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Biological pre-treatment of wastewater containing sulfate using anaerobic immobilized cells

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Abstract

Biological reduction of sulfate to sulfide using sulfate reducing bacteria (SRB) was investigated. A respirometer was used to study the sulfide toxicity in the systems fed glucose, the results showed that sulfide would start to inhibit methanogens when the dissolved sulfide and total sulfide concentrations were 276.4 and 304.6 mg/L, respectively. When chemostats were used to study the Monod kinetic coefficients, Y, k_d, K_s, and k were 0.36 mg VSS (volatile suspended solids) using SRB/mg SO_4-S, 0.05/day, 147.30 mg SO_4-S/L, and 6.50 mg SO_4-S/mg VSS using SRB-d, respectively. Using pure cultural techniques, SRB were found to be 29.45% of the VSS in the chemostats. Sulfate removal using an upflow anaerobic filter packed with immobilized cells was also investigated. Under sulfate loading rates of 0.2 and 0.4 g SO_4-S/L day, and a hydraulic retention time (HRT) of 2 days, a sulfate removal efficiency greater than 93% could be achieved. When the filter was operated under COD (chemical oxygen demand)/S from 10/1 to 5/1 and HRTs of 2, 1 and 0.5 days, sulfate removal efficiency was between 98.1 and 70.9%. It is believed that protection by the immobilized cell structure caused the microbial cells in the filter to tolerate higher dissolved sulfide (447.8 mg/L) and total sulfide (940.3 mg/L) levels, allowing a much higher biomass concentration (13.2–13.5 g VSS/L) to be reached.
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Keywords: Sulfide toxicity; Sulfate reduction; Bubble respirometer; Immobilized cells; Methane production

1. Introduction

Anaerobic biological treatment processes have been used in the treatment of sewage sludge for more than a century [1,2]. The advantages of using anaerobic processes include: (i) the excessive biomass production is only about 1/3–1/5 of the typical aerobic process allowing the subsequent sludge treatment cost to reduced; (ii) with a low assimilated biomass, the amount of nutrients required for biomass synthesis is low; (iii) no aeration is required, thus power consumption can be reduced; (iv) under standard temperature and pressure, removal of 1 kg COD can produce 0.35 m^3 methane; (v) even with no feeding for a period of time the system can remain dormant. When feeding is restored, the system can return to normal operation within a short period of time; (vi) unlike aerobic systems, anaerobic systems are not restricted to electron acceptor limitations (i.e., molecular oxygen), thus the system can be loaded under a much higher concentration than aerobic systems [2–4].

The anaerobic process has some disadvantages: (i) the reaction rate is only 1/4–1/10 of a typical aerobic process; (ii) the low growth rate from the low reaction rate requires a longer start-up time and takes a longer time to recover from fluctuations such as pH, temperature, and organic loads; (iii) with a low growth yield, a high biomass concentration is not easy to maintain when the influent concentration is low; (iv) anaerobic treatment is operated under reduced conditions and produces volatile acids, mercaptans, and hydrogen sulfide causing odor problems; (v) due to the low growth rate, anaerobic microorganisms are more sensitive to inhibitory or toxic compounds.

For some industries such as pulp, chemical, metallurgical, and sulfite liquor producing high strength sulfate wastes from flue-gas, the reduction of sulfate into sulfide causes odors, corrosion, a low methane yield, and toxicity problems during the anaerobic treatment processes. Sulfide

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accumulation in the reactor inhibits the anaerobic bacteria, and can cause a complete system shutdown. Various organic electron donors have been used to study the effect of sulfide toxicity on methanogens [5–7]. For a typical suspended growth reactor with acetate or butyrate as the electron donor, when the dissolved sulfide (DS) levels reached about 150–200 mg/L or the H_2S-S reached 60–75 mg/L, the inhibition to the system started. But, for system fed lactate or glucose, even when the DS reached 200–400 mg/L or the H_2S-S reached 100–150 mg/L, the system could still stay stable and without significant inhibition. If sulfate in the influent could be biologically converted into sulfide and then into elemental sulfur, sulfate removal from the system could be achieved and the subsequent anaerobic treatment and methane utilization could proceed.

In the design and operation of a traditional suspended growth bioreactor such as activated sludge process, one key factor to success is how to achieve a higher solid retention time (SRT) and operate under a lower hydraulic retention time (HRT). A higher SRT means a higher biomass concentration, while a lower HRT means a smaller reactor volume. This kind of operational strategy relies very much on a good settling of the final clarifier. Immobilized cell technology can be applied in biological treatment to meet this requirement [8–10]. Immobilized cell applications in biological treatment processes have the following advantages: (i) The biomass is easily retained and no recirculation is required. (ii) With a higher biomass concentration in the reactor, the system can tolerate higher hydraulic or organic loads. (iii) With a longer sludge age, a lower yield would alleviate sludge handling and treatment problems. This is estimated to account for only about 20–30% of the traditional activated sludge process. (iv) This system is easy to operate and maintain. Solids separation is easy and no final clarifier is needed. (v) Depending upon the field requirements, a specific enriched culture can be selected, processed, and applied in a target compound or wastewater. (vi) The coexistence of aerobic, anoxic, and anaerobic environments becomes possible because of the interaction between the microbial oxygen demand and molecular oxygen transfer. This method can provide for more diversified microorganism species within the system. (vii) When the system is not in use, the immobilized cells can be preserved at 3 °C for future restoration.

The objective of this study was to develop a bioreactor for sulfate reduction into sulfide. Once the sulfate is converted into sulfide, it can be easily treated chemically or biologically into elemental sulfur [11–13]. The toxicity from sulfate and sulfide to the microorganisms was investigated using a bubble respirometer. The Monod kinetic coefficients of sulfate reduction were studied using chemostats. To determine the fraction of SRB in the biomass (expressed in mg/L of VSS), the relationship between the pure culture SRB and VSS was established. To retain a higher biomass concentration in the reactor so that the system could be operated under higher hydraulic and organic loads, immobilized cells using cellulose triacetate (CTA) were used in the reactor.

2. Materials and methods

2.1. Experimental setup

2.1.1. Sulfate enriched SRB culture

The SRB seeds were taken from the supernatant of an anaerobic digestion tank from swine wastewater near the campus. A sulfate enriched culture was grown in a 10 L glass bottle under the fill-and-draw mode, and maintained at a 25-day SRT by wasting and feeding 400 mL daily. Daily wasting was done by mixing first with nitrogen gas followed by the withdrawal of 400 mL of mixed liquor. An organic loading rate (OLR) of 0.5 g COD/L day was applied using glucose. The glucose served as the sole carbon and energy source, while the sulfate served as the electron acceptor. The inorganic compounds and their concentrations in the feed solution are shown in Table 1. The COD/S was maintained at 20/1. This ratio was decreased to 10/1 and 5/1 for the chemostat and upflow filter experiments.

2.1.2. Immobilized cells

Cellulose triacetate (CTA) was procured from Showa Chemical Inc., Japan, with the formula of $[C_6H_7O_2(OH)_3 \cdot m(CH_3COO)_m]n$, where $m = 0$–3. This is a white powdered

Table 1
Nutrient compositions in the influent feed

Constituent	Concentration (mg/L)	Constituent	Concentration (mg/L)
Continuous feed			
NH_4Cl	400	NH_4VO_3	0.5
$MgCl_2$	400	$ZnCl_2$	0.5
KCl	400	$Na_2MoO_4 \cdot 2H_2O$	0.5
$CaCl_2 \cdot 2H_2O$	25	H_3BO_3	0.5
$CoCl_2 \cdot 6H_2O$	2.5	$NiCl_2 \cdot 6H_2O$	0.5
KI	2.5	Glucose	Depending on OLR
$MnCl_2 \cdot 4H_2O$	2.5	$MgSO_4 \cdot 7H_2O$	Depending on COD/S
Manual Feed (10 mL added daily)			
$(NH_4)_2HPO_4$	960	$(NaPO_3)_6$	120
$FeCl_2 \cdot 4H_2O$	480	Cysteine	120

chemical. The degree of acetylation was 66% and the average degree of polymerization was 300. In the immobilized cell preparation, 50 g of CTA was mixed with 500 mL of dichloromethane until the CTA was completely dissolved. This was then mixed with centrifuged sulfate enriched SRB grown from the fill-and-draw mode under conditions of about 85% moisture content. During the mixing process, distilled water (around 80–100 mL) was added slowly into the slurry. The slurry was then removed to soak in toluene for 24 h to become hardened. The hardened slurry was then cut into 1 cm^3 cubes. These cubes were then flushed with distilled water to remove the residual toluene. About 800 immobilized cell cubes were randomly packed into one 1.8 L filter.

2.1.3. Sulfate reduction kinetic study

Chemostat reactors were used to study sulfate reduction using SRB and Monod kinetics of Y (maximum yield coefficient), k (maximum specific substrate utilization rate, 1/day), K_s (half velocity constant, mg/L), and k_d (decay coefficient, 1/day). Determinations of these Monod kinetics using a CSTR system with no biomass recirculation can be obtained from a typical wastewater engineering textbook [14]. The chemostats were continuously stirred tank reactors (CSTR) with 4.2 L volume each operated under HRTs of 5, 10, and 15 days, respectively. An OLR of 0.5 g COD/L day from glucose and a COD/S of 10/1 was used. The nutrients in the influent included continuous and manual feeds. The composition is shown in Table 1. When these systems reached a steady state, sulfate, SS, and VSS concentrations were analyzed to determine the kinetic coefficients.

2.1.4. Sulfide toxicity experiment

Batch mode experiments were set up and a bubble respirometer was used to study the inhibition of sulfide on methanogens. Seven serum bottles (six for the tests and one as the control, each with a total volume of 1.0 L) were prepared with identical substrates (COD = 4000 mg/L from glucose) and SO_4-S concentrations (1006.5 mg/L). The nutrients were the same as in the chemostat study, except that no manual feed was added. Each bottle was filled with the same sulfate enriched SRB culture (MLVSS = 260 mg/L) with a liquid volume of 630 mL. Seven bottles were placed into a water bath at a constant temperature of 30 °C. Operation followed the bubble respirometer instruction manual [15]. Every 24 h a serum bottle was removed and the compositions were analyzed.

2.1.5. Sulfate toxicity experiment

To confirm that the microbial activity inhibition was from the sulfide, different sulfate concentrations were prepared in this study. With different gas productions and concentrations left in the serum bottles for 5 days operation, it was expected that the sulfate concentration inhibition on the microbial activity could be determined. Seven serum bottles (six for the tests and one as the control) were prepared with identical substrate (COD = 3000 mg/L from glucose) and biomass concentration (MLVSS = 350 mg/L). The nutrient compositions were the same as used in the sulfide toxicity experiment. The liquid volume, water bath temperature, and experimental procedures were the same as above.

2.1.6. Establishment of the relationship between SRB and VSS

In engineering practice, the biomass concentration is normally represented using VSS analysis. In this study, because the sulfate reduction was carried out predominately by the SRB, when VSS were used to represent the biomass, the kinetic coefficients of the Y value could be overestimated and the k value could be underestimated. It was therefore necessary to construct a relationship between the SRB and VSS. SRB were screened first and then purified. Five milliliters of pure SRB culture with 95 mL of liquid media was placed in serum bottles purged with 95% N_2 and 5% CO_2 beforehand. Ten serum bottles were prepared and cultured anaerobically under 30 °C. Every 24 h a bottle was removed and colony formation units (CFU) of SRB and VSS concentrations were determined to establish the relationship.

2.1.7. Upflow anaerobic filter experiment

One of the objectives of this study was to develop a pre-treatment system for industrial wastewater containing high strength sulfate. In this experiment, an acrylic column 70 cm high, Ø6 cm ID, with a total volume of 1.8 L was used as the reactor for immobilized cells, as shown in Fig. 1. The number of cells added was about 800 cubes with a porosity of 0.35. The filter was operated under HRTs of 2, 1, and 0.5 days with COD/S of 10/1, 5/1, and 2/1, respectively, to evaluate the sulfate removal efficiency. The inorganic nutrients in the feed were the same as for the chemostat experiment.

2.2. Analytical methods

The sulfate was measured using ion chromatography (Dionex 2000 i/sp). Sulfide was measured following 4500-S^{2-} F iodometric method of Standard Methods [16], with dissolved sulfide sample was filtered through a 0.45 μm filter first. The biomass concentration was measured as suspended solids (SS) and volatile SS (VSS) following Standard Methods 2540 D and 2540 E [16]. SRB analysis was based on the fact that under anaerobic conditions SRB will reduce sulfate into sulfide. With Fe^{2+} addition to the media. The colony formation unit could be counted based on the black FeS spots [17–19]. The glucose concentration was determined using the Nelson's method [20,21]. Organic nitrogen and COD were measured following the 4500-N_{org} B and dichromate reflux method of 5220 B in Standard Methods [16]. The pumps used in this study were Cole-Parmer MasterFlex L/S Microprocessor Pump Drive 7524-10, Multi-Channel Cartridge Pump Heads 07519-20 and Narrow Cartridge 07519-65. The hemocytometer used for counting the SRB [22,23] was from Marienfeld

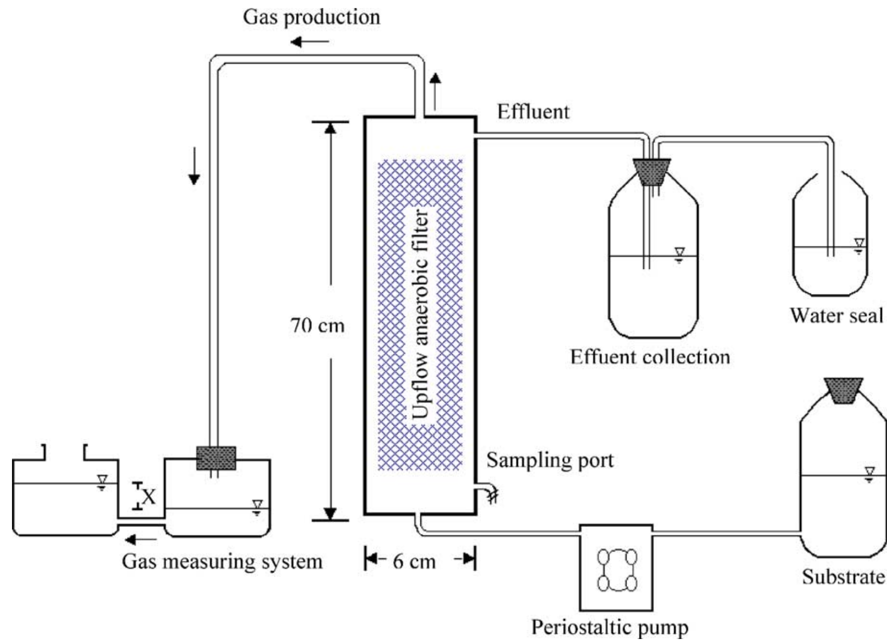

Fig. 1. Experimental setup of the upflow anaerobic filter.

(Germany). The microscope used for counting the SRB was a Nikon, model TMS. The scanning electron microscope used was a JEOL, model JXA-840 (Japan). The spectrophotometer used for the nitrogen and glucose analyses was a Hitachi spectrophotometer model U-2000.

3. Results and discussion

It has been reported that under the COD/S of 20/1, anaerobic bacteria can grow without any inhibition. In the SRB culture, the COD/S was maintained first at 20/1. When the sulfate removal rate was over 90%, COD/S was then decreased to 10/1, and then to 5/1.

3.1. Sulfide toxicity experiment

Six serum bottles with identical substrate and biomass concentrations were removed for analysis after 24, 48, 72, 96, 120, and 132 h operation, respectively. Through gas production detection using the bubble respirometer and sulfate reduction analysis and the increase in sulfide concentrations

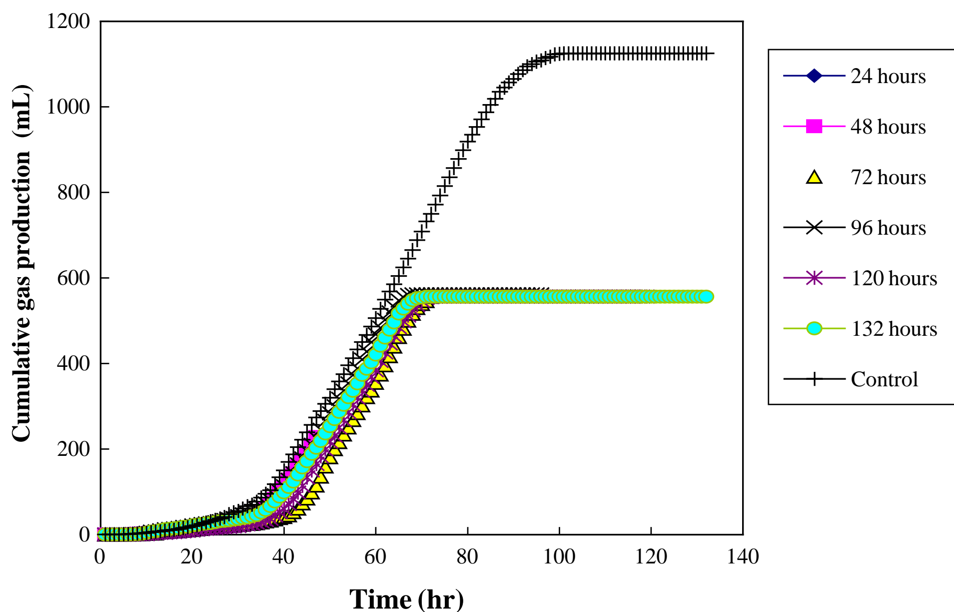

Fig. 2. Cumulative gas production of sulfide toxicity test from bubble respirometer.

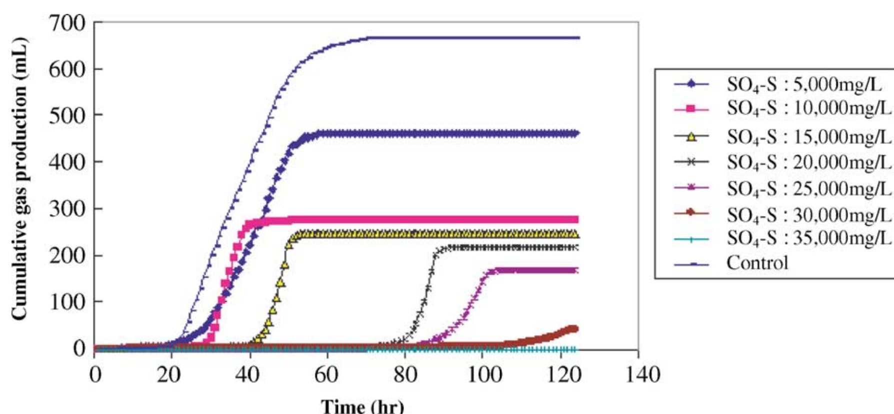

Fig. 3. Cumulative gas production of sulfate toxicity test from bubble respirometer.

after different reaction times, the inhibition of SRB by sulfide toxicity could be then evaluated.

From Fig. 2 it is clear that after about 72 h there was no further gas production. At this moment the substrates in the serum bottles were not yet used up (concentrations of COD from glucose and SO_4-S left were 830.2 and 571.1 mg/L, respectively). This indicated that the methane producing bacteria (MPB) were inhibited. This inhibition was due to the conversion of sulfate into sulfide by the SRB. Results from the serum bottles' analyses showed that when the total sulfide concentration was 304.6 mg/L and the dissolved sulfide was 276.4 mg/L (H_2S-S was 148.6 mg/L), MPB had already stopped gas production due to sulfide toxicity. However, the sulfate concentration was still decreasing and the sulfide was still increasing. At the end of the experiment (132 h), the dissolved sulfide concentration was 367.8 mg/L and H_2S-S was 200.0 mg/L, and there was no sign of SRB inhibition (the concentrations of COD from glucose and SO_4-S left were 392.1 and 389.4 mg/L, respectively). It was clear that in a system fed glucose, MPB were inhibited before SRB. These results are consistent with the previous studies on systems fed glucose or lactate under 100–150 mg/L dissolved sulfide levels and H_2S levels of 200–400 mg/L [6]. Choi and Rim [24] had a similar observation in that MPB were inhibited by sulfide toxicity before SRB.

3.2. Sulfate toxicity experiment

To further identify the inhibition of MPB and SRB either from influent sulfate or converted sulfide, a respirometer was used to monitor the gas production. In Fig. 3 there was no gas production at the SO_4-S level of 30,000–35,000 mg/L (Mg^{2+} = 22,781–26,578 mg/L), indicating that the MPB were completely inhibited. The 50% inhibition rate was around 8300 mg/L of SO_4-S (Mg^{2+} = 6303 mg/L). Analyses showed that even under these levels of SO_4-S, SRB still could convert sulfate into sulfide. At these Mg^{2+} levels, inhibition interpretation could also be due to cation toxicity [4]. Reports showed that sulfate would only cause a slight inhibition to microbial activity [25,26]. From the above there is enough evidence to say that, with the sulfate level in the previous section (∼1000 mg/L SO_4-S), it is the sulfide which is converted by SRB that causes the inhibition.

3.3. Sulfate reduction kinetic study

Chemostats were used to investigate the Monod kinetic coefficients of sulfate reduction by SRB. After at least three HRTs' operation, steady state data were analyzed and the results were: Y = 1.23 mg VSS/mg SO_4-S, k = 1.92 mg SO_4-S/mg VSS-d, K_s = 147.30 mg SO_4-S/L, and k_d = 0.05/day, as shown in Table 2. Here the biomass was measured as VSS. Since sulfate conversion into sulfide was carried out by SRB, using VSS as a biomass indication would not accurately represent the kinetic results. It was therefore necessary to establish a relationship between VSS and the active biomass. These results are shown in Table 3.

It was found that $Y = 13.224\ln(X) - 148.05$ (R^2 = 0.8644), where X and Y are the SRB concentration in terms of CFU/mL and mg VSS/L, respectively. After recalculation, as shown in Table 3, it was found that

Table 2
Results of kinetic coefficients determination using chemostats (n = sample number)

	SRT (days)		
	5	10	15
OLR (g COD/L day)	0.5	0.5	0.5
SLR (g SO_4^{2-}-S/L day)	0.05	0.05	0.05
S_0 (mg SO_4^{2-}-S/L)	250	500	750
S (mg SO_4^{2-}-S/L) (n = 6)	17.6 ± 0.3	10.0 ± 0.1	7.5 ± 0.04
VSS (mg/L) (n = 6)	230.5 ± 0.8	396.6 ± 6.6	536.0 ± 2.4
U	0.20	0.12	0.09
$1/\theta_c$	0.2	0.1	0.067
$1/U$	4.96	8.09	10.82
$1/S$	0.06	0.10	0.13
Y (mg VSS/mg SO_4^{2-}-S)		1.23	
k_d (1/day)		0.05	
k (mg SO_4^{2-}-S/mg VSS-d)		1.92	
K_s (mg SO_4^{2-}-S/L)		147.3	

Table 3
Establishment of SRB between CFU and VSS

	SRT (days)		
	5	10	15
Run #1 biomass conc. (CFU/mL)	2.45×10^7	3.75×10^8	1.24×10^9
Run #2 biomass conc. (CFU/mL)	3.90×10^7	1.25×10^8	2.10×10^9
Mean biomass conc. (CFU/mL)	3.18×10^7	2.50×10^8	1.67×10^9
Recalculated VSS (mg/L)	84.0	107.7	132.8
Directly analyzed VSS (mg/L)	230.5	396.5	536.0
Calculated/analyzed (%)	36.44	27.15	24.77

Table 4
Sulfate removal in the upflow anaerobic filter with immobilized cells

	HRT (days)								
	2	1	0.5	2	1	0.5	2	1	0.5
OLR (g COD/L day)	2	4	8	2	4	8	2	4	8
SLR (g S/L day)	0.2	0.4	0.8	0.4	0.8	1.6	1	2	4
COD/S	10/1	10/1	10/1	5/1	5/1	5/1	2/1	2/1	2/1
Inf. SO_4^{2-}-S (mg S/L)	400	400	400	800	800	800	2000	2000	2000
Eff. SO_4^{2-}-S (mg S/L)	7.5	51.1	102.1	56.4	178.3	232.8	594.7	644.3	923.3
Total sulfide (mg S/L)	254.5	238.2	215.4	382.5	391.6	398.4	923.6	938.6	940.3
Dissolved sulfide (mg S/L)	184.2	165.2	135.4	224.3	233.8	247.8	424.8	433.3	447.8
Sulfate removal eff. (%)	98.1	87.2	74.5	93.0	77.7	70.9	70.3	67.8	60.5
COD removal eff. (%)	71.4	75.3	76.7	81.4	63.1	45.4	100.0	98.8	86.0

SRB occupied an average of 29.45 ± 6.16% ($n = 3$) of the VSS in the chemostat. Based on this fraction, the kinetic coefficients can be recalculated as follows: $Y = 0.36$ mg VSS by SRB/mg SO_4-S, $k_d = 0.05$/day, $K_s = 147.30$ mg SO_4-S/L and $k = 6.50$ mg SO_4-S/mg VSS by SRB-d. Based on the stoichiometry and energetic calculations from McCarty [27,28], the biological transformation of sulfate into sulfide using glucose as the electron donor would give a theoretical yield coefficient between 0.36 and 0.50 mg VSS/mg SO_4^{-2}-S under a sludge age from 5 to 15 days. In this study, $Y = 0.36$ mg VSS by SRB/mg SO_4^{-2}-S fell into this range. The recalculated kinetic coefficients should more accurately represent the conversion of sulfate into sulfide by SRB.

3.4. Upflow anaerobic filter

In this experiment, an enriched SRB culture was entrapped in the immobilized cells and packed in the filter. This was designed to maintain a longer SRT and to support a higher biomass concentration in the filter to reach higher sulfate removal efficiency. It was also hoped that the carrier would provide a high specific surface area for microbial growth and also provide a shelter for bacteria that encountered sulfide toxicity.

In this experiment, the filter was operated under OLRs of 2, 4, and 8 g COD/L day from glucose and COD/S of 10/1, 5/1, and 2/1 with HRTs of 2, 1, and 0.5 days, respectively. The steady state data (after three HRTs) for the filter are shown in Table 4. The results show that when the sulfate loading rates (SLR) were 0.2 and 0.4 g S/L day (influent concentrations were 400 and 800 mg/L SO_4-S), the sulfate removal efficiency can reach over 90%. For influent COD/S of 10/1 and 5/1, over 70% of the sulfate can be removed. For influent COD/S of 2/1, it could be that there might not have been enough substrate for the MPB to produce methane or for the SRB to reduce the sulfate. It is possible that the sulfide reached a toxic level that immobilized the cells on the surface. Most of the sulfate reduction was converted by the biomass sheltered in the carrier. The removal efficiency therefore decreased to around 60%. Two SEM photos of different magnifications are shown in Figs. 4 and 5. As the pictures show, the immobilized cell can provide porous media for microbial growth, and this growth could be locally intense.

In the test runs shown in Table 4 it can be seen that although the sulfate removal efficiency decreased with decreasing HRT, the amount of sulfate removed increased with increasing SLR. For the substrate distribution between SRB and MPB, as the SLR increased, as high as 90% of the COD from the glucose was used by the SRB. This indicated that under high sulfate levels, SRB dominated the system and also dominated the substrate utilization. From the previous results showing that MPB ceased gas production when the total and dissolved sulfide concentrations were around 304.6 and 276.4 mg/L, respectively, the SRB were not inhibited at levels where the total and dissolved sulfide concentrations were 407.8 and 367.8 mg/L, respectively. In this

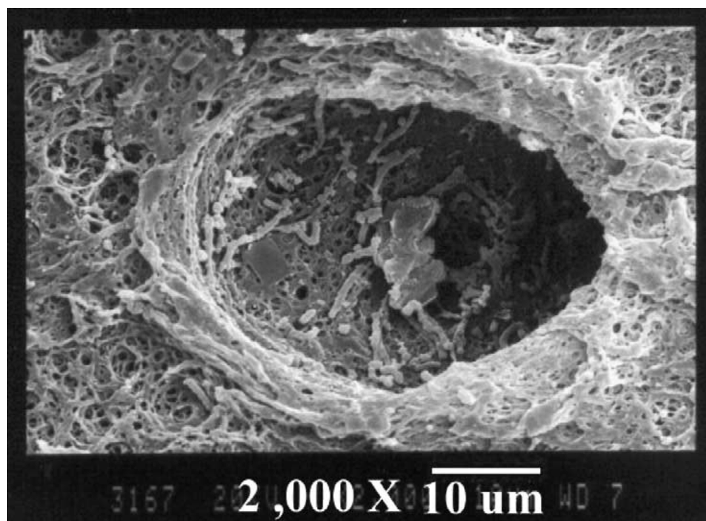

Fig. 4. SEM picture of immobilized cells.

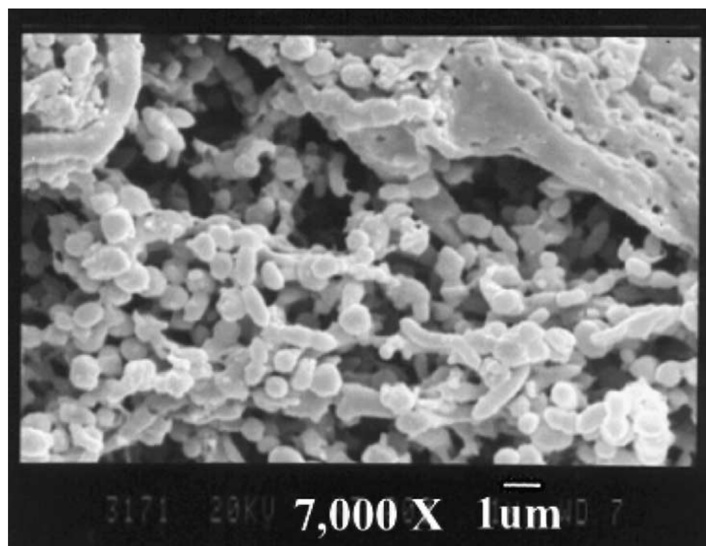

Fig. 5. SEM picture of immobilized cells in a closer view.

experiment, when the total and dissolved sulfide concentrations were 940.3 and 447.8 mg/L, respectively, the MPB were partially inhibited due to sulfide toxicity. However, gas production could still be observed and the SRB were not inhibited at all. This indicated that a system with immobilized cells could tolerate higher toxic material concentrations than a suspended growth system.

In order to estimate the biomass concentration in the filter, immobilized cells after two test runs (i.e., run #1: OLR = 2 g COD/L day, HRT = 2 days, COD/S = 10/1; run #2: OLR = 8 g COD/L day, HRT = 0.5 day, COD/S = 2/1) were removed for biomass analysis. Since there is no nitrogen in the immobilized cell composition, nitrogen analysis was used to estimate the biomass concentration. The typical organic nitrogen content in the sludge was 7–12.5%. When the mean of 9.75% was used to represent the nitrogen content, the VSS were calculated as shown in Table 5. The volume of each immobilized cell cube was 1.21 cm^3 (dimension = 1.1 cm × 1.0 cm × 1.1 cm), with the effective volume of the filter being 1800 mL with a porosity of 0.35. It was estimated that 800 cells were placed into the filter. With a dry weight of 0.26 g for each cube, the VSS in the cell was estimated to be 114.3–116.8 mg/g, and the VSS concentration in the filter can be calculated as:

$$\frac{114.3\,\text{mg VSS/g Gel} \times 0.26\,\text{g/ea.} \times 800}{1.8\,\text{L}} = 13\,208\,\text{mg/L}$$

From the above calculation, it was found that the biomass concentration in the reactor was around 13.2–13.5 g/L. This is a concentration that is not easy to reach or maintain in a typical suspended system reactor.

Table 5
Results of nitrogen analyses and VSS calculation of the immobilized cells

Sampled position	Run #1			Run #2		
	Inf.	Mid.	Eff.	Inf.	Mid.	Eff.
Nitrogen content in the carrier (mg N/g Gel)	14.0	12.74	6.66	14.20	13.38	6.59
VSS in the carrier (mg VSS/g Gel)	143.8	130.7	68.3	145.6	137.2	67.5

4. Conclusions

Based on the observations made from this research, the following conclusions are drawn:

1. Results from the toxicity experiments using a bubble respirometer showed that when the total and dissolved sulfide concentrations were 304.6 and 276.4 mg/L, respectively, the MPB stopped gas production due to inhibition from sulfide, while the SRB were not inhibited. This indicated that MPB are more sensitive to sulfide toxicity than SRB.
2. Methane production had completely stopped at SO_4-S levels of around 30,000–35,000 mg/L. This is an indication that the MPB were totally inhibited by the high sulfate concentration. Under the same conditions in which SRB could still convert sulfate into sulfide, it is believed that the typically encountered sulfate level in industrial wastewater should not cause significant inhibition. The 50% gas production inhibition rate was found to be at around 8300 mg/L of SO_4-S.
3. In the chemostat system, SRB were found to occupy $29.45 \pm 6.16\%$ ($n = 3$) of the VSS. When the kinetic coefficients were based on SRB, they were recalculated to $Y = 0.36$ mg VSS by SRB/mg SO_4-S, $k_d = 0.05$/day, $K_s = 147.30$ mg SO_4-S/L, and $k = 6.50$ mg SO_4-S/mg VSS using SRB-d.
4. In the lab-scale, upflow anaerobic filter filled with immobilized cells, when operated under HRT of 2 days and sulfate loading rates of 0.2 and 0.4 g SO_4-S/L day, the sulfate removal efficiency can be over 93%. When this system was operated under HRTs of 2, 1, and 0.5 days with COD/S of 10/1 and 5/1, the sulfate removal efficiency was between 98.1 and 70%. In the filter with total and dissolved sulfide concentrations of 940.3 and 447.8 mg/L, the COD removal efficiency could still be maintained as high as 86%. Neither MPB nor SRB were significantly inhibited. It is believed that because of the immobilized cells this system can tolerate higher sulfide concentrations and support a higher biomass concentration of 13.2–13.5 g VSS/L.

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