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Characteristics of Mixed Anaerobic Fermentation of Decomposed Cyanobacteria and Anaerobic Sludge

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Bench-scale test was employed to study the biogas yields of anaerobic fermentation on cyanobacteria. The results indicated that, after 7 days' decomposition at 30~35°C, fresh cyanobacteria achieved the highest biogas generation efficiency. 246mL/gCOD of biogas was produced at 35°C of fermentation temperature, with a biogas yield potential of 354mL/g(VS). After 15-day anaerobic reaction, the cumulative biogas, COD and VFA concentration tended to be stable. The activities of amylase and dehydrogenase were inhibited at the early stage of anaerobic reaction, while the activity of protease and the concentration of coenzyme F_{420} increased gradually, reaching to 27.66μmol/(gVS·min) on the 6th day and 0.62μmol/g(VS) on the 15th day, respectively. The period of 15 to 18 days was favorable for the anaerobic fermentation of decomposed cyanobacteria, which was less than that of anaerobic digestion with fresh cyanobacteria as stroma. The process of cyanobacteria decomposition promoted the anaerobic reaction, and 7-day decomposition of cyanobacteria promoted microbial activity and methane generation potential.

Key words: Decomposed cyanobacteria, Anaerobic fermentation, Biogas, Enzyme activity.

Taihu basin is the area with a large population and developed economy in China. Since 1990s, the eutrophication of Taihu has tended to be serious. The occurrence of cyanobacteria algae blooms affected landscapes, produced with the water body stinks. The production of microcystic toxins may cause liver dysfunction and even liver cancer (Matsushima *et al.*, 1992). To rapidly reduce cyanobacteria concentration in waters, a common method is to routinely salvage and collect cyanobacteria in order to remove a great deal of nitrogen and phosphor from water bodies and decrease the eutrophication level t (Shen *et al.*, 2004). However, a huge number of the collected cyanobacteria generally have high water contents. It is therefore difficult for the future disposition. Part of the collected cyanobacteria can be poured to landfill, ravine or lakeside depression .where they die soon, turn into decomposed biomass and severely pollute atmosphere via generating disgusting smell. In addition, decomposed cyanobacteria can be washed by rain, and the nutrition substance with nitrogen and phosphor will flew into Taihu Lake, leading to secondary pollution (Han *et al.*, 2009).

Cyanobacteria are rich in nitrogen and phosphor nutrition substance and carbohydrate. If processed by anaerobic fermentation to yield biogas, cyanobacteria can be decomposed, harmless and act as resource. Some existing researches showed that, although cyanobacteria had a great potential to yield biogas , it started slowly and took a long time for anaerobic digestion, generally more than 30d (Xu *et al.*, 2007; Dong *et al.*, 2006; Yen *et al.*, 2007). After natural

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decomposition, cyanobacteria were dissolved while cellulose was hydrolyzed, with an increased biodegradability than the fresh cyanobacteria, and suitable for microbial degradation. There were some reports about employing decomposed cyanobacteria to irrigate vegetables (Geoffrey *et al.*, 1999; Kaushal *et al.*, 2010), which could promote the growth of vegetables. However, there are few reports about anaerobic fermentation with decomposed cyanobacteria. Therefore, this study took decomposed cyanobacteria as stroma to mix with anaerobic sludge for anaerobic fermentation. The biogas yield capacity and the best decomposed time of cyanobacteria were investigated to provide theoretical basis and technical support for the utilization of cyanobacteria.

MATERIALS AND METHODS

Materials for experiment

A certain amount of fresh cyanobacteria slurry was taken and placed into the equipment for decomposition (Figure 1). The decomposition process was carried out under natural condition (30~35°C). The cyanobacteria which were respectively decomposed for 1d, 4d, 7d and 10d were sampled for the anaerobic bench-scale tests. The anaerobic sludge for inoculation was taken from the UASB reactor.

Table 1. Components of fermentation materials

Material	TS(%)	VS(%)	TN(mg/g)	TP(mg/g)
Fresh cyanobacteria slurry	2.86	2.72	31.44	3.49
Anaerobic sludge	4.58	4.06	2.53	0.56

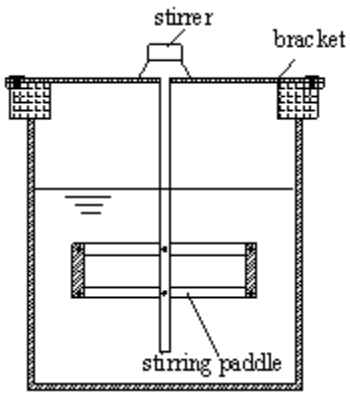

Fig. 1. Schematic diagram for cyanobacteria decomposition

Experimental method

Batch fermentation was used. The setting was showed as figure 2.Ten 500ml bottles were taken as reactors and were divided into 5 groups. Stroma and anaerobic sludge were added into the temperature controlled oscillator at the ratio of 1:5. Biogas samples were taken from the sampling outlet on the top of the bottle , while the liquid samples were taken from algae slurry sampling outlet- on the top of the bottle, and the pressure inside the setting was measured by a U-tube manometer connected with a bottle.

The volumes of decomposed cyanobacteria and anaerobic sludge added into each group were as follows: group 1, as control, was added with 80ml anaerobic sludge and 400ml of distilled water; group 2, group 3, group 4 and group 5 were added with 80ml anaerobic sludge, as well as 400ml cyanobacteria with the decomposed days being 1d, 4d, 7d and 10d respectively. Each group had two reactions. After feeding, bottles were manually oscillated for 10min to mix decomposed cyanobacteria and anaerobic sludge evenly, and the air in the bottles was excluded for 30min by blowing nitrogen. The experiment was conducted in 100r/min constant temperature oscillator at 35°C, lasting for 30d. Saturated NaCl solution was used in water-draining method to gather biogas.

Chemical analysis

COD was measured with potassium dichromate method (GB11914-89).

The content of biogas was analysed by the Gas Chromatograph(GC) (Tenghai GC 2001, Tengzhou, China) with a Thermal Conductivity Detector (TCD) and a stainless steel column TDX-01(Φ3mm×4m). Determination condition: the temperatures of the sample injector, the column and the TCD were 150, 130 and 150°C, respectively. The bridge current was 120mA. The flow velocity of carrier gas H_2 was 15mL/min. The nature determination was done in accordance of the retention time of guide sample, while the quantitative determination was done with an external standard method. The Volatile Fatty Acid(VFA) was measured by the Tenghai GC 2001

with a Hydrogen Flame ionization Detector(FID) and a SE-30 capillary column (30m×0.32mmID× 0.25μm, Agilent). Test conditions: the temperature of sample injector, the FID and the column was respectively 200°C, 230°C and 120°C, lasting for 5min. The flow velocity of carrier gas N_2 was 20mL/min, with a split ratio of 16:1. Based on the retention time of the guide sample, the nature determination was done and the quantitative determination was done with an external standard method.

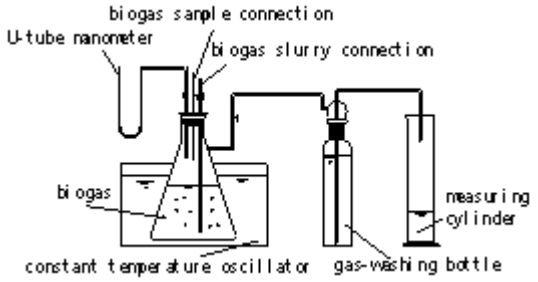

Fig. 2. Schematic diagram of bench-scale test on decomposed cyanobacteria's anaerobic fermentation to yield biogas

The detection of enzyme activity

DNS was used in detecting the amylase activity (Bernfeld, 1955); protease activity was detected with UV spectrometry (Zhang *et al.*, 2004); the content determination of coenzyme F_{420} was employed UV-visible spectrophotometry (Wu *et al.*, 1986); the TTC-dehydrogenase activity determination was adopted improved TTC-dehydrogenase activity detection (Zhou *et al.*, 1996).

RESULTS AND DISCUSSION

COD and VFA concentration variation

Figure 3a showed that with the increase of the decomposition time, the COD degradation efficiency increased. The reason probably was that most organic matters, such as protein and saccharide released from decomposed cyanobacteria(Zhu *et al.*, 2012), and were directly utilized by the anaerobe. Therefore, in the early stage of anaerobic fermentation, COD removal rate was high, and the COD removal rate of the cyanobacteria which decomposed for 7 days was the highest. The organic matters in the cyanobacteria which decomposed for 1 day continued to degrade until the 21st day, while the rests had organic matter concentrations tending to be stable after the 15th day, and COD removal rates were beyond 33%. Combined with the analysis of cyanobacteria's biogas generation performance, the conclusion was drawn that the organic matters in the cyanobacteria which was decomposed for 7d or longer time finished degradation and yielded methane on the 15th day of anaerobic fermentation to achieve efficient recycling of biomass energy. After fermentation, COD concentrations of liquid were more than 30000mg/L, the reasons were first, there were too many impurities and other matters that could not be utilized by the microorganisms in the decomposed cyanobacteria, and second, anaerobic fermentation was incomplete. In the future study, the fermentation condition requires further optimization, to increase the activity of methanogen and enable the organic matters in the cyanobacteria to be released as much as possible, and thus to improve fermentation efficiency and biogas generation potential.

Figure 3b showed that the change rules of VFA in different groups were the same as COD, but with different peak times. When anaerobic fermentation started, the experimental group with 10 days' decomposition decomposed and hydrolyzed thoroughly in the early period, so the initial concentration of VFA was the highest; the VFA concentrations of the rest groups' increases and then decreases, and finally became stable. The cyanobacteria which decomposed for 1d and 4d hydrolyzed partially in the decomposed period, and continued hydrolysis in the anaerobic system, with pH decreased to 6.12 and 6.01, respectively, while ORP grew to -150mV and -189mV, separately (Zhu *et al.*, 2012). VFA increased rapidly, reaching the peak on the 6th day. During the methanogenic period, VFA slowly decreased, consistent with the gas generation changes of all groups. During the whole reaction, the VFA peak values among all groups differed slightly, and the variation of VFA of the 7 days' decomposition group was the greatest, with a difference between the maximum value and the minimum of 2541mgAc/L. During the processes of decomposition and anaerobic fermentation, VFA increased constantly in a certain time. However, due to the degradation of nitrogen organic compounds in the cyanobacteria, alkalinity grew, which caused pH in the system to reach more than 5.79 at hydrolysis stage (Zhu *et al.*, 2012).

Thereby, the accumulation of volatile organic acids did not lead to the retardation of anaerobic fermentation.

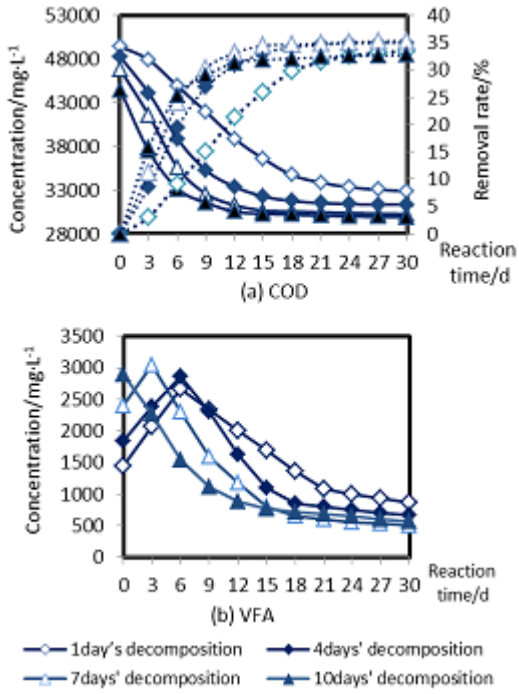

Fig. 3. Regular pattern of COD and VFA of cyanobacteria decomposed for different time changing with anaerobic reaction time

The variation of system s biogas generation performance

Figure 4a showed that during the initial period of anaerobic fermentation, the yielding rates

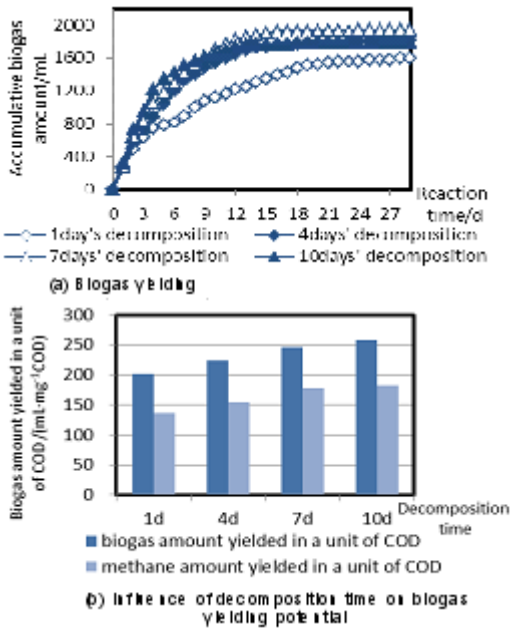

Fig. 4. Influence of decomposition time on the biogas yielding amount of anaerobic system

of biogas were fairly high, but tended to be lower from the 6th day. After 30 days' anaerobic digestion reaction, 4 experimental groups did not yield biogas.

On the 15th day of anaerobic reaction, the biogas yielding amount of the cyanobacteria which had decomposed for 4d, 7d and 10d just increased slightly, consistent with the COD removal rules.

The group of 7 days' decomposition had the highest accumulative biogas yields, and the group of 1 day's decomposition had the lowest biogas yield rates and the smallest accumulative biogas yields. The reason might be that the cyanobacteria cell wall was hard to degrade. The shorter the decomposition period was, the fewer cell walls been degraded. Thus the organic matters were hindered to release from cells. Thereby, the degradation velocity of cyanobacteria was decreased and then the anaerobic fermentation's biogas yielding velocity was affected directly. Hence, the anaerobic fermentation's biogas yielding rates were closely linked with the decomposition degree. The cyanobacteria of 7 days' decomposition yielded the maximum daily biogas, 363ml, on the 1st day of anaerobic fermentation. The accumulative biogas yield accounted for 60% of the total biogas yield on the 5th day. 90% of the biogas yield was generated in the previous 11 days. The accumulative biogas yield reached to 97.8% on the 15th day. With the increase of biogas yield, all groups' COD and VFA concentrations gradually reduced and became stable after 15 days' of reaction. Consequently, the time needed for the 7 days' decomposition cyanobacteria to be anaerobically digested would be controlled between 15 to 18 d, which was less

than the fresh cyanobacteria (Xu and Gao, 2007; Dong *et al.*, 2006; Yen *et al.*, 2007). Meanwhile, COD decreased by 7.93g, the calculated value of biogas yield was 246mL/gCOD, methanogenic yield was 167mLCH_4/gCOD, and biogas yielding potential was 354mL/g(VS). The results were almost equal to that of the anaerobic digestion system with fresh cyanobacteria as stroma (Xu *et al.*, 2007; Dong *et al*., 2006; Yen and Brune, 2007).

The variation of enzyme activity in system

Amylase is the generic term for hydrolyzed starch and glycogen enzymes. And protease is the generic term for the enzymes of hydrolyzed protein. The variations of amylase and protease's activity are the attribute of the influence placed by decomposition time on organic hydrolysis process during the anaerobic digestion process. The changes of TTC-dehydrogenase activity represent the influences of decomposition time on the enzyme activity of microorganism and the effects of microorganism on the degradation capacity of organic matters. The changes of coenzyme F_{420} concentration represent the influence of decomposition time on methanation process.

Figure 5a and 5c suggested that, amylase and TTC-dehydrogenase existed in the cyanobacteria plasma which decomposed varied time. Due to the changes of environment, the activities of amylase and dehydrogenase of all groups were inhibited in the initial period of anaerobic reaction. After 3 days' adaptation, the activities recovered. After 6 days' reaction, the activities reached the stable stage. In the experimental group of 7 days' decomposition, the activities of amylase and dehydrogenase were higher than other groups. And the group of 1 day's decomposition had the lowest amylase and dehydrogenase activity. Figure 5b showed when decomposed cyanobacteria were put into the anaerobic reactor, the activity of protease gradually increased, reaching 27.66μmol/(gVS·min) on the 6th day. Cyanobacteria were rich in protein with the content up to 61.81% (Du, 2008). During the decomposition process, cyanobacteria protein was progressively released and degraded by protease. The longer decomposition time caused the greater activity of protease reached. When the decomposed cyanobacteria were put into the anaerobic reactor, all groups had enhanced the activity of protease, showing that rigorous anaerobic condition contributed to the degradation of protein. Because the protein of the 10days' decomposition group gradually degraded during the decomposition period (Zhu *et al.*, 2012). The activity of protease in the anaerobic reactor enhanced less slightly than those of other groups. The group of 4 days' decomposition had the highest activity of protease.

Coenzyme F_{420} which plays a vital role in the formation of methane is the specific matter of methanogen, of which the biochemical effect is an electron transfer carrier with low potential played. It cannot be replaced by other electron carriers. Figure 5d made it clear that in the groups of 7days' and 10days' decomposition, the initial concentrations of coenzyme F_{420} were higher than that of groups of 1 day' and 4 days' decomposition. As anaerobic reaction was in progress, the concentrations of coenzyme F_{420} increased gradually and became stable on the 12th day. In the later period of anaerobic reaction, the group of 7 days' decomposition had a higher coenzyme F_{420} concentration than other groups, which reached 0.62μmol/g(VS) on the 15th day of anaerobic reaction. The obtained value was slightly higher than 0.56μmol/g(VS), which was measured by Hu Ping in the anaerobic fermentation system with mixed anaerobic granular sludge and cyanobacteria(Hu, 2009).Coenzyme F_{420} of 1day's fermentation group was the lowest, with the value of 0.51μmol/g(VS) on the 12th day of anaerobic reaction. The differences of coenzyme F_{420} of all groups was consistent with that of biogas yield potential, suggesting that coenzyme F_{420} content would perfectly reflect the activity of methanogen in the system.

The study results of enzyme activity demonstrated that a prolonged decomposition period benefits the hydrolytic process, and 7 days' decomposition promoted the degradation of organic matters and the biogas yield in the anaerobic system. However, when it came to more than 10 days, the anaerobic system turned into the anaerobic methanogenic process, and decreased the methanogenic efficiency. The activities of amylase, protease and dehydrogenase were familiar to the enzyme activities in the anaerobic digestion reactor with kitchen waste as stroma in this research group (Shao, 2009), manifesting that in

the anaerobic process, the hydrolysis rates of decomposed cyanobacteria and kitchen waste were close. However, the concentration of coenzyme F_{420} was much greater than that in the anaerobic digestion reactor with kitchen waste as stroma(0.083μmol/g(VS)), indicating that the anaerobic fermentation system with decomposed cyanobacteria had a greater methanogenic capacity.

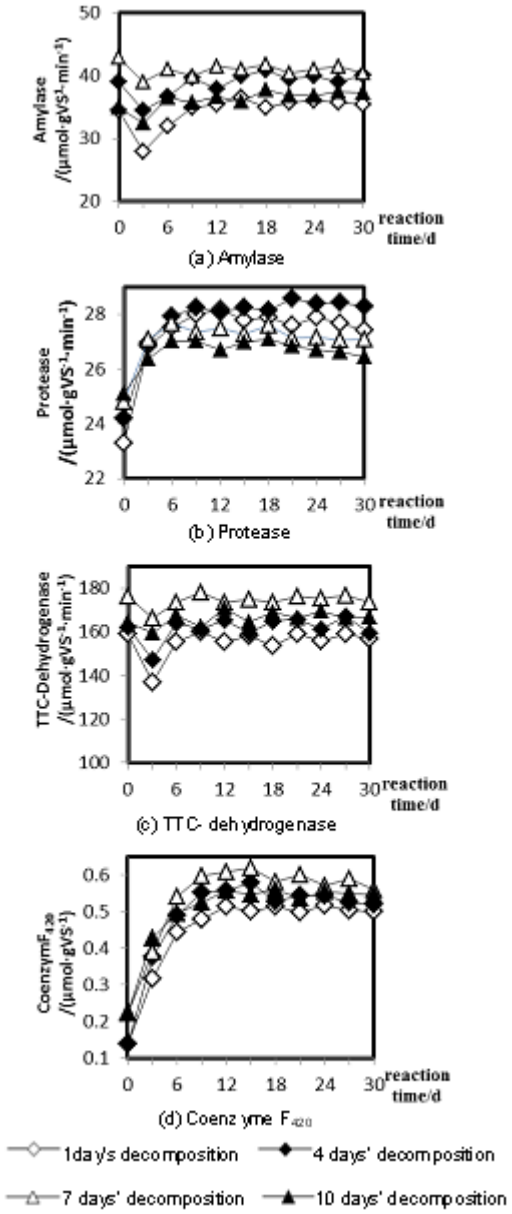

Fig. 5. Variation of enzyme activity in decomposed cyanobacteria fermentation liquid

CONCLUSION

1) When mixed with the anaerobic sludge, the decomposed cyanobacteria could effectively yield biogas through anaerobic fermentation and achieved the recycle of biomass energy.
2) Biogas yields in the anaerobic system based on the decomposed cyanobacteria were closely linked with the decomposition degree. Fresh cyanobacteria, when decomposed for 7 days at 30~35°C, could achieve the largest biogas yielding rates of 246mL/gCOD and the biogas yield potential was 354mL/g(VS). After 15 days' anaerobic reaction, the accumulative biogas yield, COD and VFA concentrations tended to be stable15 to 18 days were the appropriate anaerobic fermentation time for the decomposed cyanobacteria, being less than the anaerobic digestion time with fresh cyanobacteria as stroma. The decomposition process of cyanobacteria promoted the anaerobic reaction.
3) The activities of amylase and dehydrogenase were inhibited during the initial period of anaerobic reaction, recovered after 3 days' adaptation and became stable after 6 days' reaction. The activities of protease and the concentrations of coenzyme F_{420} gradually increased, and tended to be stable on the 6th and 12th day of reaction, respectively. The content of coenzyme F_{420} perfectly reflected the methanogen activity. The anaerobic system of cyanobacteria for 7 days' decomposition had greater microbial activity and methane yield potential than the others.

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