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RESEARCH ARTICLE

Feasibility assessment of up-flow anaerobic sludge blanket treatment of sulfamethoxazole pharmaceutical wastewater

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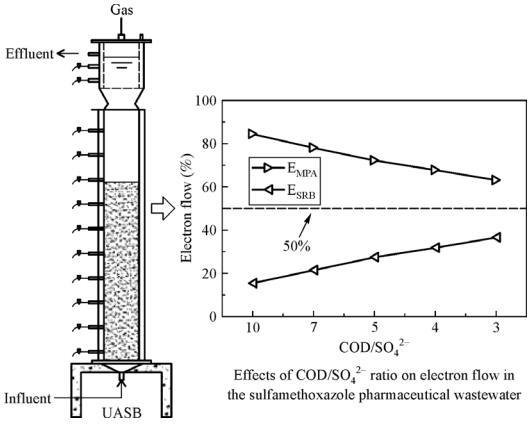
HIGHLIGHTS

- The UASB system successfully treated sulfamethoxazole pharmaceutical wastewater.
- High concentration sulfate of this wastewater was the main refractory factor.
- UASB recovery performance after a few days of inflow arrest was studied.
- The optimal UASB operating conditions for practical application were determined.

GRAPHIC ABSTRACT



Effects of COD/SO_4^{2-} ratio on electron flow in the sulfamethoxazole pharmaceutical wastewater

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ABSTRACT

Treatment of sulfamethoxazole pharmaceutical wastewater is a big challenge. In this study, a series of anaerobic evaluation tests on pharmaceutical wastewater from different operating units was conducted to evaluate the feasibility of using anaerobic digestion, and the results indicated that the key refractory factor for anaerobic treatment of this wastewater was the high sulfate concentration. A laboratory-scale up-flow anaerobic sludge blanket (UASB) reactor was operated for 195 days to investigate the effects of the influent chemical oxygen demand (COD), organic loading rate (OLR), and COD/SO_4^{2-} ratio on the biodegradation of sulfamethoxazole in pharmaceutical wastewater and the process performance. The electron flow indicated that methanogenesis was still the dominant reaction although sulfidogenesis was enhanced with a stepwise decrease in the influent COD/SO_4^{2-} ratio. For the treated sulfamethoxazole pharmaceutical wastewater, a COD of 4983 mg/L (diluted by 50%), OLR of 2.5 kg $COD/(m^3 \cdot d)$, and COD/SO_4^{2-} ratio of more than 5 were suitable for practical applications. The recovery performance indicated that the system could resume operation quickly even if production was halted for a few days.

1 Introduction

Sulfamethoxazole is used extensively to treat respiratory diseases because it is an inhibitor of *p*-aminobenzoic acid in the folic acid metabolic cycle and can inhibit the multiplication of bacteria (Jia et al., 2017). During its production process, a series of operations and chemical reactions occurs in the reactors and in different stages, which causes the release of a wide range of organic and inorganic constituents into the process wastewater including raw materials, solvents, products, intermediates, by-products, and waste products. Therefore, sulfamethoxazole pharmaceutical wastewater often contains high levels of organic pollution, biotoxicity, and salinity, which is

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difficult to be treated.

Anaerobic technology is a viable option for treating pharmaceutical wastewater owing to its advantages of high organic loading, lower sludge production, and lower operating cost (Chen et al., 2011), compared with conventional activated sludge processes. However, some antibiotics originating from production processes can severely inhibit the activities of anaerobic microorganisms (Svojitka et al., 2017). Many studies have focused solely on a single antibiotic such as sulfamethoxazole, which was added to synthetic wastewater depending on requirement of the particular study (Chen et al., 2017; Jia et al., 2017). In addition, few studies in the literature have regarded the anaerobic biodegradability characteristics of wastewater with complex organics (Das et al., 2015), including sulfamethoxazole pharmaceutical wastewater (Aydin et al., 2015; Cetecioglu et al., 2015), and little is known about the feasibility of using anaerobic digestion to deal with these types of wastewaters. Moreover, the presence of high sulfate concentrations in pharmaceutical wastewater can also affect the anaerobic digestion process. This is because the sulfate-reducing bacteria (SRB) utilize sulfate as the terminal electron acceptor and compete with methane-producing archaea (MPA) and homoacetogenic bacteria for carbon sources (Hu et al., 2015). At the same time, the sulfide converted from sulfate by SRB can be potentially toxic to MPA to thus decrease methane production (Liu et al., 2015). At present, the effect of sulfate on methanation has been researched mainly by using synthetic wastewater (Kiyuna et al., 2017). Despite the great advances achieved, few studies have focused on the anaerobic treatment of sulfamethoxazole pharmaceutical wastewater under a wide range of chemical oxygen demand (COD)/SO_4^{2-} ratio conditions. In addition, research thus far has not determined whether a UASB system fed with sulfamethoxazole pharmaceutical wastewater runs smoothly during long-term operation.

In this study, a series of anaerobic evaluation tests of different wastewater process units is conducted to evaluate the possibility of using anaerobic digestion to treat actual sulfamethoxazole production wastewater and to illustrate the key refractory factors of the anaerobic digestion. Based on the test results, a laboratory-scale UASB reactor operating for 195 days is used to investigate the purification performance under different operating conditions, including start-up and stable performance. Moreover, the recovery capacity of the UASB reactor within several days of stopping the inflow is elevated in order to simulate a production halt caused by business requirements or other reasons.

2 Materials and methods

2.1 Pharmaceutical wastewater and biomass

Wastewater was obtained from a sulfamethoxazole pharmaceutical company located in Shandong Province, China, and was stored in a 4°C laboratory refrigerator. Characteristics of the wastewater from different operating units in the sulfamethoxazole production process are shown in Table 1. The other constituents added to the wastewater included 750 mg/L KCl, 250 mg/L K_2HPO_4, 100 mg/L KH_2PO_4, 125 mg/L $MgCl_2 \cdot 6H_2O$, 15 mg/L $CaCl_2$, 42 mg/L $FeCl_2 \cdot 4H_2O$, 5 mg/L $CoCl_2 \cdot 6H_2O$, and 4.5 mg/L $NiCl_2 \cdot 6H_2O$. The sludge was drawn from a starch plant, which is also located in Shandong Province, China. The sludge was acclimated by adding sulfamethoxazole pharmaceutical wastewater for about two months.

During the experimental process, the UASB reactor influent contained a mixture of wastewater from different units according to the average proportion of an actual production process. This mixed wastewater contained approximately 10000 mg/L COD and a COD/SO_4^{2-} of 10.

Table 1 Characteristics of wastewaters originating from different units in the sulfamethoxazole production process

Wastewater	Flow rate (m^3/d)	Major ingredients	pH	COD (mg/L)	Sulfate (mg/L)	COD/SO_4^{2-}
Amide wastewater	80	Na_2SO_4, $(NH_4)_2SO_4$, $Na_2C_2O_4$, $(NH_4)_2C_2O_4$, $C_2H_4Cl_2$, amide	6.2	42000–46000	3000–5000	9.2–14
Acetylsulfanilyl Chloride (ASC) wastewater	30	Na_2SO_4, NaCl, sodium sulfanilate, ASC	1.5	15000	2000–2200	6.8–7.5
Dimethyl oxalate wastewater	80	$(NH_4)_2C_2O_4$, $(NH_4)_2SO_4$, $C_2H_4Cl_2$, CH_3OH	5	20000–24000	5000–8000	3–4
Refined wastewater	100	Na_2SO_4, NaCl, sulfamethoxazole	4.8	4400–5000	18000–20000	0.24–0.25
Amino content wastewater	60	$CHCl_3$, NaOH, NaCl, Na_2CO_3, amino compounds	14	20000–22000	200–1000	22–100
Condensation wastewater	130	Na_2SO_4, CH_3COOH, sulfamethoxazole, ammonia nitrobenzene, condensation compounds	6.2	27000–30000	16000–20000	1.5–1.7

2.2 Batch tests

A series of batch tests was conducted to evaluate the possibility of using anaerobic digestion to deal with the sulfamethoxazole pharmaceutical wastewater of different process units. The sludge was placed in a series of 250 mL vials with a total biomass concentration of 5.0 g VSS/L. The vials were inoculated with wastewater of the different units (Table 2 (a)) and different dilution ratio of raw water (Table 2 (b)). The vials were sealed with a butyl rubber stopper and an aluminum cap and were flushed with nitrogen gas for 30 min to establish anaerobic conditions. The initial pH value was fixed at 7.0–7.5. The vials were anaerobically incubated on a temperature-controlled shaker (150 rpm), with the temperature fixed at 35°C. Water samples were taken out from the vials for COD analysis, and the gas volumes were measured at a few time intervals. The batch tests were conducted in triplicate.

2.3 Experimental setup and operation

A schematic diagram of the experimental setup is presented in Fig. 1. The UASB reactor had a volume of 2.0 L, with an inner diameter of 7 cm and was covered with a black sponge to block light and to prevent the growth of phototrophic organisms. The reactor was temperature controlled at 35°C by using a thermostatic bath connected to the double wall of the reactor. The effluent from the gas–liquid–solid separator on top of the reactor was collected in a container. The produced biogas was connected to a wet gas flow meter via an alkali absorption equipment.

Table 3 summarizes the operating conditions applied to the UASB system in different experimental phases (I–V) over a period of 195 days. The influent pH value was adjusted to 7.0, and the COD/SO_4^{2-} ratio was kept at 10 in Phases I and II. The COD was increased from 2012 mg/L (raw water was diluted by 80%) to 7165 mg/L (diluted by 20%) in Phase I. In Phase II, the COD was lowered to 4983 mg/L (diluted by 50%), which was dependent on batch activity measurements and the operating conditions in Phase I. In addition, the organic loading rate (OLR) was increased from 1.7 kg COD/($m^3 \cdot d$) to 5.0 kg COD/($m^3 \cdot d$) by reducing the hydraulic residence time (HRT). In Phase III, the OLR was lowered to 2.5 kg COD/($m^3 \cdot d$), and the COD/SO_4^{2-} ratio was adjusted from 10 to 3 by adding Na_2SO_4. After 160 days of operation, the system performance decreased, and the influent was hence stopped, which simulated an actual halting of production (Phase IV). Then, the recovery experiment was initiated under the conditions of COD/SO_4^{2-} = 10, OLR = 2.5 kg COD/($m^3 \cdot d$), and COD = 4985 mg/L with 50% dilution (Phase V).

2.4 Analytical methods

The samples were filtered through a 0.45-μm polyethersulfone membrane prior to analysis. The COD, sulfate, sulfide, and volatile fatty acid (VFA) levels were measured

Table 2 (a) Sulfamethoxazole pharmaceutical wastewaters from different units in the vials

Number	Wastewater	Raw water volume (mL)	Sludge volume (mL)
1	Amide wastewater	220	30
2	ASC wastewater	220	30
3	Dimethyl oxalate wastewater	220	30
4	Refined wastewater	220	30
5	Amino content wastewater	220	30
6	Condensation wastewater	220	30
7	Condensation wastewater after desulfuration	220	30
8	Refined wastewater after desulfuration	220	30
9 (blank control)	Tap water	220	30

Table 2 (b) Different dilution ratios of raw water in the vials

Number	Raw water volume (mL)	Tap water volume (mL)	Sludge volume (mL)	Dilution ratio (%)	COD concentration (mg/L)
1	220	0	30	0	9850
2	176	44	30	20	7165
3	110	110	30	50	4983
4	66	154	30	70	2920
5	44	176	30	80	2012
6	22	198	30	90	1122
7 (blank control)	0	220	30	100	256

Table 3 Operational conditions of the UASB reactor

Period	Operating time (d)	HRT (h)	COD (mg/L)	Dilution ratio (%)	OLR (kg COD/(m^3·d))	COD/SO_4^{2-}
I	1–20	96	2012	80	0.5	10
	21–28	96	2920	70	0.7	10
	29–37	96	4983	50	1.2	10
	38–49	96	7165	20	1.8	10
II	50–57	72	4983	50	1.7	10
	58–66	48	4983	50	2.5	10
	67–79	36	4983	50	3.3	10
	80–96	24	4983	50	5.0	10
III	97–110	48	4983	50	2.5	7
	111–124	48	4983	50	2.5	5
	125–141	48	4983	50	2.5	4
	142–160	48	4983	50	2.5	3
IV	161–165	–	–			–
V	166–195	48	4983	50	2.5	10

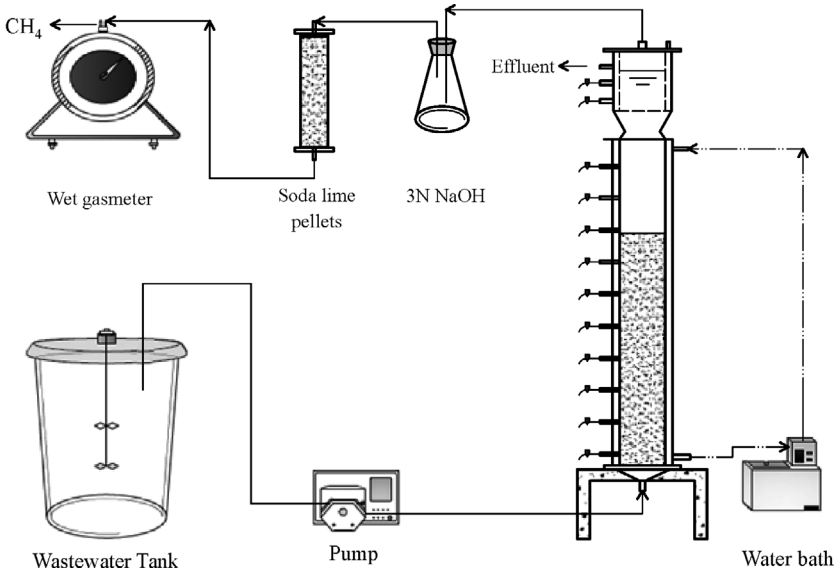

Fig. 1 Schematic diagram of the UASB system setup

according to American Public Health Association (APHA) standard methods (APHA, 2005). In addition, the aqueous H_2S concentration was calculated via Eq. (1) (Li et al., 2015). The pH was measured by using a pH meter (INESA, PHSJ-4F). The biogas production was measured via a wet gas meter (SHINAGAWA, W-NK-0.5) after washing with a 3 N NaOH solution. CH_4 was determined by gas chromatography (BFRL, SP-2100A), and H_2S in the biogas was measured by using hydrogen sulfide detecting tubes (Gastec, No. 4H). The proportions of electrons utilized by MPA and SRB were calculated via Eqs. (2) and (3), respectively (Hoa et al., 2007). All of the analytical estimates were made in triplicates; the average values are presented.

$$H_2S\ \text{fraction} = 1/(1 + (K_1/10^{-pH})) \quad (1)$$

$$\text{Percentage of eletron flow by MPA} = CH_4\text{–COD}/(CH_4\text{–COD} + H_2S\text{–COD}) \quad (2)$$

$$\text{Percentage of eletron flow by SRB} = H_2S\text{–COD}/(CH_4\text{–COD} + H_2S\text{–COD}) \quad (3)$$

In these equations, K_1 is the first ionization constant of H_2S; CH_4-COD = moles of CH_4 produced × 64 g; and H_2S-COD = moles of sulfide produced in gas and water × 64 g.

3 Results and discussion

3.1 Anaerobic evaluation tests of each part of the sulfamethoxazole pharmaceutical wastewater

3.1.1 Effect of sulfamethoxazole wastewater of different units on methane production

The methane yield and production rate were significantly affected by the wastewater of the different units and are shown in Fig. 2 (a) and (b), respectively. The yield and rate of the methane production, calculated by deducting the blank from the value, were the highest for the dimethyl oxalate wastewater. The order of methane production capability was dimethyl oxalate wastewater>amino content wastewater>amide wastewater>acetylsulfanilyl chloride (ASC) wastewater>refined wastewater = condensation wastewater. The methane production rates reached their highest values at 24 h, perhaps because the MPA had adapted to the new environment, and abundant carbon and energy were available at that moment. These demonstrate that using anaerobic digestion is a feasible method for treating wastewater, excluding refined wastewater and condensation wastewater, because MPA was severely inhibited in those two wastewaters, as indicated by the lower values than those of the blanks. The refined wastewater contained sulfamethoxazole, sodium chloride, and sodium sulfate, whereas the ingredients of the condensation wastewater included sulfamethoxazole, condensation products, sodium sulfate, acetic acid, ammonia nitrobenzene. The common characteristics of these wastewaters are high sulfate concentration, which reached 16000–20000 mg/L, and the presence of sulfamethoxazole. To clarify whether sulfamethoxazole toxicity or the sulfate effect, in the wastewater was the major restraint, further tests on the performance of the refined wastewater and condensation wastewater were performed after desulfurization; the results are shown in Fig. 2 (a) and (b). After the desulfurization process, the methane yields for the refined and condensation wastewaters increased from 0 to 75 mL and 60 mL, respectively. In particular, the methane production rate reached its maximum value (60 mL/d) in the refined wastewater after desulfurization. The following reasons may account for this phenomenon. The reduction of 1500 mg SO_4^{2-} consumes 1000 mg COD in the sulfidogenesis process (Sabumon, 2008); and SRB always predominate in carbon source utilization during high sulfate situation (Shin et al., 1997). Therefore, carbon sources were not sufficient for the MPA when high concentration sulfate exists. It could be speculated that the high sulfate concentration was the key reason for MPA inhibition. In addition, the COD/SO_4^{2-} ratio was a vital factor in the anaerobic digestion, which would be evaluated in the following study.

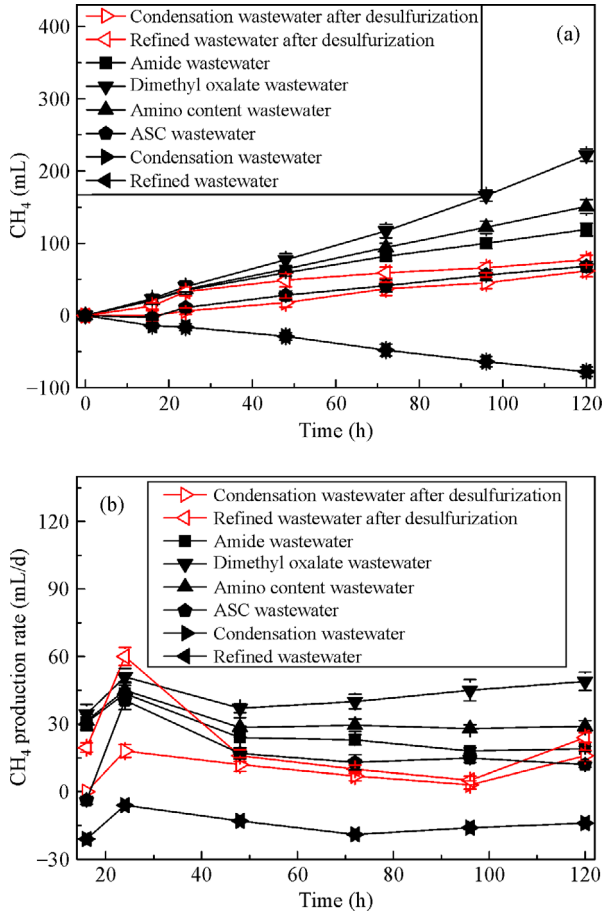

Fig. 2 Methane production (a) yield and (b) rate (deduction of blank) of wastewaters of different process units

3.1.2 Effect of different dilution ratios of wastewater on methane production

The UASB reactor influent was mixed with these wastewaters according to the average proportions of actual wastewater; the resultant mixed wastewater contained approximately 10000 mg/L COD and a COD/SO_4^{2-} ratio of 10. Considering that the raw wastewater had high levels of organic pollution, biotoxicity, and salinity, batch tests were performed to explore the MPA activity in different dilutions of the mixed wastewater.

The methane production yield under the different dilution ratios is displayed in Fig. 3 (a). After 20 days of operation, the methane production yield reached maximum values, i.e., 1080, 973, 847, 513, 487, and 440 mL CH_4/gVSS (deduction of blank) on day 24, with 0%, 20%, 50%, 70%, 80%, and 90% dilution, respectively. The slopes of the 70%, 80%, and 90% dilution ratios were similar to each other because the MPA was inhibited slightly by only a small amount of the wastewaters with low toxicity, which maintained similar activities.

The COD of the supernatant was determined at the

beginning and end of the experiments, and the relationship between the change in COD and methane production per unit volume of raw water was analyzed; the results are shown in Fig. 3 (b). The highest methane production for each unit volume of raw water had a 90% dilution ratio, whereas the lowest value was without dilution. This shows that a smaller dilution ratio is related to higher toxicity, which leads to sludge poisoning. In summary, 50% dilution of the raw wastewater (4983 mg/L COD) was chosen as the UASB system influent for the stable phase owing to better sludge activity, lower toxicity, and greater treatment capacity.

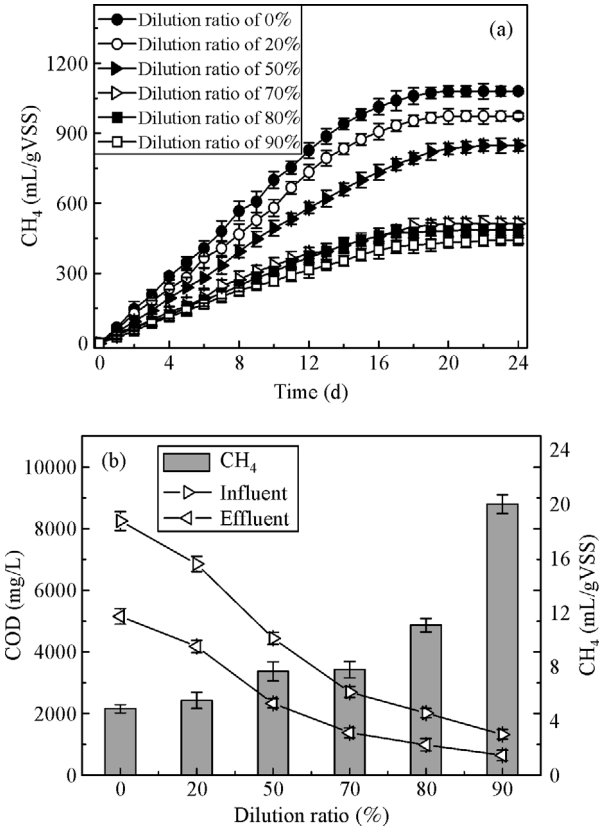

Fig. 3 Effect of different dilutions on (a) methane production yield and (b) methane production and COD per unit volume of raw water

3.2 Stable treatment performances of the UASB reactor

3.2.1 Effect of influent COD concentration on system performance (Phase I)

The system performance in terms of OLR, methane production, and COD, sulfate, sulfide, and VFA concentrations over time is displayed in Fig. 4. The start-up COD concentration of the UASB reactor was 2012 mg/L (raw water was diluted by 80%), and the HRT was 96 h. In Phase I, the influent COD concentrations increased gradually from 2012 (diluted by 80%) to 7165 (diluted by 20%) mg/L, resulting in an increase in methane production yield; however, the COD removal efficiency dropped from 61.3% to 55.6% (Fig. 5 (a)). These results are similar to those reported by Cetecioglu et al. (2015), who found that biogas production was parallel to influent COD concentrations in an anaerobic sequencing batch reactor (ASBR) fed with a selected synthetic substrate without the addition of sulfamethoxazole. As shown in Table 4, a previous study (Sponza and Demirden, 2007) reported a COD removal efficiency of 68% when using glucose as a carbon resource and adding 90 mg/L sulfamethoxazole, which is higher than our results using various organics such as acetate, dicarboxylate, trichloromethane, methanol, and sulfamethoxazole as carbon resources. The main reason for this may be that sulfamethoxazole pharmaceutical wastewater has more abundant and varied organic pollutants that are difficult to degrade. Furthermore, the addition of antibiotics such as sulfamethoxazole and benzothiazole cause system deterioration (Sponza and Demirden, 2007; Aydin et al., 2015; Cetecioglu et al., 2016; Li et al., 2017). The SO_4^{2-} was effectively removed with an increase in COD concentration which may be attributed to the abundant SRB. In summary, the COD concentrations of 4983 and 7165 mg/L had extremely significant effects on the methane yield ($P < 0.01$) compared with the COD concentration of 2012 mg/L. The batch tests also verified that a low dilution ratio was not favorable for COD removal; thus, the COD of 4983 mg/L was proved again to be an optimal operating condition for future research.

3.2.2 Effect of OLR on system performance (Phase II)

While maintaining the COD concentration at about 4983 mg/L (raw water was diluted by 50%), the HRT was shortened gradually from 72 h to 24 h to evaluate the effect of OLR on the UASB system performance. The OLR was increased from 1.7 to 5.0 kg COD/($m^3 \cdot d$), resulting in a decrease in COD removal efficiency and a slow increase in methane production (Fig. 4 (Phase II) and Fig. 5 (b)). In particular, the COD removal efficiency sharply decreased from 57% at the OLR of 1.7 kg COD/($m^3 \cdot d$) to about 36% at the OLR of 5.0 kg COD/($m^3 \cdot d$). The results are similar to those reported by Hu et al., who found that an excessive OLR results in COD removal deterioration (Hu et al., 2015). Moreover, the VFA concentration of the effluent obviously increased with an increase in OLR. The VFA value was about 340 mg/L at an OLR of 1.7 kg COD/($m^3 \cdot d$) and reached 750 mg/L at an OLR of 5.0 kg COD/($m^3 \cdot d$). These values are higher than those reported by Li et al., who found that the VFA level was approximately 220 mg/L at the OLR of 8 kg COD/($m^3 \cdot d$) and 450 mg/L at 12 kg COD/($m^3 \cdot d$) in chemically synthesized pharmaceutical wastewater (Li et al., 2015). In addition, they revealed

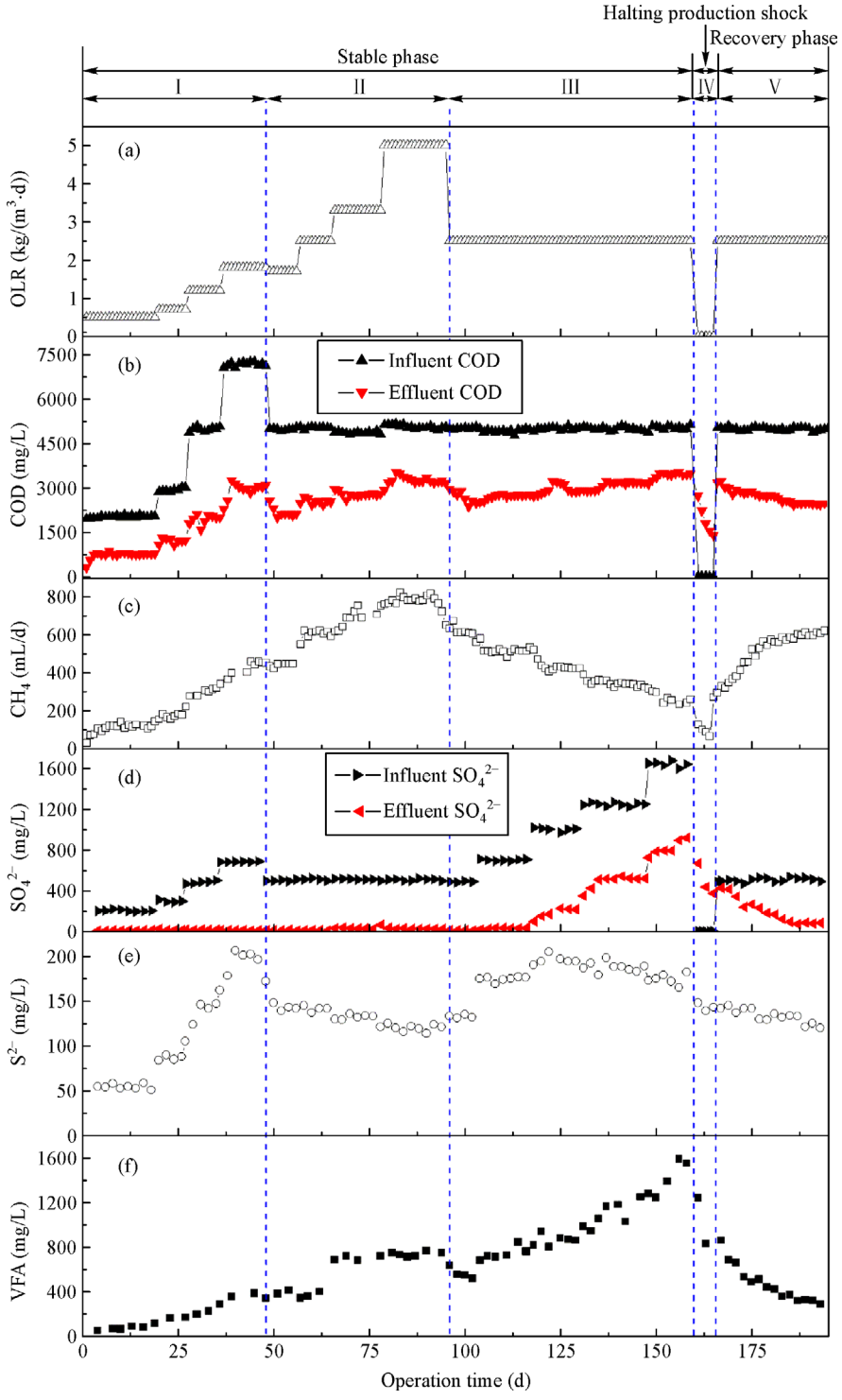

Fig. 4 Overall performance of the UASB system during continuous operation: (a) OLR, (b) influent and effluent COD concentrations, (c) methane production, (d) influent and effluent sulfate concentrations, (e) effluent dissolved sulfide concentration, and (f) effluent VFA concentration

that the increase in VFA was accompanied by a decrease in pH from 8.5 to 7.1. It could be concluded that the high VFA concentration is one of the reasons for deterioration of COD removal (Jing et al., 2013) and the sulfamethoxazole pharmaceutical wastewater was more easily acidified. Although the increase in methane production was accompanied by an increase in OLR, the uptrend slowed when the OLR exceeded 2.5 kg COD/($m^3 \cdot d$). As shown in Table 4, many researchers selected lower OLRs for pharmaceutical wastewater studies (Sponza and Demirden,

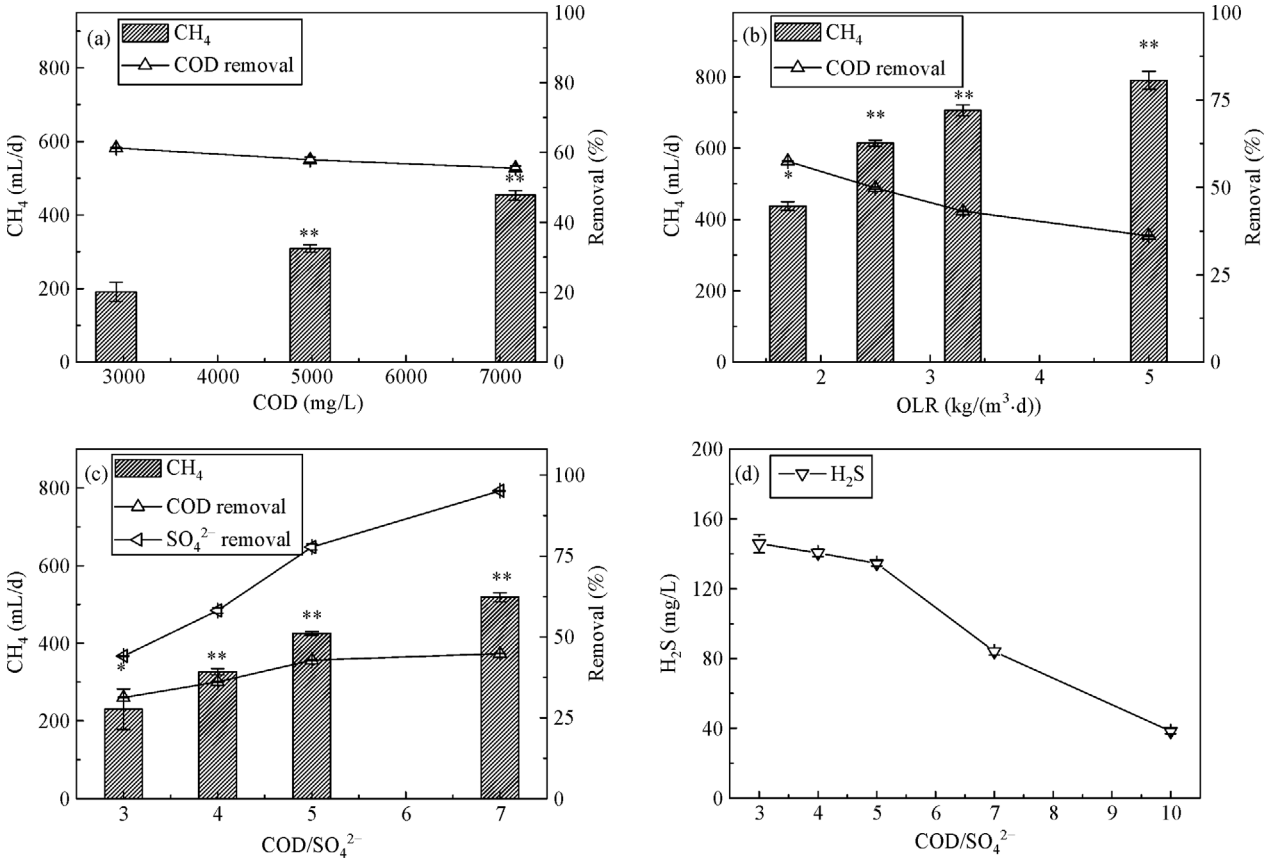

Fig. 5 Effects of (a) COD concentration and (b) OLR on COD removal efficiency and methane production and the effects of COD/SO_4^{2-} ratio on (c) COD removal efficiency, sulfate removal efficiency, methane production and (d) H_2S concentration; **, $P < 0.01$ indicates extremely significant difference whereas *, $P < 0.05$ indicates significant difference

2007; Aydin et al., 2015; Cetecioglu et al., 2016; Li et al., 2017). The COD removal efficiency was lower for the sulfamethoxazole pharmaceutical wastewater than for others because the raw wastewater (diluted by 50%) contained various organic pollutants was therefore complicated. Essentially, the OLRs of 2.5, 3.3, and 5.0 kg COD/($m^3 \cdot d$) had extremely significant effects on the methane yield ($P < 0.01$), compared with the OLR of 1.2 kg COD/($m^3 \cdot d$). Thus, in the case of COD removal and methane production, an OLR of 2.5 kg COD/($m^3 \cdot d$) is suitable for practical application in terms of sulfamethoxazole pharmaceutical wastewater.

3.2.3 Effect of COD/SO_4^{2-} ratio on treatment performance (Phase III)

The COD/SO_4^{2-} ratios of the refined wastewater and condensation wastewater were below 3, whereas those of the amide wastewater, ASC wastewater, and dimethyl oxalate wastewater were 3–14. It was necessary to research the COD/SO_4^{2-} ratio because it is an important parameter of the treatment performance of the anaerobic digestion process. This parameter it not only affects the competition between SRB and other anaerobic bacteria but also impacts the overall process performance (Lu et al., 2016). Therefore, the COD/SO_4^{2-} ratio was changed from 10 to 3 by adding Na_2SO_4 in order to test the effect of COD/SO_4^{2-} ratio on reactor performance. The results are shown in Fig. 4 (Phase III) and Fig. 5 (c) under the conditions of COD = 4983 mg/L (diluted by 50%) and OLR = 2.5 kg COD/($m^3 \cdot d$).

The COD removal efficiency gradually dropped to about 45% at a COD/SO_4^{2-} ratio of 8 and then sharply decreased to about 31% at a COD/SO_4^{2-} ratio of 3. In parallel, methane production decreased with decrease in the COD/SO_4^{2-} ratio, showing a trend similar to that for COD removal efficiency. Moreover, the effluent VFA values increased from 550 mg/L at a COD/SO_4^{2-} ratio of 10 to 1500 mg/L at a COD/SO_4^{2-} ratio of 3. These results are consistent with the observations of Li et al. (2015) in that a decrease in COD removal and methane content and an increase in VFA production (from 300 to 680 mg/L) were noticed when the COD/SO_4^{2-} ratio was further decreased to 1.5. Because the MPA were unable to utilize VFAs to produce methane, the system was obviously inhibited, which could be attributed to organic acid accumulation (Aydin et al., 2015; Cetecioglu et al., 2015). These observations indicated that the COD/SO_4^{2-} ratio exerted

Table 4 Summary of relevant literature regarding the effects of antibiotics on COD removal efficiency and methane production

Reactor	Carbon resource	Antibiotic type and concentration (mg/L)	COD concentration (mg/L)	OLR (kg COD /(m^3·d))	COD removal efficiency (%)	Methane production (mL/d)	Reference
ASBR[a]	Starch, glucose, sodium acetate, sodium butyrate, sodium propionate	/	2200	2.3	97.8	1004	(Cetecioglu et al., 2016)
		Sulfamethoxazole, 45	2200	2.3	25	96	
AFBR[b]	Glucose, acetate	/	3000	3	93	12740	(Li et al., 2017)
		Benzothiazole, 40	3000	3	80.9	11220	
SBR[c]	Starch, glucose, sodium acetate, sodium butyrate, sodium propionate	Erythromycin, 25; tetracycline, 2.5; sulfamethoxazole, 2.5	2500	1	65	600	(Aydin et al., 2015)
		Sulfamethoxazole, 2.5; tetracycline, 2.5	2500	1	10	100	
UASB + CSTR[d]	Glucose	Sulfamerazine, 90	3000	3.6–3.8	68	2850	(Sponza and Demirden, 2007)
UASB	Acetate, dicarboxyl, trichloromethane, methanol, sulfamethoxazole, etc.	Sulfamethoxazole, sodium sulfanilate, etc.	4983	1.2	58	312	This research

Note: [a] ASBR: anaerobic sequencing batch reactor; [b] AFBR: anaerobic fluidized-bed reactor; [c] SBR: sequencing batch reactor; [d] CSTR: continuously stirred tank reactor

an unfavorable effect on the removal of organics because the transfer routes of electron donors may have differed (Lu et al., 2016). It is interesting that the rise in effluent sulfate followed the increase in influent sulfate concentration. Generally, a reduction of 1500 mg SO_4^{2-} consumes 1000 mg COD in the sulfidogenesis process (Sabumon, 2008), and its consumption increases when the carbon requirement for SRB growth is considered. Therefore, the wastewater containing 4983 mg/L COD was sufficient for the competition between the SRB and MPA, which indicates that the activities of SRB and MPA were relatively stable. In addition, the sulfate removal efficiency decreased with an increase in influent sulfate concentration, which indicates that more influent sulfate was unused at a lower COD/SO_4^{2-} ratio (Hu et al., 2015). Furthermore, the increase in effluent dissolved sulfide also indicates that more sulfates had been transformed and that SRB was more favorable than the MPA to the lower COD/SO_4^{2-} ratio. Nonetheless, a mass of evidence (Tursman and Cork, 1989; Jing et al., 2013) in the literature has demonstrated the unfavorable effect of sulfide on SRB and MPA. Tursman and Cork (1989) reported that H_2S is a toxic form of sulfide because it diffuses into cell membranes. The H_2S concentrations increased with a decrease in the COD/SO_4^{2-} ratio and reached about 130–140 mg/L at COD/SO_4^{2-} ratios below 5, as shown in Fig. 5 (d). High H_2S concentrations may have been caused by the elevated influent sulfate concentration and the greater proliferation of the SRB (Lu et al., 2016). Jing et al. found that free H_2S greater than 110 mg/L caused inhibition of MPA (Jing et al., 2013). Thus, the activities of the MPA could have been inhibited at low COD/SO_4^{2-} ratios because of the high sensitivity of MPA to H_2S, which follows that reported by Jing et al.: High H_2S stops the normal growth of MPA and may even cause process failure (Jing et al., 2013). Moreover, the differences in methane yield between COD/SO_4^{2-} = 10 and COD/SO_4^{2-} = 5, 4, or 3 were extremely significant ($P < 0.01$). Hence, considering the maximization of bioenergy utilization from sulfamethoxazole wastewater and concurrent sulfate removal, the COD/SO_4^{2-} ratio should be higher than 5.

According to the CH_4 and H_2S composition in biogas and sulfide and the residual COD in the effluent, the COD conversion proportions at different COD/SO_4^{2-} ratios were obtained according to stoichiometry (Fig. 6 (a)). The data indicated that only 34.9% of influent COD was converted into methane and that 50.3% of the COD was left in the effluent at the COD/SO_4^{2-} ratio of 10. The methane conversion subsequently dropped with further decreases in the COD/SO_4^{2-} ratio. The level decreased to as low as 14.6% when the COD/SO_4^{2-} ratio was fixed at 3, with up to 69.0% of the COD retained in the effluent. In contrast, the effect of the COD/SO_4^{2-} ratio on COD utilization for sulfate reduction was almost within 5.6%–7.8%, which seemed much slighter than that in other reports, e.g., 3.6%–11.4% (Lu et al., 2016) and 5.2%–12.1% (Hu et al., 2015), when the COD/SO_4^{2-} ratio changed from 10 to 3. These diverse findings may be related to differences in the composition of the carbon source, sulfate concentration, and other environmental factors such as HRT.

The sulfate conversion was also analyzed by using the contents of different forms of sulfur; the results are shown in Fig. 6 (b). Although more than 86.4% of the influent sulfate was converted into sulfide at the COD/SO_4^{2-} ratio of 10, a sharp drop (30.6%) occurred when the COD/SO_4^{2-} ratio decreased to 3. This trend is similar to that reported by

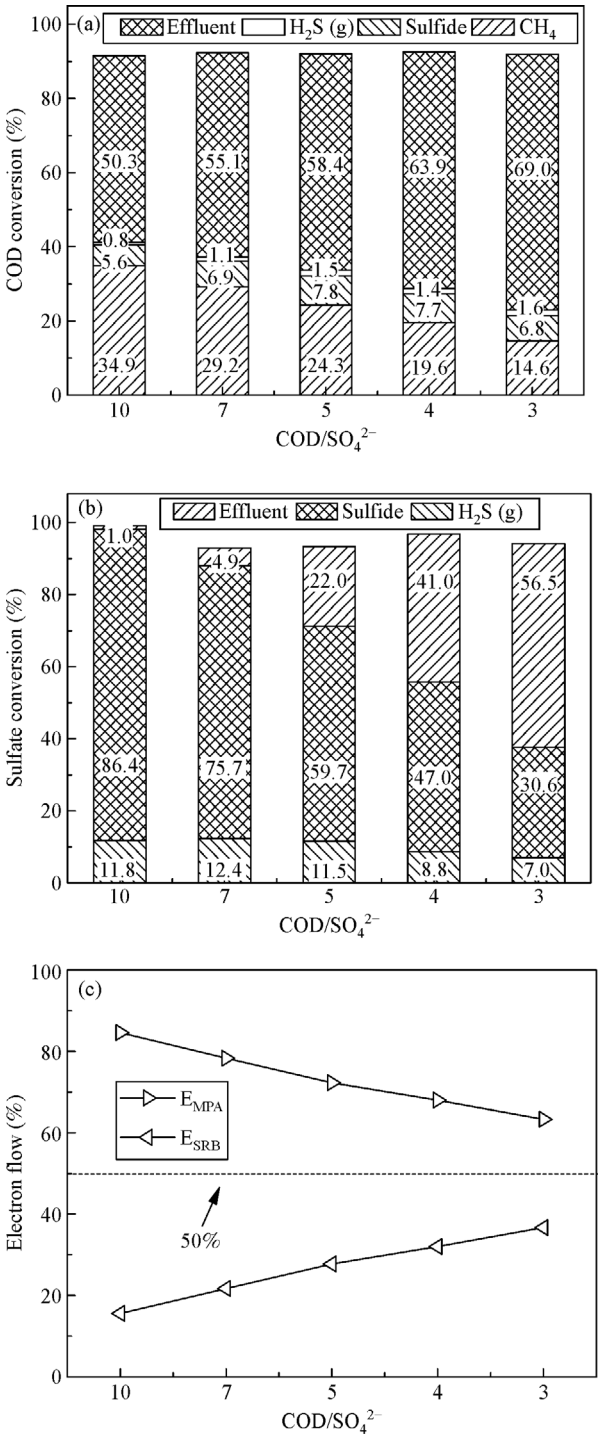

Fig. 6 Effects of COD/SO_4^{2-} ratio on (a) COD conversion, (b) sulfate conversion, and (c) electron flow

Hu et al., who used acetate and ethanol as carbon sources (Hu et al., 2015). However, the trend is different from that found by Lu et al., who used starch as a carbon source, for an uptrend from 52.7% to 77.1% (Lu et al., 2016). This may be explained by the following factor: Acetate oxidation is a rate-limiting step in the sulfate reducing reactor (Vallero et al., 2005) because the SRB utilizes acetate less efficiently than the MPA at low COD/SO_4^{2-} ratios (Weijma et al., 2000). The sulfide production recovered a very small fraction as gaseous H_2S and changed slightly, from 12.4% to 7.0%, which accorded well with the results stated by Hu et al. (2015).

Quantitative analysis of the electron flow to MPA and SRB is beneficial for evaluating their competition (Isa et al., 1986). The corresponding results of the COD/SO_4^{2-} ratio effect on electron flow, as shown in Fig. 6 (c), indicate that MPA always dominates in the UASB reactor at COD/SO_4^{2-} ratios of 10 to 3. For example, MPA utilized up to 86.3% of electrons when fed with sulfamethoxazole wastewater at the ratio of 10. This value is lower than that reported by Lu et al. (2016) who found that MPA is predominant at high COD/SO_4^{2-} ratios, utilizing up to 94.8% of electrons when fed with starch wastewater. However, the percentage of electrons utilized by MPA showed a clear drop with a decrease in the COD/SO_4^{2-} ratio, whereas the percentage of electrons consumed by SRB increased. The MPA utilized up to 62.3% of electrons at the COD/SO_4^{2-} ratio of 3, with only 37.7% left for the SRB, which indicates that the competitive advantage of MPA decreased at low COD/SO_4^{2-} ratios. These results indicate that COD biodegradation pathways, such as methanogenesis and sulfidogenesis, are closely related to the COD/SO_4^{2-} ratio (Lu et al., 2016). However, the outcome of the competition between SRB and MPA depended not only on the COD/SO_4^{2-} ratio but also was influenced by the operational conditions of the reactor, such as substance type, sludge concentration, HRT, and temperature. As shown in Table 5, some researchers found that MPA is predominant at the COD/SO_4^{2-} ratio of 3 (Jing et al., 2013; Hu et al., 2015; Lu et al., 2016), whereas others reported that SRB is predominant even at a COD/SO_4^{2-} ratio of 2 (Hoa et al., 2007; Jeong et al., 2009). Ethanol is often considered to be an excellent substrate for sulfate reduction because sulfidogenesis is always predominant in the presence of ethanol in sulfate-rich wastewater digestion (Jing et al., 2013), and MPA cannot utilize ethanol directly (Hu et al., 2015). Nevertheless, acetate favors methanogenesis over sulfate reduction under mesophilic conditions (Kaksonen and Puhakka, 2007). In addition, Hu et al. (2015) reported mutual acceleration for ethanol and acetate biodegradation in high sulfate situations. Jing et al. found that ethanol was utilized by SRB in sulfate reduction, with more energy and acetate supplied to MPA for methane production (Jing et al., 2013). The aforementioned investigations reflect the complexity and changeability of the interactions between SRB and MPA.

3.3 Halting production shock and reactor recovery (phases IV–V)

In real-world application, companies halt production to fulfill business requirements. Therefore, it was necessary to

Table 5 Summary of relevant literature on electron flow

Reactor	Substance	HRT (h)	OLR (kg COD/(m^3·d))	COD/SO_4^{2-}	MPA (%)	SRB (%)	Reference
UASB	Acetate, ethanol	4	11.9	3	82	18	(Hu et al., 2015)
UASB	Starch	6	4	3	81	19	(Lu et al., 2016)
UASB	Acetate, ethanol	4	18	1	70	30	(Jing et al., 2013)
UASB	Ethanol	5	1.6	2	21	79	(Hoa et al., 2007)
Bioreactor	Waste sewage sludge	240	1	2	4	96	(Jeong et al., 2009)
UASB	Acetate, dicarboxyl, trichloromethane, methanol, sulfamethoxazole, etc.	48	2.5	3	63	37	This research

research the performance and recovery of the reactor for such a situation. The influent was stopped for five days, from days 161 to 165, and the H_2S in the sludge was stripped by using nitrogen gas. The effluent COD, sulfate, methane, and VFA concentrations decreased further during the five-day period, as shown in Fig. 4 (Phase IV).

The recovery experiment was started under the conditions of COD/SO_4^{2-} = 10, OLR = 2.5 kg COD/(m^3·d), and COD = 4983 mg/L (raw water diluted by 50%). The system was operated for 30 days, from days 166 to 195, to evaluate the UASB performance. As shown in Fig. 4 (Phase V), the effluent COD decreased from 3170 mg/L to approximately 2470 mg/L, both values are lower than that measured before the production halting, at 3452 mg/L. In particular, after the recovery experiment was run for 20 days, the COD removal efficiency reached 50%, whereas the sulfate removal efficiency returned to 80%. This indicates that the effect of COD and sulfate degradation in the system after short-term recovery could be increased to the previous level. The methane production increased from 250 mL/d to 600 mL/d. It could be inferred that MPA activity can be restored and that MPA dominated when competing with SRB owing to the increase in COD/SO_4^{2-} ratio from 3 to 10. After the recovery experiment was run for 30 days, the VFA concentration trended downward from 1000 mg/L to 350 mg/L, which is lower than the 1582 mg/L recorded for the accumulation of VFAs in Phase III. Previous research has shown that anaerobic sludge activity is affected by VFA accumulation and that the system would not be obviously inhibited if the VFA concentration is below 400 mg/L (Mizuno et al., 1994). Because the VFAs were converted to CH_4, the removal efficiencies of COD and sulfates increased. In summary, this indicates that the system would resume quickly even if production were halted for a few days.

Additionally, continuous operation, from days 1 to 160, caused high levels of VFAs, H_2S, and COD and low COD and sulfate removal efficiencies. This occurred because production halting alleviated some problems and allowed the anaerobic sludge to rest in order to recover the activity of microorganisms. Hence, the halt of production by companies might be beneficial because the recovery experiment was positive, resulting in an improvement in the system's ability to treat more wastewater.

4 Conclusions

This study demonstrated the feasibility of laboratory-scale UASB reactors fed with sulfamethoxazole pharmaceutical wastewater and evaluated the performance of anaerobic treatment of sulfamethoxazole pharmaceutical wastewater under a wide range of COD/SO_4^{2-} ratio conditions. Anaerobic evaluation tests revealed that the main refractory factor of sulfamethoxazole wastewater from different process units was high sulfate concentration. The electron flow indicated that methanogenesis was still the dominant reaction even though sulfidogenesis was enhanced with a stepwise decrease in the influent COD/SO_4^{2-} ratio. This indicates that the outcome of competition between SRB and MPA depends on a combination of factors. A COD of 4983 mg/L (diluted by 50%), an OLR of 2.5 kg COD/(m^3·d), and a COD/SO_4^{2-} ratio exceeding 5 were suitable for practical application in terms of sulfamethoxazole pharmaceutical wastewater. This indicates that it is necessary to dilute pharmaceutical wastewater; moreover, lower OLR and higher COD/SO_4^{2-} ratio are feasible for anaerobic digestion when dealing with pharmaceutical wastewater. The recovery performance indicated that the system would resume quickly even if production were halted for a few days. These results provide new insights that reinforce the technical support and theoretical basis for the application of anaerobic digestion to pharmaceutical wastewater.

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