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Diagnosis of the acidification and recovery of anaerobic sequencing batch reactors

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ABSTRACT

This study aims to investigate the diagnosis of acidification and efficient recovery of a laboratory-scale anaerobic sequencing batch reactor (ASBR) which treats synthetic glucose wastewater under mesophilic conditions (35°C). The diagnosis of the ASBR showed that acidification occurred on the seventh day after adding 20 mmol/L of sodium 2-bromoethanesulfonate into the reactor. A three-step recovery strategy was employed to recover the acidified reactor efficiently and to study its restoration process. Results indicated that the acidified ASBR can be revived in approximately 50 d. The specific methanogenic activities of the sludge, which were based on the substrate of acetate, propionate, and butyrate, were restored at 0.85, 0.67, and 0.51 (gCOD-CH_4)/(gVSS•d), respectively. The fluorescent observation images revealed large amounts of Methanosarcina-like and rod-shaped methanogens distributed in the sludge flocs after reactor restoration, thus ensuring that the fermentative, acidogenic, and methanogenic processes proceeded effectively in the anaerobic system.

Keywords: Anaerobic sequencing batch reactor; Methanogenic inhibitor; Acidification; Three-step recovery strategy; Specific methanogenic activities

1. Introduction

As early as 1966 at the Iowa State University, Dague and his colleagues had already conducted an anaerobic experiment on a batch-feed discontinuous anaerobic reactor which was renamed as anaerobic sequencing batch reactor (ASBR) by Dague in 1991. Since then, the ASBR has gradually gained ground in scientific research and has become widely used in wastewater treatment [1]. ASBR is operated in four steps: feed, reaction, settle, and withdrawal. The substrate concentration in the reactor reaches the highest point and stimulates the highest possible metabolic activity of bacteria after the feeding period. After the reaction period, the bulk concentration becomes low which benefits bacteria flocculation. In the settling period, the mixing stops and the reactor serves as a static sedimentation tank which stimulates sludge–liquid

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separation. After settling, the supernatant is withdrawn and prepared for the next cycle. ASBR is easier and more flexible to operate than other high-rate anaerobic reactors (i.e. UASB and EGSB), because it has no solid–liquid–gas separator. Moreover, the influent can be injected directly into the reactor, and no special distribution equipment is required. These characteristics make ASBR very attractive to industries, particularly those treating small volumes of wastewater in which anaerobic digestion processes are expected to be installed [2,3]. Many reports have shown evidence for the successful application of ASBR in treating industrial wastewater, such as slaughterhouse wastewater, landfill leachate, and some low-strength industrial wastewater [4–6].

Stability is a critical factor in the operation of an anaerobic digester. Many factors which interfere with the methane formation will cause unstable performance and even failure of the anaerobic system [7]. The accumulation of volatile fatty acids (VFAs) is the main reason for the anaerobic process instability. Previous studies suggested that high VFAs accumulation, low alkalinity levels, or the loss of methanogens may result in the anaerobic system failure. Furthermore, organics, pH, and other parameters also influence the digester performance [8,9]. Inhibitors and other environmental changes result in an imbalance in the anaerobic food chain and in the accumulation of fatty acids, such as acetate, propionate, and butyrate [10].

Neutral pH conditions at a generally accepted optimum range of 6.8–7.2 are conducive to methanogens. Conditions beyond this range steeply decrease the methane production rate, which in turn causes an imbalance in the syntrophic relationship between acetogens and methanogens, and results in VFAs accumulation [11,12]. Inhibitors, including sodium 2-bromoethanesulfonate, nitro-ethane, and ethyl *trans*-2-butenoate, which interfere with methane formation cause VFAs accumulation, which in turn results in a series of problems, such as a reduction in pH and gas production, sludge rising, and deterioration of effluent quality. This type of phenomenon is known as acidification of the anaerobic reactor, an important factor which limits the development and promotion of the anaerobic process [13–16]. Therefore, exploring an effective recovery strategy for anaerobic acidification is significant for the steady operation of the anaerobic process.

This study aims to investigate the acidification and stability recovery process of a laboratory-scale ASBR. Several key parameters, particularly biogas production, methane content, pH value, VFAs concentration, and sludge-specific methanogenic activities (SMAs), are proposed to evaluate the reactor performance and diagnose the acidification of the reactor. A three-step recovery strategy was also suggested for the efficient recovery of the acidified reactor.

2. Material and methods

2.1. Inoculum and feed solution

The sludge investigated in this study was inoculated from synthetic glucose wastewater (COD_{inf} = 3,000 mg/L) treatment ASBR previously operated at a steady-state condition. The pH value of the reactor was controlled in the range of 6.8–7.2, COD removal efficiency of up to approximately 98%, biogas production of 6.2 L/d, and methane content of 54–56%. The sludge inoculum was found predominantly in flocs, and its initial SMAs in the presence of acetate, propionate, and butyrate were 0.87, 0.65, and 0.52 gCOD/(gVSS·d), respectively.

Synthetic wastewater with glucose as the sole carbon source was fed into the reactor during ASBR operation. The composition of synthetic wastewater under a steady state is as follows (in mg/L, except for the trace element solution): COD (3,000), NH_4Cl (191), KH_2PO_4 (44), $NaHCO_3$ (3,000), $MgCl{\cdot}6H_2O$ (30), NaCl (72), Na_2SO_4 (25.8), and trace element solution of 150 μL/L. During the recovery process of the reactor, each component was added to the synthetic wastewater in accordance with the proportions mentioned above. The trace element used in this study is shown in Table 1.

2.2. Experimental setup and operation

The laboratory-scale ASBR was made of tempered glass, which had a headspace of 1.5 L, liquid volume of 4.5 L, height of 30 cm, and diameter of 18 cm (Fig. 1). The liquid was mixed by an axial continuous agitation at a speed of 90 rpm, ensuring the full and timely mixing of the liquid and biogas release. The biogas production was measured by a wet gas flow meter. The ORP, temperature, and pH were monitored online by the probes (Mettler–Toledo, Switzerland), which were connected to a data acquisition system. To reduce the disturbance of the pressure fluctuations in the biological metabolism and the biogas measurement, an air dunnage bag was installed between the ASBR and the gas flowmeter.

The ASBR was operated at a constant temperature of 35 ± 1℃ for an 8 h cycle that consisted of 30 min feeding, 420 min reaction (including 30 min feeding), 30 min sedimentation, 10 min withdrawal, and 20 min idle time. The exchange ratio was controlled at 1/3, which corresponded to a 24 h of hydraulic retention time. The solid retention time covered 20 d.

Table 1
Trace element solution components

Components	Concentration (g/L)	Components	Concentration (g/L)
$FeSO_4{\cdot}7H_2O$	8	H_3BO_3	0.1
$MnCl_2{\cdot}4H_2O$	0.5	EDTA	0.05
$CoCl_2{\cdot}6H_2O$	0.88	$NiCl_2{\cdot}6H_2O$	0.036
$CuCl_2{\cdot}2H_2O$	0.035	$(NH4)_6Mo_7O_{24}{\cdot}4H_2O$	0.64
$ZnSO_4{\cdot}7H_2O$	0.1	$MgSO_4{\cdot}7H_2O$	5

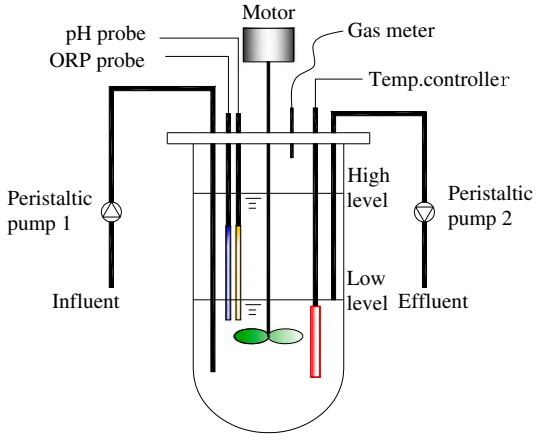

Fig. 1. Schematic diagram of the ASBR.

To investigate the acidification of ASBR by sodium 2-bromoethanesulfonate (BES) addition and its recovery process, an experimental study was designed as follows: The ASBR was initially operated at a steady state for 10 d (COD_{inf} = 3,000 mg/L) after which 20 mmol/L of BES was added into the reactor on the 11th day. The performance of the reactor was subsequently monitored, and acidification occurred in the later week. Finally, a comprehensive three-step recovery strategy (described in detail in the following section) was implemented in the acidified ASBR beginning on the 18th day.

2.3. Analytical methods

COD was determined through titration according to the standard method [17]; pH was measured by the electrode method; biogas production was measured with a wet gas flowmeter; The methane content of the biogas was analyzed using a gas chromatograph according to a previously described method [18]; The microscopic examination of the methanogens in the ASBR was performed using an Olympus-BX51 fluorescence microscope (Japan).

The composition of the VFAs was determined using a gas chromatograph (Agilent 6890 N GC) equipped with a DB-WAX capillary column (30 m × 0.32 mm × 0.50 μm) and a flame ionization detector. The temperatures of the injection port and detector were 230 and 250˚C, respectively. The oven of the GC was programmed to begin at 100˚C and stay for 2 min, increase at a rate of 3˚C/min up to 160˚C, and then remain constant at 160˚C for an additional 10 min. Nitrogen was selected as the carrier gas and the flux was 20 mL/min. The burning gases were H_2/air at 30/300 mL/min flow rate. The sample injection volume was 1.0 μL.

2.4. Specific methanogenic activity test

A serum bottle test was used to examine the variations of sludge SMAs during the acidification and recovery of the reactor. Acetate, propionate, and butyrate were used as the substrate. The initial concentration of each substrate was prepared at 2000 mg/L, and the sludge concentration in the three serum bottle was approximately 3 g-VSS/L. The pH of the substrate solution was adjusted to the range of 6.8–7.2 before it was added into the test bottles. CH_4 production was measured at a regular time interval (once every 1 h) after the test was initiated. At the end of the SMA test, the biomass was precisely quantified using the gravimetric method [17]. The SMAs were calculated according to the previously presented equation [18].

3. Results and discussion

3.1. Acidification characteristics

The pH value is a crucial indicator of the stable operation for an anaerobic digestion system. The pH in the reactor should be maintained in the range of 6.8–7.2 to ensure that the anaerobic metabolism proceeds successfully, particularly in the methanogenic stage. When a small amount of BES is added to the system, the balance of the anaerobic metabolism is disturbed, which results in decreases in the pH value, biogas production, and organic removal efficiency. Results showed that under the steady-state conditions,

the COD removal efficiency reaches up to 98%, and effluent COD concentration is about 52 mg/L. The effluent COD further increases and reaches as high as 573 mg/L on the seventh day after adding BES. The pH value of the liquid drops from 7.01 to 6.25, and the effluent VFAs concentration substantially increases. The concentration of acetate increases from 28 to 309 mg/L, and the propionate concentration also increase from 10 to 254 mg/L. These results indicate that acidification has occurred in the ASBR.

3.2. Recovery strategy

Reducing the influent organic loading is a commonly used method for restoring the acidified reactor. However, previous reports showed that this method must be applied along with the alkali dosing method, because that reducing the influent substrate concentration alone could not lead to an effective recovery [14]. The addition of $NaHCO_3$ could adjust the pH value to an appropriate range without interfering with the sensitive physical and chemical equilibrium of the microorganisms. Moreover, the CO_2 content of the biogas would also be less affected, and the fluctuation of pH is smaller than that of other chemical additions. From these findings, the following three-step remedy strategy was adopted in this study. (1) The sludge sample is elutriated with oxygen-free water to remove the residual BES. The high concentration of accumulated VFAs can also be displaced and diluted in this step, thereby reducing the toxic effects of the high concentration of VFAs on the methanogens. (2) The pH value of the mixed liquor is quickly adjusted to 6.8–7.2 through $NaHCO_3$ addition to reduce the adverse effect of low pH on the methanogens. (3) Methanogenic activity is recovered by gradually increasing the concentration of the influent substrate according to the following sequence: 500, 1,000, 1,600, 2,000, and 3,000 mg/L.

3.3. Restoration and reconstruction process

3.3.1. Reactor performance variation

Fig. 2 illustrates the variations in the performance of the ASBR in terms of COD removal, biogas production, and methane content of the biogas. Under steady-state conditions, the effluent COD concentration is as low as 52 mg/L, biogas production 6.2 L/d, and the methane content 54–56%. Adding BES into the ASBR on the 11th day affects the performance of the reactor in terms of the continuous decline in the COD removal efficiency and methane production. On the seventh day after adding BES, the effluent COD increases to as high as 573 mg/L, biogas production decreases to 1.3 L/d, and the methane content decreases to 24.5%. These changes indicate that the performance of the reactor is seriously affected and that acidification occurs in a week after adding BES.

The recovery process of the ASBR is also shown in Fig. 2. The sludge in the acidified situation is washed with oxygen-free water until the high concentration of the residual substrate is displaced. Since the COD concentration in the reactor decreases to 54 mg/L, the influent substrate concentration is reduced to 500 mg/L and then increased through five steps to restore the acidified reactor. In the early stage of each step, the effluent COD concentration increases by a certain degree. Continuous running under the same organic loading rate gradually decreases the effluent COD until it is stabilized at a relatively low level. These findings lead to the conclusion that micro-organisms are unable to adapt immediately to a rise in substrate concentration in the initial period of each stage, and the performance of the reactor can be improved gradually with the adaptation and recovery of the bacteria. Once the effluent COD is reduced and kept in a normal range, the substrate concentration increases again to initiate the next recovery stage. After about 50 d of recovery operation, the feeding concentration of the ASBR reactor increases to 3,000 mg/L, the effluent COD concentration is maintained at approximately 53 mg/L and the organic removal efficiency reaches as high as 98%. Biogas production and methane content are also continuously recovered to the normal level.

3.3.2. Change characteristics of the effluent pH value and VFAs concentration

Fig. 3 presents the pH value and VFAs concentration in the ASBR effluent during the study period. The effluent VFAs of the ASBR exhibit a profile similar to that of the effluent COD during the recovery process. Under the steady-state conditions, the effluent acetate and propionate concentration are approximately 28 and 10 mg-COD/L, respectively. After adding BES on the 11th day, the VFAs concentration constantly increases and the pH of the liquid drops from 7.01 to 6.25. Adding BES into the ASBR inhibits the activity of the methanogens, and thereby increases the concentration of acetate and H_2/CO_2 in the reactor. The high concentration of accumulated hydrogen affects the degradation process of propionate, because the anaerobic degradation of propionate into acetate and H_2 is thermodynamically unfavorable under standard conditions (Gibbs free energy, $\Delta G^0 = +76$ kJ/mol).

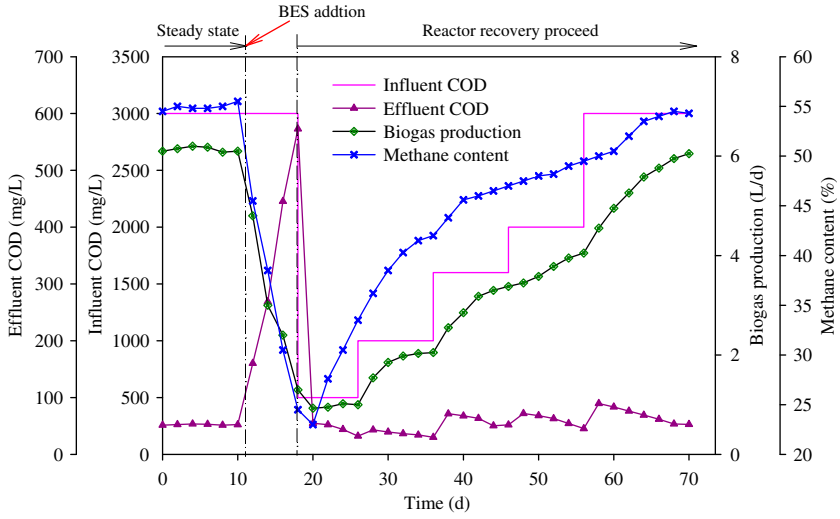

Fig. 2. Variations in the performance of the ASBR during acidification and recovery.

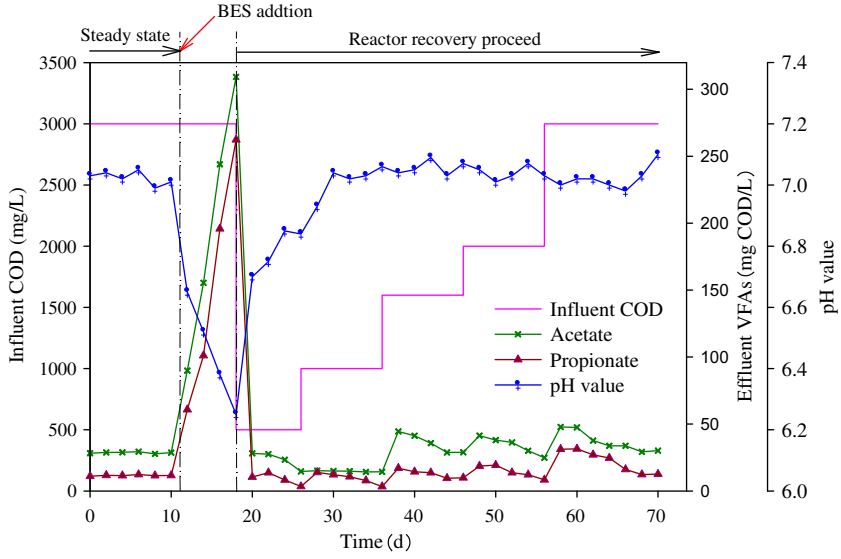

Fig. 3. Variations in the effluent VFAs and pH value during acidification and recovery of the ASBR.

The accumulation of VFAs causes decrease in the effluent pH value, which is unfavorable to the growth and metabolism of methanogens, the ideal neutral pH condition of which is an optimum range of 6.8–7.2. Therefore, $NaHCO_3$ is selected as the buffer agent to adjust the pH value to the favorable range when acidification occurs in the reactor. The pH value is always maintained in the range of 6.8–7.2 by adjusting the $NaHCO_3$ dosage later in the recovering process, thus providing an appropriate pH environment for the recovery of methanogenic activity. After the recovery of the ASBR, the concentration of the influent substrate concentration increases to 3,000 mg/L, and the acetate and propionate contents in the effluent decrease to 30 and 12 mg-COD/L, respectively.

3.3.3. Restoration of the sludge SMAs

Sludge SMAs are an important parameter in evaluating the methanogenic potential of an anaerobic digestion system. Fig. 4 shows the changes in the sludge SMAs during the study period. Under steady-state conditions (COD_{inf} = 3,000 mg/L), the sludge SMAs based on the substrate of acetate, propionate, and butyrate are 0.87, 0.65, and 0.52 (gCOD-CH_4)/(gVSS•d), respectively. After the acidification of the reactor, the sludge SMAs

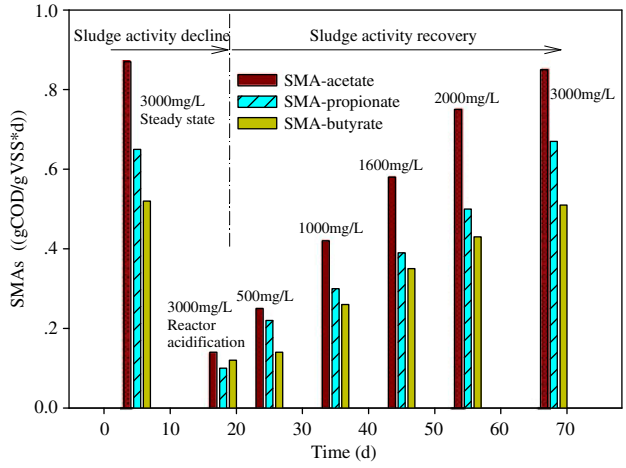

Fig. 4. Variation in the sludge SMAs (mg/L refer to COD concentration of the glucose substrate).

substantially decrease to 0.14, 0.10, and 0.12 (gCOD-CH_4)/(gVSS•d), respectively. Immediately after adding BES, the activity of methanogenic organisms is inhibited, resulting in the accumulation of VFAs. A high concentration of accumulated VFAs lead to a significant decrease in the pH of the reactor, which further impact the methane production rate of the anaerobic sludge in subsequent operations. According to the recovery strategy used in this study, the SMAs of the sludge recover to the normal level, such as that in the steady-state conditions, after about 50 d of recultivation.

3.3.4. Observation of methanogens

As a specific enzyme, the coenzyme F420 (7, 8-didemethyl-8-hydroxy-5-deazaribolfavin derivative) consists of an electron transport involved in methane formation, and it is widely distributed in

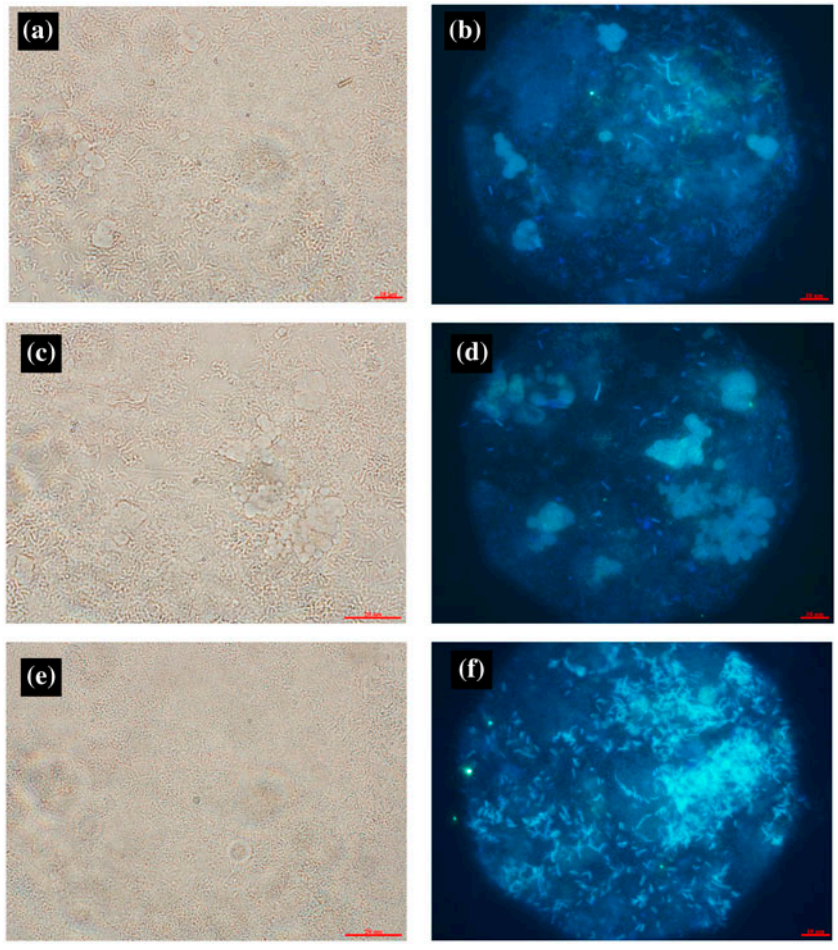

Fig. 5. Photomicrographs taken under phase contrast and then with ultraviolet excitation. (a) Typical sludge flocs under acidified conditions, phase contrast. (b) Same field as (a), photographs under ultraviolet. (c) Methanosarcina under steady-state conditions, phase contrast. (d) Same field as (c), photographs under ultraviolet. (e) Rod-shaped methanogens under steady-state conditions, phase contrast. (f) Same field as (e), photographs under ultraviolet.

methanogens [19]. Coenzyme F420 radiates a typical blue–green fluorescence with a strong absorption at a wavelength of 420 nm, which is the autofluorescence characteristic attributed to methanogens [20,21]. Therefore, the typical blue–green fluorescence exhibited by methanogenic organisms can be used to detect methanogens with a fluorescence microscope [22].

In this study, anaerobic sludge was taken from the ASBR under acidified and steady-state conditions, and was observed through the fluorescence detection method. Phase contrast and fluorescence photomicrographs of the sludge flocs are presented in Fig. 5. When the reactor acidifies, only a few of micro-organisms are detected as methanogens in the sludge flocs (Fig. 5(a) and (b)). However, Methanosarcina predominate in the ASBR under steady-state conditions (Fig. 5(c) and (d)), and certain amounts of rod-shaped methanogens are distributed in the anaerobic sludge (Fig. 5(e) and (f)). Residual bacteria in the flocs that are not fluoresced are deduced to be the fermentative and acidogenic microbes. Substantial methanogenic archaea can be found distributed in the anaerobic sludge after the reactor recovers, thus ensuring that the anaerobic digestion proceeds efficiently.

4. Conclusions

In this study, acidification occurred in the ASBR on the seventh day after 20 mmol/L of BES were added into the reactor. Several important parameters, such as the effluent COD concentration, biogas production and the methane content, pH value, and VFAs concentration, were monitored and used to estimate the acidification in the reactor. The sludge activity was initially inhibited because of the addition of BES into the ASBR, which resulted in the accumulation of VFAs. The accumulation of VFAs to a high concentration led to a significant decrease in the pH of the reactor, which further affected the methane production rate of the anaerobic sludge in the later stages of the operation. A three-step recovery strategy was presented, and results showed that the acidified reactor could be restored to a normal level within about 50 d. Sludge fluorescent images showed that much Methanosarcina-like and rod-shaped methanogenic archaea were distributed in the ASBR under steady-state conditions, thus ensuring that the anaerobic digestion proceeded efficiently.

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References

[1] R.R. Dague, C.E. Habben, S.R. Pidaparti, Initial studies on the anaerobic sequencing batch reactor, Water Sci. Technol. 26 (1992) 2429–2432.
[2] L.T. Angenent, R.R. Daugue, A laboratory scale comparison of the UASB and ASBR processes, in: Proceedings of 50th Purdue Industrial Waste Conference, Ann Arbor Press, Chelsea, MI, 1995, pp. 365–377.
[3] M. Zaiat, J.A.D. Rodrigues, S.M. Ratusznei, E.F.M. de Camargo, W. Bozami, Anaerobic sequencing batch reactor for wastewater treatment: A developing technology, Appl. Microbiol. Biotechnol. 55 (2001) 29–35.
[4] D.I. Massé, L. Masse, A. Verville, S. Bilodeau, The start-up of anaerobic sequencing batch reactors at 20˚C and 25˚C for the treatment of slaughterhouse wastewater, J. Chem. Technol. Biotechnol. 76 (2001) 393–400.
[5] J.A. Hollopeter, R.R. Dague, Anaerobic sequencing batch reactor treatment of landfill leachate, in: Proceedings of 49th Purdue Industrial Waste Conference, Ann Arbor Press, Chelsea, MI, 1994, pp. 277–284.
[6] G.C. Banik, R.R. Dague, ASBR treatment of low strength industrial wastewater at psychrophilic temperatures, Water Sci. Technol. 36 (1997) 337–344.
[7] J.D. Keenan, Multiple staged methane recovery from solid wastes, J. Environ. Sci. Heal. A. 11 (1976) 525–548.
[8] G.K. Kafle, S.H. Kim, Sludge exchange process on two serial CSTRs anaerobic digestions: Process failure and recovery, Bioresource Technol. 102 (2011) 6815–6822.
[9] C. Gallert, J. Winter, Bacterial Metabolism in Wastewater Treatment Systems, Environmental Biotechnology, Concepts and Applications, Wiley-VCH, Weinheim, 2005, pp. 1–48.
[10] C. Gallert, J. Winter, Propionic acid accumulation and degradation during restart of a full-scale anaerobic biowaste digester, Bioresource Technol. 99 (2007) 170–178.
[11] J. Ma, M. Carballa, P. Van De Caveye, W. Verstraete, Enhanced propionic acid degradation (EPAD) system: Proof of principle and feasibility, Water Res. 43 (2009) 3239–3248.
[12] R.E. Speece, Anaerobic Biotechnology for Industrial Wastewaters, Printed in the United States of America, 1995, pp. 25–59.
[13] Y.N. Su, C.S. David, Microbial response to environmental changes in an anaerobic baffled reactor (ABR), Antonie van Leeuwenhoek 67 (1995) 111–123.
[14] B. Zhang, W.M. Cai, P.J. He, Influence of lactic acid on the two phase anaerobic digestion of kitchen wastes, J. Environ. Sci. 19 (2007) 244–249.
[15] T. Hernández, G. Masciandaro, J.I. Moreno, C. García, Changes in organic matter composition during composting of two digested sewage sludge, Waste Manage. 26 (2006) 1370–1376.
[16] B.K. Ahring, P. Westermann, Thermophilic anaerobic degradation of butyrate utilizing bacterium in coculture and triculture with methanogenic bacteria, Appl. Environ. Microb. 53 (1987) 429–433.

[17] APHA, Standard Methods for the Examination of Water and Wastewater, twentieth ed., American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, 1998.
[18] Y.P. Hou, D.C. Peng, X.D. Xue, H.Y. Wang, L.Y. Pei, Hydrogen utilization rate: A crucial indicator for anaerobic digestion process evaluation and monitoring, J. Biosci. Bioeng. 117 (2013) 519–523.
[19] X.B. Yin, L.W. Lian, J.Q. Xu, Unique enzymes and biochemical monitoring methods in methanogenesis, China Biogas 10 (1998) 8–13.
[20] L.D. Eirich, G.D. Vogels, R.S. Wolfe, Proposed structure for coenzyme F420 from methanobacterium, Biochemistry 17 (1978) 4583–4593.
[21] J. Dolfing, J.W. Mulder, Comparison of methane production rate and coenzyme F420 content of methanogenic consortia in anaerobic granular sludge, Appl. Environ. Microbiol. 49 (1985) 1142–1142.
[22] H.J. Doddema, G.D. Vogels, Improved identification of methanogenic bacteria by fluorescence microscopy, Appl. Environ. Microbiol. 36 (1978) 752–754.