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Short Communication

Pre-treatment options for halophytic microalgae and associated methane production

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HIGHLIGHTS

- All anaerobic digestion successful at 7% salinity (2× seawater salinity).
- Methane production rate of 122 mL per g VS for lipid extracted *Tetraselmis* sp.
- Methane production rate of 252 mL per g VS for non-disrupted *Tetraselmis* sp.
- Methane production rate of 248 mL per g VS for pretreated disrupted *Tetraselmis* sp.
- Microbial degradation of *Tetraselmis* sp. demonstrated during anaerobic digestion.

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ABSTRACT

Methane production from lipid extracted, pre-treated disrupted and non-pretreated *Tetraselmis* spp. microalgae was investigated. The results demonstrated that 122 mL per g VS methane was produced for the lipid extracted *Tetraselmis* spp., demonstrating that lipid free *Tetraselmis* can be effectively digested in an anaerobic environment. A total of 252 mL per g VS and 248 mL per g VS of methane was reported for non-disrupted and pre-treated disrupted *Tetraselmis* sp. respectively. It was also observed that the microbial community caused cell lysis of *Tetraselmis* spp. during the anaerobic digestion process. Cell lyses can offer a direct conversion pathway of intact *Tetraselmis* spp. for energy production, thus negating the need for pre-treatment.

1. Introduction

Anaerobic digestion of residual microalgae biomass after lipid extraction for lipid based biofuel production provides the ability to recover nutrients whilst producing methane for conversion to electrical and thermal energy (Ward et al., 2014). The anaerobic digestion of intact microalgal biomass offers great potential for biogas production from microalgae based wastewater treatment systems (Golueke et al., 1964).

One of the major problems associated with the anaerobic digestion of microalgae is the need to break the cell wall allowing the cell contents to be processed by the bacterial community to form precursor chemicals for the formation of methane biogas (Chen and Oswald, 1998; Gonzalez-Fernandez et al., 2012b; Mussgnug et al., 2010; Samson and Leduy, 1983; Sialve et al., 2009; Ward et al., 2014).

Golueke et al. (1957) demonstrated the ability of *Scenedesmus* spp. and *Chlorella* spp. of microalgae to pass through an anaerobic digester intact and remain undigested. The authors noted that *Scenedesmus* spp. and *Chlorella* spp. microalgal cells are known to effectively resist bacterial attack, and the authors detected intact microalgae cells in the digestate after a 30-day hydraulic retention time.

Research undertaken by Mussgnug et al. (2010) highlighted the role of the cell wall in the digestion process. Mussgnug et al. (2010) results indicate that the highest gas production reported was due to microalgae species that had either no cell wall or a protein based cell wall. Gas production was observed to decrease for microalgal species that had a carbohydrate-based cell wall containing hemicellulose. The lowest gas production reported came from the species *Scenedesmus obliquus* that has a particular rigid cell wall containing a large proportion of sporopollenin like biopolymers.

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Little or no cell wall degradation was detected in *S. obliquus* and very low methane volumes were produced by the microalgae substrate when anaerobically digested. The authors Mussgnug et al. (2010) and Gonzalez-Fernandez et al. (2012a) both concluded that the degradation of the cell wall was strongly correlated to the amount of biogas produced during anaerobic digestion.

Multiple authors indicated the need for a pre-treatment step to disrupt the cell wall increasing bacterial hydrolysis during anaerobic digestion (Golueke et al., 1957; Mussgnug et al., 2010; Ward et al., 2014). The various mechanical, physical, thermal and chemical methods used to improve microalgae methane potential have a high energy investment (Lee et al., 2013). Lee et al. (2013) have shown that the disruption or cell lyses of *Tetraselmis* spp. of microalgae is higher than the energy content of the *Tetraselmis* spp. cell. Therefore the method of cell disruption and biomass treatment plays a critical role in energy utilisation and overall commercial feasibility of a microalgae based biofuels production and microalgae wastewater treatment systems.

This reported study investigates the role of cell disruption and biomass lipid extraction by comparing methane gas potentials from anaerobically digested *Tetraselmis* sp. with 3 different pre-treatment scenarios. The first treatment comprises microalgae that have been disrupted and the lipid content extracted with hexane, as is the standard procedure for lipid based biofuel production (Pragya et al., 2013). The second treatment evaluates the gas potential of disrupted *Tetraselmis* sp. biomass, and the third treatment investigates un-disrupted, un-treated, direct digestion of intact *Tetraselmis* sp. biomass for methane potential.

2. Methods

2.1. Source of microalgae

The microalgae *Tetraselmis* sp. (MUR 233) was grown in outdoor open raceway ponds located in Karratha, Western Australia, Australia (20S 45′47.72″, 116E 44′9.88″). Microalgae biomass was first harvested utilising electroflocculation and then further concentrated to 20% dry weight solids by centrifugation utilising a T10 Evodos centrifuge and transported frozen to the laboratory. Biomass was then defrosted and resuspended to 10% solids w/w content for experimental use. Prior to use, the disrupted and lipid extracted microalgae biomass treatments were sonicated using a Branson sonifier at a resonance of 10 kHz for 10 min to disrupt the microalgae cell wall. The lipid extracted biomass was prepared from dried *Tetraselmis* spp. that was solvent extracted (Hexane) using a soxhlet apparatus. After lipid extraction the residual biomass was dried to remove any entrained solvent and resuspended in saline water (7%) at 10% solids w/w concentration for experimental use. For the third treatment, intact *Tetraselmis* spp. was used with no pre-treatment.

2.2. Digester setup

Twelve 500 mL Schott bottles with an initial working volume of 450 mL were used for the experiment, allowing four separate treatments in triplicate. The four treatments consisted of non-disrupted *Tetraselmis* spp., sonicated disrupted *Tetraselmis* spp., extracted *Tetraselmis* spp. and a control inoculum treatment. This inoculum was sourced from a anaerobic digester operating at 7% salinity (Ward et al., 2015). The control treatment contained only the inoculum volume as used in all treatments, and biogas produced from the control treatment was deducted from the biogas volumes produced from the three experimental treatments. The Schott bottles were fitted with a stopper and an air tight tube, which was connected to an inverted measuring cylinder. The displacement of water within the measuring cylinder was used to quantify the gas volume produced, which was recorded and reset daily. The methane content was determined using a SRI 8610C gas chromatograph (SRI Instruments, Torrance, CA) fitted with a thermal conductivity detector (TCD), using helium as a carrier gas in a 0.3-m HaySep-D packed Teflon column with a capillary 6′ silica gel column and a capillary molecular sieve column (6′MS13X) (Labatut et al., 2011). A total of 2.43 g of the *Tetraselmis* spp. was fed to the digester at the start of the experimental period, which equated to a loading rate of 5.4 g VS L^{-1} per replicate. The *Tetraselmis* spp. feedstock was stored at 4 °C to prevent pre-digestion prior to use during the experimental period. The treatment digesters were placed in a water bath held at 37 °C for the duration of the experiment. The Schott bottles were shaken daily to resuspend settled material. Gas production and the corresponding methane percentage were recorded for all treatments over the experiment. The *Tetraselmis* spp. feedstock was characterised for total solids (TS) and volatile solids (VS) content. The TS content of the *Tetraselmis* spp. feedstock was determined by drying the samples at 50 °C until a constant weight was recorded. The VS content was determined using standard wastewater methods (Clesceri et al., 1998), where the oven dried samples were placed in pre weighted crucibles and then ignited for 2 h at 550 °C in a muffle furnace. The data was analysed for significant differences utilising a One-way ANOVA of variance with least significant difference test utilising the software package SPSS version 21. All data was checked for population normality and homogeneity of variance prior to analysis.

3. Results and discussion

All treatments produced biogas. The pH ranged between 7.22 and 7.88 for the control treatment. This was significantly different to the non-disrupted, disrupted and extracted treatments ($P = 0.00$), although no significant difference was found between the non-disrupted, disrupted and extracted treatments ($P = 1.00$). The pH for the non-disrupted, disrupted and extracted *Tetraselmis* spp. treatments ranged from 6.79 to 7.11 for the non-disrupted, 6.80 to 7.29 for the disrupted and 6.78 to 7.41 for the extracted biomass treatment respectively. The reduced pH within the non-disrupted, disrupted and extracted *Tetraselmis* spp. treatments was within the optimum anaerobic digestion operational pH range of 6.5–7.6 reported by Parkin and Owen (1986). This initial decrease in pH is believed to be due to the solubilisation of organic material and production of volatile fatty acids (VFA's) produced from the degradation of *Tetraselmis* spp. by the acetogenic bacteria. Increasing pH was noted in the non-disrupted, disrupted and extracted *Tetraselmis* spp. treatments during later stages of the experimental period. This indicates that the methanogenic bacteria were utilising the precursor VFA's and converting them to methane, which indicated that the acetogenic and methanogenic bacterial communities were balanced and essential for stable digester performance (McCarty, 1964; Ward et al., 2014).

A significant difference ($P = 0.00$) between treatments was found for the total biogas produced for both the disrupted and non-disrupted *Tetraselmis* spp. biomass treatments. However no significant difference ($P = 0.607$) was found between the non-disrupted and disrupted *Tetraselmis* spp. treatments. Daily biogas production over the experimental period followed a similar trend for the non-disrupted and disrupted *Tetraselmis* spp. treatments (Fig. 1). Gas production increased rapidly and remained elevated until day 15 where upon, gas production reduced to the end of the experimental period. The extracted *Tetraselmis* spp. treatment also followed a similar trend with gas productivity until day 10 before a reduction occurred. This trend was repeated in the