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ENERGY RECOVERY FROM IMMOBILISED CELLS OF *SCENEDESMUS OBLIQUUS* AFTER WASTEWATER TREATMENT

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Summary
Biomethane batch test of alginate beads and beads with algae at different stages of utilisation in the wastewater treatment plants showed that immobilised *S. obliquus* yield similar biogas and biomethane than freely suspended algae (between 60.51 ± 4.19 and 82.32 ± 2.17 mL g-1 VSadd) and that a pre-treatment stage was not necessary for the digestion process.

Introduction
Microalgae have shown to be able to remediate nutrients effectively from secondary wastewater, their use in WWT processes and biogas production by anaerobic digestion (AD) was first reported by Golueke at al. (1957) and Oswald and Golueke (1960). At the time, the authors' main conclusion was that, although the algae removed nutrients to satisfactory levels, the overall process was not economically and energetically viable, and regrettably this is still the case today (Ometto, 2014b). Process intensification can be achieved using cells entrapped into a resin or gelatinous media, such as alginate or synthetic polymers (3.3 g·L-1 DW). Ruiz-Marín et al. (2010 and 2011) and Whitton et al (2014) all demonstrated good nutrient removals with immobilised algae. However, even when immobilised, the inclusion of microalgae in the WWT process for nutrients absorption could only be justified if biomass is processed to recover energy.

Of the currently available biomass-to-energy technologies, gasification, thermochemical liquefaction, direct combustion and anaerobic digestion (AD), AD provides the most feasible process for large scale application which, depending on the chemical composition, has the potential to yield up to 800 $mlCH_4$ gVS^{-1} (Heaven *et al.,* 2011). However, microalgae species have the ability to resist microbial degradation, their structure and chemical composition identified the cell wall as the main limiting factor to microbial degradation (Atkinson *et al.,* 1972; Burczyk *et al.,* 1999). High energy (thermal and ultrasound) and low energy (mechanical and biological) pre-treatments can be used to: (1) degrade the cell wall, (2) release AOM and hence (3) enhance methane production (Alzate *et al.,* 2012; González-Fernández *et al.,* 2012b; Cho *et al.,* 2013a).

Batch anaerobic digestion experiments were used to assess the effect of thermal and biological pre-treatment on the methane production of immobilised *Scenedesmus obliquus*, after nutrients removal process.

Material and methods
<u>Algae culture and immobilisation</u>

The *S. obliquus* (276/42) culture was obtained from the Culture Collection for Algae and Protozoa (CCAP), (Oban, UK). Microalgae was cultured in batch in 100 L tanks containing 50 L Jaworski media as reported in Ometto et al., 2014c and b . Immobilisation conditions were reported in Whitton et al., 2016.

<u>Batch anaerobic digesters</u>

Five different substrates were analysed in this work. Four types of algal beads; 1) Blank Beads with no algal biomass, with only the alginate matrix (BB), 2) Clean Algal Beads (CA), fresh beads containing microalgae cells imbedded in the alginate matrix that have not been used for wastewater remediation, 3) Beads after 6 days of wastewater treatment (6-d UA), 4) Beads at 10 days usage (10-d UA), and 5) the residual algal sludge at the end of the columns experiment (AS). All substrates were characterised in terms of TS and VS before and after anaerobic digestion (APHA, 2005). The four types of beads were pre-treated and the degradation of their structure was analysed. The biomethane test was carried out on both untreated and enzymatically treated substrates. The batch tests were done as reported Ometto et al., 2014b

Pre-treatments (thermal and biological)

Thermal pre-treatment of the beads biomass was achieved using an autoclave at 121ºC and 1.06 bar for 30 min. The solid content (VS and TS) and the sCOD were measured in duplicate before and after treatment (Ometto, 2014c). Only beads pre-treated with E1 and E2 at 150 U mL^{-1} (equivalent to 7.5 U kg^{-1} TS) were used for the biomethane test.

The enzymes used in the study experiments are summarised in Table 1.

Table 1. List of enzyme used for the biomass pre-treatment

Enzyme	Commercial name	Composition	Conditions tested
E1	DepolTM 40L	Cellulase 1,200 U g^{-1} +Endogalactouronase 800 U g^{-1}	25, 50, 150, 250, and 350 U mL^{-1}
E2	LipomodTM 957	Esterase 3,600 U g^{-1} +Protease 90 U g^{-1}	150 and 250 U mL^{-1} (7.5 U kg^{-1}TS)
E3	LipomodTM 166P	Esterase 5,220 U g^{-1}	150 and 250 U mL^{-1} (7.5 U kg^{-1}TS)
E4	Lipase LT	Lipase 100,000 U g^{-1}	150 and 250 U mL^{-1} 7.5 U kg^{-1}TS)
E5	Accelerase 1500	Endoglucanase 2200 U g^{-1} + β-Glucosidase 450 U g^{-1}	150 and 250 U mL^{-1} (7.5 U kg^{-1}TS)

Results and discussion

The calculation of the biogas yield for the untreated beads were 29.73 ± 2.17 mL for DS; 160.12 ± 6.66 mL for Blank Beads (BB); 124.27 ± 15.56 mL for (Clean algae beads) CA; 179.54 ± 12.43 mL for 6-d (Untreated Algae Beads) UA; 254.17 ± 6.71 mL for 10-d UA; 175.51 ± 32 mL for AS; and 217.82 ± 24 mL for control with cellulose. Ometto et al. (2014c) obtained a biogas yield of 265.28 ± 10 mL g-1 VSadd, which is similar to the 10-d UA value.. 10-d UA beads demonstrated a similar behaviour to suspended *S. obliquus* microalgae for biogas and biomethane production. The 10-d beads were weak and misshapen due to degradation of the alginate matrix hence exposing the immobilised cells, this is likely to be the reason for the similarity to suspended calls. The lower performance of 6-d UA, and the even lower of CA, was likely due to the lower biomass concentration as a result of the reduced contact time with wastewater and reduced growth (105 microalgae cells in CA beads, 5.3x105 cells in 6-d UA, and 7x105 cells in 10-d UA). Results are reported in Figure 1.

Biogas yield of enzymatically pre-treated beads, resulted in enzymes and dosage: 1316.3 121.60 ± 32.18 mL (for BB+E1); 125.27 ± 24.01 mL (for CA+E1); 142.41 ± 3.44 mL (for 6-d UA+E1); 271.16 ± 3.04 mL (for 6-d UA+E2); 20 and 135.83 ± 9.72 mL (for 10-d UA+E1). The cumulative biogas values are lower than those reported by Ometto (2014c) for suspended *S. obliquus* with the same ± 224 mL g^{-1} VSadd with E1 at 150 U mL^{-1} and 986.34 ± 201 mL g^{-1} VSadd with E2 at 150 U mL^{-1}. About the CH_4 production, excepting BB, all substrates yielded a very similar amount of biomethane after 33 days. Results are reported in Figure 2.

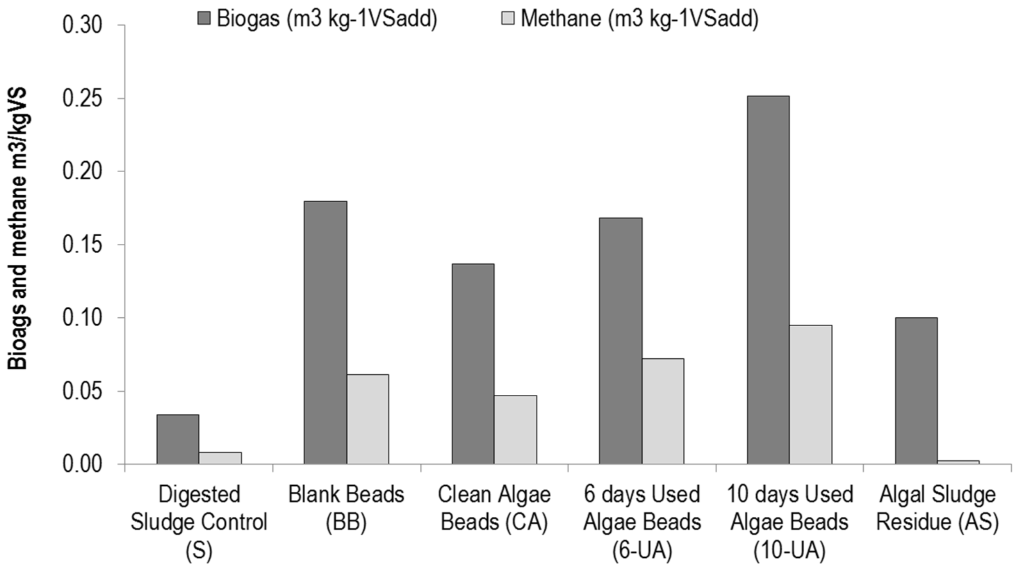

Figure 1. Biogas and biomethane production in batch tests of untreated exhausted algae beads after nutrient removal.

ESEM pictures (Figure 3) of the untreated and treated beads showed that higher biodegradability was observed after enzymatic pre-treatment in terms of alginate structure damage. The granules visible on the outside of the treated beads are likely to be non-dissolved enzymes.

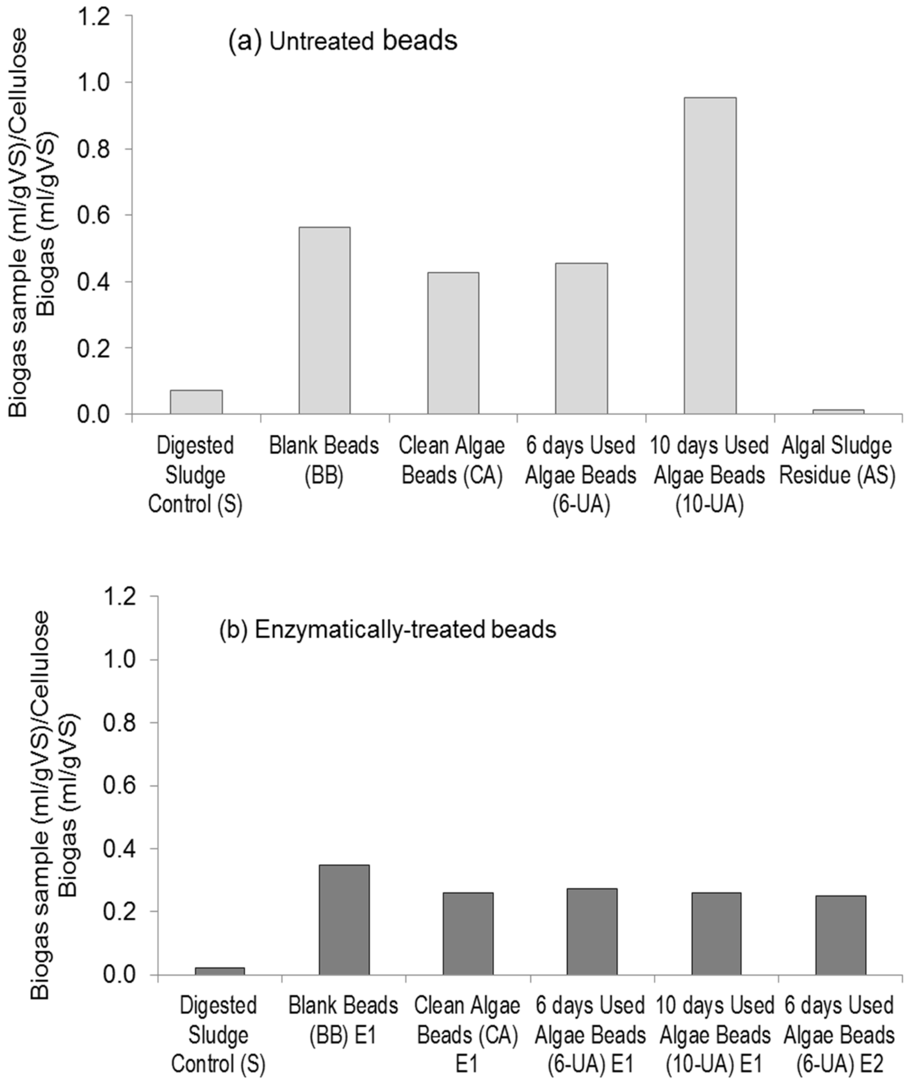

Figure 2. Comparison of biogas production of treated (enzymatically) and untreated algae beads in batch tests. Biogas values have been normalised using the value obtained for cellulose (reference material) to allow comparison amongst different batches.

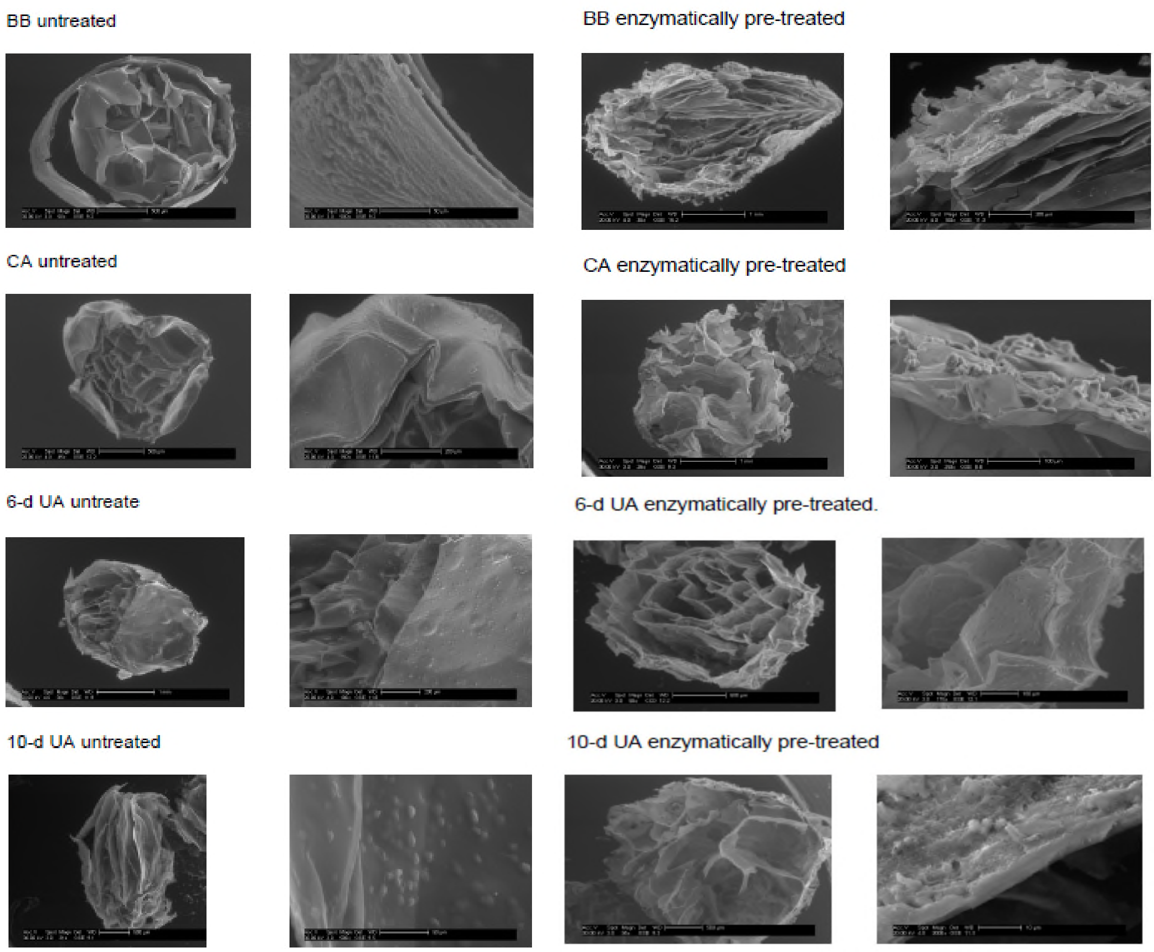

Figure 3. ESEM pictures of treated (enzymatically) and untreated algae beads.

Conclusions

- Biogas can be produced from microalgae (*S. obliquus*) after wastewater treatment process
- Immobilised *S. obliquus* used for wastewater treatment can produce similar biogas and biomethane yields compared to freely suspended *S.obliquus*.
- Specific biogas and methane values of microalgae beads gave industry-acceptable values (up to 80%).
- Pre-treatment of the algae beads to increase biogas production did not give positive results. This indicates an inhibition during AD, probably caused by the released of calcium alginate (bead matrix).

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