Enhanced methane production from algal digestion using free nitrous acid pre-treatment

Xue Bai [a], Paul A. Lant [a], Paul D. Jensen [b], Sergi Astals [b], Steven Pratt [a, *]

[a] School of Chemical Engineering, The University of Queensland, Brisbane, QLD 4072, Australia
[b] Advanced Water Management Centre, The University of Queensland, Brisbane, QLD 4072, Australia

ABSTRACT

The methane yield from the digestion of algae is typically much lower than the theoretical methane yield, and lower than yields reported for other organic substrates. This study presents a novel free nitrous acid (FNA) pre-treatment technique to improve methane production from algal biomass. The methane production yield through anaerobic digestion was found to be dramatically enhanced by FNA pre-treatment (2.31 mg HNO_2−N L^{-1}), with a 51% increase in the methane yield (from 161 to 250 L CH_4 per kg VS added). A two substrate model was used to describe the apparent presence of rapid and slowly degradable material. Model-based analysis revealed that with FNA pre-treatment (2.31 mg HNO_2−N L^{-1}), the availability of both rapid and slowly biodegradable substrates were increased. Higher levels of nitrite (159 and 1006 mg N L^{-1}) had an inhibitory/toxic effect. For this reason, coupled with the fact that denitrification of nitrite consumes organic substrate, it is concluded that pre-treatment liquor should be removed before digestion.

1. Introduction

While the potential of microalgae is more focused, at both research and commercial levels, for production of drop-in fuels, the production of bio-methane from either whole cell or algal residue has been regarded with general interest. Anaerobic digestion of microalgae has been considered as a necessary step to make algal biodiesel sustainable [1,2]. Moreover, with a higher N:C ratio composition comparing to general substrates such as pig manure, algae can also contribute to AD as a co-digestion substrate [3].

Methane yields from anaerobic digestion (AD) of algae are often relatively low [4]. A couple of factors may contribute to reduced yields, including the physical characteristics of algae as well as the inhibitory effects of digestion by-products such as ammonia [5–7]. In particular, the cell envelope (cell wall and membrane) of algae is described as a rigid barrier and therefore limits access for biological degradation.

A number of algae pre-treatment techniques to potentially increase methane yields have been investigated, including thermal hydrolysis, ultrasound, microwave, chemical and enzyme hydrolysis methods [8–14]. Rodriguez et al. reviewed and compared the pre-treatment techniques used for anaerobic digestion of algae and pointed out that more research is needed in order to overcome the disadvantages of existing pre-treatment methods and achieve more efficient methods to scale up and apply in industry [14]. Keymer et al. achieved an 83% boost with high pressure thermal hydrolysis during anaerobic digestion of Scenedesmus [8]. Cho et al. enhanced methane production from digestion of algae biomass by 8% by using pH 9 alkali pre-treatment [10]. Mahdy et al. reported a highest 51% enhanced methane production of algae biomass after protease hydrolysis pre-treatment comparing to raw algae [11]. These technologies destroy cell structures and release intracellular and/or extracellular constituents to the liquid phase. The disrupted cells and released constitutes are more readily biodegraded during AD, therefore enhancing methane production yield. However, all of the above pre-treatment techniques have high energy and/or chemical requirements [14,15].

A potential alternate pre-treatment technique that has already been demonstrated to be effective for improving anaerobic biodegradability of waste activated sludge (WAS) from wastewater treatment plants (WWTP) is free nitrous acid (FNA) pre-treatment [16]. When digesting WAS from wastewater treatment plants, FNA pre-treatment (at 1.78–2.13 mg HNO_2−N L^{-1}) led to an improved methane potential, with the highest improvement being

approximately 27% (from 201 to 255 L CH$_4$ per kg VS added) compared to untreated WAS [16]. FNA pre-treatment could be applicable for algae, especially considering recent literature demonstrating FNA pre-treatment to disrupt algae to boost lipid extraction showed that FNA, at ppm levels, can severely disrupt algal cells. Bai et al. reported that total accessible lipid content was found to increase with pre-treatment time (up to 48 h) and FNA concentration (up to 2.19 mg HNO$_2$–N L^{-1}) [17].

The aim of this paper is to quantify for the first time the improvements in methane yields and apparent hydrolysis rate constants that can be achieved by applying FNA pre-treatment to algae, and to understand the pre-treatment mechanism. Both untreated algae and FNA treated algae were tested. It is worth noting that nitrite present in the FNA pre-treatment liquor is a concern due to its potential toxicity to methanogenesis [5,18]. Thus the effect of different levels of nitrite present in anaerobic digestion of algae (from FNA pre-treatment liquor) was also evaluated.

2. Materials and methods

2.1. Microalgae harvest and sludge source

Tetraselmis striata M8 was cultured in an open algal culture pond at The University of Queensland. F/2 medium was used as the growth media according to Guillard et al. [19]. Culture pH was kept constant at 8.5 ± 0.2 by CO$_2$ injection with an electronic controller, and the depletion of nutrients (NO$_3^-$ and PO$_4^{3-}$) was tested by seawater aquaria nutrient kits (DAPI Aquarium Pharmaceuticals for NO$_3^-$ and Nutrafin for PO$_4^{3-}$). Open algae culture was maintained as a low cost mass algae production for lipids and protein accumulation. The algae was grown to a dry weight of around 1 g L^{-1} and then 300 L algal culture was concentrated to a paste by centrifugation at 5000 rpm for 3 min in 6 L batches. The wet algae paste was collected and re-dissolved into de-ionized water (DI water) prior to the FNA pre-treatment (described in Section 2.2.).

The inoculum for the biochemical methane potential (BMP) tests (described in Section 2.3.) was collected from mesophilic anaerobic digesters operating at 37 °C and treating a mixture of primary and waste activated sludge at a domestic WWTP (Queensland, Australia). Specific methanogenic activity of the inoculum was 0.2 g COD g VS^{-1} d^{-1} [20].

Total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), total solids (TS), volatile solids (VS), of the algae and inoculum are shown in Table 1, with standard errors obtained through triplicate measurements. The composition of the algae sample was also measured.

Table 1
Characteristics of algae and inoculum used in BMP tests, mean ± SE (n = 3).

Characteristics	Algae	Inoculum
pH	–	7.5 ± 0.1
TCOD (g L^{-1})	11.5 ± 0.05	24.8 ± 0.2
SCOD (g L^{-1})	2.39 ± 0.1	0.54 ± 0.05
TS (g L^{-1})	9.80 ± 0.3	24.5 ± 0.1
VS (g L^{-1})	7.40 ± 0.6	14.4 ± 0.1
Protein (% TS)	49.4 ± 0.2	–
Carbohydrates (% TS)	16.0 ± 0.3	–
Lipids (% TS)	6.40 ± 0.2	–
Ash (% TS)	24.5 ± 0.5	–

2.2. FNA pre-treatment

Contact batch tests were performed to assess the effect of FNA pre-treatment on the subsequent methane production from digestion. The experimental conditions are summarised in Table 2. Algal biomass was harvested and concentrated as per Section 2.1, the algae paste was then re-dissolved into DI water and then the mixed liquor was evenly distributed between four beakers (reactor volume 1 L) as the four tests listed in Table 2 in the "FNA pre-treatment" section. Pre-determined amounts of sodium nitrite stock solution (30 g N L^{-1}) were then added to the beakers in different volumes at the beginning of each experiment to achieve the designated initial concentrations of nitrite of 0, 100, 300, and 1900 mg N L^{-1}. Each test lasted for 48 h, during which pH was controlled constant through pre-treatment at 5.5 ± 0.2 by manually adding 0.5 M HCl. Extended contact times (48 + hours) result in severe cell disruption [17] so 48 h of contact time for this work was applied. But it is worth noting that, there is a challenge in optimising pre-treatment time with extended times resulting in cell disruption but potentially limiting industrial throughput. All samples were well mixed by magnetic stirrers at a constant speed of 350 rpm during the entire treatment. The FNA concentration was calculated using the equilibrium expression for FNA and nitrite.

$$FNA\left(mg\ HNO_2 - N\ L^{-1}\right) = \frac{S_{NO2^- - N}}{\left(K_a \times 10^{pH}\right)} \qquad \text{Eq. (1)}$$

where $S_{NO2^- -N}$ is the dissolved nitrite concentration (mg N L^{-1}) and $K_a = e^{-2,300/(273.15+T)}$; temperature T (°C) for this study was 25 °C [21].

In each experiment, mixed liquor samples were taken before and after the pre-treatment with a syringe and immediately filtered through disposable Millipore filter units (0.22 μm pore size) for the off-line analysis. The off-line analysis including TS, VS, TCOD and SCOD etc. are described in details in Section 2.5.

2.3. Anaerobic biochemical methane potential tests

The biochemical methane potential (BMP) assay provides a measure of the anaerobic biodegradability of a given substrate, and was used to determine the methane yields and process kinetics of the algal biomass with and without FNA pre-treatment (0–14.61 mg HNO$_2$–N L^{-1}, for 48 h), as described in Table 2. BMP assay is a standard method for systematically quantifying method yields and kinetic parameters of AD process. Algal biomass was digested in 240 mL sealed glass serum bottles (170 mL working volume). As shown in Table 2 in the second section (BMP test), the BMPs for algae digestion (BMP-control and BMP1-4) and three BMP blanks to test for the effect of NO$_2$–N (BLK-I-III) were operated with three serum bottles per batch as triplicate tests. Each BMP test contained 80 mL inoculum and 90 mL algal liquor from FNA pre-treatment outputs with an inoculum to substrate ratio of 1.5 on a dry VS basis. The digested sludge, used as inoculum, was degassed and kept under anaerobic conditions at 38 ± 1 °C for 6 days prior to commencing the experiments. Before sealing the serum bottles, pH was neutralized to 7.0 ± 0.2 by 0.1 M of NaOH or HCl. The anaerobic conditions were established by flushing the headspace of each serum bottle with high purity nitrogen immediately followed by sealing with butyl rubber stopper secured by an aluminium crimp cap. The batches were incubated at 38 ± 1 °C and agitated every 2–3 days. As shown in Table 1, the substrate of BMP-control was the algal liquor of the FNA-control trial; the substrate of BMP 1 was the algal pellet after FNA2.31 pre-treatment with the liquid removed by centrifuge; the substrate of BMP 2–4 was the pre-treated algae from FNA0.77, FNA2.31, and FNA14.61, respectively. Three sets of