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Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Rapid startup of a hybrid UASB-AFF reactor using bi-circulation

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article info

Article history: Received 12 April 2009 Received in revised form 6 August 2009 Accepted 10 August 2009

Keywords: Anaerobic Startup Circulation Granular sludge UASB

1. Introduction

Among the various methods used to treat wastewater from industrial processes that contain high concentrations of organic substances, anaerobic digestion has been found to be a promising technology [\[1,2\]. T](#page-4-0)he UASB (upflow anaerobic sludge blanket) reactor is considered to be one of the most representative anaerobic digesters due to its high biomass concentration and rich microbial diversity. Indeed, UASB reactors have been used for the treatment of various industrial and municipal effluents. Compared with other anaerobic technologies, the UASB reactor is highly dependent on the granulation of sludge [\[3,4\]. B](#page-5-0)ecause the development of the anaerobic granular sludge generally requires 2–8 months, an extremely long period is necessary for startup of the UASB [\[5\]. E](#page-5-0)ven though it has been demonstrated that startup can be accelerated through the addition of inert materials or inorganic precipitates or inoculation with sludge from other reactors [\[6–8\],](#page-5-0) the startup of UASB reactors using these methods is still time consuming, which has limited their wider application.

Biomass distribution is another important factor that affects the function of UASB reactors. Theoretically, anaerobic sludge is suspended in the reactor due to the hydraulic impact and the biogas lift. However, sludge is often found to be deposited in the bottom of the reactor. This low mass transfer between sludge and substrates likely leads to a decline in treatment efficiency. The EGSB (expanded granular sludge blanket) reactor is a modified UASB reactor that overcomes this poor sludge distribution. Specifically, the ESGB

ABSTRACT

The startup of a hybrid system consisting of an upflow anaerobic sludge blanket (UASB) and an anaerobic fixing filter (AFF) with internal hydraulic circulation and external sludge circulation was investigated. The reactor was rapidly cultivated using municipal sludge as a seed 38 d after a failed startup. During the operation, the average size of the granular sludge increased from 111 μ m to 264 μ m, and the sludge was uniformly distributed in the reacting region. Efficient performance was attributed to good hydraulic contact between the substrate and sludge and the low loss of sludge. However, excessive hydraulic circulation resulted in a sharp decline in the effectiveness. After a pause in the operation, a second startup was rapidly completed in 15 d, during which time the organic load reached 15.4 COD/(m^3 d).

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employs external hydraulic circulation of the drainage water [\[9\],](#page-5-0) which also dilutes the waste at the point of entry to the reactor. As a result, the EGSB reactor performs better than the conventional UASB reactor. However, the circulation that occurs in the entire EGSB reactor, including the three-phase (gas/water/sludge) separator, increases the hydraulic load of the separator, which results in poor separation of the biogas and sludge from the effluent. The internal circulation reactor (IC) is another anaerobic reactor with high efficiency that consists of two layers of UASB. The hydraulic circulation between the two layers of UASB [\[10\]](#page-5-0) results in an increased hydraulic rate. Similar to the EGSB reactor, the hydraulic circulation in an IC reactor increases the burden of the separator. Additionally, the IC reactor is quite complicated, and it is difficult to control the level of the hydraulic circulation in such reactor. A high burden on the separator in IC (and EGSB) reactors results in a great deal of sludge being washed out, therefore, sludge deposit tanks with a large volume are often employed to recycle the sludge. As a result, a considerable number of obligate anaerobic bacteria such as methanogens are exposed to the air during the process, which likely leads to decreased activity.

In this study, an anaerobic reactor in which hydraulic circulation was conducted in part of the UASB reactor and sludge circulation was employed outside the UASB reactor was designed to promote the mass transfer that occurs between the sludge and substrates and the loss of the sludge that occurs in the aforementioned reactors. Because anaerobic bio-films that intercept and bind microorganisms required for anaerobic treatment would enhance the process [\[11\], a](#page-5-0)n AFF (anaerobic fixing filter) was settled into the reactor to create a hybrid UASB-AFF reactor. For the present study, excessmunicipal excess sludge was used as the sludge seed because it is easy to obtain. The goal of this study was to investigate a new

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^{1385-8947/\$ –} see front matter © 2009 Elsevier B.V. All rights reserved. doi:[10.1016/j.cej.2009.08.005](dx.doi.org/10.1016/j.cej.2009.08.005)

Fig. 1. Schematic diagram of the experimental system.

method for rapid startup of the anaerobic reactor. To accomplish this, the startup procedure, characteristics of the sludge and effects of circulation in the reactor developed here were investigated.

2. Materials and methods

2.1. Experimental reactor

The reactor (ω 80 \times 1200) was composed of two layers of organic glass (Fig. 1) with an interlayer that was used to control the temperature by circulation of water with a temperature of 35 ± 1 °C. From bottom to top, the reactor was composed of an 800 mm region of suspended microorganisms, a bio-filter layer with a height of 200 mm and a three-phase separator with a height of 200 mm. In the suspended region, which was the primary area for anaerobic digestion, the waste was recycled from the top to the bottom using a peristaltic pump. The hydraulic circulation flow was controlled using a valve. In the bio-filter layer (φ 80 \times 200), hollow cylindrical bio-carriers composed of PE (φ 10 × 10) were loaded to intercept sludge that escaped from the suspended region. A three-phase separator was used to separate the residual sludge and biogas from the effluent. The effluent then entered a sedimentation tank for further separation of sludge. Sediments from the bottom of the sedimentation tank were pumped back to the reactor to decrease the loss of sludge.

During the initial startup, the reactor was operated in batch mode with a reaction time of 24 h, which meant that the water in the reactor was completely replaced every 24 h. After 10 d of operation in batch mode, the reactor was operated under continuous mode with a hydraulic retention time (HRT) of 24 h and a feed rate of 250 mL/h. The hydraulic circulation and sludge circulation were suspended when the reactor was operated in batch mode and during the initial continuous mode.

2.2. Materials

Wastewater used in this study was collected from the extrusion process of a fruit juice factory. The pH of the original sample was approximately 5–6, while the SS concentration was about 1200 mg/L. After deposition for 30 min, the supernatant, which had a COD ranging from 8000–12,000 mg/L, was used to feed the reactor. NaHCO₃ was used to adjust the feed water to $pH 8-9$ to prevent acidification in the reactor. To reduce the organic load impact on microorganisms during the startup period, the wastewater was diluted as the feed. The influent concentration generally increased to that of the raw wastewater as the reactor stabilized.

Sludge taken from a sludge thickening tank at Chunliu municipal sewage plant in Dalian, northeast China, was used as the seed sludge. The sludge volume index (SVI) was 28 mL/g and the ratio of volatile suspended substances to total suspended substances (VSS/TSS) in the sludge was 0.53 after removal of the debris with a sieve. The specific methanogenic activity (SMA) in the reactor was 25.4 mL/(g d), while the TSS was 38 g/L.

2.3. Analysis

COD, BOD, TSS, VSS and alkalinity were determined in accordance with the Standard Methods [\[12\]. V](#page-5-0)olatile fatty acids (VFAs) were evaluated using a HPLC (Waters 2695) equipped with an ultraviolet (210 nm) detector [\[13\]. T](#page-5-0)he composition of biogas was measured using a GC (Shimadzu, Japan) equipped with a thermal conductivity detector (TCD) [\[14\]. A](#page-5-0)n acidity analyzer (Sartorius PB-20) was used to record the pH, while the size of the granulation sludge was measured using a granularity analyzer (SA-CP3) and the volume of the biogas was determined using a wet gas flowmeter. The surface morphology of the catalyst was observed by scanning electron microscopy (SEM) using an Oxford JSM-5600LV microscope with an accelerating voltage of 20 kV.

Fig. 2. Startup of the reactor without the circulation. (A) Changes of pH, (B) removal of COD (The reactor was operated at HRT of 24 h. Prior to this experiment, the reactor was operated in a batch mode for 10 d.).

Fig. 3. Startup of the reactor with the circulation. (A) Removal of COD, (B) changes of pH, (C) Biogas production rate (The reactor was at HRT of 24 h, hydraulic recirculation ratio of 8:1, and sludge recirculation of 100%.).

3. Results and discussion

3.1. Startup without circulation

After inoculation with municipal sludge, the reactor was initially operated in batch mode with an influent COD of approximately 2000 mg/L. During operation in batch mode, waste in the reactor was displaced every 24 h. After 10 d of operation in batch mode, the removal of COD reached 48% and the VFA in the reactor reached 480 mg/L (data not shown). The operation was then transferred to continuous mode. The changes in the COD and pH before and after treatment during continuous operation are displayed in [Fig. 2. T](#page-1-0)he highest removal of COD observed was 60% at 28 d. At this time, the pH in the effluent decreased to 5.0, indicating that organic acids had accumulated. The influent COD was increased to 3000 mg/L after 30 d, which resulted in an obvious increase in the effluent COD.

In this experiment, the anaerobic sludge was found to be deposited in the bottom of the reactor. As a result, the sludge was not suspended in the reacting region, implying that not all of the space in the reactor was used to degrade the substrates. This was ascribed to low rates of hydraulic flow and biogas ascension. Specifically, the low biogas production during startup prevented effective

Fig. 4. Characterization of the granular sludge during domestication. (A) Changes of the granule size, (B) SEM of sludge granule.

lifting of sludge, which led to a low mass transfer, slow startup and low level of treatment.

3.2. Startup with circulation

After the startup failed, hydraulic circulation and sludge circulation were employed. Immediately upon the initiation of hydraulic circulation, the deposited sludge in the bottom was divided into two layers with a piston-like gas column sandwiched between the layers. This phenomenon revealed that biogas including $CO₂$ and $CH₄$ was trapped in the bottom of the reactor in the absence of the circulation. This likely resulted in saturated $CO₂$ conditions and a consequential decrease in pH in the reactor, which in turn harmed the anaerobic treatment process. During this time, the VFA increased to 1580 mg/L and the pH reached 5.5. It is interesting to note that the pH of water saturated with $CO₂$ is 5.6. After approximately 1 h, the two layers gradually disappeared, after which the gas was released from the column and the sludge was uniformly distributed throughout the entire suspended (reacting) region. Because the hydraulic circulation was only conducted in the suspended region, the hydraulic impact on the three-phase separator wasminor when the biogas production rate was low. In addition, the upper bio-filter intercepted some sludge. Thereafter, the influent concentration of COD increased gradually to the concentration of the raw waste. The effects of hydraulic circulation are shown in [Fig. 3.](#page-2-0)

The pH in the reactor ascended dramatically from 5.5 to 7.8 immediately after 1 d of hydraulic circulation. This may have occurred in part due to the release of supersaturated $CO₂$ from the reactor. In addition, the hydraulic circulation enabled microorganisms to function in a greater space, which counteracted the effects of acidification. As a result, a stable removal of COD was maintained, even as the influent COD increased. After a week of the circulation, the removal of COD reached 70% when the influent COD was 4000 mg/L. The effluent pH was maintained between 6.5 and 7.5, and gas production was gradually enhanced. When the influent COD increased from 3000 mg/L to 10,300 mg/L over 38 d, the effluent COD was approximately 1100 mg/L, which was equivalent to a COD removal rate of 89.3% under an influent organic load of 10 kg COD/ $(m^3 d)$. The biogas production rate reached approximately 1250 mL/h, which was equivalent to 0.65 m³ gas/(kg COD). The methane concentration in the biogas was about 60% at the end of the startup. Taken together, these results indicated that rapid startup of the reactor was accomplished when circulation was employed.

3.3. Characterization of sludge

The granularity distribution and SEM image of the sludge are displayed in [Fig. 4.](#page-2-0) The initial average size of the sludge granules in the reactor was $111 \mu m$. As the reaction proceeded, the size of the sludge granules increased gradually. At the end of the startup period, the average size of the granules at heights of 100 mm and 600 mm in the reactor was 264 μ m and 249 μ m, respectively ([Fig. 4A](#page-2-0)). In addition, the concentrations of acetic acid, propionic acid and butyric acid during this period were 608.4 mg/L, 117 mg/L and 54.6 mg/L, respectively, indicating that 78% of the VFA was acetic acid. It is well known that Methanothrix and Methanosarcina are the primary microorganisms responsible for the degradation of acetic acid to methane. Additionally, it has been suggested that these bacteria, especially filamentous Methanothrix, are important to granule formation [\[15\]. I](#page-5-0)ndeed, Dubourgier [\[16\]](#page-5-0) suggested that the granulation process started as a result of the bridging of microflocs by Methanothrix filaments, after which other bacteria and acidogenic bacteria colonize the flocs to form sludge with increasing granule sizes. Similarly, Bochem [\[17\]](#page-5-0) believed

Fig. 5. Influence of the hydraulic circulation ratio on the removal of COD (operation at HRT of 24 h).

that Methanosarcina was capable of producing clumps of bacteria that could reach macroscopic dimensions and contain cavities, which could be inhabited by other species [\[18\]. B](#page-5-0)ased on the SEM image ([Fig. 4B\)](#page-2-0), some filamentous bacteria intertwined with other microorganisms formed the sludge produced in this study. In addition, some of the sludge granules had deep holes visible on the surface. Hulshoff [\[18\]](#page-5-0) also suggested that compact Methanothrix granules were formed by colonization of the central cavities of Methanosarcina clumps by Methanothrix bacteria. The morphology of the sludge produced in the present study was in accordance with the characteristics of granulation described by these authors. The results of this study indicated that granulation sludge was formed. Although the size of this sludge was less than that of granulation sludge formed in other studies [\[19\]](#page-5-0) due to the short cultivation time, the reactor had a high removal efficiency of COD.

3.4. The effect of the hydraulic circulation ratio

During water treatment, the substrate is first transferred from the bulk liquid via the diffusion to the surface of the granule (i.e., external mass transfer), followed by successive intra-granule mass transfer and biochemical reaction within the granule [\[20\]. A](#page-5-0)ccordingly, low external mass transfer due to low hydraulic load in the feed would limit the performance of the treatment, including microbial growth and biodegradation. In the present study, hydraulic circulation enhanced the mass transfer between the substrate and sludge, thereby accelerating the startup procedure. To more clearly demonstrate the effect of the hydraulic circulation, the system was operated under various circulation ratios (defined as the ratio of circulation flow to feed flow, R). The results obtained when the system was operated under various circulation ratios are shown in Fig. 5. The removal of COD was maintained at about 85% when the R varied from 8 to 19 and the influent COD was approximately 10,000 mg/L. These results indicated that the removal of COD did not increase as the hydraulic circulation increased. Furthermore, a decrease in COD removal was observed when the R increased from 19 to 28. During this time, the effluent COD increased sharply from 1401 mg/L to 4263 mg/L, while the pH decreased from 6.8 to 5.5 and the biogas production rate decreased from 710 mL/h to 52 mL/h. These results suggest that excessive hydraulic circulation enhanced the mutual collisions among sludge granules, resulting in fracturing of the granular sludge. These findings are supported by a decrease in the diameter of the sludge

Fig. 6. Secondary startup experiment. (A) Changes of COD. (B) Changes of VTA and biogas (HRT of 24 h and the hydraulic circulation ratio of 8).

granules from 0.25 mm to 0.15 mm that was observed when the system was operated at an R of 28 (data not shown). In the structural model of granulation sludge provided by Mcleod [\[21\],](#page-5-0) Methanothrix was the core of the sludge granules, while acetate producers and fermentative bacteria formed the exterior layer around the core. Based on this model, fracture of the sludge in the reactor developed here likely led to the exposure of Methanothrix to an acidic environment, which led to a decrease in COD removal.

3.5. Secondary startup of the reactor

In practical applications, the operation of anaerobic reactors occasionally needs to pause for a variety of reasons. During such pauses, anaerobic bacteria become inactive and dormant. Therefore, if the conditions of the reactor (temperature, pH, etc.) are not properly controlled during such pauses, the system is likely to deteriorate. As a result, low treatment efficiency and acidification are often observed when a reactor is restarted after paused operation, which necessitates a secondary startup to restore performance. In such cases, the performance of the secondary startup is an important indicator of the overall performance of the anaerobic reactor.

The results of a secondary startup are shown in Fig. 6. The operation of the reactor was suspended for 16 d. During the pause, the feed and the circulation were simultaneously stopped, and the temperature of the reactor decreased from 35 ± 1 °C to the ambient temperature (approximately 15 $°C$). Due to the low temperature, the activity of methanogens was inhibited, which resulted in an increase in the residual VFA and a consequential decrease in the pH from 7.2 to 5.5. This decreased methanogen activity led to a decrease in the biogas production rate to 20 mL/h. When restarting the system using influent with a COD of 3900 mg/L, the COD removal rate was 41%, which was equal to 1.59 kg $\text{CD}/(\text{m}^3 \text{ d})$. At this time, the amount of biogas and VFA generated was 305 mL/h and 550 mg/L, respectively. During the restarting, the performance of the reactor recovered rapidly even as the influent COD increased. Specifically, the COD removal reached 80.5% after 15 d of operation under an influent COD concentration of 19,148 mg/L and a HRT of 24 h. The biogas production rate and VFA increased to 1420 mL/h and 4420 mg/L, respectively, during this period. These results indicate that the volume load of the reactor reached 15.4 kg COD/($m³$ d) and the gas production rate reached $0.44 \,\mathrm{m}^3$ biogas/(kg COD).

3.6. Sludge circulation effect

After completing startup, the amount of biomass on the biocarriers was 9.8 kg MLSS/ m^3 . The bio-filter layer intercepted the sludge and possibly played a role in decomposition of the substrate. As the influent organic load increased, the biogas production rate obviously increased, which resulted in sludge rising at an

Table 1

Changes of TSS in the effluent and the gas production rate under various organic loads.

Organic load ($kg\text{ COD/(m}^3\text{ d})$)	1.4	5.9	10.3	15.4
Gas production rate (mL/h)	100	600	860	1420
TSS(mg/L)	200	1230	2850	4720

increased rate. Although most rising sludge was intercepted by the bio-carriers and settled by the separator, a large amount of sludge was washed out the reactor. The changes in the TSS of the effluent and biogas production rate under various organic loads were analyzed and are shown in Table 1. The TSS of the effluent reached 4720 mg/L under an organic load of 15.4 kg $\text{CD}/(\text{m}^3 \text{ d})$. These findings clearly demonstrated that the sludge must be recycled into the reactor, otherwise the amount of biomass would be insufficient. The sludge was well precipitated in the sediment tank with a SI (sediment index) of 25% in 30 min. Therefore, the sludge circulation flow was adjusted to maintain the MLSS in the reactor at approximately 38–40 g/L.

4. Conclusions

A hybrid reactor combined with an UASB and an anaerobic fixed filter that employed hydraulic circulation and sludge circulation was investigated in this study. The reactor was rapidly cultivated in 38 d after a failed startup using municipal sludge as the seed. At the end of the startup period, the organic load approached 10 kg $\text{COD}/\text{(m}^3\text{ d})$. In addition, the average size of the sludge granules increased from 111 μ m to 264 μ m, and the sludge was well distributed in the reacting region. Good performance of the reactor occurred due to the good hydraulic conditions and the low loss of sludge. However, higher hydraulic circulation did not lead to increased COD removal, and actually caused a sharp decrease in the effectiveness of the reactor. After a pause of the operation, secondary startup was completed in 15 d. During secondary startup, the organic load reached 15.4 COD/ $(m³ d)$.

Acknowledgment

This study was conducted with financial support from National Key Scientific and Technology Project for Water Pollution Treatment of China (2008ZX07208-004, 2008ZX07208-002).

References

- [1] F.F. Ricardo, J.M. Pontesa, Pinto analysis of integrated kinetic and flow models for anaerobic digesters, Chem. Eng. J. 122 (1–2) (2006) 65–80.
- A.E. Tarek, O. Ralf, Anaerobic biodegradability and treatment of grey water in upflow anaerobic sludge blanket (UASB) reactor, Water Res. 41 (2007) 1379–1387.
- [3] M.M. Ghangrekar, S.R. Asolekar, S.G. Joshi, Characteristics ofsludge developed under different loading conditions during UASB reactor start-up and granulation, Water Res. 39 (6) (2005) 1123–1133.
- [4] Y. Liu, H.L. Xu, K.Y. Show, J.H. Tay, Anaerobic granulation technology for wastewater treatment, World J. Microb. Biotechnol. 18 (2002) 99–113.
- [5] Y. Liu, H.L. Xu, S.F. Yang, J.H. Tay, Mechanisms and models for anaerobic granulation in upflow anaerobic sludge blanket reactor, Water Res. 37 (3) (2003) 61–67.
- [6] H.H.P. Fang, H.K. Chui, Y.Y. Li, T. Chen, Performance and granule characteristics of UASB process treating wastewater with hydrolyzed proteins, Water Sci. Technol. 30 (1994) 55–66.
- [7] H.Q. Yu, H.H.P. Fang, J.H. Tay, Enhanced sludge granulation in upflow anaerobic sludge blanket (UASB) reactors by aluminum chloride, Chemosphere 44 (1) (2001) 31–36.
- [8] A. Vlyssides, E.M. Barampouti, S.Mai, Influence of ferrous iron on the granularity of a UASB reactor, Chem. Eng. J. 146 (2009) 49–56.
- [9] D. Jeison, R. Chamy, Comparison of the behaviour of expanded granular sludge bed (EGSB) and upflow anaerobic sludge blanket (UASB) reactors in dilute and concentrated wastewater treatment, Water Sci. Technol. 40 (8) (1999) 91–97.
- [10] L.H.A. Habets, A.J.H.H. Engelaar, N. Groeneveld, Anaerobic treatment of inuline effluent in an internal circulation reactor, Water Sci. Technol. 35 (10) (1997) 189–197.
- [11] G.D. Najafpour, A.A.L. Zinatizadeh, A.R. Mohamed, High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor, Process Biochem. 41 (2006) 370–379.
- [12] Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association (APHA), American Water Works Association, and Water Environment Federation, Washington DC, 1998.
- [13] S.K. Han, S.H. Kim, H.S. Shin, UASB treatment of wastewater with VFA and alcohol generated during hydrogen fermentation of foodwaste, Process Biochem. 40 (2005) 2897–2905.
- [14] S. Jiang, Y. Chen, Q. Zhou, Effect of sodium dodecyl sulfate on waste activated sludge hydrolysis and acidification, Chem. Eng. J. 132 (1–3) (2007) 311– 317.
- [15] W.J. De Zeeuw, Granular sludge in UASB reactors, in: G. Lettinga, A.J.B. Zehnder, J.T.C. Grotenhuis, L.W. Hulshoff Pol (Eds.), Granular Anaerobic Sludge: Microbiology and Technology, Pudoc, Wageningen, The Netherlands, 1987, pp. 132–145.
- [16] H.C. Dubourgier, G. Prensier, G. Albagnac, Structure and microbial activities of granular anaerobic sludge, in: G. Lettinga, A.J.B. Zehnder, J.T.C. Grotenhuis, L.W. Hulshoff Pol (Eds.), Granular Anaerobic Sludge: Microbiology and Technology, Pudoc, Wageningen, The Netherlands, 1987, pp. 18–33.
- [17] H.P. Bochem, S.M. Schoberth, B. Sprey, P. Wengler, Thermophilic biomethanation of acetic acid: morphology and ultrastructure of a granular consortium, Can. J. Microbiol. 28 (1982) 500–510.
- [18] L.W. Hulshoff Pol, S.I. Castro Lopes, G. Lettinga, P.N.L. Lens, Anaerobic sludge
- granulation, Water Res. 38 (6) (2004) 1376–1389. [19] Y-H. Ahn, Physicochemical and microbial aspects of anaerobic granular pellets, J. Environ. Sci. Health A 35 (9) (2000) 1617–1635.
- [20] H.H. Chou, J.S. Huang, J.H. Jheng, R. Ohara, Influencing effect of intra-granule mass transfer in expanded granular sludge-bed reactors treating an inhibitory substrate, Bioresour. Technol. 99 (9) (2008) 3403–3410.
- [21] F.A. McLeod, S.R. Guiot, J.W. Costerton, Layered structure of bacterial aggregates produced in an upflow anaerobic sludge bed and filter reactor, Appl. Environ. Microbiol. 56 (6) (1990) 1598–1607.