Improving fermentative hydrogen and methane production from an algal bloom through hydrothermal/steam acid pretreatment

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Abstract

Algal blooms can be harvested as renewable biomass waste for gaseous biofuel production. However, the rigid cell structure of raw algae may hinder efficient microbial conversion for production of biohydrogen and biomethane. To improve the energy conversion efficiency, biomass from an algal bloom in Dianchi Lake was subjected to a hydrothermal/steam acid pretreatment prior to sequential dark hydrogen fermentation and anaerobic digestion. Results from X-ray diffraction and Fourier transform infrared spectroscopy suggest that hydrothermal acid pretreatment leads to

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stronger damage of the amorphous structure (including hemicellulose and amorphous cellulose) due to the acid pretreatment, as evidenced by the higher crystallinity index. Scanning electron microscopy analysis showed that smaller fragments (~ 5 mm) and wider cell gaps (~ 1 μm) on algal cell surfaces occurred after pretreatment. In comparison to steam acid pretreatment, hydrothermal acid pretreatment resulted in a maximum energy conversion efficiency of 44.1% as well as production of 24.96 mL H_2/g total volatile solids (TVS) and 299.88 mL CH_4/g TVS.

Keywords: Dianchi Lake algal bloom; hydrogen; methane; hydrothermal pretreatment.

1. Introduction

Dianchi Lake, the largest freshwater lake in Yunnan Province, China, suffers from annual algal bloom outbreaks. Until December 2017, the water quality of Dianchi Lake was still in a eutrophic state. During the summer of 2017, more than 100 tons of algal biomass were salvaged every day, causing great harm to the ecological environment. However, this large amount of algae can also be used as a potential feedstock for fermentative biofuel production, as demonstrated by many studies on biohydrogen production from algal biomass [1-4].

Different algal species, such as *Chlorella* and *Arthrospira*, have been assessed for their biohydrogen potential through dark fermentation [5-7]. To further improve

the energy recovery from raw algae, various pretreatment methods, such as steam, ultrasound and microwave treatments, have been developed [8-11]. In addition, the dark fermentation process has been optimized to overcome the inhibitory effects of fermentative intermediates (such as acetic acid) on hydrogen yield [12-15]. Apart from hydrogen production during fermentation, a large amount of volatile fatty acids are generated and remain as unutilized energy. Previous studies have demonstrated that subsequent photo fermentation or anaerobic digestion could increase the biofuel yield and energy conversion efficiency from microalgal biomass [16-18].

Researchers have utilized biomass harvested from an algal bloom in Taihu Lake to produce hydrogen, yielding 1.1 kJ of hydrogen per gram of dry biomass weight (g-TVS) [19]. However, the energy conversion efficiency was very low during the one-stage dark hydrogen fermentation of the biomass. In the study, “one-stage” refers to the process of dark hydrogen fermentation or anaerobic digestion and “two-stage” refers to the combined process of dark hydrogen fermentation and anaerobic digestion. In another study, the Taihu Lake algal bloom biomass pretreated with acid-domesticated hydrogenogens resulted in a 47.0% increase in the energy conversion efficiency by cogenerating 256.7 mL/g-TVS hydrogen and 253.5 mL/g-TVS methane in a three-stage process that utilized dark-fermentation, photofermentation, and methanogenesis [20]. It is noteworthy that, although hydrogen production was improved, the input light energy was not considered when calculating the energy conversion efficiency.

Zhong found that anaerobic digestion of Taihu Lake algae was feasible in

laboratory-scale anaerobic reactors [21]. These reactors performed well at an OLR of 2.00 $gVSL^{-1}d^{-1}$ for methane production with a VS removal of 50% at an HRT of 10 days; however, the rate-limiting step was acetate and propionate degradation. There were also many studies on the co-digestion of the Taihu Lake algal bloom biomass and kitchen wastes [22] or swine manure [23]. The feasibility of adjusting the C/N with co-digestion of Taihu algae and other biomass to increase biogas production was demonstrated. However how to increase the production of hydrogen and methane of algal bloom alone was not considered.

To date, there are few studies on the utilization of Dianchi Lake algal bloom biomass for biogas production. Furthermore, an effective pretreatment method to improve biofuel yield remains unclear as unprocessed microalgae are not considered to be the best substrate for biogas production [24]. Additionally, the energy conversion efficiency of single stage hydrogen or methane generation is not high and acetate degradation limits the fermentation rate. Moreover, the degradation effect of microalgal biomass after pretreatment should be quantitatively represented.

In this study, biomass harvested from a Dianchi Lake algal bloom was used as the feedstock for fermentation. This biomass, which was mainly composed of *Microcystis*, is of a different composition than that of Taihu Lake. Hydrothermal /steam acid pretreatment was examined to improve the hydrolysis efficiency of the algal biomass. The physicochemical properties of the algal biomass before and after pretreatment were comparatively assessed using X-Ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy and scanning electron microscopy (SEM).

The biomass, which was pretreated under different conditions, was subjected to two-stage hydrogen and methane co-generation in an effort to improve the overall energy conversion efficiency.

2. Materials and methods

2.1. Substrates and characterization

The substrate used in the fermentation experiments was the algal bloom biomass harvested from Dianchi Lake (Kunming City, Yunnan Province, China). Morphological analysis revealed that *Microcystis wesenbergii* and *Microcystis aeruginosaare* were the dominant species, accounting for 40%-80% of the agal bloom biomass in the lake. The harvested algal bloom biomass was processed for further experimentation via air-floatation, drying and grinding. The raw substrates were cryopreserved at -20°C before use. The moisture content of the biomass was measured by drying the samples in oven at 100°C until the total mass was constant. The contents of TVS and ash were determined by heating at 450°C for 2 h. The total carbohydrates, lipids and heating value were determined by methods described in our previous study [25]. The microcosmic structure of the Dianchi Lake algae was observed using XRD (X-ray diffractometer, Rigaku MiniFlex 600), FTIR (gas chromatograph- Fourier infrared spectrometer, SGE, Agilent 6890, Nicolet 5700) and SEM (tabletop microscope, TM-1000, HITACHI).

2.2. Algal biomass pretreatment

Many studies have demonstrated that for a variety of algal biomass, the optimal pretreatment temperature in microwave, steam and other pretreatment methods was found in the range of 135^{o}C to 140^{o}C, and the optimal pretreatment time was 15 min to 20 min [26, 27]. When the pretreatment temperature was lower than 135^{o}C or the pretreatment time was shorter than 15 min, the damages to the recalcitrant components in biomass were insufficient, and as a result the large molecular polysaccharides such as cellulose could not be fully degraded into small molecular reducing sugars. When the pretreatment temperature was higher than 140^{o}C or the pretreatment time was longer than 20 min, the Maillard reaction between reducing sugar and protein took place, resulting in decreased yield of reducing sugar and fermentative biogas production. There were also many studies showing that for a variety of algal biomass, the optimal concentration of acid (such as sulfuric acid) in pretreatments was 1%-2% [28, 29]. After the fermentation experiments using Dianchi Lake algal biomass as the feedstock, it was found that the pretreatment with 1% sulfuric acid had insufficient strength for cellulosic composition degradation. Therefore, the sulfuric acid concentration of 2% was selected for our pretreatment experiments.

Two pretreatment methods for the Dianchi Lake algal bloom biomass were conducted: (1) Hydrothermal heating with a dilute acid, referred to as the hydrothermal pretreatment, was conducted in a hydrothermal reactor (Parr Instrument 4500, USA). In brief, 5 g of algal biomass and 100 mL of dilute H_2SO_4 (2% v/v) were

added to a 250 mL hydrothermal reactor. The mixture was then heated to 135°C for 15 min. (2) Steam heating with dilute acid, referred to as the steam pretreatment, was conducted in an autoclave (Sanyo MLS-3780, Japan). Briefly, 5 g of algal biomass and 100 mL of dilute H_2SO_4 (2% v/v) were added to a 417 mL glass fermentation bottle. The mixture was then heated in an autoclave to 135°C for 15 min.

The reducing sugar content after pretreatment was determined by the 3-5 dinitrosalicylic acid method, as described in a previous study [27]. The theoretical maximum of the reducing sugar yield was calculated using the following formula:

$$(C_6H_{10}O_5)n + nH_2O \rightarrow nC_6H_{12}O_6$$

The ratio of reducing sugar to the theoretical value (%) was defined as the weight ratio of the reducing sugars (g) after pretreatment and hydrolysis to the theoretical reducing sugar products (g) of Dianchi Lake algae.

2.3. Inocula

Inoculum for dark hydrogen fermentation was obtained from anaerobic digestion sludge collected from a biogas plant in Huzhou, Zhejiang Province, China. The original sludge contained a variety of microbes, including hydrogen-producing bacteria and methanogens. To inactivate the methanogens, the sludge was heated at 100°C for 30 min in an autoclave. The sludge was then cultured to enrich the abundance of hydrogen-producing bacteria. The major species of dark hydrogen fermentation bacteria, as identified by 16S rRNA gene analysis, was *Clostridium butyricum* [30].

Inoculum for anaerobic digestion was sourced from the same biogas plant in Huzhou, China. The original digestate was degassed in an anaerobic workstation (Whitley DG250, UK) at 35°C for 14 days to ensure depletion of the remaining substrates before the experiment. The major species of methanogenic bacteria, as identified by 16S rRNA gene analysis, were *Methanosarcina* and *Methanothrix* [20].

2.4. Dark fermentation and anaerobic digestion

Many studies have suggested that for microalgae, kitchen waste, cassava residue and other biomass, the substrate concentration in fermentation was generally in the range of 10 g/L-20 g/L [31, 32]. Some researchers set the fermentation concentration of microalgae to 3 g VS [33]. When the substrate concentration was lower than 10 g/L, the organic load was too low to provide sufficient nutrients level for hydrogen-producing microorganisms during fermentation. However, when the substrate concentration was higher than 20 g/L, the organic load might be too high for the growth and metabolism of microorganisms. Therefore, the addition of algal biomass in each fermenter was set to 3 g-TVS, which was equivalent to 10 g-TVS/L. In these reported studies, the inoculum of hydrogen-producing bacteria was generally selected to be 25 mL. The effluents were then inoculated with the methanogenic inoculum based on the TVS ratio of 1:2 (substrate to inoculum).

Biohydrogen production via dark hydrogen fermentation was conducted in 417 mL glass reactors. The substrates (100 mL of pretreated solution-5 g of Dianchi Lake algal biomass equivalent) and the hydrogen-producing inoculum (25 mL) were added

into each reactor. The total liquor volume was adjusted to 300 mL with distilled water. The initial pH was adjusted to 6.0±0.1 with 6 M NaOH and 6 M HCl. The reactors were then sealed using silicone rubber stoppers and purged with nitrogen gas for 8 min to maintain an anaerobic environment. The dark hydrogen fermentation experiment was conducted for 72 h in a water bath, which was maintained at 35.0°C.

The hydrogen production experiments were conducted in three groups, which were performed in triplicate. Group 1 contained 100 mL algal bloom suspension after hydrothermal pretreatment with dilute acid, 175 mL distilled water and 25 mL hydrogen-producing inoculum. Group 2 contained 100 mL algal bloom suspension after steam pretreatment with dilute acid, 175 mL distilled water and 25 mL hydrogen-producing inoculum. Group 3 contained 5 g algal bloom suspension without pretreatment, 275 mL distilled water and 25 mL hydrogen-producing inoculum. After dark hydrogen fermentation, the effluents were adjusted to pH 8.0 $\pm$ 0.1 using 6 M NaOH and then inoculated with the methanogenic inoculum according to the TVS ratio of 1:2 (substrate to inoculum). The total working volume of each reactor during anaerobic digestion was 300 mL, including 120 mL effluents from dark fermentation, 150 mL methanogenic inoculum and 30 mL distilled water.

The reactors were then sealed, purged with nitrogen gas for 8 min to ensure anaerobic environment, and maintained at 35.0°C. The anaerobic digestion experiments were carried out for 25 days. For each group, the experimental conditions of anaerobic digestion without dark hydrogen fermentation were separately used as the control groups.

2.5. Analytical methods and calculations

Hydrogen and methane concentrations were determined using a gas chromatography (GC) system equipped with a thermal conductivity detector (GC-TCD; Agilent 7820A, USA). Hydrogen and methane yields were calculated from the amount and composition of the headspace gas and the total volume. The soluble metabolic product (SMP) compositions were determined using a GC system equipped with a flame ionization detector (GC-FID; Agilent 7820A, USA).

Origin 8.0 software was used to fit the modified Gompertz equation (as shown below) and the hydrogen production kinetic parameters were obtained [34, 35].

$$H=H_m\exp\{-\exp[R_m e(\lambda-t)/H_m+1]\}$$

where H is the hydrogen yield (mL/g-TVS); H_m is the maximum hydrogen yield potential (mL/g-TVS); R_m is the peak rate of hydrogen production (mL/g-TVS/h); λ is the hydrogen production delay time (h); t is the hydrogen production time (h) and l is the lag-phase time (h).

The energy conversion efficiency (ECE) was defined as the ratio of the energy values of hydrogen and methane to the total heating value of the algal biomass [36, 37].

3. Results and discussion

3.1. Characterization of algal biomass before and after pretreatment

The TVS accounted for 55% of the total weight of the original algal bloom and the heating value of the algal biomass was 25.17 kJ/g. The dried algal bloom biomass consisted of 36.60% ash, 15.13% carbohydrate, 38.30% protein and 2.54% fat. The main elements detected were C_{ad} (24.09%), H_{ad} (4.93%), N_{ad} (2.59%), O_{ad} (25.26%), and S_{ad} (0.81%). After hydrothermal pretreatment, the dried algal samples consisted of 40.82% ash, 2.06% carbohydrate, 42.22% protein and 2.88% fat. The main elements detected were C_{ad} (27.12%), H_{ad} (5.94%), N_{ad} (2.93%), O_{ad} (25.33%) and S_{ad} (0.91%). In comparison, the dried algal samples after steam pretreatment consisted of 39.64% ash, 4.23% carbohydrate, 40.81% protein and 2.69% fat. The main elements detected were C_{ad} (26.06%), H_{ad} (5.14%), N_{ad} (2.79%), O_{ad} (25.88%) and S_{ad} (0.87%). It can be seen that whichever pretreatment method was applied, the proportion of carbohydrates decreased significantly but the proportion of N element increased. This was because after pretreatment, the macromolecular carbohydrates were degraded into small molecular soluble reducing sugars, which were then dissolved in the liquid fraction. As compared to steam pretreatment, hydrothermal pretreatment resulted in a much lower carbohydrate content, which indicated that hydrothermal pretreatment had a stronger effect on carbohydrate degradation. When the reducing sugar content in the liquid fraction was higher, its promotion on hydrogen production and

subsequent methanogenesis was more effective.

The yield of the reducing sugar from algae without pretreatment was very low at 3.6% of the theoretical maximum yield. After hydrothermal or steam pretreatment, the reducing sugar yield reached more than 75% of the theoretical maximum yield. The addition of dilute acid during hydrothermal pretreatment significantly improved the sugar yield to 94.5%. The original cell structure of the algal biomass was very intact and it was difficult for the macromolecular carbohydrates to be hydrolyzed. Hydrothermal and steam pretreatment significantly increased the hydrolysis rate of the algal biomass by destroying cells and hydrolyzing macromolecule sugars.

Hydrothermal treatment refers to the reaction processes of degrading, dissolving, oxidizing and synthesizing various substances that exist in high temperature and high pressure water by utilizing the special properties of the water. The fermentation effect of the algal bloom biomass after hydrothermal pretreatment was better than that of the steam pretreatment, which might be due to the following aspects. First, when treated at the same temperature, processing time and acid concentration, the pressure of the hydrothermal pretreatment was higher than that of steam pretreatment due to the smaller volume of the hydrothermal reactor, thereby promoting disruption of cell walls and efflux of cell contents. This could allow for a higher hydrolysis efficiency of macromolecules. Second, the heat transfer of the hydrothermal reaction was via conduction allowing for rapid heating and a better temperature maintenance effect. The heat transfer of the steam reaction was via conduction and convection between the biomass and the steam; therefore, the heating effect was not as fast as the

hydrothermal pretreatment. A better heating effect results in a higher hydrolysis efficiency of macromolecular polysaccharides, which is beneficial to the subsequent fermentation experiments.

To further reveal the physicochemical changes of the algae after pretreatment, the biomass was examined by XRD, FTIR and SEM. The XRD spectra were used to characterize the ratio of crystalline cellulose and amorphous cellulose of the algal biomass after hydrothermal and steam pretreatment (Fig. 1). In the spectra, 2θ = 20–21° represents the region of highly crystalline cellulose and $2\theta = 18°$ represents the region of amorphous cellulose. Since the cellulose is coated with tough lignin, the algal bloom biomass without pretreatment did not show the characteristic peaks. The cellulose crystallinity index (CrI) of the samples could be calculated using Segal's empirical formula.

$$CrI=(I_{21} - I_{am})/I_{21}$$ [38]

where I_{21} is the diffraction peak intensity of $2\theta = 21°$ and I_{am} is the diffraction peak intensity of $2\theta = 18°$. The CrI of the non-pretreated algal bloom was 14.3, whereas it was 42.7 for the hydrothermal acid pretreated algal biomass and 33.7 for the steam acid pretreated algal biomass. The higher CrI after hydrothermal or steam pretreatment indicates that the amorphous cellulose was effectively degraded while the crystalline cellulose was more difficult to degrade, thus increasing the CrI.

The FTIR spectra were used to characterize the changes in functional groups before and after pretreatment (Fig. 2). The absorption peak at 1430 cm^{-1} notably weakened after the hydrothermal or steam pretreatment. This peak was assigned to

271 CH_2 bending vibrations, which is a characteristic peak of cellulose. This observation
272 is due to the fact that part of the cellulose was degraded after pretreatment. The
273 absorption peaks at 1638 cm^{-1} and 1558 cm^{-1} were clearly present before and after the
274 pretreatment. These peaks are assigned to the stretching vibrations of acetyl or
275 carboxylic acid C=O or the stretching vibrations of aromatic C=C, which are the
276 characteristic peaks of lignin. These results show that lignin cannot be effectively
277 degraded by either hydrothermal or steam pretreatment. The absorption peak at 1430
278 cm^{-1} represents the crystalline cellulose region. The absorption peak at 898 cm^{-1}
279 represents the region of crystalline and amorphous cellulose [39]. By calculating the
280 lateral index (LI) [40], which is the absorption rate A1430 cm^{-1} /A898 cm^{-1}, the
281 proportion of crystalline cellulose in the total cellulose can be analyzed. The LI of the
282 non-pretreated algae was 0.874, while it was 0.985 for the hydrothermal heating
283 pretreated algae and 0.966 for the steam heating pretreated algae. After pretreatment,
284 the algal bloom biomass showed a high proportion of crystalline cellulose,
285 demonstrating that the hydrothermal and steam pretreatments effectively damaged the
286 easily degraded amorphous cellulose. This result was consistent with the XRD and
287 CrI analysis.

288 The microstructural properties of the algae after pretreatment were visualized by
289 SEM (Fig. 3). Before pretreatment, the algae were mainly in the form of irregular
290 clumps (60-90 mm), with the space between the clumps ranging from approximately
291 10 μm to 40 μm. The surface was rough and compact with many tiny particles
292 attached to it. These observations might be due to the loss of moisture from the

293 microalgae cells when exposed to the sun, resulting in formation of a large amount of
294 biomass fragments. Further mechanical grinding resulted in the formation of a rough
295 surface. After the pretreatment of hydrothermal heating (135°C, 15 min) with dilute
296 sulfuric acid (2%), the algal bloom biomass showed a smooth and loose surface with
297 formation of a large amount of fragments of about 5 μm and a large number of gaps
298 (~1 μm) between the fragments formed. After the pretreatment of steam heating
299 (135°C, 15 min) with dilute sulfuric acid (2%), the algal bloom biomass also showed
300 a smooth and loose surface with formation of fragments of about 10 μm. The size of
301 the gaps between the fragments was about 1-2 μm. This was due to hydrolysis of the
302 algal bloom biomass in the presence of acid. After hydrothermal or steam heating, the
303 surface structure of the algal cells became fluffy and the gaps between the fragments
304 became wider. However, steam pretreatment was not as effective as hydrothermal
305 pretreatment. This was because the pressure of the autoclave during the steam
306 pretreatment was about 0.2 Mpa, whereas the pressure of the hydrothermal reactor
307 could reach 0.25 Mpa at the same reaction temperature and time. This higher pressure
308 was more likely to cause the formation of more gaps and smaller fragments.

309 ## 3.2. Hydrogen production through dark-fermentation

310 Biohydrogen production of algal blooms through dark fermentation is shown in
311 Fig. 4. The dynamic parameters of the dark hydrogen fermentation fitted by the
312 modified Gompertz equation are shown in Table 1. After hydrothermal or steam
313 pretreatment, H_m and R_m increased substantially while λ decreased. Due to the rigid

cell structure of the algal 14bloom biomass, the reducing sugars were difficult to release. As a result, the hydrogen yield from the algal biomass without pretreatment was less than 1 mL/g TVS. The hydrogen yield from the algal biomass with hydrothermal pretreatment and dilute acid (24.96 mL/g TVS) was higher than that with steam pretreatment and dilute acid (18.63 mL/g TVS). The peak hydrogen production rate from the algal biomass with hydrothermal acid pretreatment (2.04 mL/g TVS/h) was higher than that with the steam acid pretreatment (1.83 mL/g TVS/h). This is because the reducing sugar yield from the algal biomass with hydrothermal pretreatment (94.5%) was higher than that with steam pretreatment (74.8%). The hydrogen yield of this study was much lower compared with the values reported in literature, especially using easily degradable substrates (such as starch and food wastes) [2, 7, 27, 33, 38]. For example, the hydrogen yield of pretreated algal biomass (24.96 mL/g-TVS) was lower than that (147.42 mL/g-TVS) of the food waste biomass. This was due to the fact that the concentration of carbohydrates was low and compared to glucose, lignocellulose was difficult to be degraded. Without enzyme hydrolysis or photo hydrogen fermentation, bio-hydrogen yield from the algal bloom remained low even after the hydrothermal pretreatment.

The SMP compositions of the dark fermentation effluents of algal bloom biomass after pretreatment are shown in Fig. 5. The main components of the liquid phase metabolites were acetic acid and butyric acid, which accounted for 88.8%-90.7% of the total soluble metabolites. There were also small amounts of ethanol, propionic acid, isobutyric acid, isovaleric acid, valeric acid and caproic acid. This result is

consistent with the fermentation metabolic pathway (i.e., acetate/butyrate pathway) of hydrogen-producing bacteria [41]. After dark fermentation, the total soluble metabolites from the algal biomass without pretreatment was 52.93±10.10 mM. The pretreatment with hydrothermal heating and dilute acid increased the total soluble metabolites to 369.14±33.33 mM, whereas pretreatment with steam heating and dilute acid increased the total soluble metabolites to 310.86 ± 42.62 mM. This result suggests that pretreatment with hydrothermal heating and steam heating can promote the hydrolysis of the algal bloom, thus increasing the utilization of substrates and the fermentation efficiency by hydrogen-producing bacteria. As a result, approximately 6-7 times more soluble metabolites can be produced than that without pretreatment. Compared to the steam heating pretreatment, hydrothermal heating pretreatment of the algal bloom biomass showed more efficient hydrolysis and saccharification, which enabled the hydrogen-producing bacteria to efficiently the utilize organic components of the hydrolyzed biomass for fermentation, thus greatly promoting the production of soluble liquid phase metabolites.

3.3. Methane production during anaerobic digestion

Second stage anaerobic digestion was employed to further produce methane from the residual hydrogen fermentation solutions. For comparison, one-stage anaerobic digestion of the algal biomass with and without pretreatment was also employed to assess the methane production. The maximum methane production rate occurred within the first two days (Fig. 6). After approximately 25 days, methane yield did not

vary substantially; thus, the methanogenic phase was considered to have ended. The two-stage cogeneration of hydrogen and methane generally resulted in a higher methane yield and a peak production rate, as compared to the one-stage anaerobic digestion. The one-stage anaerobic digestion of untreated algal biomass resulted in the lowest methane yield (206.82 mL CH_4 /g TVS) with a peak production rate of 26.91 mL CH_4 /g TVS/d. The highest methane yield (299.88 mL CH_4 /g TVS) from the two-stage fermentation was achieved with the hydrothermal acid pretreatment. Correspondingly, the peak methane production rate was 49.91 mL CH_4 /g TVS/d. For the one-stage anaerobic digestion, the maximum methane yield (246.13 mL CH_4 /g TVS) and peak production rate (39.27 mL CH_4 /g TVS/d) were observed after the hydrothermal pretreatment. Similarly, two-stage fermentation after the steam acid pretreatment led to better methane production, when compared to the one-stage anaerobic digestion. These trends were due to the fact that (1) deep acidification during hydrogen fermentation had an enhanced effect on the degradation of algal cell macromolecules; and (2) formation of intermediate substrates, such as acetic acid and butyric acid, could be easily used by methanogens.

It was noted that the hydrothermal acid pretreatment outperforms steam acid pretreatment during two-stage fermentation. This might be due to the following reasons: (1) compared with the steam acid pretreatment, more gaps and smaller fragments of algae were formed after the hydrothermal acid treatment; and (2) the hydrothermal acid pretreatment led to a higher production of total soluble metabolites (369.14 mM) during fermentation than the steam acid pretreatment (310.86 mM).

3.4. Energy conversion efficiency

A comparison of previously published fermentative gaseous biofuel production and energy yields from pretreated algal bloom biomass is shown in Table 2. The energy conversion efficiency was calculated based on the heating values of hydrogen (242 kJ/mol), methane (801 kJ/mol) and algal bloom biomass (25.17 kJ/g TVS). In this study, the energy conversion efficiency of hydrogen production remained low (0.4%), even after hydrothermal or steam pretreatment. However, following methane production from the residual hydrogen fermentation solutions, the energy conversion efficiency significantly increased to 44.10% after hydrothermal pretreatment and 39.38% after steam pretreatment. In the study that used the Taihu Lake algal bloom biomass, photo hydrogen fermentation was used to further improve hydrogen production after dark fermentation [20]. Although hydrogen production was improved, the input energy of light was not considered when calculating the energy conversion efficiency. When only hydrogen production by photosynthetic bacteria is considered, the energy conversion efficiency of light energy to hydrogen is only 1%. In this study, the hydrogen yield of pretreated algal biomass was lower than that of the Taihu Lake algal bloom biomass because there was no enzyme pretreatment or photo hydrogen fermentation. However the energy conversion efficiency was almost equal to the three-stage cogeneration of hydrogen and methane from the pretreated algal biomass of Taihu Lake [20].

4. Conclusions

Hydrothermal acid pretreatment of algal bloom biomass from Dianchi Lake could significantly increase the energy conversion efficiency during a two-stage process comprising of dark hydrogen fermentation and anaerobic digestion. XRD and FTIR analysis showed that the cellulose crystallinity index of the algal biomass pretreated with hydrothermal acid was significantly higher than that pretreated with steam acid, thereby suggesting that the hydrothermal acid pretreatment has a stronger degradation effect on hemicellulose and cellulose than the steam acid pretreatment. The energy conversion efficiency of the hydrothermal heating pretreated algal biomass remarkably increased to 44.1% by cogenerating 24.96 mL/g TVS hydrogen and 299.88 mL/g TVS methane during the two-stage process. The effective conversion of algal bloom waste into biofuel demonstrates a promising future for industrial applications.

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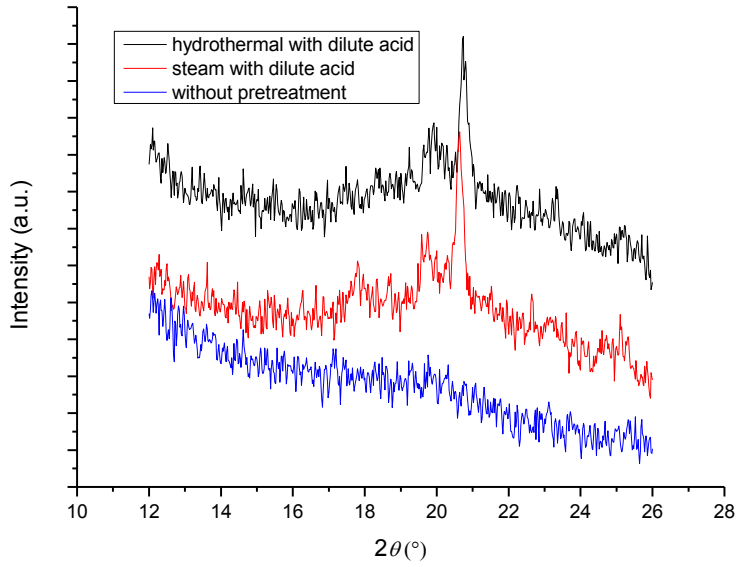
List of figures and tables:

Fig. 1 XRD spectra of algae biomass pretreated by hydrothermal heating and steam heating with dilute acid for 15 min.
Fig. 2 FTIR spectra of algae biomass pretreated by hydrothermal heating and steam heating with dilute acid for 15 min.
Fig. 3 SEM images of algae biomass pretreated by hydrothermal heating and steam heating with dilute acid for 15 min.
Fig. 4 Hydrogen production from pretreated algae biomass by dark fermentation.
Fig. 5 Soluble metabolite byproducts from dark hydrogen fermentation of pretreated algae biomass.
Fig. 6 Methane production from the residues of hydrogen production.

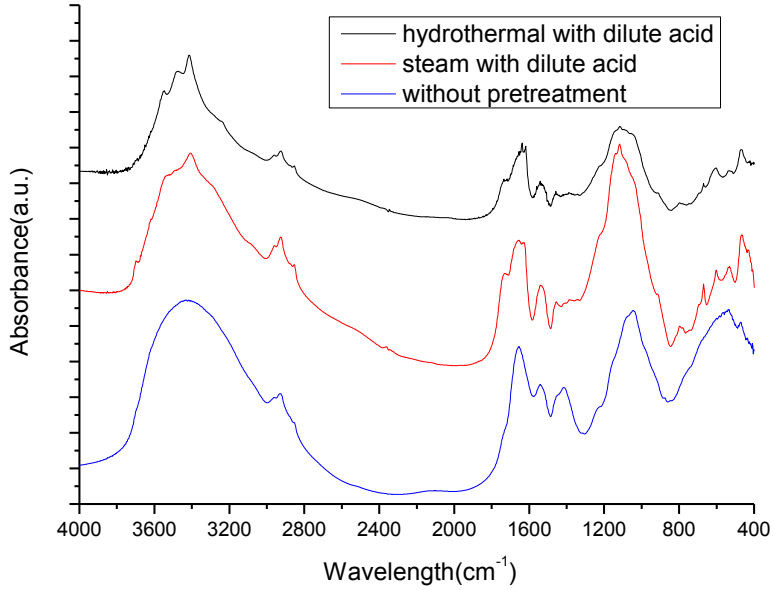
Table 1 Dynamic parameters of hydrogen production in dark fermentation.
Table 2 Comparison of the energy conversion efficiency of hydrogen and methane cogeneration from pretreated algae biomass in literature.



545

546 **Fig. 1 XRD spectra of algae biomass pretreated by hydrothermal heating and**

547 **steam heating with dilute acid for 15 min.**



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549 **Fig. 2 FTIR spectra of algae biomass pretreated by hydrothermal heating and**

550 **steam heating with dilute acid for 15 min.**

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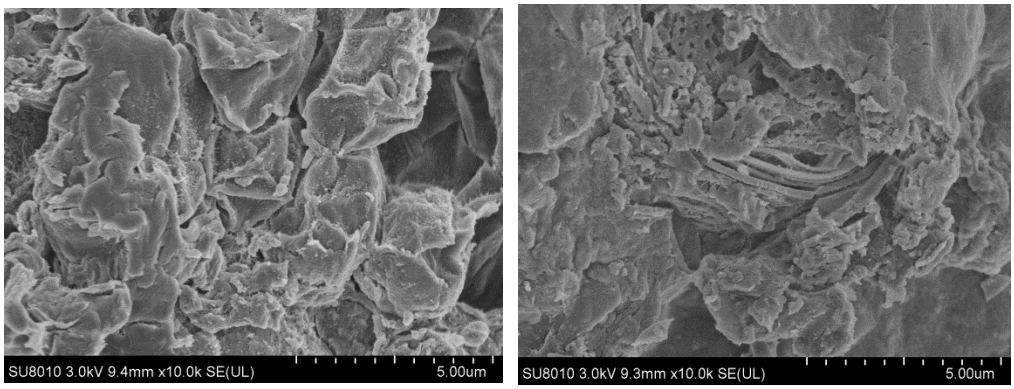
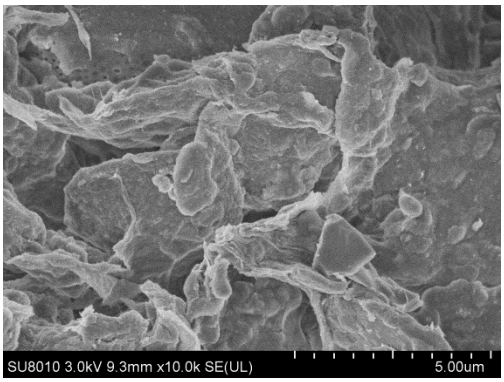
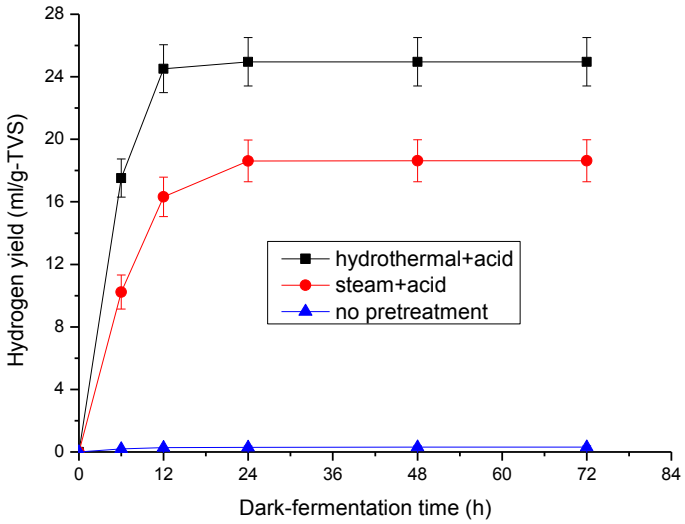


(a) without pretreatment (×10000) (b) hydrothermal with dilute acid (×10000)



(c) steam heating with dilute acid (×10000)

Fig. 3 SEM images of algae biomass pretreated by hydrothermal heating and steam heating with dilute acid for 15 min.



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563 **Fig. 4 Hydrogen production from pretreated algae biomass by dark**

564 **fermentation.**

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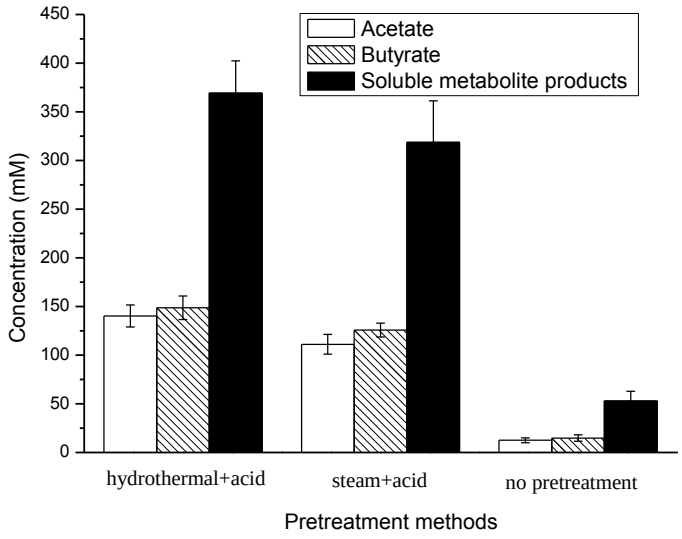
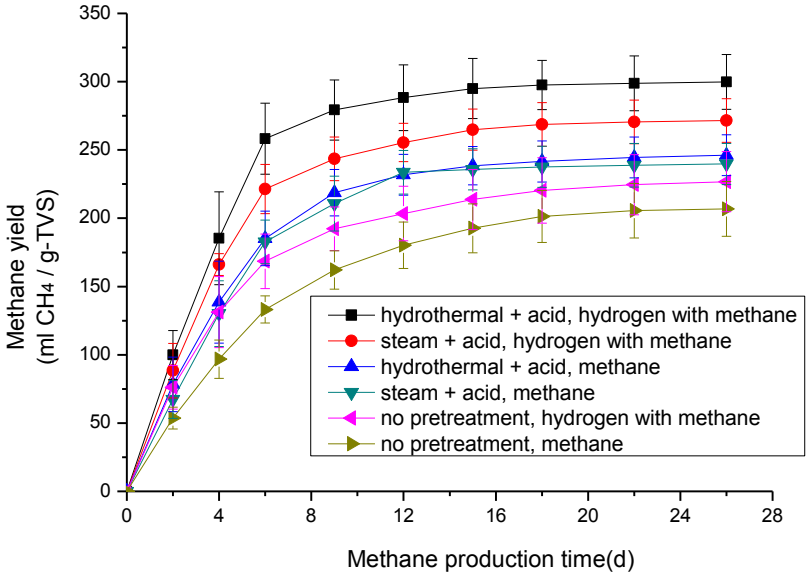


566

567 **Fig. 5 Soluble metabolite byproducts from dark hydrogen fermentation of**

568 **pretreated algae biomass.**

569



570

571 **Fig. 6 Methane production from the residues of hydrogen fermentation.**

Table 1 Dynamic parameters of hydrogen production in dark fermentation.

Pretreatment method	H_2 yield (mL/g-TVS)	H_2 production peak rate (mL/g-TVS/h)	Kinetic model parameters			
			H_m (mL/g-TVS)	R_m (mL/g-TVS/h)	λ(h)	R^2
No pretreatment	0.30	0.03	0.31	0.05	5.08	0.982
Hydrothermal heating +acid	24.96	2.04	24.96	3.05	3.40	0.993
Steam heating +acid	18.63	1.83	18.63	2.46	4.42	0.999

Table 2 Comparison of the energy conversion efficiency of hydrogen and methane cogeneration from pretreated algae biomass in literature.

Feedstock	Carbohydrate content (% of dried biomass)	Feedstock pretreatment	Process	Bacteria	H_2 yield （mL/g TVS）	CH_4 yield (mL/g TVS)	Energy yield of only H_2 (kJ/g TVS)	Energy yield of H_2 and CH_4 (kJ/g TVS)	Total energy conversion efficiency (%)	References
Taihu Lake algal bloom	N/A	Alkaline pretreatment	Dark fermentation (H_2)	Anaerobic granular sludge	105.0	/	1.1	1.1	N/A	[19]
Taihu Lake algal bloom	12	Steam heating with dilute H_2SO_4	Dark fermentation (H_2) + Photo fermentation (H_2) + Methanogenesis (CH_4)	HPB + PSB + MPB	256.74	253.53	2.77	11.84	47.04	[20]
Taihu Lake algal bloom	12	Microwave heating with H_2SO_4	Dark fermentation (H_2) + Photo fermentation (H_2) + Methanogenesis (CH_4)	HPB + PSB + MPB	283.41	166.83	3.06	9.03	35.88	[20]
Dianchi Lake algal bloom	13	Steam heating with dilute H_2SO_4	Dark fermentation (H_2) + Methanogenesis (CH_4)	HPB + MPB	18.63	271.51	0.20	9.91	39.38	In this study
Dianchi Lake algal bloom	13	Hydrothermal with dilute H_2SO_4	Dark fermentation (H_2) + Methanogenesis	HPB + MPB	24.96	299.88	0.28	11.10	44.10	In this study

			(CH_4)							

HPB: Hydrogen producing bacteria; PSB: Photosynthetic bacteria; MPB: Methane producing bacteria.