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Enhanced the energy outcomes from microalgal biomass by the novel biopretreatment

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ABSTRACT

Microalgae have been considered as one of the most promising biomass for the generation of biofuels. The anaerobic digestion (AD) has been proved to be a promising technique to transfer the microalgal biomass into biofuels. Previous study demonstrated that anaerobic pretreatment of microalgae biomass by *Bacillus licheniformis* could improve methane production. In this study micro-aerobic bio-pretreatment of microalgal biomass by the facultative anaerobic bacteria *Bacillus licheniformis* was invested with different loads of oxygen supplied. The bio-hydrogen and biomethane productions were tested to calculate total energy outcomes. The transmission electron microscope (TEM) photographs suggested that the novel micro-aerobic bio-pretreatment (MBP) could effectively damage the firm cell wall of algal cells. The processing time of the novel method (24 h) was less than the previous anaerobic pretreatment (60 h). Results showed that the group with 5 mL oxygen/g VS_{fed} had the highest total energy outcomes, which was 17.6% higher than that of the anaerobic pretreatment.

1. Introduction

Fossil fuels have been meeting the increasing energy needs of the world for centuries [1], whereas the overuses result in exacerbating climate change, energy crisis and environmental pollution [2]. As biofuels generated from biomass are excellent for the sustainable and renewable energy producing potential, they are considered as a potential alternative of fossil fuels in the long term [2]. Microalgae, capable of fixing carbon dioxide and providing algal biomass concurrently, have aroused considerable interest among researchers [3,4]. Biofuels, such as biodiesel, bioethanol, bio-hydrogen, and biomethane, have been successfully generated from microalgal and macroalgal biomass [5–10]. However, indispensable fertilizers and energy consumptions have impeded the application of the microalgal biofuels [11]. It was demonstrated that AD would be a necessary technique to generate more biofuels from microalgae and realize the recycling nutritive elements [12,13].

Besides, the microalgal cell walls are the main barriers for maximizing energy conversion efficiency [14]. Generally, breakup of the rigid and tough cell walls by different kinds of pretreatment techniques is an effective strategy to improve the bioavailability of organic matters in the algae cells [15]. Currently, the physical, chemical and enzymatic methods are the most widely used solutions [16]. Mendez et al. validated that thermal pretreatments could increase microalgal biodegradability by 50% [17]. Spiden et al. revealed that acidic and thermal treatments damaged microalgal cell walls [18]. Passos et al. proved that mix-enzymolysis improved methane production by 15% [19]. But, physicochemical methods are constrained by high cost or high energy consumption, and studies on novel, economical methods are scarce [16].

Some researchers have been making attempts to reduce the microalgal pretreatments cost and energy consumption [20]. Prajapati et al. verified that fungal crude enzymes including cellulase and xylanase were successfully used to elevate the methane production from *Chroococcus* sp. [21]. However, to produce fungal enzymes, the long-time cultivation of fungi was necessary. Muñoz et al. found that "whole-cell" cellulolytic pretreatment by some marine bacteria could effectively improve the biogas production from *Nannochloropsis gaditana* [22]. Ahmed showed that the methane productions of *Chlorella vulgaris* were 190.6 mL CH_4 g COD_{fed} without hydrolytic enzymes pretreatment and 299.6 mL CH_4 g COD_{fed} with pretreatment [23]. Notwithstanding these

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attempts, to date, reports about using proteolytic bacteria to enhance methane production from *Chlorella* sp. are few.

In addition, some studies have evidenced that the methane production can be enhanced by adding certain amount of oxygen through a pretreatment process [24–26]. Previous researches demonstrated that micro-aerobic bio-pretreatment (MBP) improved the hydrolytic enzymes activities [25], promoted the growth of facultative bacteria [27] and stimulated the formation of some metabolics benificial for the anaerobes [26].

Our previous work demonstrated that microbial pretreatment with proteolytic *Bacillus licheniformis* was an eco-friendly method to break the microalgal cell and to enhance corresponding methane yields [28]. But, for this kind of bio-pretreatment under anaerobic condition, the process time was as long as 60 h, which was much longer than the cost by physical, chemical and enzymatic methods, in which, the treatment time was only several h or scores of min [29–31]. Therefore, exploring an efficient solution is crucial for practical application of the novel microbial pretreatment [32].

As the facultative anaerobic bacteria, *B. licheniformis* grows fast and needs less doubling time in the presence of oxygen than under the anaerobic atmosphere [33]. Amutha verified that *B. licheniformis* could ferment biomass and produce biohydrogen [34]. In the present study, we investigated the effects of MBP by *B. licheniformis* on biohydrogen and biomethane production from *Chlorella* sp. For the first time to our knowledge, little studies on the enhancing effects of MBP by the pure bacteria *B. licheniformis* on energy outcome from microalgae were conducted [23,35].

2. Methods

2.1. Substrates, inocula and microorganisms

Chlorella sp powder (Fangsheng Co., Shanxi, China) was used as substrates. The inocula were anaerobic digested mesophilic granular sludge taken from a brewery plant (Qingdao, China). The granular sludge was stored at 4 °C refrigerator and was activated under 37 °C before inoculation. The chemical parameters of substrates and inocula were tested through the following methods and the results were shown in Table 1. TS and VS were determined by standard method [36]. Protein content in microalgae was estimated according to the total elemental nitrogen measurement with the conversion factor 6.35 [37]. Carbohydrate and lipid concentration were analyzed through phenol sulfuric acid method [38] and cold extraction using chloroform/methanol [39], respectively. The ratio of inoculum to substrate (VS/VS) was set as 1:1.

Bacillus licheniformis 21,886 was kindly provided by Prof. Xiangzhao Mao (Ocean University of China). The *B. licheniformis* 21,886 was cultivated in the modified nutrient broth medium as described previously [28].

2.2. MBP of microalgae biomass with B. licheniformis

The optimal concentration of 8% (v/v, bacterial culture/working volume) was set as the dosage of bacteria [28]. For pretreatment, the 8 mL bacteria culture, 1.283 g microalgae powder and 92 mL sterile water were mixed in the 250 mL bottles with 100 mL working volume. The bottles only containing bacteria culture and sterile water were set as the blanks. The pH value was adjusted to 7.1 with 2.0 M HCl and NaOH. In order to quantitatively investigate the oxygen effects on the pretreatment, firstly, all bottles were sealed with butyl rubber stoppers and flushed with pure nitrogen to replace the air in bottles. And then, A syringe (5 mL) with fine needle was used to inject 0, 1, 2, 5, 10 mL of oxygen into each bottle to reach the oxygen loads of 0, 1, 2, 5, 10 mL/g VS substrate (marked as M0, M1, M2, M5, M10), respectively. Batch pretreatments were conducted at 37 °C for 140 rpm. After the total 24 h MBP process, biogas production was measured. To analyze the morphological change of *Chlorella* sp. cells, 1 mL solution were sampled before and after the pretreatment. Net H_2 (mL/g VS added) produced from microalgae was calculated by the following equation:

$$H_2(\mathrm{mL/g\,VS}) = \frac{H_2(\mathrm{mL, algae\ and\ bacteria\ culture}) - H_2(\mathrm{mL, bacteria\ culture})}{\mathrm{microalgae\ (g\,VS)}} \quad (1)$$

2.3. Batch AD experiments

After the MBP, 5.87 g granular sludge was added into these bottles. To obtain the biogas yield from the non-treated microalgae (marked as N), another group consisting of crude algae powder and corresponding inoculum blanks were employed. The working volume and initial pH value of all bottles was adjusted to 100 mL and 7.1 with 2.0 M HCl or NaOH. Then, all bottles were sealed and flushed with N_2:CO_2 (80:20, v/v) to keep anaerobic conditions. All bottles were placed at 37 °C constant temperature incubator without shaking. Biogas production was measured periodically (the 1st, 3rd, 5th, 7th, 10th, 13th, 21th, 25th day). All groups were performed in duplicates. Net CH_4 (mL/g VS added) produced from microalgae was calculated by the following equation [40]:

$$CH_4(\mathrm{mL/g\,VS}) = \frac{CH_4(\mathrm{mL, algae\ and\ bacteria\ culture}) - CH_4(\mathrm{mL, bacteria\ culture})}{\mathrm{microalgae\ (g\,VS)}} \quad (2)$$

The VS degradation efficiency of algal biomass was calculated as the formula [24]:

$$\text{VS degradation efficiency}(\%) = \frac{\text{Initial VS(g)} - \text{Final VS(g)}}{\text{Initial VS(g)}} \quad (3)$$

2.4. Analytical methods

The volume of biogas was measured by water displacement method and its composition was detected by a gas chromatograph (SP 6890, Lunan Inc., China), equipped with Porapak Q stainless steel column (180 cm × Ø 3 mm) and a TCD (thermal conductivity detector). The temperatures of injector, detector and oven were 50, 100 and 100 °C, respectively.

For the morphological observation, samples were centrifuged at 1000*g* for 3 min and the precipitate was washed three times with 0.1 M PBS buffer (pH 7.2). Then, the precipitate was immersed into 2.5% glutaraldehyde dissolved in 0.1 M PBS buffer for two h. After washing with PBS buffer for three times, algal cells were further subjected to osmic acid fixation (2%, 1 h). Again algal cells were washed for three times. Then, the gradient 30%, 50%, 70%, 80%, 90%, 95% concentrations of acetone were used to dehydrate algae

Table 1
Chemical characterization of *Chlorella* sp. and granular sludge (Average ± standard deviation).

Chemical parameters	*Chlorella* sp. (%)	Granular sludge (%)
TS	92.9 ± 0.2	8.8 ± 0.1
VS (based on TS)	83.9 ± 3.4	77.5 ± 0.1
Protein (based on TS)	65.8 ± 0.3	–
Carbohydrate (based on TS)	16.0 ± 0.6	–
Lipids (based on TS)	17.0 ± 0.3	–