Enhanced sulfate reduction with acidogenic sulfate-reducing bacteria

Aijie Wang [a], Nanqi Ren [a],
Xu Wang [a], Duujong Lee [a,b,*]

[a] *School of Municipal & Environmental Engineering, Harbin Institute of Technology,
P.O. Box 2614, 202 Haihe Road, Harbin 150090, China*
[b] *Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan*

Abstract

Sulfate reduction in a continuous flow, acidogenic reactor using molasses wastewater as the carbon source was studied at varying chemical oxygen demand/sulfate (COD/SO_4^{2-}) ratios. At a critical COD/SO_4^{2-} ratio of 2.7, neither COD nor sulfate were in excess for extra production of ethanol or acetate in the reactor. An acetic-type microbial metabolism was established with sulfate-reducing bacteria (SRB) significantly consuming hydrogen and volatile fatty acids produced by acidogenic bacteria and hydrogen producing acetogens in degrading COD, thereby yielding sulfate removal rate >94.6%. A low critical COD/SO_4^{2-} ratio of 1.6 was also observed with the enriched ASRB population in reactor which overcomes the barrier to the treatment capability of sulfate-laden wastewater treatment with limited COD supply.
© 2007 Elsevier B.V. All rights reserved.

Keywords: Sulfate-reducing; COD/SO_4^{2-} ratio; Acetate; Metabolism

1. Introduction

The sulfate-reducing bacteria (SRB) present a unique microbial group responsible for the noted sulfate reduction capability with organic matters or hydrogen as electron donors. The SRB can suppress methane-producing bacteria (MPB) through competition for the same substrates such as hydrogen and acetate, and consequently result in a primary inhibition to MPB [1,2]. Moreover, the SRB can convert sulfate into sulfide which can poison MPB and decrease methane production [3–5]. The chemical oxygen demand (COD)/SO_4^{2-} ratio in influent significantly affected the metabolic pathways of SRB [6,7].

Five main microbial groups co-exist in the acidogenic sulfate-reducing reactor: acidogenic bacteria (AB), hydrogen producing acetogens (HPA), hydrogen-utilizing SRB (HSRB), acetic acid-utilizing SRB (ASRB), and fatty acid-utilizing SRB (FSRB). The FSRB includes propionic acid-utilizing SRB (p-SRB), butyric acid-utilizing SRB (b-SRB), and lactic acid-utilizing SRB (l-SRB). The SRB manipulate hydrogen transfer in the overall anaerobic metabolic processes [8]. Furthermore, the SRB compete better than the HPA for the same substrate, such as propionate, butyrate, and benzophenone [9]. The knowledge on how different co-existing microbial groups interact in such a complicated ecosystem is limited.

Wang [10] noted that the metabolites of sulfate-reducing reaction are mainly composed of acetate, indicating that the metabolic pathway resembles an "acetic acid-type microbial metabolism". Wang et al. [11] indicated that the electron-flow to SRB decreases with increasing COD/SO_4^{2-} ratio. These authors claimed that the SRB populations consumed hydrogen to a low level for achieving high sulfate removal efficiency of sulfate in the reactor.

This work aims at exploring the metabolic pathways of SRB in an acidogenic sulfate-reducing bioreactor and demonstrating how the acetic acid-type microbial metabolism was possibly formed in response to complicated interactions between different microbial populations. The accumulation of acetate as end product limits the sulfate treatment efficiency in the reactor. Therefore, this study enriched ASRB in the reactor to achieve

* Corresponding author at: Department of Chemical Engineering, National Taiwan University, Taipei 10617, Taiwan. Tel.: +886 2 23625632; fax: +886 2 23623040.
E-mail address: djlee@ntu.edu.tw (D. Lee).

Table 1
Operational conditions of continuous tests

Experiment phase	Operational conditions				
	COD/SO$_4{}^{2-}$ ratio	COD (mg l^{-1})	SO$_4{}^{2-}$ (mg l^{-1})	Ns (kg SO$_4{}^{2-}$ m^{-3} d^{-1})	HRT (h)
Phase a	4.5	3220	715	1.4	10.6
Phase b	3.6	3220	894	1.9	10.6
Phase c	2.7	3220	1190	2.5	10.6
Phase d	1.9	3220	1490	3.6	10.6
Phase e	0.9	3220	3580	7.5	10.6

complete oxidation of COD to effectively reduce sulfate at a low COD/SO$_4{}^{2-}$ ratio.

2. Materials and methods

2.1. Substrates and continuous tests

The acidogenic sulfate-reducing reactor, a continuous stirred tank reactor (CSTR) with an internal gas–liquid–solid three-phase separator used in Wang et al. [11], was the testing reactor. The reactor had an effective volume of 9.7 l. Sodium sulfate was the electron acceptor and molasses wastewater from a beet sugar refinery was the electron donor. Diammonium phosphate supplemented nitrogen and phosphate sources in molasses wastewater.

The waste activated sludge collected at the secondary clarifier in the wastewater treatment plant a petroleum refining plant was incubated in the molasses wastewater dosed with sodium sulfate without mixing for 1 month. This inoculum was seeded in the reactor at volatile suspended solids of 11.7 g l^{-1}. Since the generation time for SRB is generally larger than the HRT applied in this study (10.4 h) [12], hence, 1200 g of activated carbon (size 0.3–0.5 mm, specific gravity of 1.41 g cm^{-3}) was added to the reactor for biofilm development. On day 151 the alkalinity of influent was adjusted at 700 mg l^{-1} by supplying NaHCO$_3$. The reactor was design to be able to keep an anaerobic atmosphere but still with easy H$_2$S gas release. Temperatures was kept at 35 ± 1 °C in all tests. Nitrogen gas purged the bottles before the tests.

The reactor was started up with influent of COD/SO$_4{}^{2-}$ = 5/1, SO$_4{}^{2-}$ = 660 mg l^{-1}, and sulfate loading rate (Ns) = 1.4 kg SO$_4{}^{2-}$ m^{-3} d^{-1}. The hydraulic retention time (HRT) of the wastewater was set at 23.3 h during day 1–10, then the HRT was decreased to 10.6 h since day 11 and onward. After 30 days of start-up, the sulfate removal ratio reached up to 98%, pH dropped from 6.6 to 5.4–5.7, alkalinity increased from around 140 to 650–800 mg l^{-1}, and the oxidation reduction potentials

(ORP) dropped from −200 to −320 mV, S^{2-} concentration increased from 0 to 64 mg l^{-1}, and total volatile fatty acids (VFA) increased from around 100 to 1500 mg l^{-1}. The reactor was considered successfully started up when its performance was stable over 2 weeks.

After successful start-up of the reactor, the influent was adjusted according to the five phases indicated in Table 1. Restated, the reactor was operated sequentially at COD/SO$_4{}^{2-}$ ratio of 4.5, 3.6, 2.7, 1.9, and 0.9, with fixed COD (3220 mg l^{-1}) and HRT indicated. The operation was performed until the reactor had reached a steady-state, regarding the sulfate removal rate and the distributions of end products.

2.2. Enrichment test of ASRB

The waste activated sludge collected for the above continuous tests was incubated in a still suspension with added acetate and sulfate for 1 month. Then a CSTR of effective volume 1.0 l was seeded with the incubated sludge (VSS of 8.7 g l^{-1}) and was fed with an influent containing of acetate as sole carbon source (in COD of 1000 mg l^{-1}) and sulfate (500 mg l^{-1}) at an HRT of 9.6 h. Diammonium phosphate supplemented nitrogen and phosphate sources in molasses wastewater. Temperatures was kept at 35 ± 1 °C.

The sulfate removal ratio noted for the enriched bacteria using acetate as the sole carbon source increased with time, and reached over 80% after 20 days of enrichment (data not shown). Hence, the ASRB was enriched and activated in the bioreactor. Other SRB populations should be significantly inhibited owing to substrate limitation.

2.3. Substrates and batch tests

Parallel batch fermentation tests were conducted in 100 ml culture bottles, three parallel bottles under each experimental

Table 2
Comparison of metabolites from batch test A

Batch Test	End products (mg COD l^{-1})						Acetate/EtOH	EtOH/EtOH + VFA (%)	Acetate/EtOH + VFA (%)
	EtOH	Acetate	Propionate	Butyrate	Valerate	EtOH + VFA			
Test A-1	601	637	172	224	54.1	1690	1.1	35.6	37.7
Test A-2	283	1040	224	182	28.1	1750	3.7	16.1	59.0
Test A-3	414	934	219	225	43.2	1840	2.3	22.5	50.9

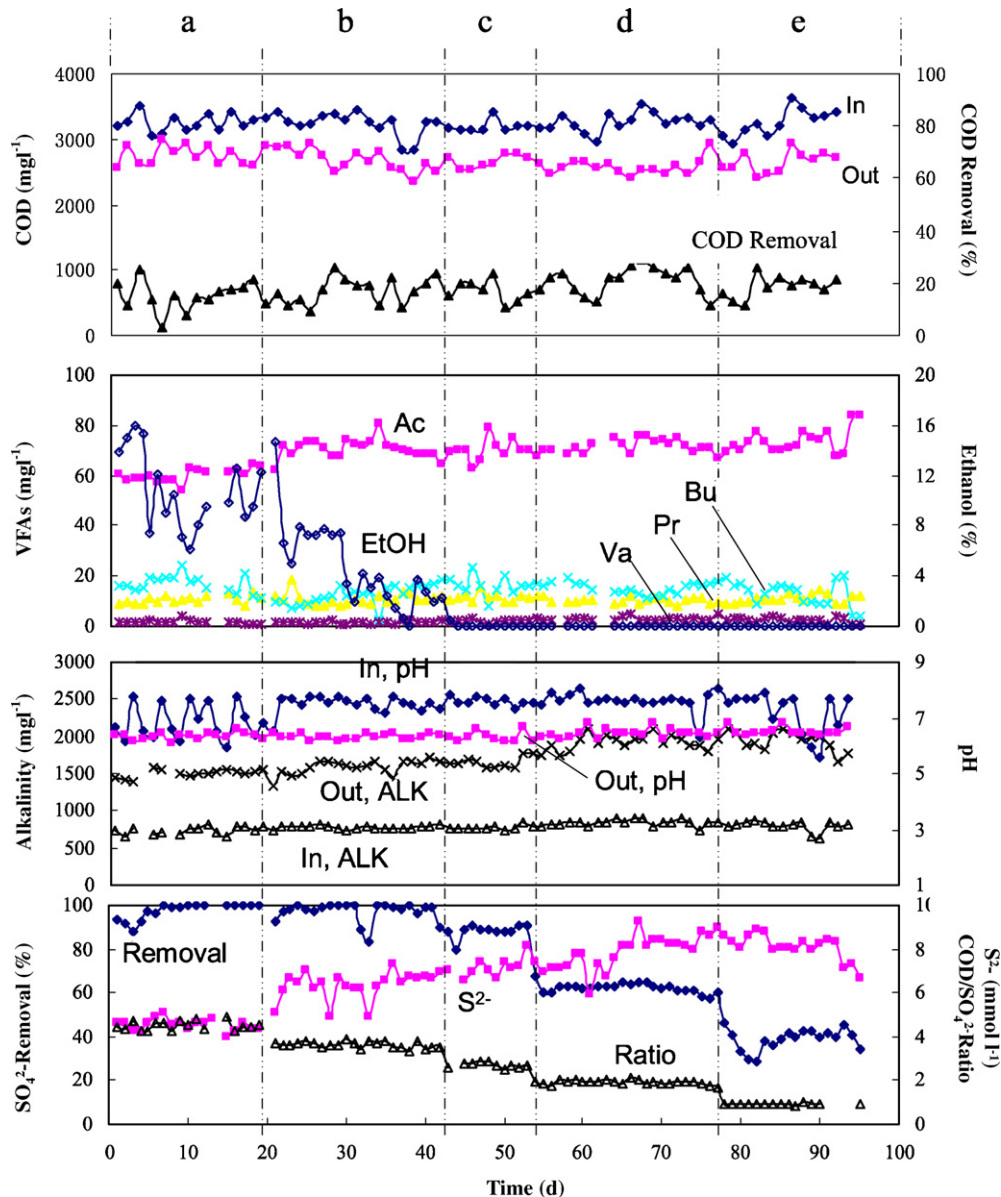COD/SO$_4{}^{2-}$ = 4.35, VFA is the sum of acetate, propionate, butyrate, and valerate.

Fig. 1. Parametric changes in continuous test with molasses wastewater. COD = 3220 mg l^{-1}. HRT = 10.6 h. Influent alkalinity = 670 mg l^{-1}. Phases (a–e) correspond to COD/SO$_4^{2-}$ ratio of 4.5, 3.6, 2.7, 1.9, and 0.9, respectively.

condition. Sludge samples collected from the mentioned acidogenic sulfate-reducing reactor under steady-state operation of COD/SO$_4^{2-}$ = 5/1 was collected and centrifugated (3000 rpm and 10 min) to remove supernatant. The obtained sediment was washed twice using milli-Q water.

Two sets of batch tests were performed. In batch test A, 25 ml sediment with 25 ml substrates (molasses wastewater, acetate, ethanol, propionate, butyrate, or lactate of initial concentrations listed in Table 3) were fed to the culture bottles with sulfate concentration fixed at 742 mg l^{-1}. In batch test B, the test was started with 25 ml molasses wastewater of COD 3220 mg l^{-1} dosed with 25 ml above-mentioned sediment. In test B-1 no sulfate was added. In test B-2, sulfate of concentration 742 mg l^{-1} was added initially. In test B-3, sulfate of concentration 742 mg l^{-1} was added only on hour 16 of fermentation. All bottles were sealed and incubated on shaken table at 35 °C and sampled regularly.

To investigate the utilizing capability of the enriched ASRB on ethanol and the VFAs, sludge samples collected from the ASRB enriched bioreactor under steady-state operation of COD/SO$_4^{2-}$ = 1/1 was collected and centrifugated (3000 rpm and 10 min) to remove supernatant. The obtained sediment was washed twice using milli-Q water and tested as those for test B (named test C).

2.4. Analytical methods

The chemical oxygen demand (COD), biochemical oxygen demand (BOD), mixed liquor suspended solids (MLSS), MLVSS, and pH of suspensions were determined according to Standard Methods [13]. The H$_2$S gas was analyzed using a GC-122 gas chromatography (Shanghai Analytical Apparatus Corporation, Shanghai, China) with a thermal conductivity

Table 3
Metabolites for batch test B

Substrate	Initial concentration (mg l^{-1})		End product (mg COD l^{-1})						SO$_4{}^{2-}$ removal ratio (%)	S^{2-} (mg l^{-1})
	COD	SO$_4{}^{2-}$	EtOH	Acetate	Propionate	Butyrate	Valerate	EtOH + VFA		
EtOH	4150	742	–	969	57.6	13.6	7.60	1050	99.9	28.2
Acetate	3020	742	5.61	–	156	64.2	25.7	252	7.5	0.64
Propionate	3420	742	16.9	121	–	53.6	12.3	203	22.2	3.84
Butyrate	3780	742	0.0	76.5	39.8	–	7.26	124	12.0	0.64
Lactate	3500	742	14.8	525	405	22.7	13.8	980	97.8	26.2

The substrate indicates the sole carbon source used in the test. VFA is the sum of acetate, propionate, butyrate, and valerate.

detector. Ethanol and volatile fatty acids (VFA) were also analyzed by a GC-122 gas chromatography using a flame-ionization detector (FID). The separation was accomplished by a 2.0-m stainless steel column packed with GDX103 (60/80 mesh). Nitrogen was the carrier gas at a flow rate of 60 ml min^{-1}. The flow rates of hydrogen and oxygen were 50 and 490 ml min^{-1}, respectively. The temperatures for detector and oven were 210 °C and 190 °C, respectively [14]. Sulfate concentration in suspension was determined using an ion exchange chromatography (CDD-6A, Shimadzu, Shimpack IC-AI, column temperature 40 °C, mobile phase 2.5 mM of potassium hydrogen phthalate). Sulfide concentration in suspension was determined with the methylene blue colorimetric method [13]. The H$_2$ and CO$_2$ contents in biogas were analyzed by a gas chromatography (Model SC-2 Shanghai Analytical Apparatus Corporation, Shanghai) using a 2.0-m stainless steel column packed with TDS-01 (60/80 mesh) and a thermal conductivity detector (TCD). Nitrogen was used as carrier gas at a flow rate of 70 ml min^{-1}. The temperatures for detector and oven were both at 150 °C. The oxidation–reduction potential (ORP) in the suspension (E_h) was calculated by the equation: $E_h = E_c + 249.1$ [mV], where E_c was the observed ORP measured by an acidity voltmeter (pHS-25, Shanghai Analytical Apparatus Corporation, Shanghai), and 249.1 [mV] was the potential of saturated calomel electrode.

3. Results and discussion

3.1. Continuous tests

The performance of the acidogenic sulfate-reducing reactor at HRT = 10.6 h, influent alkalinity of 670 mg l^{-1}, and

COD = 3220 mg l^{-1}, was shown in Fig. 1 at COD/SO$_4{}^{2-}$ ratio of 4.5, 3.6, 2.7, 1.9, and 0.9. At COD/SO$_4{}^{2-}$ = 4.5, the sulfate supply was stoichiometrically insufficient hence leading to almost complete removal of sulfate, low level of produced sulfide (4.5 mmol l^{-1}), and excess production of ethanol. At COD/SO$_4{}^{2-}$ = 3.6, the removal rate of sulfate was higher than 97%, the sulfide level was increased to 6.4 mmol l^{-1}, and the ethanol content was largely reduced. The corresponding acetate content was increased to above 70%. At COD/SO$_4{}^{2-}$ = 2.7, the removal rate of sulfate was reduced to around 88%, the sulfide level was increased to 7.2 mmol l^{-1}, while the ethanol content was reduced to zero. COD/SO$_4{}^{2-}$ = 1.9 and 0.9, the removal rate of sulfate was further reduced to 65% and 39%, respectively. The corresponding ethanol contents were all zero.

Hence, the above-mentioned tests revealed that at COD/SO$_4{}^{2-}$ = 2.7, the production of acetic acid was significantly enhanced, while that of ethanol was depressed to a rather low level. Ethanol presented in excess as intermediate when sulfate was insufficient in tests with COD/SO$_4{}^{2-}$ > 2.7. Meanwhile, with COD/SO$_4{}^{2-}$ < 2.7, sulfate was in excess to yield acetate was main final product. The metabolic pathway changed from the ethanol-type [14] to the acetate-type fermentation metabolism at COD/SO$_4{}^{2-}$ = 2.7.

3.2. Batch tests

The hydrogen contents detected in batch test A were shown in Fig. 2. Table 2 lists the corresponding end product distribution.

In test A-1, since no sulfate was present, SRB could not compete with other strains. Table 2 shows that the ethanol and acetate contributed 35.6% and 37.7%, respectively, to the end

Table 4
Metabolites for batch test C with enriched acetate-utilizing SRB

Substrate	Initial concentration (mg l^{-1})		End product (mg l^{-1})						SO$_4{}^{2-}$ removal ratio (%)	S^{2-} (mg l^{-1})
	COD	SO$_4{}^{2-}$	EtOH	Acetate	Propionate	Butyrate	Valerate	EtOH + VFA		
EtOH	756	400	451	158	38.3	6.57	0.89	655	96.2	7.36
Acetate	443	400	0	135	12.4	9.07	0.83	157	96.4	7.68
Propionate	786	500	0	210	450	36.1	2.04	698	94.6	7.04
Butyrate	897	500	0	78.9	20.4	365	2.21	467	95.9	7.04
Lactate	800	500	0	171	37.7	15.3	2.82	227	96.9	7.68
Molasses wastewater	800	500	130	554	103	57.6	10.5	855	97.4	9.28

The substrate indicates the sole carbon source used in the test. VFA is the sum of acetate, propionate, butyrate, and valerate.
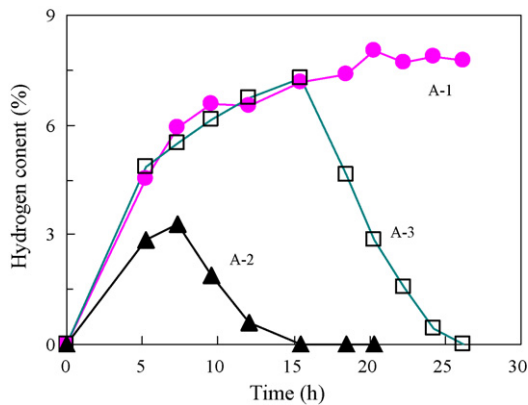
Fig. 2. Hydrogen content in batch test A. (A-1): No sulfate was added. (A-2): Sulfate of 600 mg l^{-1} was added. (A-3) Sulfate of 600 mg l^{-1} was added on hour 16.

liquid products, accounting for more than 70% of the total liquid products. AB produced hydrogen along with acid formation; hence, the hydrogen content in the headspace of culture bottles increased with fermenting time to a plateau of about 7.5%.

In test A-2, with supplied sulfate in the influent, the hydrogen in headspace increased to about 3% at 7 h of fermentation, then dropped to very low level at $t > 15$ h. In test A-3, hydrogen was formed and accumulated in the first 15 h without sulfate in influent. Then hydrogen was consumed once sulfate was added at $t > 15$ h. The hydrogen produced by AB group was consumed by the SRB group when sulfate was present.

The end product distributions in tests B and C differed markedly from in test A (Table 2). The ratios of (acetate/ethanol) were 3.7 and 2.3 for tests A-2 and A-3, higher than 1.1 for test A-1. This occurrence corresponded to the continuous-flow experiments (Fig. 1) that the co-cultured AB + SRB system utilized ethanol in sulfate-reducing reaction.

Table 3 lists the sulfate removal ratio and metabolites of batch test B. These tests with different pure substrates corresponded to COD/SO$_4^{2-}$ ranged 4.1–5.6. As shown in the continuous tests with molasses wastewater (Fig. 1), this sulfate supply should be insufficient, hence leading to almost complete removal of sulfate, low level of produced sulfide, and excess production of ethanol.

When supplied with ethanol and lactate as sole carbon resources, the sulfate removal rates and the total amount of VFAs produced were evidently higher than those supplying with acetate, propionate and butyrate as electron donors. When supplied with ethanol as sole carbon resource, most of the ethanol was converted into acetate. When lactate was fed instead, the end products were mainly acetate and propionate. On the other had, when supplied with acetate, propionate and butyrate as electron donors, few of sulfate was removed while few of fed substrate was converted into other kinds of VFAs, but they could be consumed by SRB population directly. Therefore, ethanol and lactate were SRB population's easily utilized substrate, but acetate, propionate and butyrate were not. More specifically, the utilizing capability of SRB followed ethanol > lactate > propionate > butyrate > acetate. It was indicated that from the viewpoint of thermodynamics, lactate,

propionate and butyrate were more available substrates for SRB than acetate [15].

3.3. Acetic-acid type microbial metabolism

In the acidogenic sulfate-reducing reactor, VFAs, ethanol and lactic acid could be converted to acetic acid quickly by FSRB and HPA, yielding more acetic acid as the terminal product. A so-called acetic-acid type microbial metabolism was thereby established in the studied acidogenic sulfating-reducing reactor [11].

An interpretation of such an occurrence is as follows. The H$_2$ produced by AB at degrading COD could be utilized by HSRB, while the VFAs and lactate produced by AB could be utilized by FSRB and HPA. Similarly, the acetate produced by FSRB and HPA could be utilized by ASRB, and H$_2$ produced by FSRB and HPA could be utilized by HSRB. Therefore, AB, FSRB, HSRB, ASRB and HPA formed bio-chains via the relationship of substrate provision. AB was at the first level of the bio-chains, FSRB and HPA were at the second, and HSRB and ASRB the third. The HSRB population in acedogenic sulfate-reducing reactor acted as an H$_2$-consumer and resulted in low hydrogen partial pressure. Since the available carbon resources (COD) were not limited in acedogenic sulfate-reducing reactor, in order to reserve more energy and get faster growth rate, FSRB competed against other populations and became dominate one. So, FSRB tended to utilize lactate, propionate and butyrate through non-complete-oxidation type metabolic pathway, and produce more acetic acid in the end products. The number and/or activity of acetic acid-utilizing SRB (ASRB) are apparently low in the studied microbial community.

Since acetate is one of preferred substrates for methanogenesis, the increased production of this compound could enhance efficiency of anaerobic treatment in a two-phase acidogenic bioreactor. Restated, if the herein studied sulfate-reducing reactor was adopted as the first phase reactor of the two-phase digesters, the noted acetic-acid type microbial metabolism assists the overall efficiency of the digestion process. However, the possible dampening effects of reduced pH to the second reactor should be taken into care. Apparently, the accumulated acetate is not beneficial to the treatment capability of sulfate-laden wastewater treatment with limited COD supply.

3.4. Enrichment of acetate-utilizing SRB

The ASRB population was enriched in a sulfate-reducing acidogenic bioreactor to improve the removal capability of sulfate from wastewater.

The enriched reactor was started up using acetate as the sole carbon source. With influent concentration of acetate (in COD of 1000 mg l^{-1}) and sulfate (500 mg l^{-1}) at an HRT of 9.6 h, the sulfate removal rate reached over 80% on 20 days of enrichment (data not shown).

Table 4 lists the sulfate removal ratio and metabolites of batch test C, with sludge samples collected from the enriched reactor under steady state. The present tests with various pure substrates, which differ from tests A and B, had a COD/SO$_4^{2-}$ ratio of

1.1–1.8. Restated, the present study should be COD-insufficient with reference to the results by continuous tests with molasses wastewater (Fig. 1). Moreover, the studied FSRB could not effectively utilize acetate, propionate, or butyrate as sole carbon source to reduce sulfate. However, the present, enriched ASRB effectively reduced sulfate using each of the five added substrates as the sole carbon source at a COD/SO$_4^{2-}$ ratio of 1.1–1.8. As Table 4 lists, the sulfate removal rates thus tested ranged 94.6–96.9%. The acetate contents were high in metabolites in all tests. Hence, the present ASRB could yield acetate as a product by degrading other VFAs, and further utilized the produced acetate to carbon dioxide to reduce sulfate.

The batch test using molasses wastewater (COD = 800 mg l^{-1}) dosed with 500 mg l^{-1} sulfate revealed a high removal rate of sulfate (97.4%) and high sulfide concentration (9.3 mg l^{-1}). The main metabolites were acetate (64.8%), ethanol (15.2), and propionate (12.0%). Restated, with the present ASRB population, COD was found in excess at a low COD/SO$_4^{2-}$ = 1.6, significantly lower than the critical value of 2.7 in Fig. 1. Hence, the adoption of enriched ASRB effectively overcomes the barrier to the treatment capability of sulfate-laden wastewater treatment with limited COD supply.

4. Conclusions

This work investigated the sulfate reduction reactions in an acidogenic sulfate-reducing reactor at varying COD/SO$_4^{2-}$ ratios. Continuous test with molasses wastewater as the carbon source revealed that a critical COD/SO$_4^{2-}$ ratio near 2.7 existed, above which COD in excess, the sulfate was nearly completely removed, yielding extra production of ethanol; below which sulfate was in excess to reduce sulfate removal rate and acetate was largely yielded. Since the SRB consumes hydrogen and volatile fatty acids produced by acidogenic bacteria and hydrogen producing acetogens in degrading COD, an acetic acid-type microbial metabolism was established. The abundant acetate in end product is beneficial to the methanogenesis efficiency in a subsequent methane digester, but limits the sulfate reduction efficiency in utilizing COD. The ASRB was enriched in the reactor and was noted to degrade ethanol, acetate, propionate, butyrate, and valerate to yield sulfate removal rate above 94.6%. Using

the enriched ASRB population a low critical COD/SO$_4^{2-}$ ratio of 1.6 was achieved.

References

[1] P.H. Nielsen, Bio-film dynamics and kinetics during high-rate sulfate reduction under anaerobic conditions, Appl. Environ. Microbiol. 53 (1987) 27–32.

[2] E. Chio, J.M. Rim, Competition and inhibition of sulfate reducers and methane producers in anaerobic treatment, Water Sci. Technol. 23 (1991) 1259–1264.

[3] J.P. Maree, W.F. Strydom, Biological sulfate removal in an up-flow packed bed reactor, Water Res. 19 (1985) 1101–1106.

[4] S. Kalyuzhnyi, V. Fedorovich, P.N.L. Lens, L.W.H. Pol, L. Gatze, Mathematical modeling as a tool to study population dynamics between sulfate reducing and methanogenic bacteria, Biodegradation 9 (1998) 187–199.

[5] L.W.H. Pol, P.N.L. Lens, A.J.M. Stams, G. Lettinga, Anaerobic treatment of sulphate-rich wastewaters, Biodegradation 9 (1998) 213–224.

[6] G.F. Parkin, Anaerobic filter treatment of sulfate-containing wastewaters, Water Sci. Technol. 23 (1991) 1283–1291.

[7] O. Mizuno, Y.Y. Li, T. Noike, The behavior of sulfate-reducing bacteria in acidogenic phase of anaerobic digestion, Water Res. 32 (1998) 1626–1634.

[8] Y.Y. Li, H.H.P. Fang, Interactions between methanogenic, sulfate-reducing and syntrophic acetogenic bacteria in the anaerobic degradation of benaozte, Water Res. 30 (1996) 1555–1562.

[9] O. Mizuno, Y.Y. Li, T. Noike, Effects of sulfate concentration and sludge retention time on the interaction between methane production and sulfate reduction for buturate, Water Sci. Technol. 30 (1994) 45–54.

[10] A.J. Wang, Quantification and control of restrictive ecological factors in acidogenic de-sulfate bioreactor, J. Harbin Inst. Technol. 9 (2002) 32–37.

[11] A.J. Wang, N.Q. Ren, Q.L. Zhao, J.C. Huang, Role of sulfate-reducing bacteria in acidogenic bioreactor. Int. J. Environ. Pollut., in press.

[12] S. Vladislav, T. Sava, Application of fluidized carrier to bacterial sulphate reduction in industrial wastewaters purification, Biotechnol. Tech. 6 (1992) 91–96.

[13] APHA, Standards Methods for the Examination of Water and Wastewater, 19th ed., American Public Health Association, Washington, DC, USA, 1995.

[14] N. Ren, B. Wang, J.C. Huang, Ethanol-type fermentation from carbohydrate in high rate acidogenic reactor, Biotechnol. Bioeng. 54 (1997) 428–433.

[15] L.L. Barton, Sulfate-Reducing Bacteria, Plenum Press, New York, 1995.