculum	2.7	29.3	9.1	4.1

COD – Chemical Oxygen Demand, TS – total solids, VS – volatile solids

Table 3: Anaerobic digestion: removal capacity

Pre-treatments	COD removal (%)	TS removal (%)	VS removal (%)
autoclave	26.7	32.3	40.9
control	29.4	28.3	35.1
freeze/heating	15.6	23.2	32.6
ultrasound	2.9	9.5	16.6
microwave	16.4	16.7	25
inoculum	0	0	0

COD – Chemical Oxygen Demand, TS – total solids, VS – volatile solids

Methane yield achieved in this experiment ranged from 0.030 to 0.044 L CH4 g-1 COD influent, 0.139 to 0.235 L CH4 g-1 TS influent and 0.199-0.298 L CH4 g-1 VS influent (Table 4), having the highest efficiencies been achieved in the autoclave and control assays. In fact and according to González-Fernández et al. (2012) and Jankowska et al. (2017), the thermal pre-treatment is the method that seems to give the best results for anaerobic digestion of microalgae biomass. An increase of 123 % was achieved in methane yield with the thermal methods. Kinnunen et al (2014) reported that using freeze-thaw pre-treatments, it improved methane/biogas production by enhancing microalgae digestibility (32-50 %).
The results obtained with the present work indicate that the pre-treatment induce organic matter loss, leading a decrease on the available amount for anaerobic digestion conversion to biogas. In fact, the highest losses were recorded in the ultrasounds and microwave assays which were characterized by the lowest organic load and methane production.
The comparison of the VFA concentrations present in the samples resulting from each pre-treatment, shown in Table 5, are confirming this finding and indicate that substrates are undergoes different structural changes in their composition according the methodologies applied. As example, the autoclave and control pre-treatments kept the organic material in concentrations not so far from the freeze/heating unit (101, 109 and 90 g L^{-1} COD, respectively) but distinguished in terms of composition. The occurrence of different concentrations of acids to be digested anaerobically among the diverse samples were noticed according the registered values of 11, 31 and 53 mg L^{-1} HAc in autoclave, control and freeze/heating substrates, respectively. Furthermore, in term of each acid participation in the VFA total, it was observed that the acetic acid is the main component in the case of autoclave and freeze/heating samples, while it corresponded only about half of the VFA concentration in the control substrate pre-treatment (100 % versus 49 %, Table 5). The pH alteration to more neutral values, observed in all cases, reveals the absence of any inhibition during the digestion process and can be inferred that all pre-treated substrates were likely to be valued by anaerobic digestion.

Table 4: Anaerobic digestion: methane yield

Pre-treatments	CH_4 yield ($L.g^{-1}$ COD_{in})	CH_4 yield ($L\ g^{-1}$ TS_{in})	CH_4 yield ($L.g^{-1}$ VS_{in})
autoclave	0.044	0.235	0.298
control	0.037	0.205	0.264
freeze/heating	0.033	0.174	0.226
ultrasound	0.028	0.153	0.213
microwave	0.030	0.139	0.199
inoculum	0.002	0.007	0.016

COD – Chemical Oxygen Demand, TS – total solids, VS – volatile solids, in – influent

Table 5: Anaerobic digestion: pH and Volatile fatty acids

Pre-treatments	pH_{in}	pH_{out}	VFA_{in} (mg L^{-1} HAc)	VFA rem. (%)	HAc (% VFA_{in})
autoclave	8.3	7.3	10.93	61.1	100
control	8.5	7.4	30.53	32.1	48.5
freeze/heating	8.0	7.3	53.20	88.0	100
ultrasound	8.1	7.2	12.91	67.9	95.9
microwave	8.5	7.1	4.48	13.9	100
inoculum	8.5	7.7	6.71	-72.3	100

VFA – volatile fatty acids, in – influent, out – effluent, rem. – removal

4. Conclusions

Disruption pre-treatment of *Chlorella vulgaris* were successfully applied, compared to the untreated sample, as they provided cellular material release to the medium, such as proteins and carbohydrates, and damage of cell membranes higher than 50 %. However, the amount of released material was not directly related to the cell damage effect.
Methane volumes of 178 mL was recorded in the autoclave assay, along of the 32 days of experiment. Comparatively, untreated microalgae biomass (control) exhibited a good behaviour given a methane production of 163 mL.

All pre-treated substrates were easily digested under anaerobic conditions without any apparent inhibiting effect. The methane productions are in accordance with the organic loads available in each assay for anaerobic process.

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References

Batista A., Lópes E., Dias C., Lopes da Silva T., Marques I., 2017, Wastes valorization from *Rhodosporidium toruloides* NCYC 921 production and biorefinery by anaerobic digestion, Bioresour. Technol., 226, 108-117

Collet P., Hélias A., Lardon L., Ras M., Goy R.A., Steyer J.P., 2011, Life-cycle assessment of microalgae culture coupled to biogas production, Bioresour Technol., 102, 207–14.

D1946 – 90, reapproved 2000, Standard Practice for Analysis of Reformed Gas by Gas Chromatography**,** ASTM, West Conshohocken, USA.

Frigon J.-C., Matteau-Lebrun F., Abdou R., McGinn P., O'Leary S., Buiot S., 2013, Screening microalgae strains for their productivity in methane following anaerobic digestion, Appl. Energy, 108, 100-107.

González-Fernández C, Sialve B, Bernet N, Steyer JP. Thermal pre-treatment to improve methane production of Scenedesmus biomass. Biomass Bioenergy 2012;40:105–11.

Günerken E., D'Hondt E., Eppink M.H.M., Garcia-Gonzalez L., Elst K., Wijffels R.H., 2015, Cell disruption for microalgae biorefineries, Biotechnology Advances, 33, 243–260. Doi.10.1016/j.biotechadv.2015.01.008

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, 692–709

Kinnunen V, Craggs R, Rintala J. Influence of temperature and pretreatments on the anaerobic digestion of wastewater grown microalgae in laboratory-scale accumulating-volume reactor. Water Res 2014;57:247–57.

Miller G.L., 1959, Use of dinitrosalicylic acid reagent for determination of reducing sugar, Analytical Chemistry, 31, 426–8.

Passos F., Solé M., García J., Ferrer I, 2013, Biogas production from microalgae grown in wastewater: Effect of microwave pretreatment, Applied Energy, 108, 168–175

Passos F., Uggetti E., Carrére H., Ferrer I, 2014, Pretreatment of microalgae to improve biogas production: a review, Bioresour Technol,172, 403-412.

Rice E.W., Baird R.B., Eaton A.D., Clesceri L.S., Greenberg, A.E., 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., American Public Health Association, American Water Works Association, Water Environment Federation, Washington DC, USA.

Uggetti E., Sialve B., Trably E., Steyer J, 2014, Integrating microalgae production with anaerobic digestion: a biorefinery approach, Biofuels, Bioprod. Bioref., 8, 516-529. DOI: 10.1002/bbb.1469.