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The effects of alternative pretreatment strategies on anaerobic digestion and methane production from different algal strains

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HIGHLIGHTS

- Anaerobic digestion of microalgae can be hampered by low biodegradability.
- We examined the effect of pretreatment on algal solubilization and methane yields.
- Chemical or thermal pretreatments were ineffective on methane yield.
- Thermochemical pretreatment improved methane yield by 30–40% from specific species.
- Algal type and the cell-wall nature strongly impacts on biogas and methane yields.

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ABSTRACT

The effect of various pretreatment strategies on methane yields following anaerobic digestion (AD) of five different microalgal strains was investigated. *Pavlova_cf* sp., *Tetraselmis* sp. and *Thalassiosira weissflogii* exhibited substantial methane yields of 0.4–0.5 L/g volatile solids (VS) without pretreatment, providing up to 75–80% of theoretical values. In contrast, methane yields from *Chlorella* sp. and *Nannochloropsis* sp. were around 0.35 L/g VS, or 55–60% of the theoretical values, respectively. Alkali treatment was not effective and thermal pretreatment only enhanced *Nannochloropsis* methane yields. Thermochemical pretreatment had the strongest impact on biomass solubilization with methane yields increasing by 30% and 40% for *Chlorella* and *Nannochloropsis*, respectively. The lipid content had a strong beneficial impact on the theoretical and observed methane yields as compared to protein and carbohydrate content. Other features such as cell-wall composition are also likely to be important factors dictating algal biodegradability and methane yields addressed in part by thermochemical pretreatment.

1. Introduction

Algal biofuel technologies hold much promise as a source of non-fossil fuel with lower greenhouse gas emissions. Anaerobic digestion (AD) may be economical for methane production from the low-quality and low-cost algal biomass generated during either wastewater treatment or harvested from the eutrophic water bodies. In addition, it may be coupled with biodiesel production for utilization of post-extracted algal biomass otherwise lost as waste or low-value by-products. Indeed, the potential energy obtainable from conversion of whole algae with a low lipid content to biogas is higher than from biodiesel alone, and coupled biodiesel and biogas production produces the highest energy output (Harun et al., 2011). Moreover, biogas production through anaerobic digestion (AD) avoids several energy intensive steps including drying and extraction. While biogas production from algae by AD has been the subject of research for more than 50 years, several key obstacles to biogas production remain, especially limitations in the biodegradability of algal biomass. Such bioprocessing bottlenecks reduce the economic feasibility for moving this technology forward.

Biomass biochemical composition may have a major impact on methane and biogas yields (Sialve et al., 2009). However, biodegradability, or the amount of volatile solids (VS) reduced (converted to biogas) during AD, represents another critical element of the overall process. Most of the simple organic compounds including sugars and acids, building blocks of the cells, are readily biodegradable. However, organic compounds organized into polymers and structurally complex parts of the cells such as cell wall may be significantly less biodegradable because of limited accessibility of enzymes to the substrates. As a result, the hydrolysis of particulate organic matter can be a rate-limiting step of AD (Eastman and Ferguson, 1981). Algal cells are encased in a cell wall

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that may be resistant to biodegradation, resulting in a required retention time as long as 20–30 days for conventional anaerobic digestion (Afi et al., 1996). Moreover, the amount of algal VS reduced ranges from 20% to 60% for most macro- and microalgae (Bohutskyi and Bouwer, 2013). Maximization of biomass conversion is crucial for the economic viability of the process. Several methods of algal biomass pretreatment (hydrolysis) have the potential to increase biodegradability, enhance production rate, and improve yields of CH_4. While substantial research has focused on pretreatment of lignocellulosic biomass, livestock manure, primary sewage sludge and waste activated sludge, less data are available regarding the pretreatment of microalgal biomass prior to AD even though this represents one of the most promising strategies to increase the algal digestibility, conversion rates, and methane yields.

The pretreatment methods applied to enhance biomass solubilization and biodegradability include: mechanical (grinding, milling, homogenization, sonication, maceration, liquid shear); thermal (hydrothermal, drying, treatment by steam); chemical (acid or alkali hydrolysis, ozone, hydrogen peroxide); biological (enzymatic); electrical (electro-Fenton); irradiation (gamma-ray, electron-beam, microwave); and combination treatments (e.g., thermo-chemical). Pretreatment methods aim to rupture the cell wall, to reduce the size of particulate matter and crystallinity of the structural material, and to hydrolyze biomolecular polymers. Chemical pretreatment by sodium hydroxide addition alone or in combination with thermal pretreatment was found to increase significantly the soluble chemical oxygen demand (sCOD) of microbial biomass such as that in waste activated sludge (Kim et al., 2003; Penaud et al., 1999). Indeed, the sCOD of microbial biomass increased from 8% to 20% for untreated systems to up to 60–70% of the total COD following treatment. Moreover, biogas and methane production has been improved by 30–45% (Kim et al., 2003; Tanaka et al., 1997; Valo et al., 2004). Similarly, hydrothermal alkali pretreatment prior to AD can also be effective for enhancing biogas and methane production from lignocellulosic biomass (Sambusiti et al., 2013).

Previously, the impact of a limited number of pretreatment methods including sonication, drying, thermal, hydrolytic pretreatment in percolators, enzymes, chemical, and thermochemical treatments were tested on algae (Bohutskyi and Bouwer, 2013). However, a majority of these studies were focused on macroalgae (seaweed or kelp) and cyanobacteria while only a few studies investigated pretreatment of microalgae. Furthermore, the authors used different pretreatment protocols and often reported contradictory results. For instance, thermal pretreatment of *Chlorella* sp. and *Spirulina maxima* was shown to have either no or a negative effect on methane production (De Schamphelaire and Verstraete, 2009; Samson and Leduy, 1983) while other studies showed minor or substantial improvement of methane production from *Scenedesmus* sp. (González-Fernández et al., 2013), *Nannochloropsis salina* (Schwede et al., 2013) and microalgal biomass grown in wastewater (Passos et al., 2013). Similarly, a combined thermo-chemical pretreatment of *S. maxima* caused inhibition of methanogenesis compared to untreated fresh algae (Samson and Leduy, 1983) while other authors reported methane production improved as a result of thermochemical pretreatment (Alzate et al., 2012).

Finally, a vast diversity in morphology and biochemical composition of different algae may be another reason for the inconsistency in results. The structural components of the cell wall can include crystalline or amorphous cellulose, hemicellulose, laminarin, sulfated polysaccharides (agar, carrageenan, alginate, fucoidan or ulvan), glycoproteins, $CaCO_3$, (silica) or be absent in some cases (Bohutskyi and Bouwer, 2013).

Therefore, the goal of the current study was to explore the effect of different pretreatment methods on a large variety of diverse algae species than have been investigated in the past. Specifically, the current study compared solubilization aspects of thermal, chemical and thermochemical pretreatment of different microalgae in the presence or absence of thermal pretreatment and its effect on biogas and methane yields. Pretreatment strategies improve solubilization and methane yields for some species but the methodology and impact appears to be highly dependent on the specific algae strains evaluated.

2. Methods

2.1. Algal biomass characterization

Five algal species, such as *Chlorella* sp., *Nannochloropsis* sp., *Thalassiosira weissflogii, Tetraselmis* sp., and *Pavlova_cf* sp. (CCMP459), were purchased from the Reed Mariculture Inc., and according to the supplier information, original algal biomass was harvested using centrifugation to obtain dry matter content of 6–18%. All algal samples were diluted by Milli-Q® water to obtain VS (volatile solid) content of ~2.6%. Table 1 shows the average values of algal biomass characteristics with standard deviation of two measurements used in the present study.

2.2. Biomass pretreatment and solubilization evaluation

2.2.1. Alkaline treatment

Various amounts of 50% NaOH were added to 300 ml of algal biomass solution to achieve final concentrations ranging from 0 to 21 g NaOH L^{-1}. The samples were mixed carefully and 150 ml of biomass were transferred to other bottles following thermal pretreatment. The pH in the remaining solution was measured and neutralized to 7.2 ± 0.1 by adding 37% HCl slowly. Then, the carefully mixed solutions were transferred into 50 ml centrifuge tubes. All samples were centrifuged at 5200 rpm and 4 °C for 5 min, and then the liquid phase was separated for chemical oxygen demand (COD) analysis.

2.2.2. Thermal and thermochemical treatment

Both, untreated samples and samples with NaOH added as described above were autoclaved at 121 °C and 10 bar for 30 min. The treated samples were cooled to the ambient temperature for about 1 h. pH was measured and neutralized to 7.2 ± 0.15 by adding 37% HCl slowly. Then, the carefully mixed solutions were centrifuged in 50 ml tubes at 5200 rpm and 4 °C for 5 min. Finally the liquid phase was separated for chemical oxygen demand (COD) analysis.

2.2.3. Solubilization evaluation

Soluble COD (sCOD) was defined as a fraction of COD in the liquid phase after centrifugation of pretreated sample to total COD of diluted untreated sample and was used to monitor the extent of algal biomass solubilization. The COD analysis was repeated twice and standard deviation was calculated from the two analyses for all samples.

$$\text{sCOD}\ (\%) = 100\ (\%) \times \frac{\text{supernatant COD}\ (\text{g L}^{-1})}{\text{COD}\ (\text{g L}^{-1})} \quad (1)$$

Soluble COD yield was calculated as difference between $sCOD_i$ of pretreated sample and $sCOD_0$ of untreated control.

$$\text{sCOD yield}\ (\text{g L}^{-1}) = \text{sCOD}_i\ (\text{g L}^{-1}) - \text{sCOD}_0\ (\text{g L}^{-1}) \quad (2)$$

Finally, the influence of pretreatment on CH_4 production and the conversion rate was measured using BMP tests.