Methane production from lipid-extracted algal residues

Yan Li\textsuperscript{a,b}, Dongliang Hua\textsuperscript{a,b}, Jie Zhang\textsuperscript{a,b}, Yuxiao Zhao\textsuperscript{a,b}, Hui Mu\textsuperscript{a,b}, Haipeng Xu\textsuperscript{a,b}, Xiaohui Liang\textsuperscript{a,b}, Xiaodong Zhang\textsuperscript{a,b*}

\textsuperscript{a}Key Laboratory for Biomass Gasification Technology of Shandong Province, Jinan 250014, China
\textsuperscript{b}Energy Research Institute of Shandong Academy of Sciences, Jinan 250014, China

Abstract:

Waste-grown microalgae is a potentially important biomass for wastewater treatment, meanwhile lipid extracted from the algae could be further converted into biofuels. The lipid-extracted algal residues as major by-products mainly consist of carbohydrate and protein, which makes anaerobic digestion an efficient way to recover energy. The conversion of lipid-extracted algal residues into methane plays dual role in renewable energy production and sustainable development of microalgal biodiesel industry. Therefore, an anaerobic fermentation process for investigation of methane production potential of algal residues was conducted in this paper. The effect of inoculum to substrate ratios (ISRs) on the methane production by anaerobic digestion of \textit{Chlorella} sp. residue in single stage was evaluated. The stability and progress of the reaction from algal residues to methane were monitored by measuring the pH, volatile fatty acids (VFAs), total ammoniacal nitrogen (TAN), methane volume. Due to the characteristic of high content of protein in algal residues, the inhibition would occur resulting from the generation of ammonia. Thus two stage technology was proposed and more suitable for high organic load.

Key words: Biogas, methane, algal residues, one stage, two stage

Introduction

The potential of microalgae as a source of biofuels is subject to increasing academic and industrial research [1]. Lipid-extracted algal residues (LARs) are the residual biomass from biodiesel production processes that are rich in carbohydrates and proteins. LARs could be used as high-protein animal feed and, possibly, as a source of small amounts of other high-value microalgal products [2]. The compositions of LARs make anaerobic digestion an efficient way to recover energy, which will also reduce the cost of microalgal-biodiesel production [3].

The technology for anaerobic digestion is well developed. The effects of fermentation conditions such as various materials, parameters controlled, inoculum and substrate concentrations on biogas production have been studied [4-6]. Considering the substrates relevant to this paper, there are some researches on the algal species such as \textit{Spirulina maxima} [7], \textit{Chlorella vulgaris} [8], \textit{Scenedesmus obliquus} and \textit{Phaeodactylum tricornutum} [9]. The intact algae was used in those investigations for the methane production, as a result of which the resistance of cell wall limited the hydrolysis step and fermentation period was prolonged. The method prior to accession into the digester was adopted to treat the algae. Chisti in a review discussed the recovery of energy from the microalgae residues after biodiesel production, highlighting its potential to meet most of the energy demands [10]. Using post-transesterified algal residues, Ehimen found the co-digestion of the microalgae residues with glycerol would increase by 50\% for CH\textsubscript{4} production [11,12].

There was little information in literatures on the performance of anaerobic
digestion of lipid-extracted algal residue. The aim of this study was to investigate one and two stage anaerobic digestion of LARs. The effect of inoculum to substrate ratios on methane production from algal residue under mesophilic condition was studied. The operation of two stage digestion system with high organic load was carried out.

Materials and methods

2.1 Substrate and inoculum characteristics

Dried and disrupted *Chlorella* sp. (Tianjian Co., Binzhou, China) after lipid extraction was used as substrate. The main parameters were listed in Table 1. The C/N ratio of *Chlorella* sp. was 5.3 obtained from the Table 1. Anaerobic digested sludge collected from digester operating at 35 °C in Binzhou Xiangchi corporation was used as inoculum. The TS and VS contents of sludge were 7.85 % and 86.33 %-TS, respectively.

<table>
<thead>
<tr>
<th>Table 1 Characteristics of lipid-extracted algal residue</th>
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<tr>
<td><strong>Parameters</strong></td>
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<tr>
<td>Total solid(TS,%)</td>
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<tr>
<td>Volatile solid(VS,-TS)</td>
</tr>
<tr>
<td>Total carbon(%)</td>
</tr>
<tr>
<td>Total nitrogen(%)</td>
</tr>
<tr>
<td>Protein(%)</td>
</tr>
<tr>
<td>Lipid(%)</td>
</tr>
</tbody>
</table>

2.2 One stage experiments

Batch anaerobic digestion of algal residue was performed in 1 L bottles. The mixture of sludge and algal residues were added to get a final volume of 0.8 L with fresh water supplement. The outlet of the reactor gap was connected to the airbag. Then, the set-up was placed in water bath at 35 °C and shaken at intervals. Headspace was purged with nitrogen every time the cap was opened. Tests were run as duplicates to test statistical reliability. Inoculum without substrate was used as control.

The five different ISRs in test were 3:1,2:1,1:1 and 1:2. They were achieved by keeping a constant inoculum concentration (20 g VS/L, an empirical value for seed concentration) and varying the substrate concentrations according to the ISRs. For the investigated ISRs, the TS concentrations didn’t exceeded the maximum empirical value of 10%. Anaerobic digestion of algal residues was operating for 15 days.

2.3 Two stage experiments

The operation of acidogenesis was the same as the one in one stage digestion just with the ISR of 1:3 and pH controlled at 8. UASB, made of organic glass, was used as methanogenic reactor with working volume of 2.5L. During the start-up, UASB reactors were inoculated with granular sludge and acclimated with glucose solution (1.5 g/L) at an organic loading rate (OLR) of 1.0 g COD/L·d for one month.

The UASB reactors were continuously fed with synthetic medium at an initial OLR of 2.0 g COD/L·d for 10 days and then increased to 3, 4, 6, 8, 10, 12 g COD/L·day at 10 days intervals. To achieve different OLRs, HRT varied with the control of influent volume. The chemical composition of the synthetic medium was as follows (g/l): acetic acid 0.9, propionic acid 0.225, butyric acid 0.178, valeric acid...
0.0225, iso-butryic acid 0.102, iso-valeric acid 0.068. Sodium nitrate and potassium dihydrogen phosphate were supplemented to maintain the COD:N:P at the level of 100:4:1. Experiments were run for a total of 60 days under controlled room temperature (35°C).

2.4 Analytical methods

The compositions of biogas was measured using double channel infrared technique by biogas analyzer (GA2000, Geotech), which showed the desired methane content. pH values were measured with pH meter. Total ammoniacal nitrogen (TAN, NH₃ and NH₄⁺) was analysed according to Nessler's colorimetry with 5B-2(N) ammoniacal nitrogen analysis meter (Lianhua Technology Company, China). COD was measured by COD analyzer (Lianhua Technology Company, China). The free ammonia concentrations (i.e. unionized NH₃) are a function of the total ammoniacal nitrogen concentration, the pH, dissociation constant and formulae for the calculation of free ammonia concentrations are available in the literature [13]. TS and VS were determined according to APHA Standard Methods (2005).

The VFAs (acetic, propionic, butyric, valeric, iso-butryic and iso-valeric acids) concentrations were determined using an Agilent 7890 series gas chromatograph (GC) system. The column of HP-FFAP (50 m×320μm×0.5 μm) was selected. Flame ionization detector (FID) was adjusted to 300 °C as operating temperature. Nitrogen was used as carrier gas with a constant flow rate of 30 ml/min and the inlet temperature was kept at 250 °C. Oven temperature was initially set to 60°C and then increased to 100 °C with 10 °C/min ramping. After 2 min holding time at 100 °C, the oven temperature was gradually increased to 250 °C at the rate of 10 °C/min, holding 2 min.

Results and discussions

3.1 one stage anaerobic digestion
3.1.1 biogas and methane production

Fig. 1 shows the cumulative methane production, as a function of time with different ISRs used, corrected taking the control production into account. As indicated in Fig.1, methane production started immediately on the first day of digestion. Methane production almost reached a maximum on day 7 and leveled off thereafter at all ISRs because the substrate was almost completely consumed by the bacteria consortium. The methane production was proportional to the VS load applied. As can be seen from Fig.1, the methane production increased as the ISR decreased. The cumulative methane volumes after 15 days of digestion for the ISRs of 1:2, 1:1, 2:1 and 3:1 were 850 ml, 3066 ml, 1565 ml and 1116 ml, respectively.
The methane yield was calculated for each ISR by dividing the final methane volume by the VS weight of substrate added. Table 2 indicates that the methane yield after 15 days of digestion reached high levels of 191.6 ml CH₄/g VS, 195.6 ml CH₄/g VS and 210.6 ml CH₄/g VS as the ISRs was 3:1, 2:1 and 1:1 (initial substrate VS loads of 16 g VS, 8 g VS and 5.3 g VS), but decreases when the substrate load (32 g VS for ISR of 1:2) was further increased. The result gave a methane yield higher than that found in batch experiments with *Microcystis* spp. carried out by Zeng, who obtained a yield of 132.44 ml CH₄/g VS at ISR of 1:1 [14]. As can be seen from Table 2, the methane yield decreased from 210.6 to 26.6 ml/g VS when the ISR decreased from 3:1 to 1:2. The same conclusion was previously achieved by other researchers using different substrates [15,16]. With ISR of less than 1:1, methane yields significantly decreased and reached the lowest level of 26.6 ml CH₄/g VS at ISR of 1:2, which yield were approximately one-tenth of those obtained at other ISRs (Table 2). Combined with Fig.2, though, the pH value for the whole process at ISR of 1:2 was in an appropriate range, which was the contribution of the coexistence of VFA and ammonium. The yield decrease was perhaps due to the weak methanogenic activity in the digesters, resulting from the inhibition caused by ammonia [17].

### Table 2 Performances of reactors at ISRs of 1:2, 1:1, 2:1 and 3:1

<table>
<thead>
<tr>
<th>ISRs</th>
<th>TS loaded (g/L)</th>
<th>VS-loaded of substrate (g/L)</th>
<th>Operating time (d)</th>
<th>Methane yield (ml CH₄/g VS-add)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>72.4</td>
<td>40</td>
<td>15</td>
<td>26.6</td>
</tr>
<tr>
<td>1:1</td>
<td>45.6</td>
<td>20</td>
<td>15</td>
<td>191.6</td>
</tr>
<tr>
<td>2:1</td>
<td>35.5</td>
<td>10</td>
<td>15</td>
<td>195.6</td>
</tr>
<tr>
<td>3:1</td>
<td>31.3</td>
<td>6.7</td>
<td>15</td>
<td>210.6</td>
</tr>
</tbody>
</table>

It was observed that operating time of 15 days was a little longer for the digestion. The methane production was almost finished in 10 days. The practical period for the digestion could be shortened from the perspective of methane production and energy conservation. Taking the methane yield and appropriate organic load into considerations, ISR of 1:1 was the optimum for anaerobic digestion with algae alone, organic loading of which was 2 g VS/(L·d).

#### 3.1.2 pH

The variation of pH over the period of digestion is shown in Fig.2 and ranges from 6.7 to 7.7. When measured at days for unit, the pH climbed and finally reached a steady state in all reactors with the time increasing. It was obtained (not listed in Fig.2) that the pH decreased at the first few hours of day 1. Organic acid productions were generally initiated from the onset of the reactor operations which resulted in these
expected pH drops. The short-chain fatty acids as important intermediates was then quickly converted to methane by methanogens. The ammonium was simultaneously produced and drove the pH upwards.

![Fig.2 pH variation for different ISRs during anaerobic digestion](image)

Poor performance of methane production was obtained at the ISR of 1:2. At the ISR of 1:2, pH stayed in the narrow range between 6.7 and 7.2, which was compatible with the normal growth of anaerobic microorganisms. However, as can be seen from Fig.4, VFAs were accumulated to a high level, but pH didn’t drop accordingly due to the simultaneous presence of high level of ammonium (Fig.3). The stability in pH can be attributed to the high buffering capacity of the reactor.

### 3.1.3 TAN

Total produced ammonium of different ISRs are indicated in Fig.3. An increment on TAN was observed at the experimental ISRs. This fact was predictable since it is widely known that some organic nitrogen (mainly protein) is converted to ammonium during anaerobic digestion [18]. The final ammoniacal nitrogen concentrations increased from 2115 mg/L to 13200 mg/L as the ISR values decreased from 3:1 to 1:2.

![Fig.3 NH₄⁺-N variation for different ISRs during anaerobic digestion](image)

Ammonia, known as one of the inhibitors of methanogens may be thought as a problem in anaerobic digestion, especially those digestion using protein-rich feedstocks as substrates [19]. Ammonia could mainly influence the anaerobic digestion by affecting acetate-utilizing methanogenic Archaea, hydrogen-utilizing methanogens and syntrophic bacteria. Though the inhibitory concentrations of ammonia varied due to different experiments, TAN of 1.7–5 g/L, corresponding to 0.4–1 g/L free ammonia, is the most acceptable concentrations inhibiting the anaerobic digestion [20]. As the ISRs was 3:1, 2:1 and 1:1, the observed TAN
concentrations in this study appeared not to directly affect the process. This suggested that the initial and final ammonia were too little to inhibit the anaerobic digestion.

When ISRs was 1:2, accumulation of TAN of 4.3 g/L at day 2 was far above the threshold concentration (4 g/L) reported in literature [20], therefore the relatively lower methane yield at ISR of 1:2 was ascribed to the presence of large amount of NH$_4^+$-N. Ammonia exerts negative effects on microbial cells and the concentration must be monitored in order to prevent toxic amounts to be reached. Methanogens have been proved particularly sensitive to ammonia [21,22]. In terms of anaerobic digestion, a low C/N ratio can compromise the efficiency of the process, especially when high input and/or long retention times are applied.

For the ISRs of 1:1, 2:1 and 3:1, the maximum free ammonia was about 148 mg/L, 102 mg/L, 88 mg/L, respectively. The TAN concentration reached 13200 mg/L at the ISR of 1:2, corresponding to 240 mg/L free ammonia. It was found from Fig.4 that VFAs were accumulated to a high level, which was possibly due to the poor methanogenesis inhibited by ammonia. Because the inhibitory effect of ammonia was associated with many factors such as pH, temperature and acclimation of microorganisms, the free ammonia concentration for inhibition couldn’t be determined in this study.

3.1.4 VFAs

Volatile fatty acids (VFAs), as one of the most important parameters for the accurate control of anaerobic digestion, have a direct correlation with the digester performance. The variations of VFAs concentrations during the course of the digestion at different ISRs are shown in Fig.4. The initial values of VFAs increased with the amount of algae added. As indicated in Fig.4, the VFAs concentrations showed declining tendency at ISRs of 1:1, 2:1 and 3:1, and low levels of VFAs were detected after 10 days of digestion, which means that the anaerobic digestion process was complete. The decrease of VFAs concentrations indicated that the production of VFAs was relatively slower than consumption by methanogenesis, which corresponded to the high output of methane. For the ISR of 1:2, unstable operation occurred as indicated in Fig.4. VFAs weren’t consumed but accumulated to a level as high as 36240 mg/L with small amount of methane produced. Associated with Fig.3, it could be explained that the high level of TAN caused inhibition on methanogenesis activity of methanogens, thus directly led to the accumulation of VFAs.

![Fig.4 VFAs variation for different ISRs](image_url)

3.2 Two stage anaerobic digestion

Bad performance of biogas production occurred from the above-mentioned
results in one stage operation, which was mainly due to the system overload. Accumulation of high concentration of VFAs and ammonia during the initial stage of anaerobic digestion subsequently affect the methanogenic stage in single stage anaerobic reactors. To overcome these problems, two-phase anaerobic digestion systems were developed to permit different bacterial enrichment in two different reactors by providing optimal growth conditions for initial acidogens and later methanogens [23].

3.2.1 Acidogenesis

Strong pH dependency of VFAs production during acidogenesis was investigated in our previous research [24]. It was found that at the pH of 8, the acidogenesis could performed better than those at other pH values.

![Fig.5 VFAs distribution and total VFAs concentration at pH 8](image)

As indicated in Fig.5, total VFAs concentration reached 37.2 g/L when the pH of acidogenic reactor was controlled at 8. This was mainly attributed to the dissolution of protein to different extents under alkaline conditions, which was beneficial for the protein degradation to fatty acids. However, according to the study by Liu et al., the output of VFAs produced at pH 10 was higher than those at other pH [25]. The discrepancy was caused by the presence of different microbial consortium, which changed with the variation of substances and environment. It shows that acetate accounts for 56.8% of total VFAs. The next important VFAs were propionic acid and butyric acid. Valeric acid, iso-valeric acid and iso-butyric acid were found at lower percentages. The maximum total VFAs concentration and individual VFAs concentration were almost stabilized after 108h.

3.2.2 Methanogenesis

Of the several intermediate steps in anaerobic digestion, methane formation from VFAs is often the critical pathway that limits the overall reaction rate. In this study, the authors operated UASB reactors at different organic loading rates using an artificial medium containing acetic acid, propionic acid, butyric acid, valeric acid, iso-butyric acid and iso-valeric acid.

| Table 3 Performance of UASB reactors under different OLRs |
|----------------|----------------|----------------|----------------|----------------|
| OLRs (g/L·d)  | HRT (h)        | COD of Influent (g/L) | COD of effluent (g/L) | COD removal efficiency (%) | Methane yield (ml CH₄/g COD) |
| 2             | 24             | 2                | 0.041           | 97.9           | 336             |
| 3             | 16             | 2                | 0.051           | 97.4           | 332             |
A quite stable performance of UASB reactors under fluctuating organic loads was observed (Table 3). Parawira \textit{et al.} reported that the UASB can provide stable process conversion rate up to an OLR of 6.1 g COD/L·d \cite{26}. In the present study, the OLR reached 12 g COD/L·d with specific VFAs compositions, which provided high COD removal efficiency (>80\%) throughout the operational periods. Based on the influent COD of UASB, the methane yield for the test was 319 mL CH₄/g COD. On the other hand, the UASB performance was testified with the genuine diluted acidic liquid, showing almost the same profile of methane production.

\textbf{Conclusions}

Anaerobic digestion of LARs has been carried out using one and two-phase system. At high organic loads two stage system seems to be very effective in comparison with one stage system. To upgrade yields in a two stage system, more investigations would be needed concerning the system set-up, microbial consortium in both reactor, etc., in order to optimize conditions in both hydrolyser and methanizer. However, if the two stage system is operated in a straightforward manner, like the one-phase system, this latter would be the best choice. It is simpler and can be applied successfully to the treatment of this type of waste with higher methane yield.

\textbf{Acknowledgement}

This study was funded by National High Technology Research and Development Program of China (863 Program) under Grant No. 2012AA101803, Key Projects in the National Science & Technology Pillar Program during the Eleventh Five-year Plan Period (No.2011BAD14B03) and Natural Science Foundation of Shandong province (ZR2012BL16).

\textbf{References}

