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Treatment efficiency and characteristics of bacterial community structure of two-stage and two-phase anaerobic process

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ABSTRACT

The treatment efficiency of two-stage and two-phase (TSTP) anaerobic process composed of hydrolytic acidification reactor, first-order and second-order external circulation (EC) anaerobic reactors were investigated and the characteristics of microbiological population composition in different reactors were also studied through scanning electron microscope. Results showed that by seeding anaerobic granular sludge, controlling start-up volume loading rate and the type of loading rate increasing, hydrolytic acidification reactor quickly started up in 34 d and formed stable ethanol-type fermentation. Under the conditions of organic loading rate (OLR) = 46.78 kgCOD/m^3 d, hydraulic retention time (HRT) = 5.62 h, chemical oxygen demand (COD) removal rate of first-order EC reactor was 93.2%, effluent COD was 700 mg/L, and that of second-order EC reactor was 83.3% and 110 mg/L. Furthermore, TSTP process presented strong anti-shock loading capability. Dominant bacteria were different in different reactors. *Bacillus brevis* and Bacillus were main microbial population existed on mature acidizing granular sludge. *Methanosarcina barkeri* were dominant bacteria at the bottom of 1# reactor. *Methanosaeta* were dominant bacteria in the whole 2# reactor.

Keywords: Two-stage and two-phase anaerobic process; External circulation anaerobic reactor; Hydrolytic acidification reactor; Dominant bacteria; Anti-shock loading capability

1. Introduction

Anaerobic process is being widely used in high strength organic wastewater [1,2], hazardous industrial wastewater [3], livestock wastewater [4,5] and even low concentration or low temperature wastewater [6,7] treatment plants as its high treatment performance, well running stability, powerful anti-shock loading capability and low operating cost. However, there is an obvious defect of single phase anaerobic reactor when treating high-strength wastewater under rapid changes of water quality. The reactor is easy to present acidification phenomenon which leading to collapse. Two-phase anaerobic process and staged multi-phase anaerobic (SMPA) reactor system provides an effective method to solve this problem [8,9]. Phase separation technique makes most of the acid forming organisms and methane forming organisms exist in different reactors which are convenient to build up optimal habitat conditions individually to enhance the anti-shock loading capability of process and improve its treatment efficiency. SMPA reactor system approaches more plug

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flow conditions in the whole process and continuous stirred flow conditions for each reactor. It forces the process of anaerobic reaction occurring in different reactors or different independent space of one reactor which not only be convenient to build up optimal habitat conditions for different functional bacteria, but also be beneficial to reducing the impact of water quality fluctuating by "stage by stage" results in a high treatment efficiency and powerful stability. Based on it, some new processes emerged such as anaerobic baffled reactor (ABR) and upflow staged sludge bed (USSB) [10–12].

Controlling the fermentation type of acidogenic phase reactor of two-phase and SMPA process is another important method to keep anaerobic process avoiding acidification risk. Ethanol-type fermentation is considered as the optimal fermentation type, which not only provides suitable liquid terminal products such as ethanol and acetic acid which can be easily utilized by methane forming organisms but also avoids the propionic acid accumulation [13,14]. To achieve this optimal fermentation type, many impact factors are considered such as the configuration of reactor, the seed sludge, pH value, oxidation–reduction potential (ORP), etc. [15,16].

However, many studies on SMPA, two-phase and its optimal fermentation type have been carried out individually, but there have been few studies focused on the performance and stability of an anaerobic process combined by the processes stated as above. Two-stage and two-phase anaerobic process (TSTP) composed of hydrolytic acidification reactor, first-order and second-order external circulation (EC) anaerobic reactor is a new anaerobic treatment process based on the concept of two-phase and SMPA [17–19]. In this study, it was utilized to treat high concentration artificial wastewater and its treatment efficiency and characteristics of microbiological population composition was investigated.

2. Material and methods

2.1. Experimental system

High concentration artificial wastewater from regulation water tank was pumped into hydrolytic acidification reactor; first-order and second-order EC anaerobic reactor in sequence, as shown in Fig. 1. The effective volumes were 6 L, 7.85 L and 7.85 L. A submersible mixer was fixed at the bottom of hydrolytic acidification reactor.

EC reactor contained external circulation system, cyclone-flow water distribution system and multiple-stage three-phase separator. Each reactor was heated by heating wire which controlled by temperature controller at 32 ± 2 °C. The gas came from each reactor was calculated by wet-type gas flow meter.

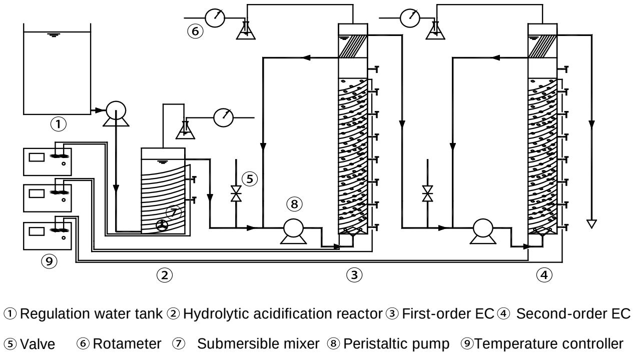

Fig. 1. Flow diagram of TSTP process.

2.2. The characteristics of wastewater

The artificial wastewater was made up of tap water, cane sugar, sodium acetate, NH_4Cl, KH_2PO_4 to keep COD:N:P = 200:5:1, and the COD concentration was controlled between 3000 and 15,000 mg/L. Microelement such as $FeCl_2{\bullet}4H_2O$, $CoCl_2{\bullet}6H_2O$, $NiCl_2{\bullet}6H_2O$ were added into water in proportion of $P:Fe^{2+} = 6:1$, $Fe^{2+}:Co^{2+}:Ni^{2+} = 10:1:2$. In addition, some $NaHCO_3$ was necessary to ensure the pH value was appropriate in reactor.

2.3. The seed sludge

Hydrolytic acidification reactor was seeded with 30 g/L of granular sludge obtained from sludge reservoir in brewery. The volatile suspended solid (VSS)/suspended solid (SS) was 0.52, the settlement ratio was 75–80%. Two-stage anaerobic reactors were seeded with 60 mg/L, 20 mg/L of granular sludge obtained from expanded granular sludge bed (EGSB) reactor in brewery. The VSS/SS was 0.63, and the settlement ratio was 85–90%.

2.4. Experimental design

Firstly, hydrolytic acidification reactor was started up with organic loading rate (OLR) of 10.45 kgCOD/m^3 d, corresponding to a hydraulic retention time (HRT) of 9.19 h. In this period, increased loading rate was mainly achieved by increasing feed concentration with the range about 7 kgCOD/m^3 d. When it formed stable ethanol-type fermentation it was considered that start-up process had been accomplished.

Then two-stage anaerobic system was start-up and combined with hydrolytic acidification reactor. This process operation period could be divided into four periods as showed in Table 1. Period 1 was sludge naturalization period. Other three periods were loading rate increasing period. The differences were that the inflow COD concentration was remained stable while HRT was decreased in periods 2 and 4. In period 3, HRT was remained stable while COD concentration was increased.

2.5. Analytical items and methods

COD, SS, VSS were determined according to Standard Methods [20]. Bicarbonate alkalinity and volatile acids were measured by the distillation method [21]. pH measurements were performed with a pH meter (pHS-3C, Leici, China). The liquid terminal products were analyzed by gas chromatography method [22]. The characters of microbiological population composition were investigated by scanning electron microscope (SEM).

3. Results and discussion

3.1. Treatment efficiency of hydrolytic acidification reactor

The start-up period of hydrolytic acidification reactor was 34 day. The content of ethanoic acid and ethanol increased obviously in liquid terminal products, and the mass concentration percentage was up to 75.9%.

In order to construct appropriate start-up conditions of two-stage anaerobic reactor [23], the OLR of hydrolytic acidification reactor was controlled at 10.4 kgCOD/m^3 d and the increased the loading rate stage by stage (Table 1). The changes of COD removal rate are shown in Fig. 2. The changes of effluent VFA are shown in Fig. 3.

In periods 1 and 2, the COD removal rate was stable as 18.1–19.2%. Then it increased obviously with increasing feed concentration and the maximum value reached to 26.9%. During the course of HRT decreased from 9.18 h to 6 h, although OLR increased to 58.7 kgCOD/m^3 d, the growth extent of COD removal rate was only 1%. When HRT decreased from 6 h to 4.28 h, COD removal rate declined to 25.4%. In the course of HRT decreased to 4 h, COD removal rate declined rapidly and the minimum value was 20.9%. Then HRT was adjusted to 5.4 h, COD removal rate restored gradually to 23.9%.

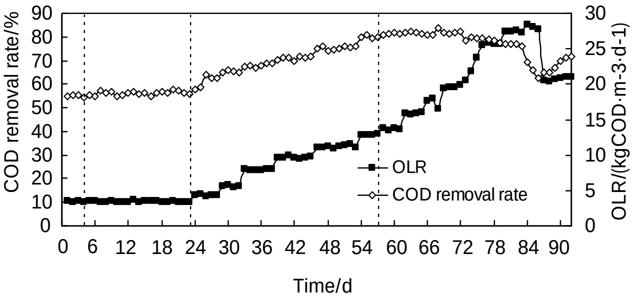

Fig. 2. Changes of COD removal rate of hydrolytic acidification reactor.

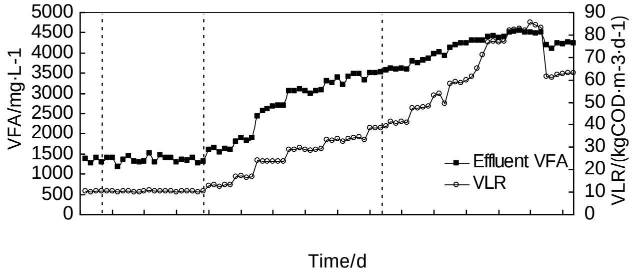

Fig. 3. Changes of effluent VFA of hydrolytic acidification reactor.

It was considered that the number of acidogenic fermentation bacterium was increasing with increasing feed concentration. Nutrient substrates were used for bacterium growth and metabolism [15]. In the prophase of period 4, although HRT continued decreasing, the contact time of sludge and nutrient substrates was adequate. The rising of OLR had less effect on COD removal rate. In the later period 4, declining HRT reduced the contact time of sludge and nutrient substrates. Some influent COD cannot be degraded completely and flowed out from the reactor along with the effluent. So in this period, COD Rrmoval rate dropped obviously. Furthermore, the pH value also declined

Table 1
Operation period of two-stage anaerobic reactor

Operation parameter	Period 1	Period 2	Period 3	Period 4
Time (d)	1–4	5–23	24–57	57–92
COD (mg/L)	3173–3280	3113–3407	3789–10,998	9989–10,981
OLR (kgCOD/m^3 d)	2.42–2.51	3.14–6.57	7.56–31.96	22.9–50.38
HRT (h)	31.4	12–31.4	12	5.2–12

and the minimum value was 3.89. To avoid the risk of over-acidification, moderate $NaHCO_3$ was added into inflow to keep appropriate alkalinity levels and HRT was adjusted to 5.4 h [24]. Then the COD removal ability recovered gradually. Hydrolytic acidification reactor presented well ability of resisting shock loading.

As can be seen from Fig. 3, effluent VFA increased with increasing feed concentration. The analysis of liquid terminal products showed that ethanol concentration exceeded ethanoic acid and became the most products when OLR reached to 33.2 kgCOD/m^3 d. At the end of period 3, the ratio of ethanol to ethanoic acid raised to 1.32:1. In period 4, although OLR increased rapidly, the growth extent of VFA turned to be gradual. This phenomenon can be interpreted as acidification bacterium producing more ethanol to keep appropriate pH levels [25].

3.2. *Treatment efficiency of two-stage anaerobic reactor*

The start-up method was controlling two-stage anaerobic reactor at high level loading and recirculation ratio. The first-order reactor (1# reactor) hydraulic upflow rate was controlled within 2.0 m/h, and that of second-order reactor (2# reactor) was 1.5 m/h. The changes of 1# and 2# reactors COD removal rate are shown in Fig. 4.

Although COD removal rate declined in every prophase of loading-raised, it increased rapidly in a short time with increasing number of methanogens bacterium and keeping appropriate alkalinity levels. At the end of period 2, 1# reactor OLR arrived to 6.38 kgCOD/m^3 d and the COD removal rate was up to 92.3%. The effluent COD concentration was 24 6mg/L. In the course of OLR increasing to 21.96 kgCOD/m^3 d, COD removal rate declined lightly, and the average value was around 85.9%. The reason can be interpreted as flocculent and small granular sludge being washed out of reactor which leading VSS continued to decrease [26].

In later period 3, hydraulic upflow rate was controlled within 2.5 m/h. Although influent COD reached up to 11,000 mg/L, COD removal rate increased further and the maximum value was 96.7%. The effluent COD concentration was 309 mg/L.

When HRT declined from 11.21 h to 7.13 h, effluent COD concentration was 752 mg/L and COD removal rate maintained at a high level as 93%. In this stage, the gas production was high and accumulated within the sludge bed without released in time which leaded to sectionalization of sludge bed [27]. To avoid it, hydraulic upflow rate was controlled within 3.5 m/h.

As HRT fell to 5.62 h, OLR reached up to 46.78 kgCOD/m^3 d and hydraulic upflow rate was controlled 4.5 m/h. With well hydraulic mixing, easily degradable influent and high activity of granular sludge, 1# reactor presented high removal ability under the condition of high loading and low HRT levels. Effluent COD concentration was around 700 mg/L and COD removal rate maintained at a high level as 93.2%.

Further reducing of HRT (to 5.23 h) bringing about abnormal operation of hydrolytic acidification reactor which caused a large quantity of unacidified substrates inflow to 1# reactor rapidly, which led to rapid proliferation of acidogenic fermentation bacteria on granular sludge. The accumulation of organic acids lowered the pH value quickly which inhibited the activity of methanogenic bacteria. Effluent COD concentration rose up to 4028 mg/L and the removal rate declined to 61.7%. To relieve to effect of shock loading, moderate $NaHCO_3$ was added into recirculation water to keep appropriate alkalinity levels and HRT was adjusted [26]. After 6 d, COD removal rate recovered to 86.9% gradually with high effluent COD concentration as 1430 mg/L (Fig. 5).

Due to low inflow substrates, granular sludge adapted to the water quality and the COD removal rate rose up to 80.3%. In period 2, inflow COD concentration maintained within 330–240 mg/L. As running at low loading level, granular sludge begun to disintegrate from the 7th day. There were a lot of flocculent sludge in recirculation water and effluent [28]. As OLR rising to 1.78 kgCOD/m^3 d, although water quality fluctuated strongly, COD removal rate rose up to 88.5% with improvement

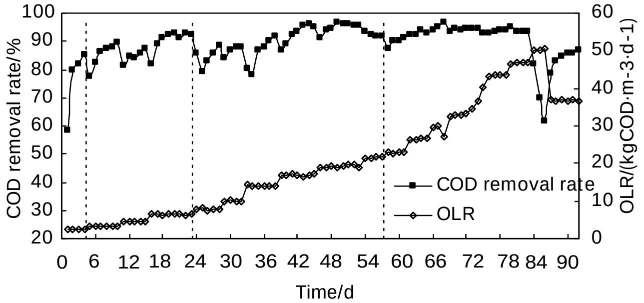

Fig. 4. Changes of COD removal rate in 1# reactor with OLR

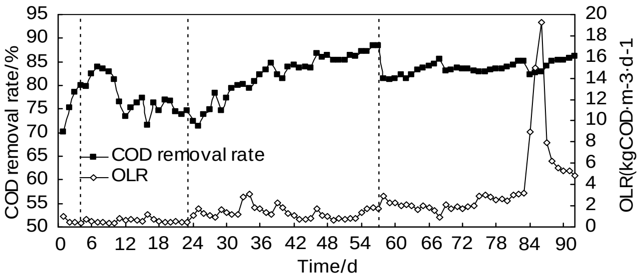

Fig. 5. Changes of COD removal rate of 2# reactor with OLR.

of sludge activity and well mass transfer efficiency by adjusting hydraulic upflow rate (1.5–2.5 m/h).

In period 4, hydraulic upflow rate was adjusted to 3.2 m/h. From the 63th day, the interface between sludge and water became clear. In this period, 2# reactor had experienced steady loading-raised, shock loading and recovery stage. In steady loading-raised stage, because of high treatment efficiency of 1# reactor, the 2# reactor OLR maintained within 2.52 kgCOD/m^3 d and COD removal rate was around 83%. It presented well resisting ability during shock loading. Although OLR rose up to 19.29 kgCOD/m^3 d rapidly, the removal rate maintained 82%. Along with loading decreased, COD removal rate arrived to 86% and the effluent COD concentration kept below 200 mg/L.

3.3. Changes of pH value in TSTP

The changes of pH value in TSTP process in different stages is shown in Fig. 6.

Inflow pH value fluctuation of hydrolytic acidification reactor had less effect on effluent. It maintained within 4.0–4.6 which was beneficial to stable operation of two-stage anaerobic reactors. The pH value of 1# reactor was within 6.51–6.72. It was mainly due to the high concentration of inflow VFA [25]. Furthermore, the ethanoic acid produced by hydrogen-producing acetogenic microflora existed on granular sludge when they metabolized ethanol also lowered the pH value. As most of VFA had been degraded in 1# reactor, the influent substrates of 2# reactor mainly included undergraded cane sugar and organic acid. Under the conditions of low concentration, these substrates can be converted to methane and discharged out of reactor quickly. According to this reason, the pH value of 2# reactor was a little higher than 1# reactor within 7.03–7.21.

3.4. Characters of microbiological population composition in TSTP

S SEM was used to investigate the characters of microbiological population composition in TSTP at different stages.

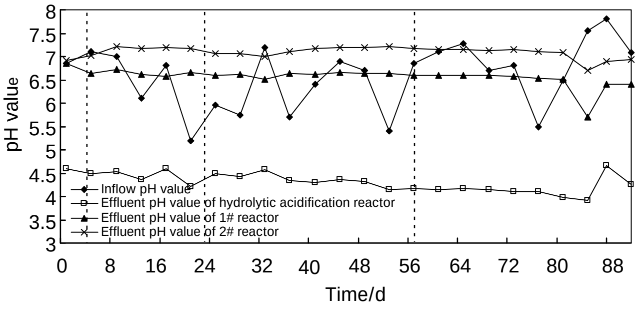

Fig. 6. Changes of effluent pH value in TSTP.

Bacillus brevis and Bacillus were main microbial population existed on mature acidizing granular sludge after 40 days running (Fig. 7). The bacteria grew closely on the surface and interior of granular sludge and intertwined with each other. This construction was beneficial to the well mechanical strength and settle ability. The surface of granular sludge was smooth which probably caused by EPS of bacteria [29].

Methanosarcina barkeri wass the dominant bacteria on granular sludge at the bottom of 1# reactor (Fig. 8). The distribution range of *Methanosaeta* was small and they existed internally presented filamentous. *Methanosarcina barkeri* was also the dominant bacteria on granular sludge at the top. The distribution range of Methanosaeta became larger. *Methanosarcina barkeri* and *Methanosaeta* intertwined with each other on the surface and interior of granular sludge.

These phenomena probably related to the inflow pH levels and substrates variety. The ethanol, ethanoic acid and butanoic acid were main inflow substrates and accumulated at the bottom which leaded to the low pH levels

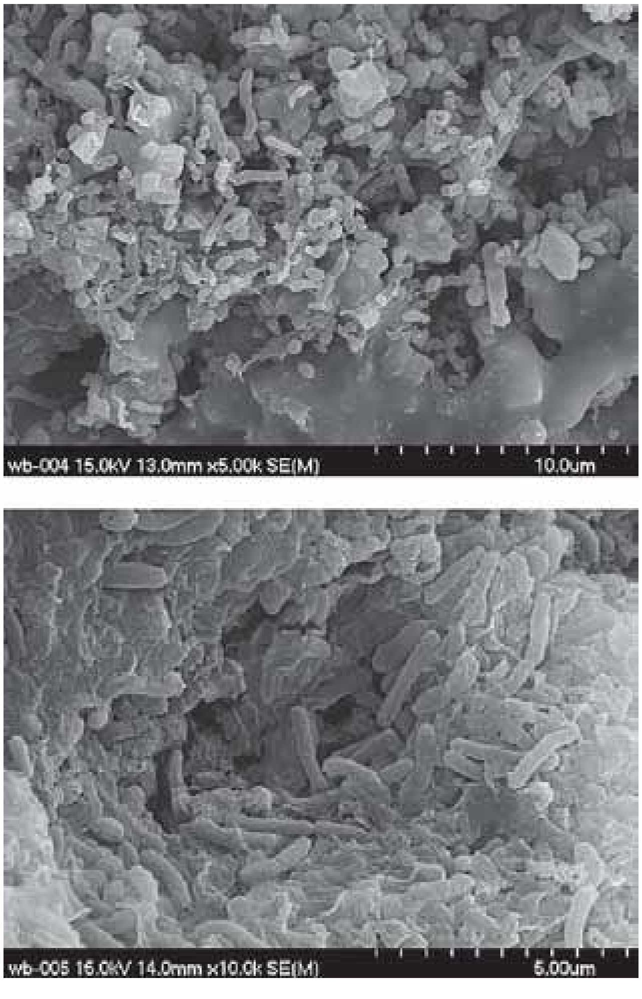

Fig. 7. Microbial phase of mature acidizing granular sludge.

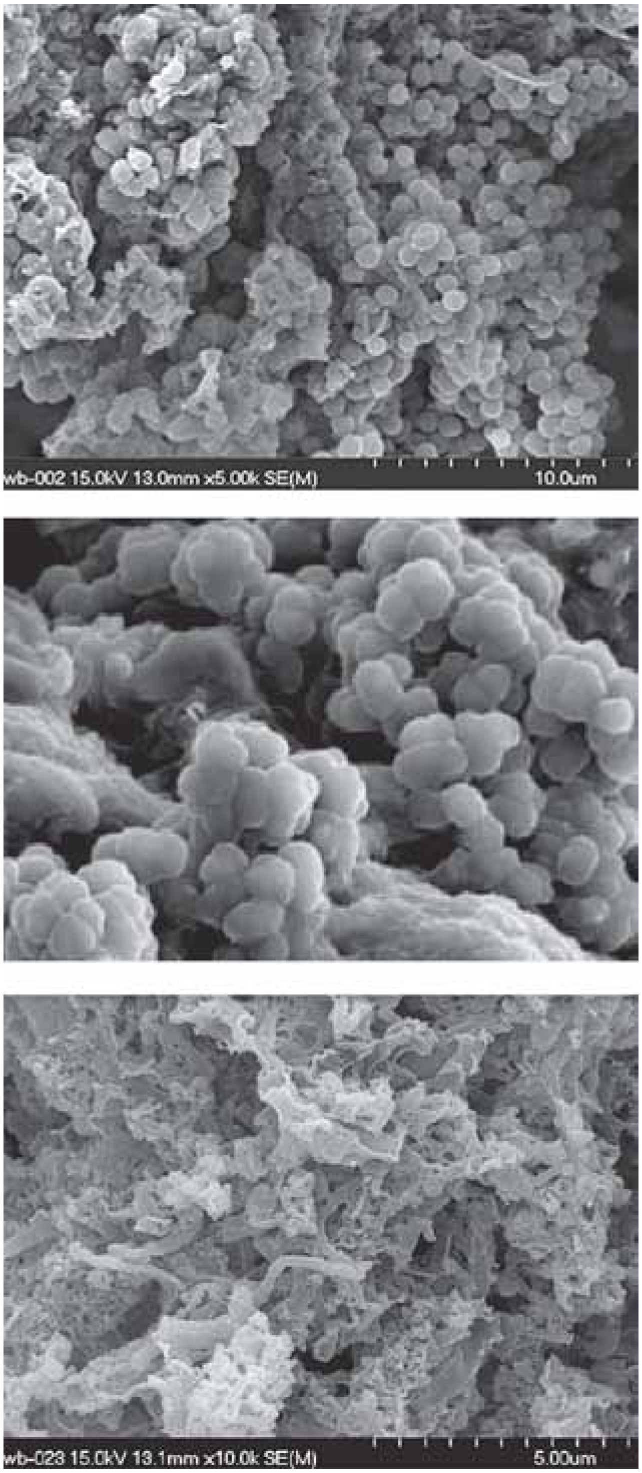

Fig. 8. Methanogen of granular sludge in 1# reactor.

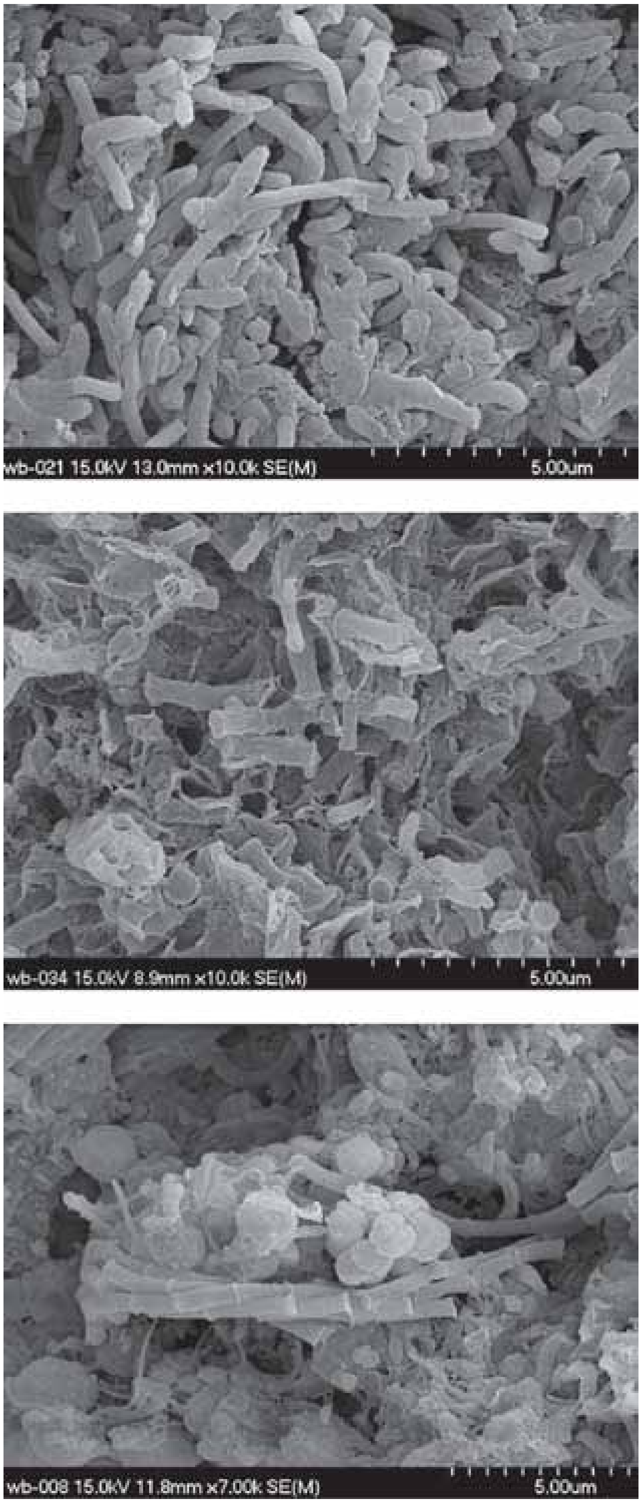

Fig. 9. Methanogen of granular sludge in 2# reactor.

of 1# reactor. In this situation, *Methanosarcina* barker with high acid resistance turned into dominant bacteria easily than others [30]. Along the height direction of 1# reactor, the ethanoic acid was degraded rapidly and the VFA concentration declined. *Methanosaeta* adapted to low ethanoic acid concentration condition multiplied rapidly which leaded to coexistence on the top.

Methanosaeta was the dominant bacteria on granular sludge in the whole 2# reactor (Fig. 9). The quantity of *Methanosarcina barkeri* became less and they mainly existed on the surface. The reason of these phenomena was that the activity of *Methanosarcina barkeri* was badly restrained under low ethanoic acid levels. And the effect of inhibition turned more obvious when nearer to the core of granular sludge. *Methanosaeta* with long hypha and large specific surface area had stronger affinity with substrates than others under low concentration level [26].

4. Conclusions

TSTP presented well treatment performance and ability of resisting shock loading. It probably related to several factors as follows.

Firstly, granular sludge selected as seed sludge provided plenty of high activity functional bacteria and natural carriers. Although some of them disintegrated during start-up period, new granular sludge re-formed in a short time. Abundant granular sludge existed in reactor guaranteed the well efficiency under high loading and low HRT.

Secondly, appropriate hydraulic upflow rate of two-stage anaerobic reactors was selected. On the one hand, when inflow substrates concentration was high, it provided well hydraulic dilution which avoided local acidification risk caused by VFA accumulating at inlet position. On the other hand, when inflow substrates concentration was low, appropriate hydraulic upflow rate promoted the touch between water substrates and sludge and alleviated the mass transfer limitation. Furthermore, high hydraulic upflow rate washed out flocculent and dead sludge which was beneficial to re-form granular sludge.

Thirdly, hydrolytic acidification reactor was start-up before two-stage anaerobic reactors and formed stable ethanol type fermentation.

The main production of hydrolytic acidification reactor was micromolecule organic matters such as ethanol, ethanoic acid and butanoic acid which can be degraded by methanogen to carry out methanogenesis.

Finally, series operation of each reactor in TSTP made the strength of shock loading weaken step by step when shock loading occurred. In addition, single-control mode of each reactor was beneficial to adjust operating parameters in time. So the treatment ability of TSTP recovered in a short time.

In addition, dominant bacteria were different in each reactor of TSTP. *Bacillus brevis* and Bacillus were main microbial population existed on mature acidizing granular sludge and they grew closely on the surface and interior of granular sludge. *Methanosarcina barkeri* were the dominant bacteria on granular sludge at the bottom of 1# reactor. *Methanosaeta* were the dominant bacteria on granular sludge in the whole 2# reactor.

Acknowledgements

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References

[1] E.K. Hala, T. Ahmed, M. Mohamed and A.H. Hisham, Treatment of high strength wastewater from fruit juice industry using integrated anaerobic/aerobic system. Desalination, 253 (2009) 158–163.
[2] R. Sowmeyan and G. Swaminathanb, Evaluation of inverse anaerobic fluidized bed reactor for treating high strength organic wastewater. Bio. Technol., 99 (2008) 3877–3880.
[3] D. Li and D.W. Li, Removal efficiency of hazardous material in cyclohexanone production wastewater applying anaerobic upflow bed filter. Adv. Mater. Res., 160–162 (2010) 1440–1444.
[4] S.M. Oh, M.H. Kim, Y.S. Bae, S.M. Hong and C.H. Park, Estimation of biodegradability and biogas recovery from a two-phase anaerobic process treating piggery wastewater. Desal. Water Treatment, 2 (2009) 30–38.
[5] Y.M. Wu, J.B. Wang, J.H. Xiong, S.F. Wang and P. Gu, Investigation of a full-scale anaerobic–aerobic and constructed wetlands integrated system treating livestock wastewater. Adv. Mater. Res., 113–116 (2010) 1189–1194.
[6] Y.Z. Lin, X.K. Han, J. Yin, P.H. Shao and S.S. Ai, Modified two-phase anaerobic baffled process for low-concentration wastewater treatment. J. Harbin Inst. Technol., 3 (2009) 326–330.
[7] Q.X. Li, S.Y. Liu, L.H. Li and D.Y. Wang, Effect of anaerobic baffled reactor treating domestic sewage under low temperature condition. China Water Wastewater, 24 (2008) 72–74.
[8] F.G. Pohland and S. Ghosh, Developments in anaerobic stabilization of organic wastes: the two-phase concept. Environ. Lett., 1 (1971) 255–266.
[9] G. Lettinga, J. Field, J. van Lier, G. Zeeman and L.W.H. Pol, Advanced anaerobic wastewater treatment in the near future. Water Sci. Technol., 35 (1997) 5–12.
[10] A. Bachmann, V.L. Beard and P.L. McCarty, Performance characteristics of the anaerobic baffled reactor. Water Res., 19 (1985) 99–106.
[11] J.B. Van Lier, F.P. Van Der Zee, N.C. Tan,S. Rebac and R. Kleerebezem, Advances in high-rate anaerobic treatment: staging of reactor systems. Water Sci. Technol., 44 (2001) 15–25.
[12] J.B. Van Lier, F. Boersma, M.M.W.H. Debets and G. Lettinga, High rate themophilic anaerobic wastewater treatment in compartmentalized upflow reactors. Water Sci. Technol., 30 (1994) 251–261.
[13] F. Ma, A.L. Dai, N.Q. Ren, L. Zeng and Y.S. Cui, Optimal fermentation type of the acidogenic phase. J. Harbin University Architec. Eng., 28 (1995) 63–68.
[14] J.Z. Li and N.Q. Ren, The operational controlling strategy about the optimal fermentation type of acidogenic phase. China Environ. Sci., 15(1998) 398–402.
[15] J.Z. Li, N.Q. Ren, and S. Guo, Rapid start-up of acidogenic reactor and acclimatization of dominant ethanol-type fermentation population. J. Harbin Inst. Technol., 34 (2005) 591–594.
[16] D. Zhao, N.Q. Ren and A.J. Wang, Fermentation type and climax community controlled by pH and ORP. Chongqing Environ. Sci., 25 (2003) 31–34.
[17] H.J. Han, W. Wang, W.C. Ma, M. Yuan and H.Q. Li, Treatment of Lurgi coal gasification wastewater in external circulation anaerobic process. J. Harbin Inst.Technol. (New Series), 42 (2010) 907–909, 924.

[18] H.J. Han and W.C. Ma, Treatment of methanol wastewater with two-stage and two-phase anaerobic process. J. Harbin Inst. Technol. (New Series), 17 (2010) 65–69.

[19] H.J. Han, W. Wang, K. Ao and S. Lin, Quick start-up of external circulation (EC) anaerobic reactor. J. Harbin Insti. Technol., 42 (2010) 580–583.

[20] F.S. Wei, W.Q. Qi, Z.G. Sun, Y.R. Huang and Y.W. Shen, Water and Wastewater Monitoring and Analysis Method, China Environmental Science Press, Beijing, 2002, 4th ed.

[21] G.K. Anderson and G. Yang, Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. Water Environ. Res., 64 (1992) 53–59.

[22] Y.L. Liu, N.Q. Ren, M. Liu, A.J. Wang, J.Z. Li and Y.N. Wu, Analysis of volatile fatty acid (VFA) in anaerobic bio-reactor by gas chromatography. J. Harbin University C.E. Architec., 33 (2000) 31–34.

[23] N.Q. Ren, B.Z. Wang and J.C. Huang, Ethanol-type fermentation from carbohydrate in high rate acidogenic reactor. Biotechnol. and Bioeng., 5 (1997) 428–433.

[24] Z. Qin, N.Q. Ren, J.Z. Li and X.F. Yan, Superacid state of acidogenic phase and controlling strategy for recovery. J. Harbin Inst. Technol., 35 (2003) 1105–1108.

[25] Y.L. Liu, Substrate convertion and community succession in two anaerobic bio-treatment system [D]. Harbin Inst. Technol., 2001.

[26] J.C. Hu, Anaerobic Wastewater Treatment Theory and Techniques. China Architecture & Building Press, 2003.

[27] H.J. Han and C.Y. Xu, Treatment of brewery waste water with UASB anaerobic reactors. J. Harbin Inst. Technol., 36 (2004) 440–442.

[28] Y. Hu, L.S. Shang, Y.H. Liu and C.H. Yu, Sludge wash-out from anaerobic granular sludge bed reactor and countermeasure thereof. Indus. Water Wastewater, 38 (2007) 6–8.

[29] H. Yang, Study on anaerobic acidogenic sludge granulation in UASB reactor, J. Shanghai Jiaotong Univ., 29 (1995) 165–169.

[30] X.F. Zhou and N.Q. Ren, Acid resistance of methanogenic bacteria in two-stage anaerobic process treating high concentration methanol wastewater. Acta Scientiae Circumstantiae, 24 (2004) 633–636.