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PERFORMANCE OF TWO-PHASE UASB REACTOR IN ANAEROBIC TREATMENT OF WASTEWATER WITH SULFATE

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Abstract : Two phase UASB reactors for treating wastewater with sulfate were operated to assess the performance and competition of organics between sulfate reducing bacteria(SRB) and methane producing bacteria(MPB), and the change of characteristics of microorganisms. The reactors were fed in parallel with a synthetic wastewater of 4,000-5,000 mgCOD/L and sulfate concentration of 800-1,000 $mgSO_4/L$. In the MPR(methane producing reactor) and CR(control reactor), COD removal efficiencies were 90% and 60%, respectively, at the OLR(organic loading rate) of 6 gCOD/L, while the amount of biogas and methane content were 6.5 L/day and 80%, and 3 L/day and 50%, respectively. However, the portion of electron flow used by SRB at the OLR of 6 gCOD/L · day in MPR and CR was 3% and 26%, respectively. This indicated that the increase of OLR of wastewater containing high sulfate like CR resulted in activity decrease and cell decay of MPB, while SRB was adapted immediately to new environment. The MPB activities in MPR and CR were 2 and 0.38 $kgCH_4$-COD/gVSS · day at the OLR of 6 gCOD/L · This indicated hat SRB dominated gradually over MPB during long-term operation with wastewater containing sulfate as a consequence of outcompeting of SRB over MPB. In addition, the solution within AFR was maintained around pH 5.0, the MPB such as *Methanothrix* spp. which was very important to formation of granules was detached from the surface of granules due to the decrease of activity by limitation of substrate transportation into MPB. Therefore, a significant amount of sludge was washed out from the reactor.

Key Words : Anaerobic treatment, UASB, Sulfate reducing bacteria, Methanogens, Sulfate

INTRODUCTION

Industrial wastewater from eatable oil, fermentation, molasess, chemical and tannery processes contains high sulfates and sulfides. In anaerobic treatment of wastewaters with sulfates, there were known to be occurred methanogenesis and sulfate reduction as the final step in the anaerobic process.[1] The presence of sulfate in wastewater results in a competition among the different groups of anaerobes. SRB competes with MPB for acetate and hydrogen, and also acetogens for substrate such as propionate and butyrate.[2] Consequently, sulfate reduction is the major end-step in the anaerobic mineralization process for wastewater with high levels of sulfate. It was reported that sulfides which caused inhibition of methane production were affected to MPB in the reactor.[3,4] Generally, the inhibition of methanization results in the inabilities of cell' function and substrate competition between SRB and MPB.[5] Therefore, the lack of methane results from deterioration of effluents as well as the loss of economics. However, a research for important gain of methane activity

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and methane production now was not achieved almost in anaerobic treatment of wastewater containing sulfate so far.

Therefore, two phase anaerobic processes will be possible by effective method that can restrain the inhibition of methanogenses caused by sulfides toxicity. Acidification and methanization are separately proceeding in two phase anaerobic process, while acidification and methanization are simultaneously proceeding in single phase.[6] Generally, soluble sulfides formed by SRB in AFR are removed from reaction as converted into gaseous sulfides under acid condition (pH: 5.2-5.5). The tolerance of acidogens on the toxicity of soluble sulfide has been known as very stronger than that of MPB.[7] Because the application of AFR can minimize toxicity effect of MPB by sulfide, it is possible for system to operate in stable conditions.[8]

In this study, for expanding the applicability of two phase UASB reactor to various industrial wastewaters with sulfate, the performance of UASB reactor was investigated in continuous experiment. In addition, tests were carried out to investigate the influence of reactor types like two phase reactor and single control reactor on the competitive electron utilization by SRB and MPB, and on the characteristics of granules.

MATERIALS AND METHODS

Experimental Apparatus

Figure 1 was shown a schematic diagram of the laboratory-scale two-phase UASB system used in this study. It was consisted of both reactors named as AFR and MPR. Also, control reactor (CR) for comparing the performance of two-phase UASB reactor was used, which had a identical volume with MPR. The both reactors were manufactured in acrylic with liquid volume of 2.0 L in AFR and 2.5 L in MPR.

The UASB systems were located in thermostatically controlled chamber with temperature of 35°C for this study. The influent was kept to refrigerator of 4°C to prevent decomposition of substrate. Gas produced from reactors was measured according to the change of water level saturated salt and sulfuric acid in gas collector.

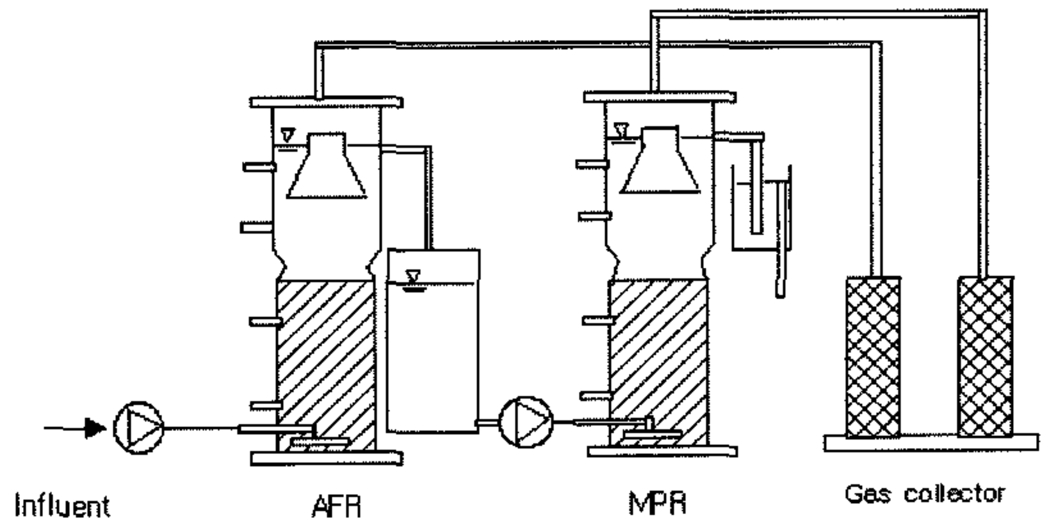

Figure 1. A schematic diagram of laboratory scale two-phase UASB systems.

Experimental Materials

The reactors were seeded with granular sludge taken from a UASB reactor located at K brewery wastewater treatment plants. For the stability of reactor after seeding, any substrate was not added to adapt in new conditions during 5 days. Total suspended solids (TSS) concentration and VSS/TSS ratio of the sludge were 16 g/L and 0.8, respectively. Initial sludge loading rates in AFR, MPR, and CR were 0.18, 0.06, and 0.06 gCOD/gVSS · day, respectively.

Substrate concentration was between 4,000 and 5,000 CODmg/L with glucose. Composition of nutrient and mineral elements added to wastewater was as follows (mg/L): NH_4Cl, 0.53; $CaCl_2 \cdot 2H_2O$, 0.075; $MgCl_2 \cdot 6H_2O$, 0.1; $FeCl_2 \cdot 4H_2O$, 0.02; $MnCl_2 \cdot 4H_2O$, 0.5; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2$ 0.03; $NaMo_4 \cdot 4H_2O$, 0.01; $CoCl_2 \cdot 6H_2O$, 0.5; $NiCl_2 \cdot 6H_2O$, 0.05; Na_2SeO_3, 0.05.

Table 1 showed the operational conditions of the UASB reactors in this study. The pH of solution in AFR was adjusted automatically by HCl to be maintained in the range between 5.2 and 5.5, while pH in MPR and CR was adjusted by $NaHCO_3$ to keep the value of 7.0-7.4. The rate of SO_4^{2-}/COD was 0.2.

Activity Test of Sludge

Activities of MPB in MPR and CR were determined by the specific activity test. Microorganism used to evaluate toxicity effect of MPB by soluble sulfides produced from reactor operation was taken from MPR and CR at the

Table 1. Experimental conditions

	Two-phase UASB		Control reactor (CR)
	AFR	MPR	
SO_4^{-2}/COD	0.2	-	0.2
pH	5.0-5.2	7.0-7.4	7.0-7.4
Alkalinity ($mgCaCO_3$/L)	500-800	2,500-3,000	2,500-3,000
Organic loading rate (gCOD/L · day)	6-10	2-6	2-6

organic loading rate(OLR) of 2, 4, and 6 gCOD/L · day, respectively. Organics in sludge was removed by using a washing method presented by Tramper.[9] All sludge used in test was homogenized as crushing perfectly by injector under anaerobic conditions, and the amount of sludge was added 10% of effective volume (55 mL). All of the batch experiment were measured two times and used the mean value. Substrate concentration injected to test bottles was 1,200 mg COD/L containing 400 mgCOD/L as acetic acid, 400 mgCOD/L as propionic acid, and 400 mgCOD/L as butyric acid.

Analytical Methods

Samples were taken under the settling zone of UASB reactor. Biogas produced from reactor was measured a time every day. Then COD, TSS, VSS, sulfate, sulfide, and gas composition were measured at intervals of 2-3 days.

The gas composition was analyzed to use Porapak Q column (80/100 mesh) under condition of column 50°C, injector 80°C, detector 90°C and carrier gas (He) of 30 mL/min.(Gow-Mac, 5860). The COD of effluent was measured after add 1-2 drops of sulfuric acid and flushing by nitrogen gas to remove disturbance by sulfide. Sulfate was measured as turbidimetric method, sulfide as titration method. All experiments was measured by Standard Methods.[10]

Calculation of Substrate used by SRB and MPB

In the anaerobic digestion of wastewater containing sulfate, the substrate electrons (in terms of COD) are partitioned between the SRB and MPB. The substrate utilization by SRB and MPB can be calculated from the following stoichiometric equations.[11]

By MPB

Methane production:

$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$

The COD of CH_4 produced can be calculated by: $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$

Thus, 1 mol of CH_4 produced $\equiv$ 2 mol of COD $\equiv$ 64 g of COD.

Electron flow by the MPB = moles of methane produced × 64 g = A

By SRB

Sulfate reduction:

$4H_2 + H^+ + SO_4^{2-} \rightarrow HS^- + 4H_2O$

$CH3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$

The COD of H_2S produced is given by: $H_2S + 2O_2 \rightarrow H_2SO_4$

Thus, 1 mol of SO_4^{2-} reduced $\equiv$ 1 mol of H_2S produced $\equiv$ 2 mol of COD 64 g of COD

Electron flow by the SRB = moles of sulfate S reduced × 64 g = B

Therefore:

Percent electron flow by MPB

$= [A/(A+B)] \times 100$

Percent electron flow by SRB

$= [B/(A+B)] \times 100$

RESULTS AND DISCUSSION

Performance of UASB Reactor

Figure 2 showed the change of the OLR for experimental period. The OLR in AFR maintained at 6-7 gCOD/L · day until 105 days and increased up to 10gCOD/L · day until 120 days,

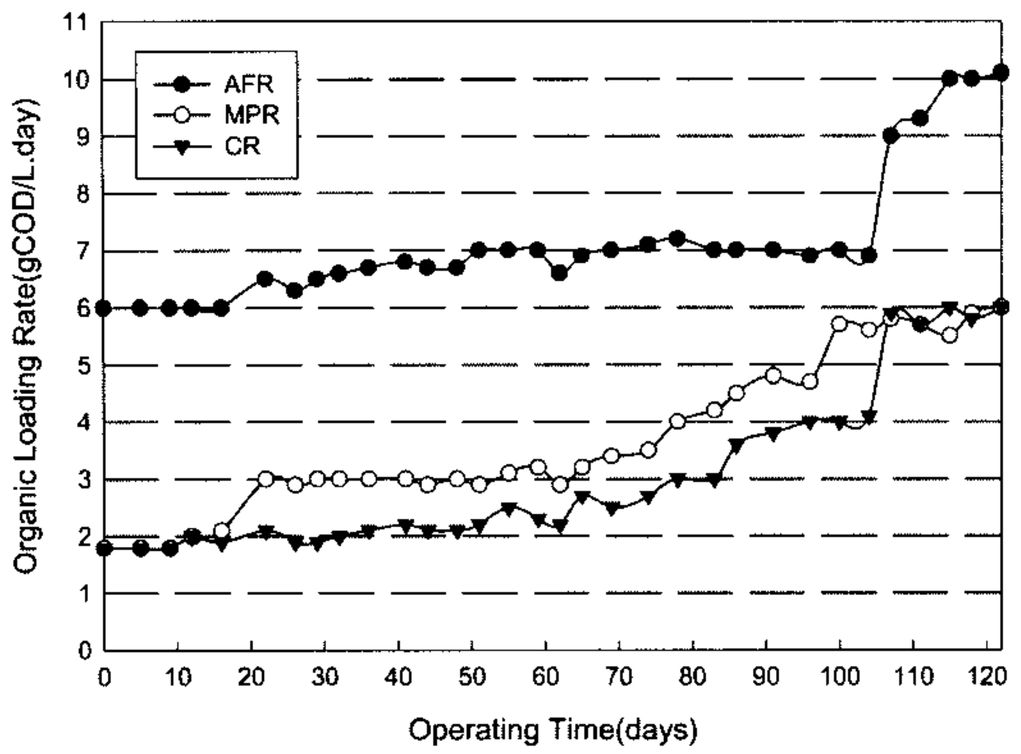

Figure 2. Change of organic loading rate in the UASB reactors. [AFR; acid fermentation reactor, MPR; methane producing reactor, CR: control reactor].

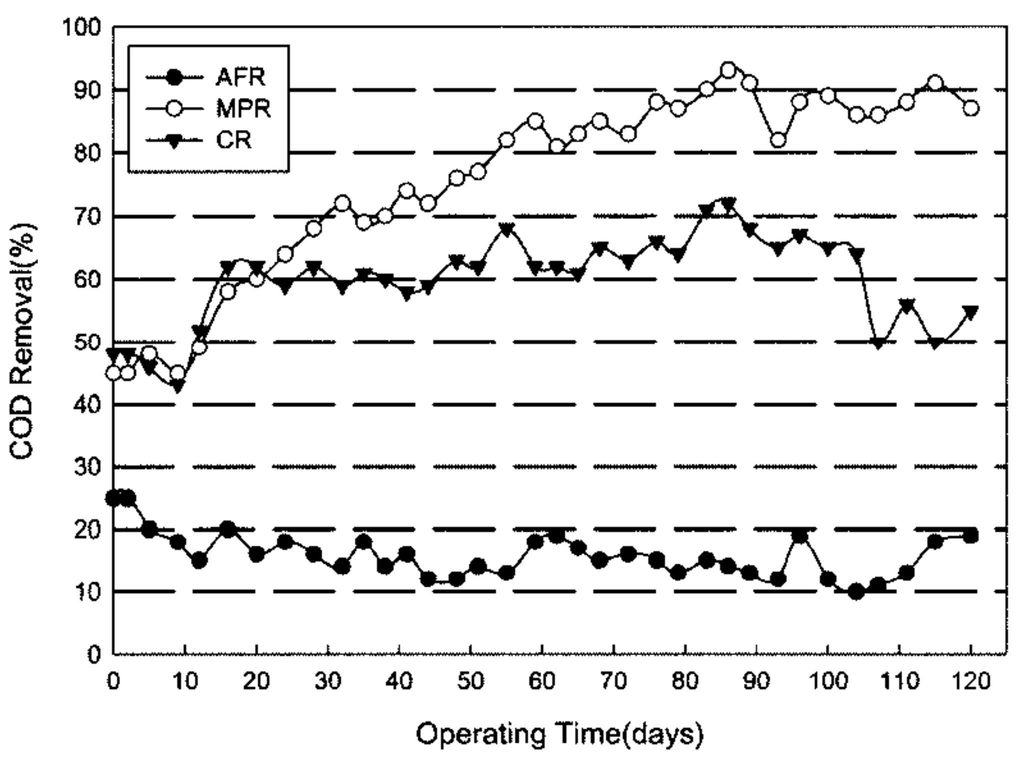

Figure 3. Change of COD removal efficiency in UASB reactors.

while the OLR in MPR and CR increased up to 2-4 gCOD/L · day until 105 days, and followed 4-6 gCOD/L · day until 120 days.

Figure 3 indicated the COD removal efficiency of two-phase UASB(AFR + MPR) and CR. The COD removal efficiency in AFR was kept 10-20% regardless of the change of the OLR. The volatile fatty acid(VFA) concentration increased up from 100 mg/L at initial stage to 1,400-1,500 mg/L at the OLR of 10 gCOD/L · day (Figure 4). This phenomenon indicated that most of the removed COD was converted into VFA by acid producing bacteria(APB) existed in AFR. The COD removal efficiency in MPR which was taken in effluent of AFR was increased up to 90% at the OLR of 6 gCOD/L · day. At this time, total VFA concentration was considered as 50 mgHAc/L (Figure 4). However, the COD removal efficiency of CR in comparison with MPR maintained between 60 and 70 % at the OLR of 4 gCOD/L · day and decreased below 50% at the OLR of 6 gCOD/L · day. At this time, the VFA concentration was increased up to 400 mgHAc/L, which indicated the decrease of MPB' activity with increase of OLR (Figure 4).

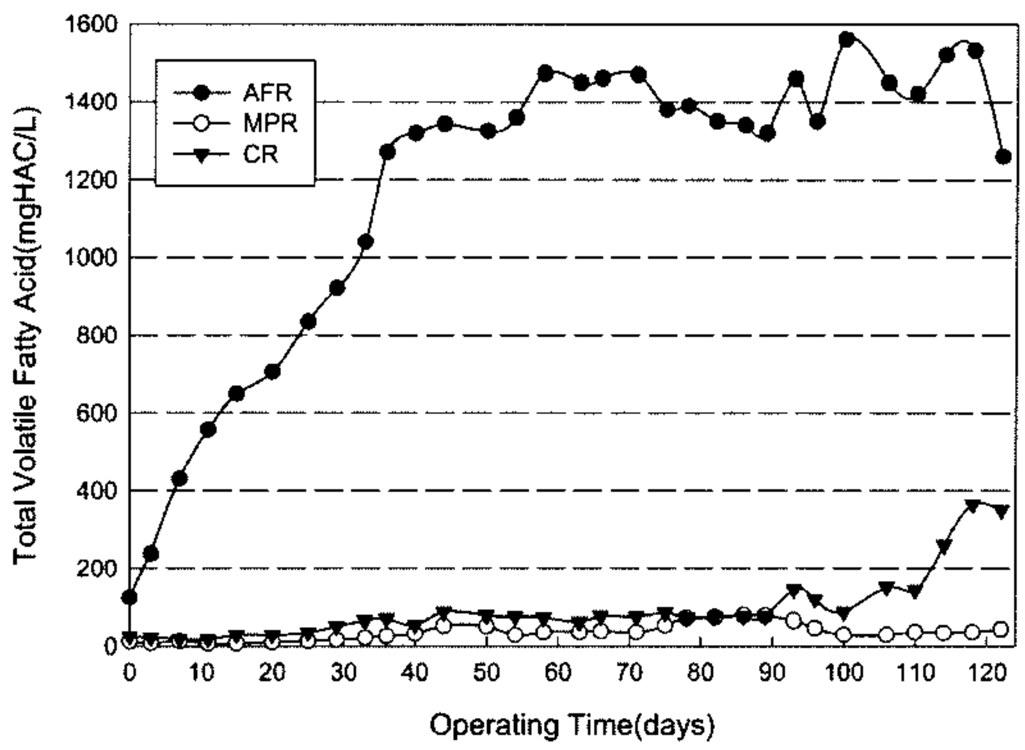

Figure 4. Change of total volatile fatty acid in UASB reactors.

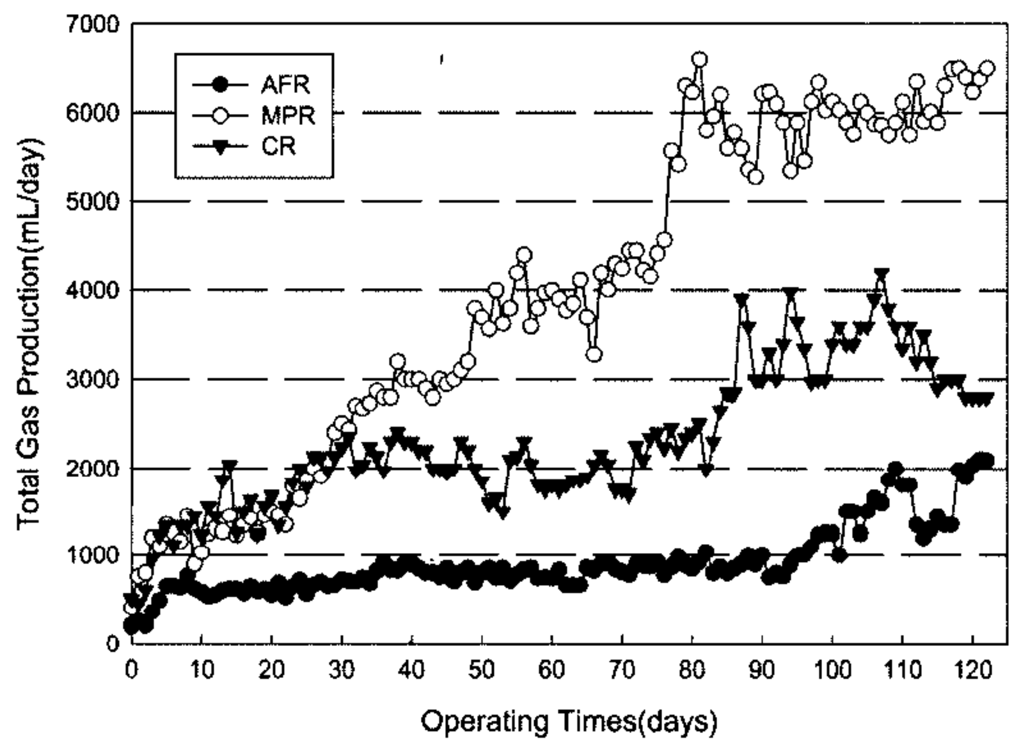

Figure 5. Change of total gas production rate in UASB reactors.

Figure 5 and Figure 6 showed the relationship between amount of total gas and methane content of the UASB system for experimental period. The amount of total gas from AFR maintained about 1.0 L/day and methane content decreased down from 52% to 2 % at the OLR of 6-7 gCOD/L · day. Because AFR had pH suitable to optimal growth of APB, the most of MPB was almost killed due to lower pH of 4.0-4.5. At the moment, the gas composition

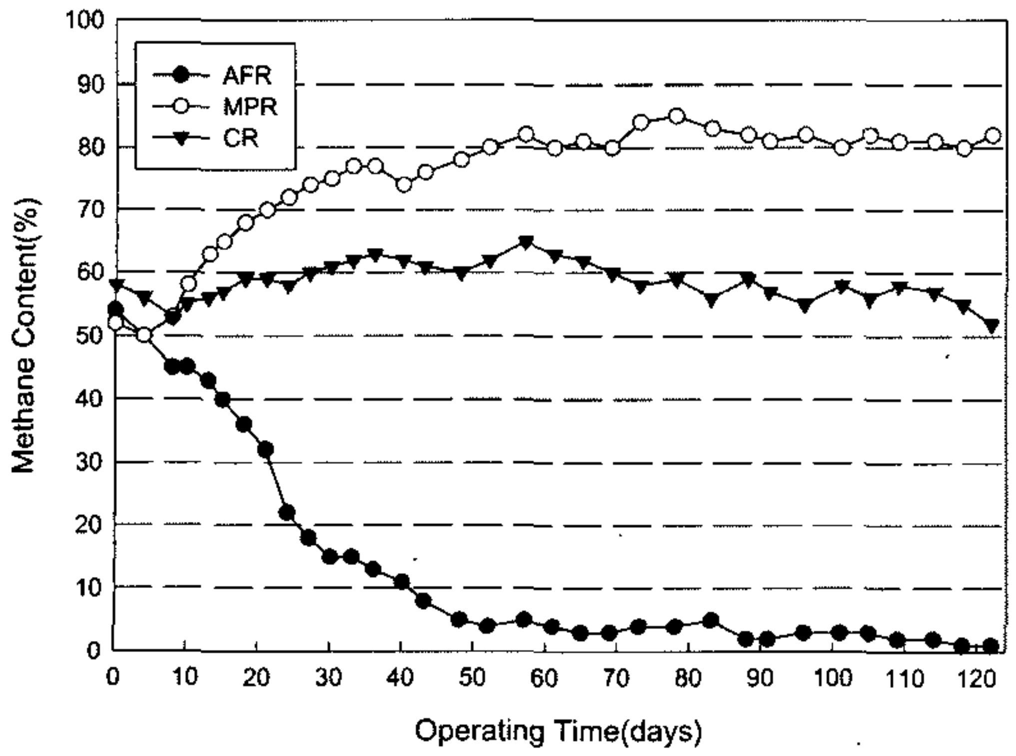

Figure 6. Change of methane content in UASB reactors.

consisted mainly of carbon dioxide (97%) and a small amount of gaseous hydrogen sulfide. The amount of biogas increased up over 6.0 L/day at the OLR of 6 gCOD/L · day in MPR. During this periods, methane content was increased from 54% to more than 80%. On the other hand, the amount and methane content of biogas in CR were 3.5 L/day and 60%, respectively, at OLR of 4 gCOD/L · day and 3.0 L/day and less than 50%, respectively, at OLR of 6 gCOD/L · day. This indicated that sulfate reducing bacteria(SRB) within AFR reduced sulfate into sulfide using electron produced from oxidation of COD. Also, lower pH converted soluble sulfide into gaseous sulfide, which emitted from reactor into air. It should be noted that MPR can obtain much better biogas than CR.

Substrate competition between SRB and MPB

The material balance of COD for experimental period was summarized in Table 2 in case of MPR and CR. The amount of COD consisted of the COD in the effluent, recovered CH_4-COD, and COD utilized sulfate reduction (SO_4-COD). Table 2 presented that the portion of electron flow used by SRB in MPR and CR was 1 and 6%, respectively, at the OLR of 2 gCOD/L · day, while the portion of electron flow used by SRB at the OLR of 4 and 6 gCOD/L · day in MPR and CR was 2 and 19%, and 3 and 26%, respectively.

This indicated that the increase of OLR of wastewater containing high sulfate like CR resulted in activity decrease and cell decay of MPB, while SRB was adapted immediately to the new environment. According to kinetics of competition for the electron doners between SRB and MPB. SRB have a higher affinity (lower Km) for hydrogen and acetate which are major methane precursor in comparison to MPB as seen in Table 3.[4,12] Therefore, SRB become dominant over MPB with time in the presence of excess sulfate.

Table 3. Kinetic constants for acetate and hydrogen of MPB and SRB

Bacteria	Substrate	K_m (mV)	V_{max} (mM/g · d)
SRB	Acetate	0.2	74
	Hydrogen	0.001	112
MPB	Acetate	3.0	45
	Hydrogen	0.006	123

Sulfide Toxicity between MPB and SRB

Several mechanisms have been proposed to explain the inhibitory effect of sulfate on metha-

Table 2. COD material balance used by SRB and MPB

OLR (gCOD/L · day)	Reactor	HRT (hrs)	COD_{load} (g)	COD $/SO_4^{-2}$	Effl.-COD (g)	CH_4-COD (g)	SO_4-COD (g)	Recovery (%)	Electron Flow(%)	
									SRB	MPB
2	MPR	13	12.0	0.2	3.8	7.1	0.1	11.0 (92)	1	99
	CR	17	8.4	0.2	2.5	4.9	0.3	7.7 (92)	6	94
4	MPR	12	16.8	0.2	1.8	14.2	0.4	16.4 (97)	2	98
	CR	16	12.0	0.2	3.9	6.2	1.4	11.5 (96)	19	81
6	MPR	8	24.0	0.2	2.3	20.2	0.6	23.1 (96)	3	97
	CR	8	24.0	0.2	10.2	9.2	3.5	22.9 (95)	26	74

* Parathesis indicates percentage
** MPR: methane producing reactor, CR: control reactor

nogenesis in anaerobic digestion. It has been found that soluble sulfides or free hydrogen sulfides produced by the microbial reduction of sulfate were toxic to the *microorganism.*[7] There were two types of inhibitions on methanogenesis as a result of sulfate reduction: primary inhibition due to competition for substrate with SRB, and secondary inhibition resulting from the decline of methanogenic population due to direct inhibition of cell functions by soluble sulfides.[13] Figure 7 showed the change of sulfide souble concentration in MPR and CR. Sulfide soluble concentration in MPR and CR was 15 and 90 mgS/L, respectively, at OLR of 6 gCOD/ L · day. Free sulfides(H_2S) among soluble sulfides(H_2S, HS^-, S^{-2}) were known as most toxicity to MPB and calculated from soluble sulfides and pH[14,15]. Since the solution pH in the MPR and CR ranged from 7.2 to 7.6 throughout the entire experiment, free hydrogen sulfide concentration was calculated as to range from 14 to 20% of the soluble sulfides concentration.

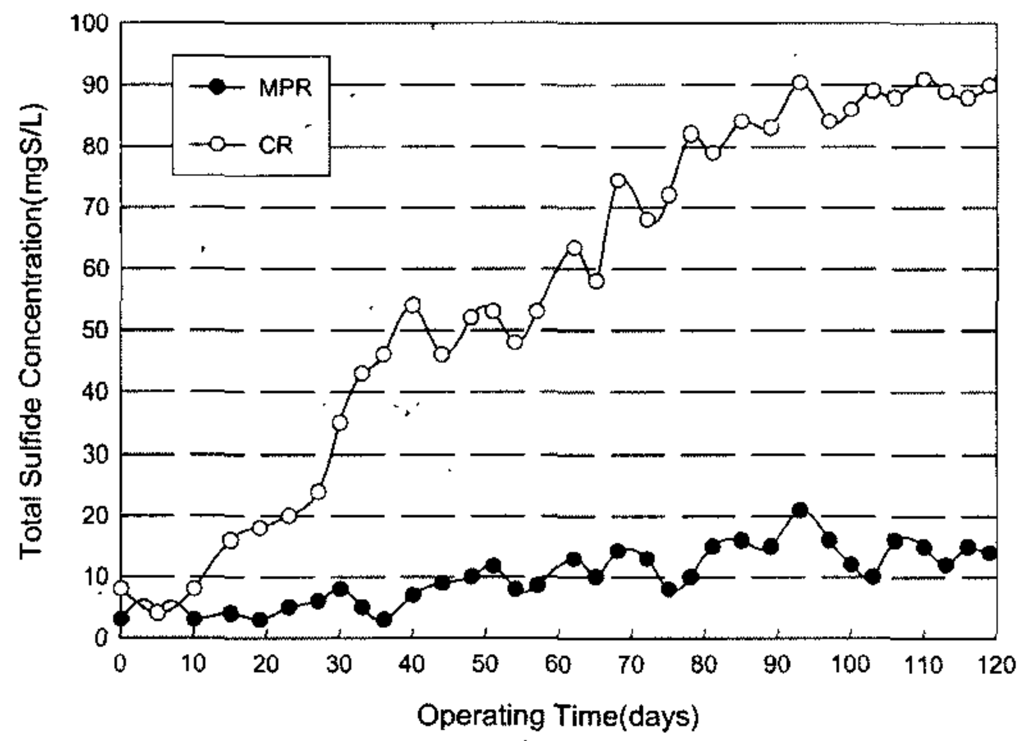

Figure 7. Change of total sulfide concentration in UASB reactors.

Therefore, the value of free hydrogen sulfide concentration in MPR and CR was maximum 2-3 mg/L and 18 mgS/L, respectively. Also, MPB' activities in MPR and CR was 0.72 and 0.38 $kgCH_4$-COD/gVSS · day at the OLR of 6 gCOD/ L · day (Table 4). This phenomenon indicated that SRB dominate gradually over MPB during the long-term operation with wastewater containing sulfate. Since free hydrogen sulfide in the reactor was less than the threshold values of its

Table 4. Specific Methanogenic Activities according to ORL.

OLR (gCOD/L · day)	Reactor	Activities ($kgCH_4$-COD/gVSS · day)
2	MPR	0.35
	CR	0.35
4	MPR	0.67
	CR	0.43
6	MPR	0.72
	CR	0.38

toxicity reported in the literature (50 mg/L[10]; 90-250 mg/L[11]; over 1,000 mg/L[7]), a severe *suppression* of MPB in CR was not caused by free hydrogen sulfide toxicity, but was simply a consequence of outcompeting of SRB over MPB.

Sludge Characterization

As shown in Figure 8, volatile suspended solid(VSS) concentration of effluent from AFR was increased from 120-150 mgVSS/L at the beginning to 200 mgVSS/L at the end of experiment, while that in MPR and CR was maintained a constant value with time. The granulation was mainly composed of mixtures of MPB and APB[14], if solution within AFR maintained below pH 5.0, MPB such as Methanothrix spp., which was abundant in the initial granules could be detached from the surface of granules due to the decrease of activity by limitation of substrate transportation into MPB. Therefore, a significant amount of sludge was washed out from the reactor.

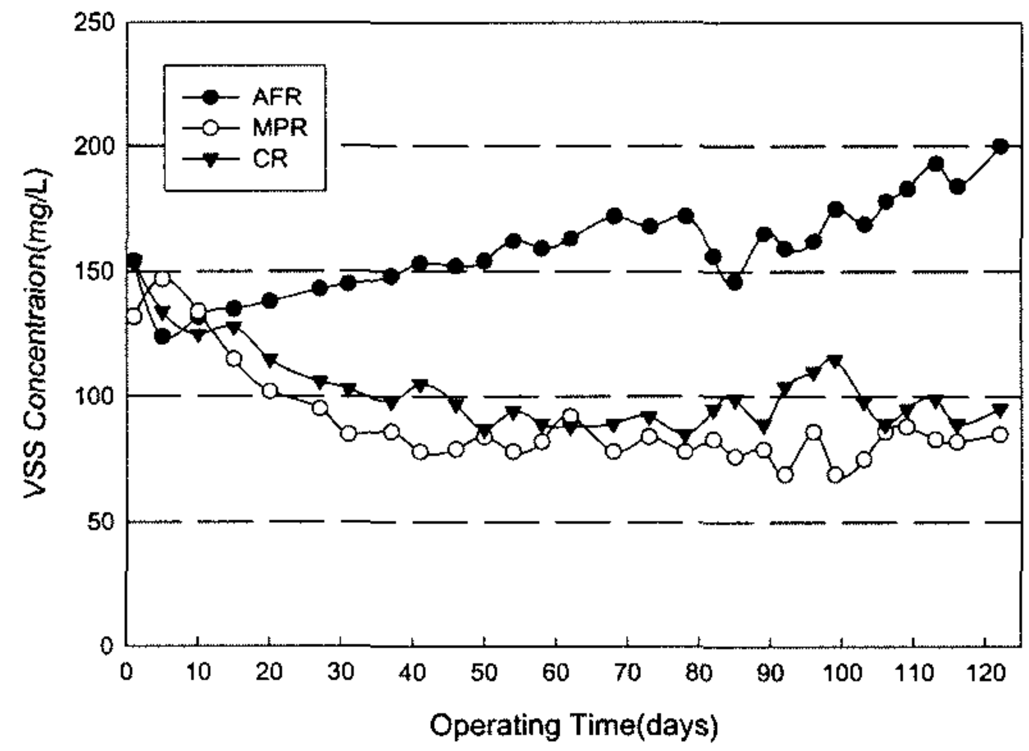

Figure 8. Change of volatile suspended solids concentration during operation periods.

CONCLUSION

1) In the MPR and CR, COD removal efficiencies were 90 and 60%, respectively, at the OLR of 6 gCOD/L, while the amount of biogas and methane content were 6.5 L/day and 80%, and 3 L/day and 50%, respectively.
2) The portion of electron flow used by SRB at the OLR of 6 gCOD/L · day in MPR and CR was 3 and 26%, respectively. This indicated that increase of OLR of wastewater containing high sulfate like CR resulted in activity decrease and cell decay of MPB, while SRB was immediately adapted to the new environment.
3) MPB' activities in MPR and CR were 0.72 and 0.38 $kgCH_4$-COD/gVSS · day, respectively, at the OLR of 6 gCOD/L · day. This indicated that SRB dominated gradually over MPB during the long-term operation with wastewater containing sulfate as a consequence of outcompeting of SRB over MPB.
4) The solution within AFR was maintained below pH 5.0, the MPB such as Methanothrix spp. which is very important to formation of granules was detached from the surface of granules due to the decrease of activity by limitation of substrate transportation into MPB. Therefore, a significant amount of sludge was washed out from the reactor.

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