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Full Length Research Paper

Effects of ethanol/SO_4^{2-} ratio and pH on mesophilic sulfate reduction in UASB reactors

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The effects of ethanol/SO_4^{2-} ratio and pH on mesophilic sulfate reduction were investigated using three UASB reactors fed with sucrose at an organic loading rate of 5.0 gCOD/L·day. When pH was at 7, ethanol/SO_4^{2-} ratio rose from 0 to 5, and sulfate reduction rate went up from 5 - 7% to 85 - 89%; the removal efficiency of COD from 82 to 89%. When sucrose was the only organic carbon source, the sulfate reduction rate was 55 - 57% with pH at 6 and 47 - 49% with pH at 5. Correspondingly, the COD removal efficiencies were 46 - 49% and 8 - 10%, respectively. When ethanol was added as part of the carbon source, the sulfate reduction rates rose to 88 - 91% and 60 - 71% respectively, and COD removal efficiencies were 56 - 61% with pH at 6 and 27 - 29% with pH at 5. Decrease of pH resulted in significant decline of specific methanogenic activity and high accumulation of volatile fatty acid. Addition of ethanol promoted sulfate reduction rate and facilitated good synergetic metabolism of sulfate-reducing and methane-producing bacteria. The results presented in this paper provide some useful information for the optimization of sulfate-reducing processes in wastewater treatment.

Key words: UASB, SRB, MPB, sulfate reduction, ethanol, methanogenic activity.

INTRODUCTION

Wastewater from starch fermentation, seafood preparation, chemical industries or pulp and paper industries contains high concentrations of sulfate and organic compounds (Omil et al., 1996; Lopes et al., 2007a; Portillo and Gonzalez, 2009). Anaerobic digestion technique has been widely used to treat sulfate-containing wastewater (Kim et al., 1999; Waybrant et al., 2002; Vallero et al., 2005; Daniel et al., 2008). With organic matters as electron donor and sulfate as electron acceptor, sulfate reducing bacteria (SRB) can transform sulfate to hydrogen sulfide. In anaerobic bioreactors, competition between SRB and methane-producing bacteria (MPB) for utilization of hydrogen and acetate often leads to decrease of methane production rate and even failure of treatment process (Janssen et al., 2009; Ikbal et al., 2003; Lehua et al., 2008). An effective solution is needed to avoid competition reaction and promote tolerance of the bacteria in reactors to sulfide toxicity.

Previous studies showed that sulfate reduction often occurred in the acidification stage (Mizuno and Noike, 1998; Demirel and Yenigün, 2002; Lens et al., 2003). Substrate concentration (Boshoff et al., 2004a, b; Teclua et al., 2009) and pH (Visser et al., 1996) were considered as the two important parameters affecting sulfate reduction rate of SRB in the system. When electron donor is abundant in the system, competition between SBR and MPB will be inhibited, especially for the wastewater with high concentration of sulfate (Bhattacharya et al., 1996). Methylamine and methanol were considered as specialized substrates (Vallero et al., 2003; Paz et al., 2009), but little information is available about the effect of ethanol on sulfate reduction, which is used by SRB easily and directly (Zhao et al., 2009; Martins et al., 2009). Variation of pH affects microbial

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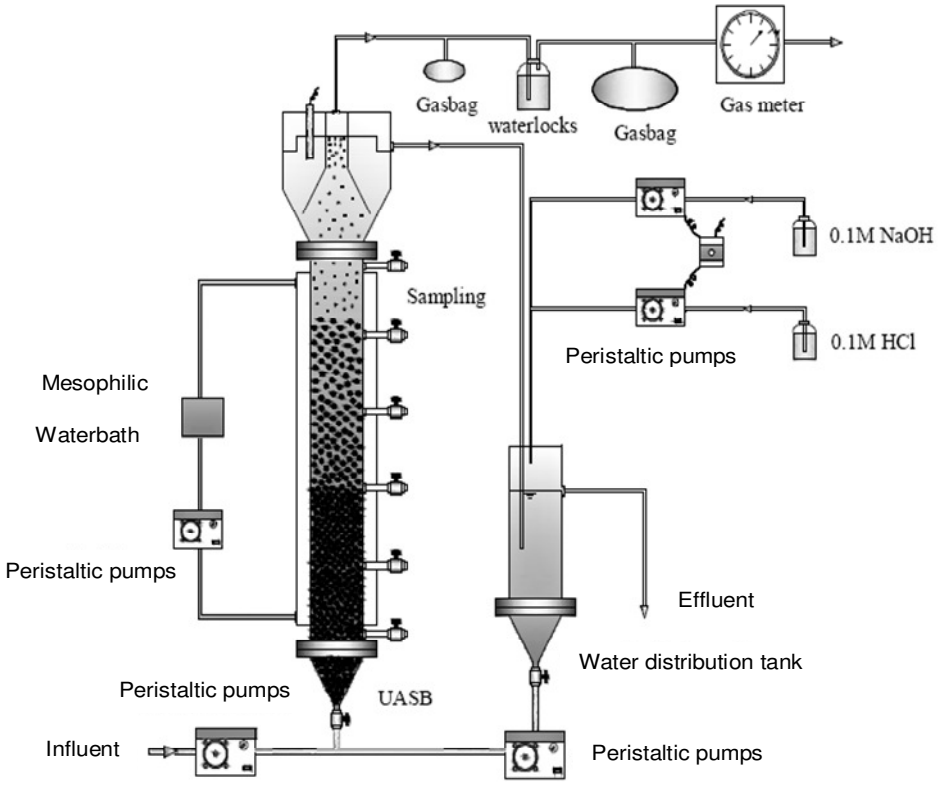

Figure 1. Schematic diagram of the USAB reactors for sulfate reduction.

metabolism and the produced sulfide and ammonia have toxic effects on these bacteria. SRB and MPB presented different capacities in resistance against hydrogen sulfide, and the optimal pH values were found to be 6.8 - 7.2 and 6.4 - 6.8, respectively (Lopes et al., 2007b). Therefore, Down-regulation of pH and electron donor concentrations in the acidification stage could result in the improvement of methanogenic activities and sulfate reduction rate.

This study aims to investigate the effects of ethanol/SO_4^{2-} ratio and pH values on mesophilic sulfate reduction using three mesophilic (35°C) upflow anaerobic sludge bed (UASB) reactors, which were operated at a consistent organic loading rate (OLR) and different ethanol/SO_4^{2-} ratios and pH values. Sulfate and COD (chemical oxygen demands) removals and metabolite production in the bioreactors were analyzed in order to provide some useful information for the optimization of sulfate-reducing processes.

MATERIALS AND METHODS

Experimental set-up

Three cylindrical double-wall UASB reactors (R1, R2 and R3) with a working height of 1.8 m and an internal diameter of 0.08 m were used in this study. The three experimental set-ups shared the same structure and dimensions (Figure 1), but pH values in R1, R2 and R3 were kept at 7, 6 and 5 (±0.3), respectively. The reactors were operated at 35°C for 240 days with a hydraulic retention time (HRT) of 9.6 h and an organic loading rate (OLR) of 5.0 gCOD/L·d. The temperature of the reactors was controlled by a circulating water bath in the double-wall interlayer (HH-4, Jiangxing Co., Shanghai, China). Wastewater was fed to the reactors by the bottom using peristaltic pumps (BT-600C, Huxi Co., Shanghai, China). Each UASB reactor was equipped with a water distributing tank with an effective height of 0.8 m and an internal diameter of 0.08 m. The UASB effluent entered the water distributing tank to separate the suspended solids from water. Supernatant overflowed from the top of the water distributing container, and the mixture of sedimentation and water returned to UASB together with the influent so as to keep the upflow velocity at 2 m/h. The pH in the reactors was measured with a pH electrode (Mettler Toledo FE20, Shanghai, China) and was regulated by adding NaOH (0.1 M) or HCl (0.1 M). The produced biogas flew through the waterlocks filled with NaOH (1 M) and zinc acetate (0.5 M) to remove CO_2 and H_2S.

Bacterial source

Each of the three reactors was inoculated with wet granular sludge (approximately 3000 g) collected from a wastewater treatment plant of Ruifeng Paper Industry Co. (Henan Province, China). An internal circulation anaerobic reactor (IC) was used in the plant to treat paper manufacturing wastewater. During the start-up period (32 days), the granular sludge was cultivated in the three UASB reactors and the reactors were fed with sucrose at an organic loading rate of 5.0 gCOD/L·day with pH at 7. At the end of the start-up period, CODcr removal in each reactor was obtained to be over 80%.

Table 1. Operational conditions of the three UASB reactors R1, R2 and R3.

Reactor	Period	Days	COD/SO_4^{2-} (mg/mg)	SO_4^{2-} (mg/L)	OLR gCOD/L·day	Ethanol /SO_4^{2-} (mg/mg)	pH
R1	Ⅰ	1 - 30	20,10	100, 200	5	0	7
	Ⅱ	31 - 140	10	200	5	0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 5	7
	Ⅲ	141~170	10,5	200, 400	5	0	7
	Ⅳ	171~240	5	400	5	0.25, 0.5, 0.75, 1, 1.5, 2, 2.5	7
R2	Ⅰ	1-30	20,10	100, 200	5	0	7
	Ⅱ	31-140	10	200	5	0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 5	6
	Ⅲ	141~170	10,5	200, 400	5	0	6
	Ⅳ	171~240	5	400	5	0.25, 0.5, 0.75, 1, 1.5, 2, 2.5	6
R3	Ⅰ	1-30	20,10	100, 200	5	0	5
	Ⅱ	31-140	10	200	5	0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 3, 4, 5	5
	Ⅲ	141~170	10,5	200, 400	5	0	5
	Ⅳ	171~240	5	400	5	0.25, 0.5, 0.75, 1, 1.5, 2, 2.5	5

Wastewater characteristics

The reactors were fed with a synthetic wastewater consisting of sucrose and ethanol as a model carbohydrate (sole electron donor and carbon source). As listed in Table 1, the concentration of sulfate was added as sodium sulfate into the bioreactors according to the ethanol/ SO_4^{2-} ratios.

Experimental design

In order to investigate the effect of ethanol/ SO_4^{2-} ratio and pH on mesophilic sulfate reduction, R1, R2 and R3 were operated continuously for 240 days, which was divided into four periods (Table 1). During the whole experimental period, concentration of ethanol was gradually raised from 250 to 1000 mg/L, and concentration of SO_4^{2-} was elevated from 200 to 400 mg/L. After the initial start-up, the pH values of the three reactors were adjusted to 7 for R1, 6 for R2 and 5 for R3.

Chemical analyses

COD in the water samples were analyzed according to the USA Standard Methods (APHA, 1998). Because of the sulfide effect, the COD was calculated by subtracting the corresponding part generated by theoretical sulfide effect according to the sulfide concentration. Sulfate was measured according to the turbidimetric method (Kolmert et al., 2000) using a spectrophotometer (UV-1100 spectrophotometer, Shanghai Mapada Inc., China), and the absorbance was determined at a wavelength of 420 nm. Sulfide was fixed with zinc acetate and measured by Iodometry (APHA, 1998). Sugars (sucrose, glucose and fructose) and lactate were measured by high-pressure liquid chromatography (Agilent 1100, USA) according to van Lier et al. (1997). Volatile fatty acid (VFA), alcohols and biogas composition were measured using gas chromatography (Agilent 6890, USA) according to Weijma et al. (2000). The production of biogas was measured by gas meters (Milligascounter, Ritter MGC-1, Bochum, Germany).

RESULTS

Sulfate reduction

In Table 1, pH value in R1 was kept at 7 for each of the four periods. In the Period I, COD was kept at about 2000 mg/L with COD/SO_4^{2-} ratio at 10 - 20. After operation for 30 days, the sulfate reduction rate was still low and showed no significant change when influent sulfate was increased from 100 to 200 mg/L (Figure 2). In Period II, ethanol was gradually added to increase influent COD; sulfate reduction and sulfide concentration rose significantly until day 101. When ethanol concentration was increased to 400 mg/L, the highest sulfate reduction rate and effluent sulfide concentration were achieved at 89% and 30.1 mg/L, respectively. However, with a further increase of ethanol concentration, no significant change was found for the sulfate reduction rate and effluent sulfide concentration. In Period III, ethanol was not added, and the sulfate reduction rate fell sharply to 5% with the sulfide concentration at about 3.5 mg/L. In Period IV, sulfate reduction rate went up after ethanol concentration was increased in R1 influent.

In R2, the pH was adjusted to 6, and the other operational conditions were the same as those in R1. In Period I of R2, the sulfate reduction rate stayed steady at 57%, and the sulfide concentration of effluent reached 20.2 mg/L which was much higher than that of R1, indicating that pH in the bioreactor was a key factor for sulfate reduction. In Period II, the addition of ethanol significantly increased sulfate reduction rate, which was similar to R1. The sulfate reduction rate went up to 87% and sulfide concentration was elevated to 34.9 mg/L when influent ethanol concentration was increased to 200

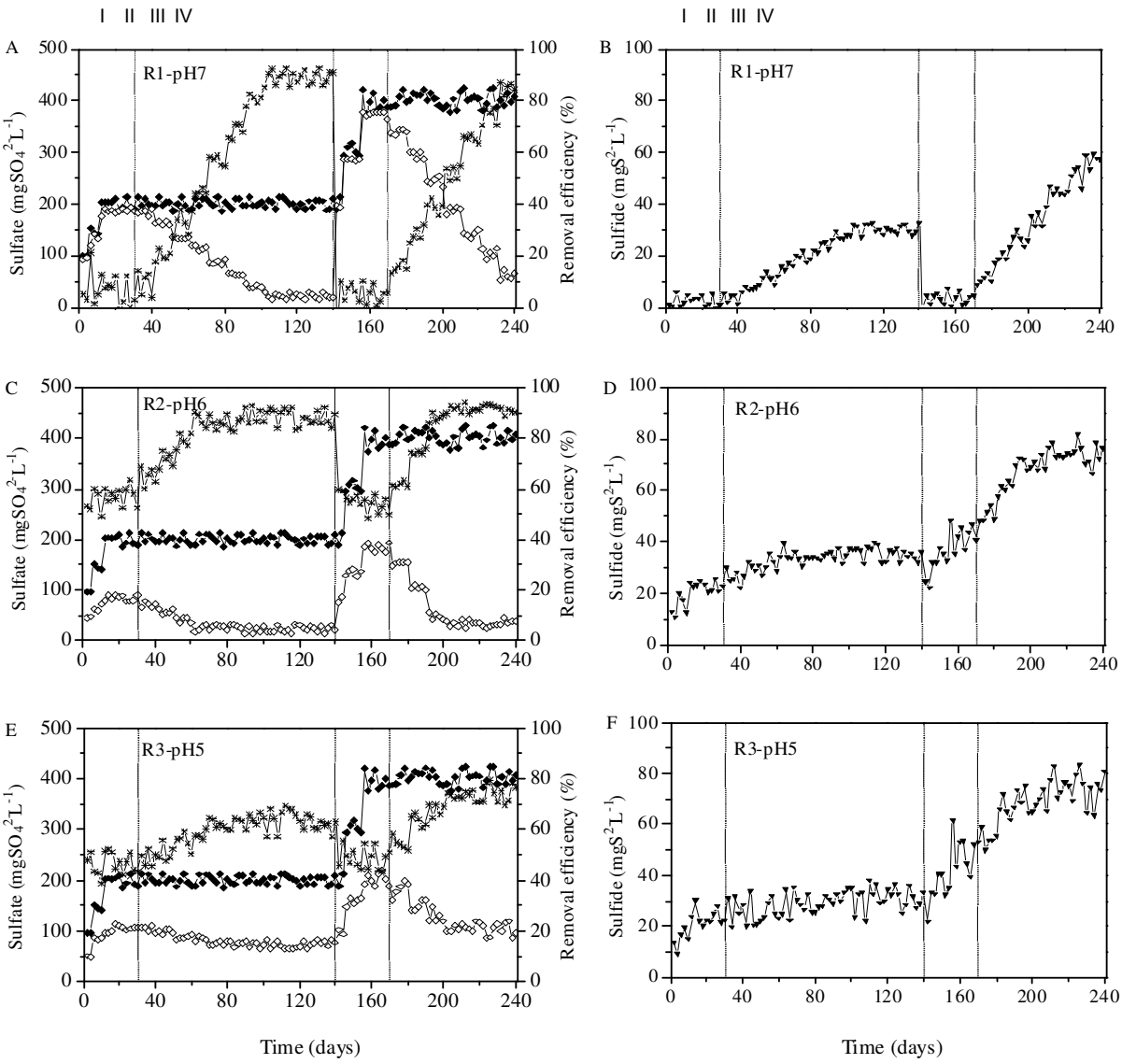

Figure 2. Effects of ethanol/SO_4^{2-} ratio and pH on mesophilic sulfate reduction. Sulfate removal efficiency **(*);** SO_4^{2-} in the influent **(◆);** SO_4^{2-} in the effluent **(◇);** S^{2-} in the effluent **(▼)**

mg/L in R2 with pH at 6. In Period III, the sulfate reduction rate fell back to 55% and the sulfide concentration increased to 42.7 mg/L in the effluent. In Period IV, the added ethanol (300 mg/L in influent) stimulated the sulfate reduction; sulfate reduction rate finally arrived at 91% and the effluent sulfide concentration fluctuated between 68 - 79 mg/L.

In R3 with pH at 5, sulfate reduction rate and effluent sulfide concentration in Period I were obtained to be 47% and 20.8 mg/L, respectively. As R1 and R2, the sulfate reduction rate and effluent sulfide level were both elevated by the addition of ethanol in R3 (In Periods II and IV). These results suggest that the level of ethanol in influent and pH value in UASB reactor can pose a significant effect on sulfate reduction.

Acidification products

The concentration of acidification products in the effluent of R1, R2 and R3 differed greatly from each other (Figure 3). The concentration of each acidification product (acetate and propionate) in the effluent slightly increased with a decrease in pH. The addition of ethanol also caused a decrease in the concentration of acidification products. In Period II of R1, VFA concentration in the effluent fell from 178 to 125 mgCOD/L and acetate concentration was reduced from 113 to 78 mgCOD/L. In Period IV, VFA concentrations went down from 217 to 133 mgCOD/L, while acetate level decreased from 142 to 72 mgCOD/L. The concentration of the propionate in the effluent also fell slightly during the period.

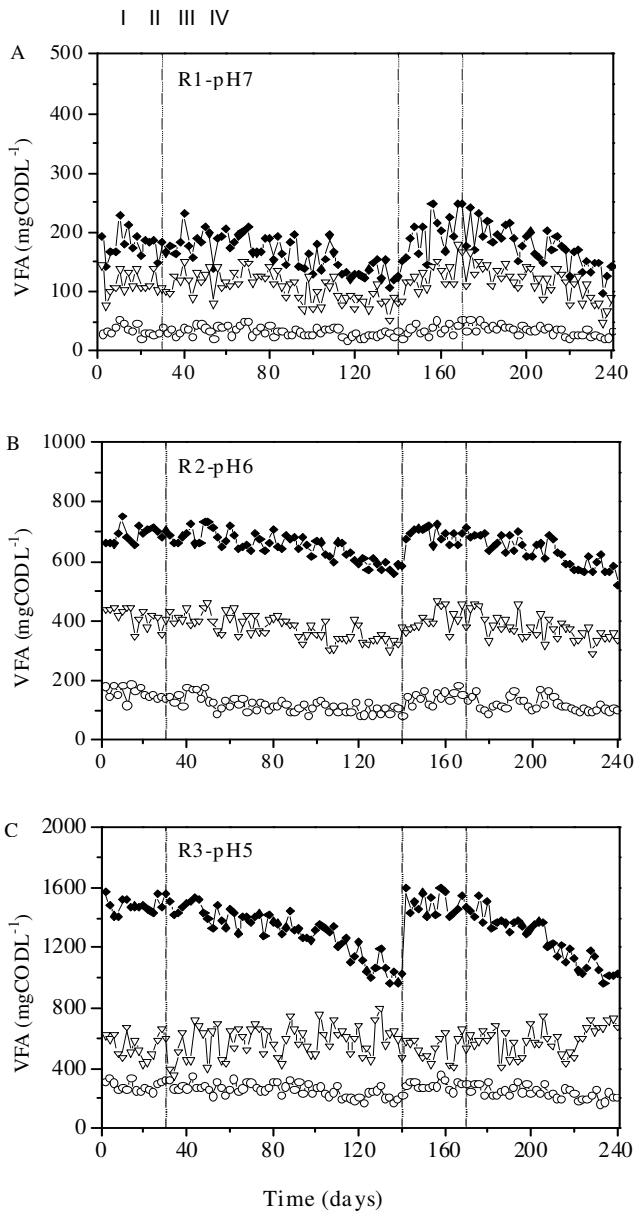

Figure 3. Effects of ethanol/SO_4^{2-} ratio and pH on acidification products of microbial metabolites. VFA **(◆);** acetate (▽); propionate (○).

During the four periods of R2 with pH at 6, the concentrations of various acidification products in the effluent showed the same variations as those in R1. The levels of acidification products in the effluent show no significant change with the variations of sulfide concentration and amount of added ethanol. However, the concentration of VFA and propionate was lowered after ethanol was added in Periods II and IV.

In R3, the concentration of acetate was about 645 mgCOD/L with pH at 5. However, during Periods I to II, VFA and propionate concentrations decreased from 1497 to 1016 mgCOD/L and from 287 to 202 mgCOD/L, respectively. They were further reduced during Periods III to IV (Figure 3).

COD removal

As shown in Figure 4, the decrease of pH led to a obvious decline of organic removal efficiency and specific methanogenic activity in the system. During the 240-days operation, the average COD removal efficiencies were obtained to be 86, 58 and 25% in R1, R2, R3, with specific methanogenic activity at 1.51, 0.95 and 0.36 L (L d) $^{-1}$, respectively. With the decrease in pH, alkali needed to be added in the system in order to keep acidity-alkalinity balance to avoid the further decrease of pH, which resulted from the over-high acidification rate. A smaller amount of VFA was converted to methane when pH was maintained at 5.

When ethanol concentration rose in the three bioreactors, the variation range of COD removal in R1 was the smallest. At the end of Periods I, II, III and IV of R1, COD removals were obtained to be 84, 89, 82 and 88% and the specific methanogenic activities were found to be 1.43, 1.55, 1.41 and 1.37 L (L d) $^{-1}$, respectively. The addition of ethanol led to a slight increase of COD removal. In R2, COD removal percentages increased from 46 to 61% and specific methanogenic activity was enhanced from 0.78 to 0.86 L (L d) $^{-1}$during the 240-days operation. The metabolic substrate was superabundant and close to one half of the substrate was not removed in R2, so that SBR competing against MPB for carbon source and the subsequent decrease of the specific methanogenic activity did not take place in the bioreactor. In R3, COD removal efficiencies and specific methanogenic activity were lower than R1 and R2, but the added ethanol could effectively promote the specific methanogenic activity in Periods II and IV.

DISCUSSION

In this study, it was found that methane was produced most efficiently in R1 with pH at 7. However, when sucrose was used as the single organic substrate, the sulfate reduction rate was relatively reduced in this reactor. With an increase of ethanol/SO_4^{2-}, the sulfate reduction rate was enhanced significantly, and the organic removal rate went up slightly in the whole process, suggesting that ethanol facilitated sulfate reduction and methane production. After pH was adjusted to 6 or 5, an increase in ethanol/SO_4^{2-} ratio promoted the sulfate reduction and improved the specific methanogenic activity.

In the course of substrate competition for anaerobic bacteria in the bioreactors, MPB mainly used acetate and H_2/CO_2 as substrates (Flaherty et al., 1998), while SRB utilized ethanol and lactate more effectively (Tatton and

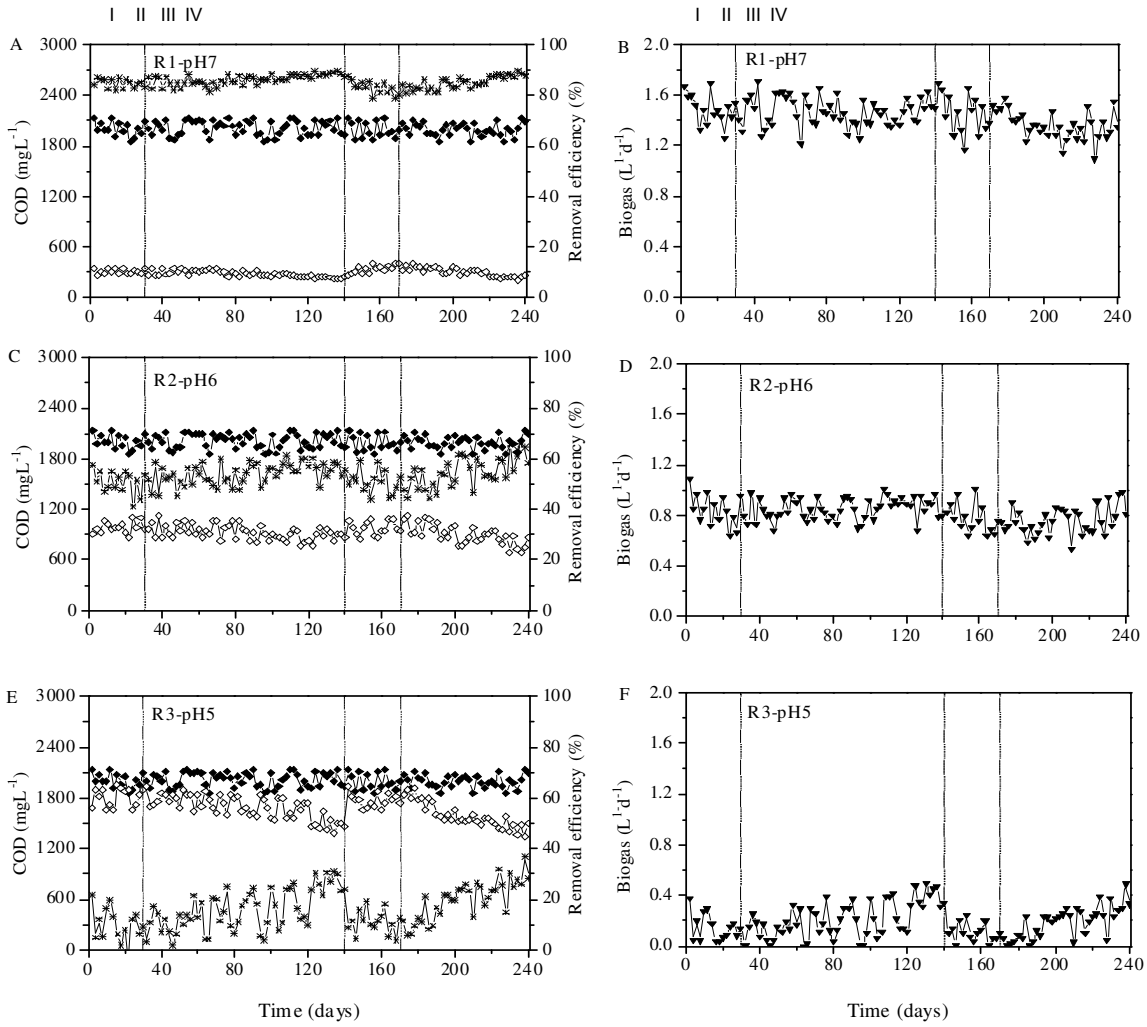

Figure 4. Effects of ethanol/SO_4^{2-} ratio and pH on COD removal efficiency and specific methanogenic activity. COD removal efficiency (*); COD in the influent (◆); COD in the effluent (◇); Biogas production (▼).

Parker, 1989). The intermediate products from the degradation of macromolecule organic by acidogenic bacteria (AB), e.g. propionate, could be utilized by AB and SRB (Flaherty et al., 1998). However, when the system contained high concentration of SO_4^{2-} and was in lack of preferential substrate, SRB would compete against MPB for acetate and H_2 (Henze and Harremoes, 1983). SRB utilized acetate and H_2/CO_2 less efficiently than MPB (Weijma et al., 2000). Moreover, most kinds of SRB could not degrade organic compounds completely, resulting in competition inhibition in the bioreactor, low efficiency and even failure of the system. Therefore, optimization of substrate ingredients could be employed to reduce the severe competition among various bacteria.

When COD/SO_4^{2-} was high and sucrose was used as the organic substrate, SRB and AB were found to compete for propionate, ethanol and lactate which were generated by sucrose acidification. Also, SRB and MPB were found to compete for acetate and H_2 which were generated by organic acidification. Although SRB had higher affinity for H_2 and acetate (Flaherty et al., 1998), its maximum substrate utilization rate was comparatively low. The high value of COD/SO_4^{2-} resulted in comparatively weak competition inhibition by SRB. AB and MPB would predominate in the bioreactor containing the substrates (Stefane and Oude, 2004; Jeong et al., 2008). However, once SRB are accessible to enough preferential substrates, that is, ethanol, it would be unnecessary for SRB to compete for carbon substrates, avoiding the inhibition reaction and promoting the sulfate reduction prompted by the competition against MPB. Moreover, in the system, the disappearance of competition pressure facilitated the utilization of acetate and H_2/CO_2 by MPB to produce methane. Part of the acetate generated by the incomplete metabolism of SRB could be further used by MPB. Thus, a substrate chain of co-metabolism was formed among SRB, AB and MPB. As a result, the sulfate reduction rate and the specific

methanogenic activity were significantly improved.

The specific methanogenic activity was reduced dramatically when pH values were decreased from 7 to 5. However, with the pH at 6 and no ethanol added in R2, the sulfate reduction rate was higher than that in R3 (pH 5), but lower than that in R1 (pH 7). A high level of sulfate reduction rate could be achieved when a proper amount of ethanol was added. Here, the adjustment of pH resulted in different effect for the improvement of the relationship between various bacteria caused by the substrate changes. The changes might inhibit the activity of some bacteria but promote the competitive advantages of other bacteria. The optimal pH range for MPB was rather narrow, but SRB and AB could work effectively in a wider range of pH (Tony and David, 2006). Therefore, decrease in pH resulted in severe inhibition of MPB activities, enabling SRB to gain advantages in the competition for H_2 and acetate. AB was also slightly influenced by the changes of pH. As a result, the specific methanogenic activity was evidently reduced but the sulfate reduction rate rose to some degree. However, the low pH had limited effect on the promotion of sulfate reduction rate for AB. Furthermore, decrease of pH could also lead to the inhibition of SRB activities.

The acidification efficiency and products of the reactor are considered as key factors influencing the treatment efficiency and the working stability of the sulfate reduction systems (Lopes et al., 2007a). With the pH at 7, the main acidification products were acetate, which decreased when ethanol was added. Addition of ethanol improved methanogenic activities and sulfate reduction capacities. When the pH was decreased to 6, the concentration of various acidification products rose to some degree, of which acetate increased most quickly. This maybe caused by the inhibition of MPB and subsequent block of metabolic pathways. At the same time, the acidification process of the organic retained a normal operation by SBR and AB and the general products were acetate. Addition of ethanol promoted the sulfate reduction rate and the methanogenic activities. Thus, the increase of ethanol/SO_4^{2-} ratio did not result in the obvious decrease of the amount of VFA.

When pH was adjusted to 5, the concentration of each acidification product (especially propionate) went up. These results suggested that the incomplete acidification of the organic by SBR and AB resulted in the rise of the acid with high molecular weight (Lopes et al., 2007b; Weijma et al., 2000). However, addition of ethanol improved SBR activities and reduced the amount of propionate. In addition to the incomplete acidification of the ethanol with the pH at 5, the amount of VFA was reduced in the reactor.

Conclusions

The adjustment of pH values could inhibit the activities of various bacteria to change the competitive advantages. The specific methanogenic activity was reduced evidently with a decrease of pH value from 7 to 5. The maximum sulfate reduction rate was achieved when pH was adjusted to 6.

Addition of ethanol can improve the microbial competitive capacity, methanogenic activities and promote the SO_4^{2-} reduction rate; and this effect was most remarkable when the pH was at 7. With pH at 7 in the bioreactor, the main acidification product was acetate and its amount decreased after ethanol was added. A decrease of pH significantly enhanced the proportion of propionate in VFA. However, addition of ethanol led to a decrease in the concentration of propionate, resulting in the improvement of competitive environment among SBR and MPB.

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