Research on soybean protein wastewater treatment by the integrated two-phase anaerobic reactor

Yaqin Yu *

Dept. of Civil Engineering, Yancheng Institute of Technology, Xiwangdadao 1#, Yancheng 224003, China

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Abstract The start-up tests of treating soybean protein wastewater by the integrated two-phase anaerobic reactor were studied. The results showed that the soybean protein wastewater could be successfully processed around 30 days when running under the situation of dosing seed sludge with the influent of approximately 2000 mg/L and an HRT of 40 h. When the start-up was finished, the removal rate of COD by the reactor was about 80%. In the zone I, biogas mainly revealed carbon dioxide (CO₂) and hydrogen (H₂). Methane was the main component in the zone 2 which ranged from 53% to 59% with an average of 55%. The methane content in biogas increased from the zone I to II. It indicated that the methane-producing capacity of the anaerobic sludge increased. It was found that the uniquely designed two-phase integrated anaerobic reactor played a key role in treating soybean protein wastewater. The acidogenic fermentation bacteria dominated in the zone I, while methanogen became dominant in the zone II. It realized the relatively effective separation of hydrolysis acidification and methanogenesis process in the reactor, which was benefit to promote a more reasonable space distribution of the microbial communities in the reactor. There were some differences between the activities of the sludge in the two reaction zones of the integrated two-phase anaerobic reactor. The activity of protease was higher in the reaction zone I. And the coenzyme F₄₂₀ in the reaction zone II was twice than that in the reaction zone I, which indicated that the activity of the methanogens was stronger in the reaction zone II.

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1. Introduction

The amount of the wastewater containing soybean protein is huge in China, with the great development of the soybean protein (Su and Yu, 2005). Soybean protein wastewater is the high organic wastewater, which contains organism, nitrogen and phosphorus (Bao et al., 2009). Therefore, it is crucial to develop an innovative appropriate anaerobic reactor for treating soybean protein wastewater.

Most of the treatment studies on soybean protein processing wastewater have focused on aerobic and membrane technology (Su and Yu, 2005). However they have the shortages...
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such as sludge yield, membrane pollution and high cost, which restrict the utilization of pond treatment system. It is regarded that anaerobic biological treatment technology is the most efficient method to deal with the high concentration of organism wastewater, which can also gain biomass energy (Maroun and Fadel, 2008; Hong and David, 2007a,b; Barber and Stuckey, 1999). Therefore, it is crucial to develop an innovative appropriate anaerobic reactor for treating and gaining biomass energy from soybean protein wastewater.

Although, a lot of anaerobic reactors such as upflow sludge blanket reactor (UASB), anaerobic filter (AF), and anaerobic continually stirred tank reactor (CSTR) have been developed, the two-phase anaerobic process has many advantages compared to those anaerobic reactors. The process of anaerobic decomposing organic substrate can be simplified into two phases of acid production and the methane production (Yu et al., 2014). The microbial activity is directly influenced by the temperature in the anaerobic reactor (Speece, 1996; Barber and Stuckey, 1999; Angenent et al., 2002). The two-phase anaerobic technology which improved separation of phases (acidogenic and methanogenic) possessed stable and high removal rate, and stronger ability to resist impact load than common anaerobic devices. The literature survey shows that it lacks on the anaerobic treatment of soybean protein wastewater by the two-phase anaerobic technology. Therefore, the purpose of this study is to determine the feasibility for treating soybean protein wastewater, but also elucidated the microbial phase in the reactor during the experiments.

2. Materials and methods

2.1. Devices

The integrated two-phase anaerobic reactor was made by UPVC, which was cylindrical with the effective volume of 60 L. It was divided into two anaerobic reaction zones, whose volume was about 20 L and 40 L, respectively. The soybean protein wastewater entered into the anaerobic reaction zone I by uniform water distribution system, through the inlet pipes which were placed at the bottom of reactor. The effluent entered into anaerobic reaction zone II through the down flow weir which was placed at the top of the baffle plate. The outlet pipe was at the bottom of the anaerobic reaction zone II. The reactor was equipped with three phase separators. The anaerobic reaction zone I was equipped with auto stirring device and auto heating apparatus. The sludge was discharged through the mud pipe placed at the bottom of the reactor. The methane production was measured by the wet-type gas flow meter. The operating temperature of the reactor was maintained constant at 30 °C by the auto heating apparatus in the anaerobic reaction zone I. The reactor was wrapped with insulating sponge, aiming at reducing the loss of heat. The soybean protein wastewater was pumped into the reactor in the way of continuous feeding by peristaltic pump. The integrated two-phase anaerobic reactor is shown in Fig. 1.

2.2. Wastewater and the seed sludge characteristic

The soybean protein wastewater used in this research was taken from a local soybean protein productive company, and the wastewater quality is shown in Table 1. The average chemical oxygen demand (COD) was about 8500 mg/L, and the biochemical oxygen demand (BOD)/COD value was about 0.6. The soybean protein wastewater was diluted using tap water to required concentration.

The seed sludge was taken from the upflow anaerobic sludge blanket (UASB) in a local foodstuff wastewater treatment plant. The ratio of mixed liquor volatile suspend solid (MLVSS) to mixed liquor suspend solid (MLSS) was about 0.7 in seed sludge. The quantity of the seed sludge in the reaction was 30 g MLVSS/L. The hydraulic retention time (HRT) of this reactor was 40 h.

2.3. Analytical methods

Analysis of COD, total nitrogen (TN), total phosphorus (TP), MLVSS, suspended solids (SS), and pH were conducted in accordance with standard methods (China, 2004). The amount of collected gas was determined by the wet corrosion gas meter (Changchun Automobile Filter Co., Ltd.). The biogas composition (CH4, CO2 and N2) was analyzed using gas...
chromatography on a Multigas analyzer, Model GC-2001 (Zhu et al., 2008). Sludge samples were taken from the reactor after the operation of 35 days and the biomass was examined by scanning electron microscopy (SEM) with model JSM 5600LV, Shimazu, JAP. At first, sludge samples were prepared at 25°C for 4 h with 2.5% (w/v) glutaraldehyde in Sorenson phosphate buffer, and then dehydrated through a graded series of water-ethanol mixtures (10%, 25%, 50%, 75%, 90%, and 100%), finally, brought to equilibrium in each mixture for 10 min and dried by the frozen drying method before sputter coating with gold particles (Rong et al., 2011). The protease was analyzed using the McDonald Chen spectrophotometry, and the coenzyme F420 was analyzed using the spectrophotometry (Whitmore et al., 1986; Whiteley et al., 2002).

It was common to use fluorescence in situ hybridization (FISH) technology to analyze the microbe in the granule sludge (Liu et al., 2012). After collecting from the reactor, the sludge sample was fixed with 4% paraformaldehyde solution and washed with PBS buffer solution. After these operations, it was kept at −20°C in the solution which was mixed with the same volume PBS buffer solution and 100% alcohol. Afterward, the sample was scribbled on the slide glass and dehydrated with alcohol.

Then, the common practice is to hybridize the sample at the temperature of 46°C for 5 h with EUB338(5c ACTCCT ACG GGA GGC AG3c) probe which was signed with CY3 (red) and ARCH915 (GTGCTCCCCCGCATACTC) probe signed with ARCH915 (yellow) (synthesized by ShangHai biotechnology company). Unhybridized probe and hybridized buffer solution were washed off from the hybridized sludge sample with hybridized cleaning solution at 46°C. Then it was rinsed 3 times with sterilization ultrapure water at the temperature of 46°C and air-dried. Finally through the fluorescence microscope, we scanned imagery and observed it.

### 3. Results and discussion

#### 3.1. COD removal of reactor

The efficiency of the anaerobic reactor was reflected by the removal of pollutant at per volume and per time and the efficiency of biomass energy transferring (Yu et al., 2013a). The COD removal is shown in Fig. 2 during the start-up of the reactor. In order to culture the seed sludge, the reactor run at the low organic load rate (OLR) in the start-up. The soybean protein wastewater was diluted using tap water. The influent COD concentration was approximately 2000 mg/L. After the acclimatization of the seed sludge in 10 days, the removal of total COD from the wastewater was 65% to 70% in the integrated two-phase anaerobic reactor. The concentration of the influent COD dropped from 1821 – 2104 mg/L to 1288 – 1560 mg/L when the wastewater flow past the reaction zone I. The efficiency of COD removal was about 25%–30%. At the same time, when the wastewater flow past the reaction zone II, the effluent COD drop to 550 – 772 mg/L.

During the initial stage, activity of the seed sludge was poor due to environmental change. And microbe needed to adapt to the new situation. Therefore, the COD concentration of the effluent was high, and the total removal efficiency was just about 56%. With the operation of the reactor, the activity of the sludge would be stronger, and the COD of the effluent of the soybean protein wastewater in the reaction zone I and II was about 1100 mg/L and 400 mg/L, respectively. The total COD removal efficiency was about 80% in the integrated two-phase anaerobic reactor.

The results showed that the soybean protein wastewater could be successfully processed by the integrated two-phase anaerobic reactor. The stirring equipment set in the reaction zone had a significant effect on the removal of COD.
zone I not only accelerated the mass transfer in the reactor, but also increased the hydrolysis acidification rate. It could enhance the treatment effect of the system by heating anaerobic reaction zone I and keeping the anaerobic reaction zone II in the mesotherm condition.

3.2. Gas production

The volumetric gas production rate was one of the sensitive indexes, which could directly reflect the reactor operation condition (Yu et al., 2013b). The change of gas production rate is shown in Fig. 3. During the 1–10 day, the reactor gas production rate was low. The gas production rate in the reaction zone I and II was 4 – 7 L/d and 6 – 10 L/d, respectively. The biogas production rate in the reactor was low, which was caused by the weak activity of the sludge during the 1–10 days. After 20 days, the gas production rate of the reaction I and II was steady at 11 L/d and 10 L/d, respectively. The main reason was considered as that the granule sludge became mature in the integrated two-phase anaerobic reactor and the space distribution of microbe tended to be rational in the reactor.

Biogas compositions of each zone in the integrated two-phase anaerobic reactor were monitored on the 30th day. There were mainly CO2 and H2 in the zone I (CO2 53%, H2 25%, methane 12%). Methane was the main component of all samples in the zone II which ranged from 53% to 59% with an average of 55%. The methane content in biogas increased from the zone I to II. It indicated that the methane-producing capacity of the anaerobic sludge increased.

It is well known that the process of anaerobic degrade organic matter can be divided into acid production phase and the methane production phase. Therefore, it was crucial to create the best environment conditions for hydrolysis acidification bacteria and methanogen bacteria in one reactor. The integrated two-phase anaerobic reactor can effectively separate acid production phase and methane production phase. The effective volume ratio between the two reaction zones was 1:2. And the HRT ratio between the two reaction zones was 1:2. The soybean protein wastewater was hydrolyzed and acidulated in the reaction zone I. A large amount of organism was decomposed into volatile fatty acid which could increase the substrate concentration for the methanogenesis process. At the same time, it is unlikely that a complete separation of phases (acidogenic and methanogenic) occurred in the integrated two-phase anaerobic reactor because biogas production was observed in the reaction zone I and II.

3.3. Biological features of the granular sludge

The sludge in the reactor was observed the fifth day and the thirtieth day using FISH by bacteria probe EUB338-CY3 and ancient bacteria probe ARC915-FITC in this study, as illustrated in Figs. 4 and 5. From the picture of the fifth day, fluorescence intensity was too weak to prove that the number of bacteria and ancient bacteria was small in the reaction zone I and II. The relative abundance of bacteria in the reaction zone I was 21.1% and the relative abundance of the ancient bacteria was 23.5%. The relative abundance of bacteria and ancient bacteria in the reaction zone II was 19.6% and 27.5%, respectively. Meanwhile, it was found that relative abundance of bacteria plus ancient bacteria was less than 50% in each reaction zone on the fifth day. This phenomenon indicated that the microbe’s activity was weak in the reactor because of the change in the environment. The seed sludge needed some time to adapt the new environment during the initial stage of the start-up operation.

At the later stage of the start-up operation, it was found that the number of the bacteria was more than that of the fifth day from the fluorescence intensity. From the FISH pictures hybridized by the ancient bacteria ARC915-FITC probe at the thirtieth day in the reaction, it was found that fluorescence intensity in the reaction zone II was stronger than that in the reaction zone I. The number of the ancient bacteria in the reaction zone II was more than that of the reaction zone I. It was because that methanogen could not use big molecule directly in the soybean protein wastewater but just the metabolin of the hydrolysis of acid producing bacteria. On the thirtieth day, the relative abundance of the bacteria and the ancient bacteria was 57.3% and 39.5% in the reaction zone I, respectively. And the relative abundance of the bacteria and the ancient bacteria was 32.4% and 56.5% in the reaction zone II, respectively. It was also found that the rate of the amount of the bacteria and the ancient bacteria was diffluent in the two reaction zones. The rate of the amount of the bacteria was bigger in the reaction zone I, while the rate of the amount of the ancient bacteria was bigger in the reaction zone II.

The unique structure of the reactor played an important role in the success of the start-up of treating the soybean protein wastewater in the integrated two-phase anaerobic reactor. The structure design of two zones could separate the process of hydrolysis acidification and methanogen, since acid-producing bacteria and methanogen had different requirements on the environment in the process of the anaerobic digestion (Mensah and Forster, 2003; Ren et al., 2002). The integrated two-phase anaerobic reactor had some advantages in the structure as follows.

Zone I in the reactor acted as a buffer zone in which the inhibitory material in the wastewater could have been degraded thus allowing the zone II to be loaded with a relatively harmless and mostly acidified influent. So that, there was much active populations of the relatively sensitive methanogenic bacteria in zone II and explained why the higher ethane yield was obtained in zone II. The acid-producing bacteria would be reproduced quickly in the reaction zone I and the activity of the ancient bacteria would be stronger in the reaction zone II. The integrated two-phase anaerobic reactor with the obvious phase separation feature could make acid-producing bacteria and methanogen to be rationally distributed during the anaerobic digestion processes. It was a benefit to form a well distribution condition of the microbe to start up the reactor. But it is unlikely that a complete separation of
phases (acidogenic and methanogenic) occurred in the integrated two-phase anaerobic reactor.

3.4. The activity of the sludge enzymes in the reactor

The enzyme secreted by the anaerobic microbe took part in different processes of digestion and methanation. The activities of enzymes, such as those of protease, TTC-dehydrogenase and coenzyme F420, have been extensively studied in anaerobic digestion. These activities play a significant role in the decomposition of substrates, thus affecting nutrients and nutrient availability (Whiteley et al., 2002; Aoki et al., 1995; Whitmore et al., 1986). The coenzyme F420 is widely distributed in methanogenic activity. Microbial enzyme activities are associated with microbial metabolism.

Figure 4  Fluorescence images of sludge samples in the reactor by EUB338 (×1000).

Figure 5  Fluorescence images of sludge samples in the reactor by ARCH915 (×1000).
After 20 days of the start-up operation, the activity of the sludge was detected every two days in the two reaction zones. It was found that the dehydrogenase activity was strong in the integrated two-phase anaerobic reactor, and the activity of the dehydrogenase in the reaction zone I and II was 131.4 – 142.3 μg/(g min) and 152.2 – 171.6 μg/(g min), respectively. The protease activity decreased longitudinally along the reactor from zone I to zone II. Then the activity of protease in the two reaction zones were 25.35 – 27.56 mol/(gvs min) and 17.31 – 23.12 mol/(g min), respectively. The protease activity demonstrated that hydrolysis and acidogenesis were the main biochemical reactions occurring in the zone I. And the hydrolysis acidification effect of the soybean protein wastewater was enhanced in the reaction zone I accelerating the process of the start-up operation.

The coenzyme F₄₂₀ in the reaction zone I and II was 0.35 – 0.42 μmol/g and 0.87 – 0.96 μmol/g, respectively. The coenzyme F₄₂₀ in the reaction zone II was twice than that in the reaction zone I, indicating a stronger activity of the methanogens in the reaction zone II. It indicated that the integrated two-phase anaerobic reactor separated the phases.

4. Conclusions

The main objectives of this study are to study treatment of soybean protein wastewater using the integrated two-phase anaerobic reactor and elucidate the microbial phase in the reactor during the experiments. Based on the data from the experiments, the following conclusions are drawn:

The soybean protein wastewater could be successfully processed around 30 days when running under the situation of dosing seed sludge with the influent approximately 2000 mg/L and an HRT of 40 h. When the start-up finished, the removal rate of COD by the reactor was about 80%. In the zone I, biogas mainly revealed carbon dioxide (CO₂) and hydrogen (H₂). Methane was the main component in the zone 2 which ranged from 53% to 59% with an average of 55%. The methane content in biogas increased from the zone I to II. It indicated that the methane-producing capacity of the anaerobic sludge increased.

There were some differences between the activities of the sludge in the two reaction zones of the integrated two-phase anaerobic reactor. The activity of protease was higher in the reaction zone I and the coenzyme F₄₂₀ in the reaction zone II was twice than that in the reaction zone I, which indicated that the activity of the methanogens was stronger in the reaction zone II. The integrated two-phase anaerobic reactor in this study was simple in structure avoiding several equipments of the traditional two-phase anaerobic system. It was observed to be an efficient reactor configuration for the treatment of the soybean protein wastewater.

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