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Enhanced biohydrogen and biomethane production from *Chlorella* sp. with hydrothermal treatment

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ABSTRACT

This study was the first attempt to investigate the effect of hydrothermal treatment on energy recovery of *Chlorella* sp. via two-stage anaerobic fermentation (TSAF). A maximum biohydrogen yield of 8.29 ± 0.33 mLH_2/gVS was achieved at the control group (CG), and a highest biomethane yield of 434.38 ± 5.72 $mLCH_4$/gVS was obtained for the group at the weakest hydrothermal treatment severity (HTS) (2.49). Compared with CG, the energy recovery was increased by 12.78% for the group at HTS 2.49, reduced by 6.05% and 32.09% for groups at HTS 4.06 and 5.21, respectively. Compared to single anaerobic digestion, TSAF significantly increased the energy recovery by 22.23–146.78%. 5-HMF and furfural were degraded by 17.65–71.08% and 46.58–82.20%, respectively, after first-stage biohydrogen fermentation. The analysis of microbial structure revealed that *Peptococcaceae* and *Desulfovibrio* related to inhibitors degradation were enriched with increasing HTS during first-stage fermentation. During the second-stage biomethane fermentation, the family *Enterobacteriaceae* was reduced as a symbiosis with hydrogenotrophic methanogens, accompanied with a decrease of *Methanobacteriaceae*. In comparison, the family *Methanosaetaceae* and *Methanosarcinaceae* belonging to acetoclastic methanogens, were remarkably increased due to the VFAs-rich effluents.

1. Introduction

Biofuels, produced from renewable biomass resources, are becoming a potential alternative to substitute fossil fuels resources due to the renewability, eco-friendly characteristics, feasibility and economic prospects [1–3]. Microalgae-based biofuels attract great attention due to the unique properties of microalgae, such as higher growth potential, nutrients absorption efficiency and carbon fixation ability compared to terrestrial plants [4]. Moreover, no arable land requirement and little fresh water demand make microalgae an ideal candidate feedstock for biofuels production [5]. Microalgae may also exhibit the potential to recover nutrients from wastewater during their growth and reproduction [6]. There are two main processes for microalgae conversion to various biofuels, which are identified as biochemical processes (e.g. dark fermentation and anaerobic digestion, generally with the participation of microorganisms) and thermochemical processes (e.g. pyrolysis, hydrothermal liquefication and gasification) [7,8]. Getting much attention, biochemical processes show significant advantages over thermochemical processes, since its less energy intensive, wider applications and more economic viability [9]. Various liquid (e.g. bioethanol) and gaseous biofuels (e.g. biohydrogen, biogas and biohythane) can be obtained via biochemical processes [1,2]. Liquid biofuels take advantages over gaseous biofuels in some aspects, including higher energy densities, more convenient for storage and transportation [10]. However, bioethanol fermentation requires a relatively pure and carbohydrate rich feedstock, whereas biohydrogen and biomethane fermentation can deal with a variety of waste materials via microorganisms in the natural environment [11,12]. Generally, microalgae consist of different biochemical components, including proteins, carbohydrates and lipids, which are more suitable for biohydrogen and biomethane fermentation [3,4,13]. If the target product is ethanol, one should employ carbohydrates rich algae, which needs to be specifically cultivated.

Biohydrogen, as a carbon-free energy, can be produced through dark fermentation which is operated under mild condition without light. However, bottlenecks still exist in limited energy gain due to an abundance of energy remains in the effluents which contain lots of soluble metabolic products such as volatile fatty acids (VFAs) and alcohols [14]. Biomethane production through anaerobic digestion could effectively utilize the soluble metabolic products in dark fermentation

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effluents to greatly improve the energy gain. Therefore, the two-stage anaerobic fermentation (TSAF) which is a combination of first-stage biohydrogen fermentation and second-stage biomethane fermentation, shows the potential to enhance gaseous biofuels production from microalgae [9,15].

Despite the non-lignin structure available, the recalcitrant cell walls structure of microalgae block intracellular substances from microbial attacks [16]. To tackle this problem, hydrothermal treatment (HTT) has been accepted as an optimal method in the field of anaerobic digestion especially for wet microalgal biomass as it avoids several energy intensive processes (e.g. dehydration and extraction) required for other treatment methods and can effectively release fermentable reducing sugars and amino acids [17]. HTT refers to the multi-stage processes of degradation, dissolution, oxidation and polymerization by using the unique properties of high temperature (100–260 °C) and high-pressure water without any chemical addition [18]. The addition of dilute acid would provide a convenience for lower reaction temperature and shorter retention time due to the effectiveness of crystallized cellulose removal [19]. Therefore, there are few studies on microalgae HTT combined with acid catalyst for coproduction of biohydrogen and biomethane, while HTT alone for it has not been reported. Cheng et al. [18] reported that hydrothermal acid treatment (2% v/v H_2SO_4, 135 °C for 15 min) strongly damaged the amorphous structure of the algal bloom cell in Dianchi Lake and resulted in a maximum energy conversion efficiency of 44.1% by cogeneration of 24.96 mLH_2/gVS and 299.88 mLCH_4/gVS. Sun et al. [11] found that HTT under acid catalysis (1% v/v H_2SO_4, 140 °C for 10 min) was an effective method for the mixture of rice residue and *Chlorella pyrenoidosa* to obtain a desired biohydrogen production (223.1 ± 8.8 mLH_2/gVS) during 144 h biological acidification, whereas the subsequent biomethane generation was unfavorable (183.7 ± 1.4 mLCH_4/gVS) due to the toxic by-products. Both HTT and dilute acid treatment can break cell walls, and hydrothermal acid treatment will lead to subsequent environmental problems due to the addition of acid [19,20]. While, the toxic inhibitors such as furfural and 5-HMF which are byproducts of reducing sugars are preferable to form during biomass is heated at high temperature (150–200 °C) under acidic conditions, accompanied by carbohydrates loss and equipment corrosion [21,22]. So, we attempt to build a hydrothermal treatment system without dilute acid addition, which has a wider application scope and a milder condition than hydrothermal acid treatment. To get a balance between thermal hydrolysis rate and side reactions, we have proposed the term hydrothermal treatment severity (HTS) to assess the treatment severity [23] and it helped to build the relationship with energy recovery.

We have previously studied the effect of HTS on microalgae anaerobic digestion and its connection with microbial functions and biochemical metabolism. The detailed objects of this work are to (1) attempt to assess the effect of HTT on the TSAF performance of *Chlorella* sp., (2) evaluate the microbial response to biochemical metabolism in TSAF system and (3) compare the energy recovery at different HTS.

2. Materials and methods

2.1. Substrates and inocula

The *Chlorella* sp. was provided by Fuqing King Dnarmsa Spirulina Co., Ltd., Fujian Province, China. The *Chlorella* sp. was dried by a centrifugal spray dryer without cell walls disruption. The proximate analysis and biochemical analysis of *Chlorella* sp. are as follows, total solids (TS): 96.98 ± 0.07%, volatile solids (VS): 87.33 ± 1.65%, proteins: 55.98 ± 0.86%, carbohydrates: 20.06 ± 0.91%, lipids: 3.99 ± 0.58%.

The inoculum for first-stage fermentation was originally collected from anaerobic sludge (TS: 4.45 ± 0.02%, VS: 2.23 ± 0.02%) in Xiaohongmen Sewage Treatment Plant, Beijing, China. The collected raw sludge was then heated at 100 °C for 15 min to deactivate biohydrogen consumers. The inoculum for second-stage fermentation was sourced from the same anaerobic sludge as first-stage fermentation.

2.2. Hydrothermal treatment procedure

Stainless steel reactor (500 mL, 4574, Parr Instrument Co., Moline, USA) was used to perform HTT as previously described according to an orthogonal experiment design (treatment temperature: 150 °C, 180 °C and 210 °C, retention time: 0 min, 30 min and 60 min, TS content: 10%, 15% and 20%) [24]. HTS methodically evaluates the severity of HTT by considering parameters of treatment temperature and retention time according to Eq. (1) [23]. In this study, the *Chlorella* sp. with the weakest (2.49), the middle (4.06) and the strongest (5.21) HTS was adopted for subsequent research. Characteristics of the treated *Chlorella* sp. have been previously elucidated, and some adopted in this study can be found in Supplementary Information.

$$HTS = \log \int_{t_1}^{t_2} \exp\left(\frac{T-100}{14.45}\right) dt \quad (1)$$

where T is the treatment temperature (°C); t_1 and t_2 are the time when the temperature increases and drops to 100 °C, respectively (min).

2.3. Two-stage anaerobic fermentation

TSAF of *Chlorella* sp. was performed in triplicate by an automatic methane potential test system-II (AMPTS II) (Bioprocess Control AB, Lund, Sweden). A water bath at 37 °C and a periodic agitation with a stirring rate of 100 rpm switch on 1 min and off 4 min were provided by the system. The system can absorb carbon dioxide produced during fermentation by 80 mL of 3 M NaOH solution with thymolphthalein as an indicator and then automatically record the biohydrogen/biomethane gas flow. All tests should be terminated when the gas production for three consecutive intervals was less than 1% of cumulative gas production [25]. Three groups of treated *Chlorella* sp. with the weakest (2.49), the middle (4.06) and the strongest (5.21) HTS were adopted for TSAF, and the untreated microalgae as control group (CG). Before fermentation, the above microalgae should be diluted to a mixture of 30 gTS/L with deionized water, respectively. Each bottle was fed with an initial VS of 3 g and had a working volume of 450 mL with 150 mL headspace.

For first-stage fermentation, microalgae mixtures were added into glass bottles and seeded with heat-treated anaerobic sludge up to 6.7 gVS/L [26]. A blank control with only heat-shock inoculum was also conducted to determine the endogenesis of inoculum. The initial pH was adjusted to 6.0 ± 0.1 with 1 M HCl and 1 M NaOH. All bottles were sealed with rubber stoppers and purged with nitrogen for 3 min to maintain anarobic conditions. First-stage fermentation started when all the bottles were connected and the system was ready. Biohydrogen production was recorded hourly by the system, and aqueous samples were taken regularly (every 2 h for the first 8 h and then every 8 h until the end).

The effluents of first-stage fermentation were used as substrates for subsequent second-stage fermentation. The effluents were seeded with anaerobic sludge at the substrate to inoculum ratio of 0.5 (VS), and adjusted to pH 7.0 ± 0.1 using 1 M HCl and 1 M NaOH. Other operations of second-stage fermentation were consistent with first-stage fermentation. Biomethane production was recorded daily by the system, and aqueous samples were taken regularly (every 2 days for the first 10 days and then every 4 days until the end).

2.4. Analytic methods

The higher heating value (HHV) of *Chlorella* sp. was measured by a calorimeter (Parr 6200, USA). Gas volumes were automatically normalized to standard conditions (273.15 K, 101.325 kPa) by the AMPTS II. Aqueous samples should be centrifuged at 4000 rpm for 12 min