Biogas yield by co-fermentation of Scenedesmus and solid waste collected from sturgeon culturing system

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Abstract. Co-fermentation of Scenedesmus cultured in waste water and the solid waste collected from sturgeon culturing system was studied for biogas yield. The biogas yield efficiency could be increased obviously by adding a certain amount of aquaculture solid waste. With mass ratio of Scenedesmus to aquatic solid waste of 1:2, total biogas yield and the methane content in biogas were 6329.5mL and 48.2% during 30 days’ fermentation, while the highest biogas yield per unit mass of VS of 499.7mL/g was obtained with mass ratio of Scenedesmus to solid waste of 1:1. However, the biogas yield was not influenced significantly by treatment method of algae cells. Total biogas yield of Scenedesmus of untreated, heat treated and ultrasonic treated were 3451.2mL, 3854.5mL and 3854.5mL, respectively, during the fermentation process, and the corresponding methane content and per unit VS biogas yield in the biogas of 49.6% and 440.8mL/g, 49.4% and 492.2mL/g, and 48.4% and 467.6mL/g, respectively. It was also shown that higher biogas yield could be obtained by increasing the ratio of inoculum. With mass ratio of Scenedesmus to the inoculum of 2:3, the highest total biogas yield and biogas yield per unit VS of 3654.5mL and 478.3mL/g were achieved, and methane content in the biogas reached 57.2%. The co-fermentation process of Scenedesmus and aquaculture solid waste for biogas yield generally lasted for 25~30 days.

1. Introduction
Sturgeon culturing has developed rapidly during the late two decades in China. The yield of Sturgeon in 2016 was 89773 tons in China, accounting for 80% of the total yield in the world [1]. However, the discharging of great deal of waste water from sturgeon culturing system has led to serious environmental pollution, depletion of fresh water resource, and increasing in costs. Therefore, waste water treatment and utilization has been the critical issue for the development of sturgeon culture enterprises [2]. On the other hand, micro-alga has higher growth rate, environmental adaptability and photosynthetic efficiency, and it can uptake nitrogen and phosphorus, and capture CO₂ effectively from waste water [2,3]. What’s more, those micro-algae could be treated as biomass and transferred into bio-energy. Therefore, as an effective alternative for bio-remediation of aquatic ecosystem, micro-algae has been used for treatment of waste water in aquaculture [4]. Additionally, compared with terrestrial biomass plants, micro alga is thought to be the most promising biomass resource, due to its higher productivity, lower lignin content, efficient energy conversion and landless cultivating [5, 6]. Since early 1980s, researches on waste water purification and bio-energy yield by microalgae have been carried out world widely [7-11]. However, the conversion of microalgae to bio-energy has been
mainly focused on biodiesel and biological hydrogen yield, few works on biogas yield of microalgae cultured in aquaculture waste water were carried out[12-15]. It was mainly because that the moisture content of micro-algae liquid was high, and organics content was relatively lower, which led to low anaerobic digestion efficiency. In aquaculture, large amount of aquatic solid waste which contained high concentration of organic acid and proteins was produced. If the aquatic solid waste was discharged directly, it would cause serious environmental pollution. But if those solid waste was codigested with micro-algae, it not only could improve the substrate for anaerobic digestion of micro-algae, but also could transfer the aquatic solid waste into bio-energy[16].

To investigate a better anaerobic digestion performance, the biogas yield by co-digestion of Scenedesmus and solid waste collected from sturgeon culturing effluents was studied. the effects of different mass ratios of micro-algae to solid waste, treatments of algae cells, and inoculation ratios on the methane yield was studied.

2. Materials and methods

2.1. Materials and pre-treatment

Scenedesmus was cultured in a pilot scale raceway bio-reactor which contented 10L of waste water from sturgeon culturing system. Scenedesmus was harvested by settlement of 48h with algae cell density of 5.36×10^7/mL in the liquid and stored at 4℃.

Inoculum: The anaerobic sludge used as an inoculum was collected from a biogas fermentation system (temperature:35±1℃ and pH:7.5±2) at Dalian Dongtai Sludge Treatment Company. The inoculum was acclimated as the follows: 200g of anaerobic sludge was put into a conical flask, and 2.5g of Scenedesmus liquid was added to the flask every day and mixed with a magnetic stirrer for 20d. The anaerobic sludge was taken as inoculum after they finish aerogenesis.

Solid waste was collected from a sturgeon culturing system in the lab and kept at 4℃.

Main components of Scenedesmus, inoculum, and aquatic solid waste were listed in table 1.

| Items          | TS/ % | VS/ % | N/ % | C/N | VS/TS/ % | pH  |
|----------------|-------|-------|------|-----|----------|-----|
| Scenedesmus    | 0.18  | 0.09  | 0.01 | 4.31| 50.00    | 8.32|
| Inoculum       | 7.70  | 2.84  | 0.05 | 3.58| 40.35    | 7.35|
| Solid waste    | 11.37 | 5.90  | 0.37 | 7.58| 51.90    | 7.10|

Notes: The components were calculated by wet weight.

TS-total solids
VS-volatile solids

2.2. Fermentation system

The anaerobic co-fermentation system of Scenedesmus and solid waste was similar to that of Li, et al[17]. Fermentation flasks were put into thermostat water bath at setting temperature. Different fermentation materials were put into different groups of flasks separately. The produced biogas got in air collectors with equal volume of water discharged from the air collector. Biogas yield could be calculated as the amount of discharged water and the composition was analysed by gas chromatograph and so on.

(1) Fermentation with different mass ratios of Scenedesmus to solid waste: 100g inoculum and 100g Scenedesmus liquid were put into each reactor, and then adding solid waste of 50g, 100g and 200g to each reactor, respectively, and mixed up by a magnetic stirrer. The above groups were marked as mass ratios of Scenedesmus to solid waste of 2:1, 1:1, and 1:2, respectively.

(2) Fermentation with differently treated Scenedesmus: 100g inoculum and 100g solid waste were put into each reactor, and adding 100g Scenedesmus liquid of untreated(N), heated in water bath at
85°C for 25 min (H), treated by ultrasonic at 50 Hz for 15 min (U), respectively, and mixed up with a magnetic stirrer.

(3) Fermentation with different mass ratios of *Scenedesmus* to inoculum: 100 g solid waste and 100 g *Scenedesmus* liquid were put into each reactor, and adding 50g, 100g and 150g inoculum to each reactor and mixed up by a magnetic stirrer, which marked as mass ratios of algae to inoculum of 2:1, 1:1, and 2:3, respectively.

100 g inoculum and 100 g *Scenedesmus* liquid without solid waste were set as a control group for the above 3 groups of experiments, marked as 1:0. Some distilled water was added if necessary to ensure that the quantities of fermentation liquor in the control and experimental groups remained consistent.

The fermentation temperature of all the experiments was 35°C. A parallel sample was taken for each approach.

2.3. Analysing methods and instruments

The daily biogas yield was calculated by the volume of water discharged from the anaerobic fermentation system because of the pressure change. TS and VS were measured by weighing (Muffle furnace, SX2-4-10; precision electronic balance, MP1100b) [18]; Total carbon content was calculated by 47% VS [19]; Volatile fatty acid (VFA) and pH were measured by colorimetric (UV spectrophotometer, UV–7504) and pH meter (PHS-3E) [20]; Methane concentration in biogas was measured by gas chromatograph (GC9890) after fermentation finish. Ultrasonic cleaning machine (KQ3200E) was used to rupture cell walls of *Scenedesmus*.

3. Results and discussions

3.1. The effects of different mass ratios of *Scenedesmus* to solid waste on fermentation characteristics

Fermentation characteristics with different mass ratios of *Scenedesmus* to solid waste were shown in Figure 1. It was clear that in this experiment, the fermentation performance could be improved by adding solid waste. The ratio 2:1 group showed relative quick increasing in daily biogas yield after starting fermentation of 5d and kept higher biogas yield during 5–9d. The peak biogas yield reached 248 mL at 11d, but dropped consequently and obtained lower biogas yield after 16d. In addition, the ratio 1:1 group achieved relatively quick increasing in daily biogas yield during 10–14d and reached its peak biogas yield of 413 mL at 14d. Significant drop in biogas yield appeared at 17d and showed lower biogas yield after 22d. Furthermore, fast growing in daily biogas yield in the ratio 1:2 group was found after starting fermentation of 14d and its peak biogas yield reached 540 mL in 18d. It kept relativity higher daily biogas yield during 15–25d. During the fermentation of 30d, the above three groups gained total biogas yield of 2838.5 mL, 4412.5 and 6329.5 mL, and the corresponding methane content in the biogas was 46.65%, 47.38%, and 48.17%, respectively (see Figure 1). It is clearly that the biogas yield was influenced by mass ratios of *Scenedesmus* to solid waste. Raising the content of solid waste was benefit for the increasing of total biogas yield and methane content. That may be that the VFA which could be utilized by methanogenic bacteria would increase with the raising of solid waste content (see Figure 1). However, the starting time for biogas yield was delayed and the biogas yield of per unit mass of VS decreased if excessive solid waste was added to the fermentation process (see Figure 1). The produced VFA did not cause obvious dropping in pH (see Figure 1), which would be beneficial to the activity of methanogenic bacteria and fermentation.
3.2. The effects of different treatment of alga on fermentation characteristics

The fermentation and biogas yield of Scenedesmus treated differently were showed in Figure 2. It could be known that untreated group obtained fast growing in daily biogas yield after starting 6 days and reached its peak biogas yield of 336mL at 10 d, while heated and ultrasonic treated groups exhibited relative quick increasing in daily biogas yield after starting of 3 days and reached their peak biogas yield of 372 mL and 365mL in 10d and 12d, respectively. The fermentation process lasted about 24 days with Scenedesmus untreated or treated, and the corresponding total biogas yield was 3512.5mL, 3915.0mL, and 3629.0mL for untreated, heated, and ultrasonic treated groups, respectively. Additionally, the biogas yield per unit mass VS and methane content in biogas were heating or ultrasonic treatment of 448.6mL/g VS, 500.0mL/g VS, 463.5mL/g VS and 48.4%, 49.6%, 49.4%, respectively. The results indicated that the treated Scenedesmus showed positive effect on methane content and biogas yield per unit VS when it was used for co-fermentation with solid waste. This could also be noted from Figure 2 that relatively higher VFA concentration and pH stability were found in heated or ultrasonic treated groups after starting fermentation of 7d. The reason may be that the cell walls of Scenedesmus could be disrupted by heating or ultrasonic treating, which was much helpful to the digestion of the microalgae. Particularly, heat treated group showed more preferable to the fermentation and the biogas yield. This was well agree with the result of González et al.[21]. Additionally, the pre-treatment of Scenedesmus had not increased the VFA concentration (see Figure 2).
3.3. The effects of different amounts of inoculum on fermentation characteristics

The amount of inoculum had significant effect on the fermentation of Scenedesmus and solid waste (see Figure 3). The ratio 2:1 group obtained its quick growth in biogas yield after starting fermentation of 6d, but the daily biogas yield was the lowest during fermentation of 12d. Although it subsequently achieved relative higher biogas yield and reached its peak daily biogas yield of 310.0mL in 13d, the total biogas yield (2686.2mL) during the fermentation of 26 d was much lower than that of the other groups. In addition, the biogas yield per unit VS (414.0mL/gVS) and the methane content in the biogas was also lower than the comparative groups (see Figure 3). It was also indicated that the daily biogas yield of ratio 1:1 group was slightly higher than that of group 1 after the starting of fermentation, but its quick increase in biogas yield was found after 6d. Group 2 gained its peak daily biogas yield (379.0mL) in 11d, but the total biogas yield (2962.5mL) during after fermentation of 26d was still much lower than that of group 3 (ratio 2:3) (see Figure 3). Meanwhile, the biogas yield per unit VS (419.0mL/gVS) and methane content (56.2%) in the biogas were in the less than that of group 3. It was clear that group 3 started much quickly and achieved markedly higher daily biogas yield during the fermentation of 10d. Additionally, the highest daily biogas yield of 450mL in 9 d and total biogas yield of 3654.5 mL in 26 d were obtained in this group. Furthermore, group 3 gained higher biogas yield per unit VS (478.3mL/gVS), and methane content (57.2%) in the biogas than that of comparative groups during the fermentation (see Figure 3). The results demonstrated that the higher the inoculum content in the fermentation, the favorite to the biogas yield. Liu, et al. [22] also found that increase the amount of inoculated sludge could increase the daily biogas yield for cabbage leaves. The reason could be that sufficient methanogens bacteria were needed during the fermentation of Scenedesmus. It could be noted correspondingly by the VFA concentration and pH in the fermentation liquid (see Figure 3), where the VFA concentration in group 3 was much lower and the pH in the liquid was relatively stable than that of group 2 and group 1. That means that much VFA could be utilized for biogas yield by adding sufficient inoculum.
Figure 3. Fermentation property with different ratios of Scenedesmus to inoculum.

4. Conclusions
Decrease the mass ratio of Scenedesmus to aquaculture solid waste could increase total biogas yield and methane content in the biogas, while the biogas yield of per unit mass of VS was not correspondingly increased as well. The highest total biogas yield and methane content in the biogas were 6329.5mL and 48.2% by co-fermentation of Scenedesmus and aquaculture solid waste at 1:2 of mass ratio, while the highest biogas yield per unit VS (499.0 mL/g VS ) was obtained with mass ratio of Scenedesmus to aquaculture solid waste of 1:1. On the other hand, the total biogas yield of co-fermentation of Scenedesmus and solid waste was 3451.2mL, 3854.5mL and 3854.5mL, respectively, if the Scenedesmus was handled as untreated, heated and ultrasonic treated, the methane content in the corresponding biogas were 49.6%, 49.4% and 48.4%, respectively. It was therefore that heated treated Scenedesmus was preferable to the biogas yield. Furthermore, the co-fermentation of Scenedesmus and solid waste could be promoted by adding sufficient amount of inoculum. The daily biogas yield, biogas yield per unit VS and methane content were 3654.5mL, 478.3mL/g and 57.2%, respectively with mass ratio of Scenedesmus to inoculum of 2:3. The biogas yield of co-fermentation of Scenedesmus and aquaculture solid waste lasted generally for 25~30 days, which has demonstrated as a promising approach for efficient utilization of microalgae cultivated in waste water and the solid waste in aquaculture system.

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