CHAPTER V

OPTIMUM REACTOR CONDITIONS FOR SIMULTANEOUS CONTROL OF SULFATE AND HEAVY METALS IN COMPLEX WASTEWATER STREAMS

5.1 Introduction and Purposes of this Chapter

During anaerobic treatment of high sulfate (SO_4^{2-}) content wastewater, large amounts of sulfide are produced causing unfavorable and undesirable conditions. The high SO_4^{2-} level stimulates the growth of sulfate-reducing bacteria (SRB) that out-compete methane–producing bacteria (MPB) for substrate (Kristjanson et al., 1982). There is substrate competition between SRB and MPB, with the SRB prevailing because they achieve a high energy profit. The level of SO_4^{2-} reduction has to be controlled not only to limit the amount of sulfide production in an anaerobic system but to avoid competition between SRB and MPB as well in order to promote methanogenesis.

The occurrence of SO_4^{2-} reduction in anaerobic bioreactors at limiting amounts of SO_4^{2-} is intriguing for the potential application of anaerobic digestion involving simultaneous sulfide detoxification and heavy metal remediation processes. Start–up of the anaerobic digestion of sulfate-rich wastewater is a critical step in determining the course and level of any problem within the process. Overall process failure of high SO_4^{2-} content anaerobic digestion is driven by the COD:S ratio (Isa et al., 1986). Different seed sludges showed different outcomes. The actual outcome, under varying influent organic compositions and strengths as well as different SO_4^{2-} concentrations that prevail during digestion of industrial wastewaters, may be quite different from that predicted by thermodynamic or kinetic considerations. Percheron et al., (1997) reported the effect of the sludge and showed that when using an appropriate sludge, the effluent was biodegradable.

One purpose of the experiments was to investigate the ratio of COD:S necessary for controlling the level and mechanism of SO_4^{2-} reduction. In this study, the range of COD:S within which SO_4^{2-} reduction was controlled by the amount of

SO_4^{2-} was investigated. The microbial assemblages were obtained from a glucose acclimated mixed microbial culture from a brewery waster treatment plant (obtained from Phase 1). Another purpose is to find the ratio of COD:S that promote methanogenesis. The COD:S that give rise to the maximum specific methanogenic activity (SMA) in order to promote methanogenesis for this selected consortium was obtained. The key parameter to indicate the mutualism of the bacterial cultures is the percentage of electron flow to MPB and SRB. The COD used for CH_4 production and for SO_4^{2-} reduction at this optimum condition was reported. This combination of factors offers the promise of quite exact results, which can be shown quantitatively and comparably.

5.2 Materials and Methods

Detailed procedure for the study on controlling the level of SO_4^{2-} reduction and the experimental method are provided in Chapter 3 (Section 3.2). Figure 3.6 shows the laboratory scale batch apparatus for this study.

Experiments were performed in reaction bottles by a batch test with temperature controlled at 35 ± 1 ^0C. The initial biomass concentration was around 10,000 mg/l. The initial COD was around 3,000 mg/l. The initial loading factor (COD/MLVSS) in each bottle was equally controlled. SO_4^{2-} was added at the concentration between 0-2,500 mg/l. Assays were run for a 120 hour period.

The competition between SRB and MPB and the inhibition of methanogenesis were determined. Inhibition was quantified by determining the doses of the SO_4^{2-} and the COD:S ratios that caused major trouble conditions during the anaerobic digestion over a fixed period of time. The parameters used in measuring the effects of SO_4^{2-} and COD:S ratios were the SO_4^{2-} reduction, CH_4 production, and the percentage of COD removal.

5.3 Results and Discussion

Methane production of the entire batch test was reported. The percentage of COD removal and specific methanogenic activities (SMAs) at different levels of SO_4^{2-} were investigated. The relationship between the percentage of COD removal,

and SO_4^{2-} content was shown as graphs. The COD balances were calculated. The recovered CH_4-COD in gas phase (CH_4gasCOD), and the COD used for SO_4^{2-} reduction (ΔSO_4^{2-}-COD) were estimated. The percentage of electron flow to SRB and MPB which showed the competition between SRB and MPB at different COD:S were reported.

5.3.1 The Concentration of Sulfate and the COD:S Ratios for Controlling the Level and Mechanism of Sulfate Reduction

Sulfate reduction is expected to proceed efficiently with no great competitive effects on acidogenesis and methanogenesis. The effect of initial SO_4^{2-} was tested under sulfate reducing conditions. In this study, the high SO_4^{2-} content caused major trouble during the anaerobic reaction. Table 5.1 displays the variables used in the test and the percentage of COD and sulfate removal in the process. The time course of SO_4^{2-} present in the effluent with various SO_4^{2-} starting concentrations is shown in Figure 5.1 (Table G-1). The results that show a comparison among reactors containing different amounts of SO_4^{2-} in terms of SO_4^{2-} reduced is illustrated in Figure 5.2 (Table G-2).

Table 5.1 Variables used, sulfate, COD:S and the percentage of COD and sulfate removal.

Influent COD (mg/l)	Initial SO_4^{2-} (mg/l)	COD:S	Effluent COD (mg/l)	Effluent SO_4^{2-} (mg/l)	%COD removal	%SO_4^{2-} removal
3,200	700	13.7	560	0	82	100
3,200	1,100	9	445	118	86	89
3,200	1,300	7.4	526	106	83	92
3,200	2,100	4.5	526	207	83	90
3,200	2,600	3.7	775	1,167	75	55

Sulfate reduction occurred in both acidogenic and methanogenic phases from the beginning up to 120 hours in this test (Figure 5.1 and Table G-1). With initial SO_4^{2-} levels of 700, 1,100, 1,300 mg/l, the initial (up to 72 hours) SO_4^{2-} reduction rate increased from 13.0 to 13.7 and 18.8 mg SO_4^{2-}/g MLVSS-day, respectively.

Whereas, an initial 2,100 mg/l level had a smaller reduction rate (4.9 $mgSO_4^{2-}$/gMLVSS-day) in this acidogenic phase. Moreover with even higher initial SO_4^{2-} concentration of 2,600 mg/l, there was no SO_4^{2-} reduction (0 mg SO_4^{2-}/gMLVSS-day) within the first 72 hours. The calculation was shown in Appendix A-2.
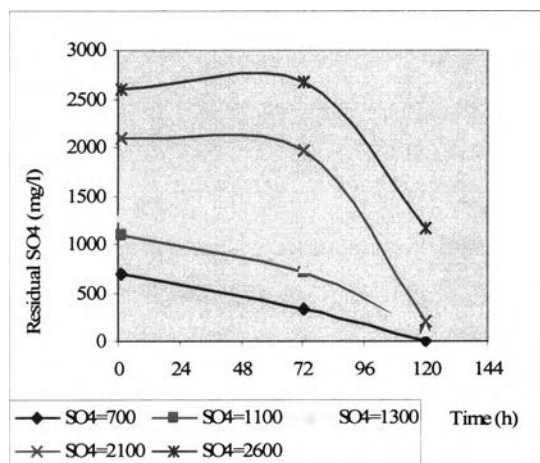
Figure 5.1 Time course of sulfate presented in the effluent.
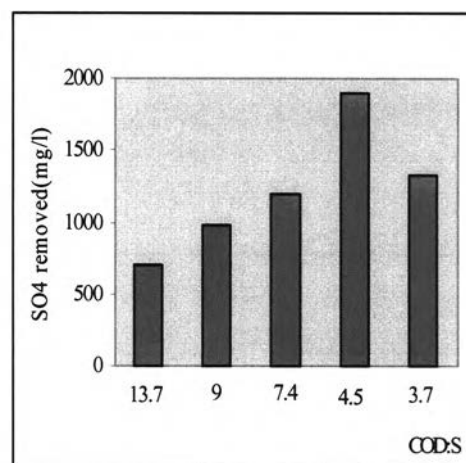
Figure 5.2 Relationship between sulfate removed and COD:S.

However, sulfate reduction occurred later in the methanogenic phase (after 72 hours) with the much higher rate of 90.7 and 77.8 mg SO_4^{2-}/g MLVSS-day for the reactor containing initial SO_4^{2-} of 2,100 and 2,600 mg/l (Appendix A-2). Within the range of SO_4^{2-} 700 to 2,100 mg/l, the maximum sulfate reduction could be expected. Sulfate was removed both in acidogenic and methanogenic phases. The acidogenic phase had the ability to remove SO_4^{2-} at the lower initial concentration. The occurrence of SO_4^{2-} reduction in methanogenic bioreactors is, in most cases, unwanted because large amounts of sulfide are produced and cause unfavorable conditions (Stefanie et al., 1994). The result indicated that the reduction of high SO_4^{2-} concentration occurred in the methanogenesis phase at greater levels than occurred with the lower SO_4^{2-} concentration. The initial higher SO_4^{2-} had more effect on SO_4^{2-} reduction in the methanogenesis phase than in the acidogenic phase. This suggested that in a system containing high SO_4^{2-}(>2,000mg/l), the optimum operational approach should be the 2 stage anaerobic reactor in order to reduce SO_4^{2-} to some extent in the acidogenic stage with a subsequent methanogenic stage to enhance CH_4 production. Mizuno et al. (1998) studied the SO_4^{2-} reduction by SRB in the acidogenic phase. The sucrose acclimated seed sludge from the primary settling tank

in a municipal sewage treatment plant was used. Similar results were reported, where SO_4^{2-} reduction of the lower SO_4^{2-} concentration (600 mg/l) occurred at hydraulic retention time (HRT) as short as 2 hours in an acidogenic reactor. At the high SO_4^{2-} concentration (2,400 mg/l), the SO_4^{2-} in the wastewater was effectively removed with a higher HRT of 8 to 10 hours. Ghigliazza et al. (2000) reported that SRB from an anaerobic treatment plant had the ability to degrade SO_4^{2-} with an HRT of 2 days in a fed batch reactor using propionate as electron donor.

Sulfate reduction was controlled by the amount of sulfate. The SO_4^{2-} concentration affected the SO_4^{2-} removal efficiency. The effects of COD:S showed the limitation of SO_4^{2-} reduction. When initial SO_4^{2-} concentrations were in the range of 700 to 2,100 mg/l, SO_4^{2-} reduction drastically increased and reached maximum at SO_4^{2-} of 2,100 mg/l or COD:S of 4.5 (Figure 5.2). Under these conditions, SO_4^{2-} concentration would never limit the reduction reaction. Almost all SO_4^{2-} was reduced. 89-100 % SO_4^{2-} removal rates were obtained (Table 5.1). However, at an initial SO_4^{2-} concentration of 2,600 mg/l (COD:S=3.7), the SO_4^{2-} reduction decreased. The percentage of SO_4^{2-} removal decreased to 55% due to excessive SO_4^{2-}. Under these conditions, SO_4^{2-} concentration would limit the reduction reaction. This indicated that either the SO_4^{2-} concentration or the COD:S (or both) affected SRB. This present study found that SO_4^{2-} reduction can be controlled by the amount of SO_4^{2-} when initial SO_4^{2-} concentrations are lower than 2,100 mg/l (COD:S higher than 4.5).

Mizuno et al. (1988) reported the SO_4^{2-} removal efficiency of the chemostat systems in which over 90% removal was obtained at SO_4^{2-} concentrations of 600 and 1,200 mg/l, while only 60 % was obtained at 2,400 mg/l.

5.3.2 The Concentration of Sulfate and the COD:S Ratios to Promote Methanogenesis

The effects of initial SO_4^{2-} concentration on CH_4 production were of interest. The total gas that was recorded at 35^0C was calculated to the corrected amount after the mixed liquor had been taken for analysis during the experiment (raw data not shown) and then it was converted to the amount of total gas at STP. The total gas at STP and the percentage of CH_4 quantified were recorded (Table G-3). The amount of

CH_4 produced and the accumulated CH_4 (at STP) were calculated and recorded (Table G-3).

The time course of CH_4 produced with various SO_4^{2-} starting concentrations is show in Figure 5.3 (Table G-4) and the results showing a comparison among reactors containing different amounts of SO_4^{2-} in terms of CH_4 produced are illustrated in Figure 5.4 (Table G-4).

The COD:S also affected CH_4 production. For COD:S at 13.7, 9, 7.4, 4.5 and 3.7 (corresponding to SO_4^{2-} concentration at 700, 1,100, 1,300, 2,100, and 2,600 mg/l), the CH_4 quantities produced were 24, 54, 50, 37 and 36 ml at STP, respectively (Figure 5.4 and Table G-4). In the present of SO_4^{2-} at 700, 1,100 mg/l (COD:S 13.7 and 9, respectively), the CH_4 gas composition analyzed was up to 68 % and 79%, respectively (Table G-4). On the other hand, with SO_4^{2-} at 2,600 mg/l, the composition decreased to 59 % (Table G-4).
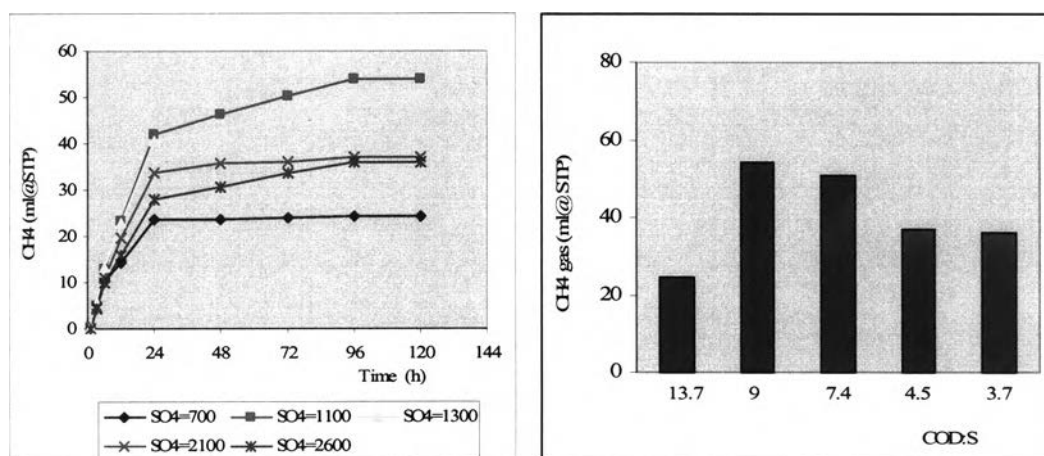

Figure 5.3 Time course of methane production. **Figure 5.4** Effects of sulfate on methane.

Reactors which contained COD:S 13.7 and 9 increased CH_4 production (Figure 5.4). The maximum CH_4 yield was shown at COD:S of 9. With SO_4^{2-} at 1,100 mg/l (COD:S of 9), the amount of CH_4 production was higher than that of any other SO_4^{2-} concentrations. The highest CH_4 production was 196 ml at STP (the theoretical amount of CH_4 of 350 liter at STP per gram of glucose utilized is reported by Tchobaglous and Burton, 1991) and the SO_4^{2-} reduced was 0.356 g, expressed per gram of COD removed. The calculation was shown in Appendices A-3 and A-4. On the other hand, at the higher SO_4^{2-} levels (COD:S in the range 7.4 to 3.7) CH_4 production gradually decreased. This phenomenon was attributed to the increment in SO_4^{2-} reduction of initial low SO_4^{2-} concentration leading to the use of organic waste

as electron donor by MPB. While the latter can explain that the increment in SO_4^{2-} reduction of initial high SO_4^{2-} concentration caused SRB to be more competitive in using organic waste, irrespective of CH_4 production.   Mizuno et al. (1998) found from their study that in the absence of SO_4^{2-} the percentage of CH_4 was higher than that of the system containing SO_4^{2-} of 2,400 mg/l.

The COD used for CH_4 production (CH_4gas-COD) was calculated (Table G-5). The MLVSS was reported (Table G-5). The specific methanogenic activities (SMAs) of this acclimated sludge from a brewery wastewater treatment plant were determined in terms of grams of recovered CH_4-COD in the gas phase per gram of COD removed per gram of MLVSS (Table G-5). Comparison of SMAs from glucose of this selected sludge at various COD:S is shown in Figure 5.5.
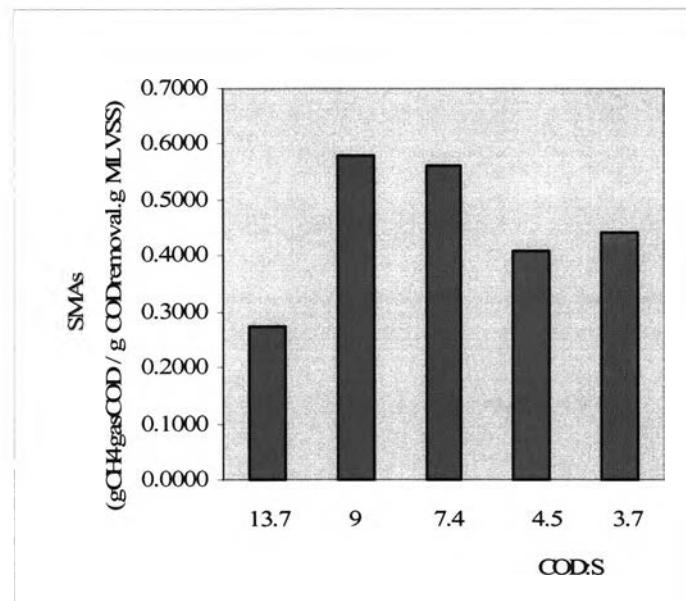

Figure 5.5 Specific methanogenic activities (SMAs) from glucose.

Within the reaction bottles that contained the COD:S ratios of more than 9 (SO_4^{2-} less than 1,100 mg/l), the SMAs of this sludge from glucose increased with increasing SO_4^{2-}. On the other hand, when COD:S ratios of less than 9 (SO_4^{2-} exceeded 1,100 mg/l) were added in the bottle, the SMAs decreased with increasing SO_4^{2-}. MPB increased their activities to the maximum level when COD:S ratios were at 9. The maximum SMA was 0.5815 g CH_4gasCOD/(g COD removal-gMLVSS). (Calculation was shown in Appendix A-5.)   Harada et al. (1994) had tested the SMAs of the sludge originating from MSW from UASB reactors that contained COD

500 mg/l and SO_4^{2-} 150 mg/l giving rise to COD:S 10 using glucose as synthetic waste. He provided different levels of SO_4^{2-} in the vial bottles and found that the SMAs from glucose increased with increasing SO_4^{2-} added to the vial within the range of the test. Their results also indicated that an optimum amount of SO_4^{2-} in the bacterial reactor, the SMA reached level higher than observed when either higher or lower amounts of SO_4^{2-} were provided. This suggested that SMAs depend on the amount of SO_4^{2-} added to the bioreactor. Thus, the optimum amount of SO_4^{2-} in order to increase SMAs needs to be investigated not only to prevent the inhibition of SO_4^{2-} reduction, but also to find the optimum amount of SO_4^{2-} to promote methanogenesis.

Sulfate concentration affected the effluent COD discharge. The influent COD (3,200 mg/l) and effluent COD were recorded and the percentage of COD removal was calculated and reported (Table G-6). Substantial differences in COD removal efficiency were observed among the reactors that contained different levels of SO_4^{2-} as shown in Figure 5.6. When the COD:S was in the range 13.7 to 4.5 (initial SO_4^{2-} concentrations was in the range of 700 and 2,100 mg/l), the percentage of COD removal was 82-86 %. Within this range, COD:S at 9 (SO_4^{2-} at 1,100 mg/l) gave the highest system performance for COD removal (86%). However, at COD:S of 3.7 (SO_4^{2-}2,600 mg/l) COD removal decreased to 75%.
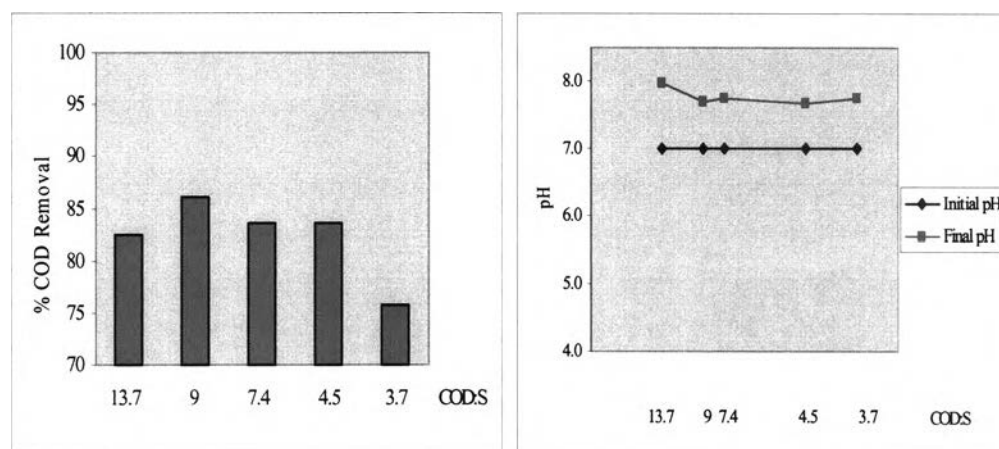
Figure 5.6 The percentage of COD removal   **Figure 5.7** The relationship between
and the COD:S ratio.   pH and the COD:S ratio.

In this experiment, the final pH of all reactors was elevated. However, the final pH in all cases did not exceed 8 (Figure 5.7 and Table G-7). pH is also of importance, because an acidic pH increases sulfide toxicity through increase in sulfide

sulfide concentration. On the other hand at pH > 8 methanogenesis is inhibited resulting in predominance of SRB (Visser et al., 1993). These experimental results elucidated the performance of the reactor involved in sulfide detoxification and heavy metal remediation processes.

### 5.3.3 COD Balance and Electron Flow to MPB and SRB

The material balance of COD around each reactor for the whole experiment period was summarized in Table 5.2. The amount of COD that leaves the reactor mainly consists of:(1) soluble COD in the effluent, (2) recovered CH_4-COD in the gas phase (CH_4gas-COD) plus dissolved CH_4-COD in the water (CH_4aq-COD, in this case = 0, because acidic water was used for water replacement in the gas collection system) and (3) COD used for SO_4^{2-} reduction (ΔSO_4^{2-}-COD). The numbering from (1) to (3) corresponds to that given in Table 5.2. CH_4 gas-COD can be calculated from

$$CH_4 + 2O_2 \longrightarrow CO_2 + 2H_2O \qquad (5.1)$$
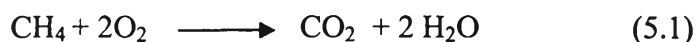
CH_4 16 g reacts with O_2 (COD) 64 g to form CO_2 and H_2O. CH_4 gas-COD received by measuring the amount of total gas and determining the percentage of CH_4. The volume and the number of moles of CH_4 was then calculated at room conditions and changed to the conditions at STP. From the number of moles, CH_4 gas was calculated to gram or milligram basis. From equation (5.1), 1 mg of CH_4 corresponds to 4 mg of COD. Thus, the conversion factor to change mg CH_4 to CH_4 gas-COD is 4 (mgCOD/mgCH$_4$). The computation table to determine CH_4 gas-COD is in Table G-8.

The recovered CH_4-COD in the gas phase (CH_4gas-COD) and COD used for SO_4^{2-} reduction (ΔSO_4^{2-}-COD) were calculated. Then the balance of COD and distribution of electrons to methanogenesis and SO_4^{2-} reduction were determined.

ΔSO_4^{2-}-COD can be calculated from the stoichiometric equation:

$$SO_4^{2-} + 8H^+ + 8e^- \longrightarrow S^{2-} + 4H_2O \qquad (5.2)$$

$$2H^+ + 1/2 O_2 + 2e^- \longrightarrow H_2O \qquad (5.3)$$
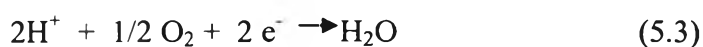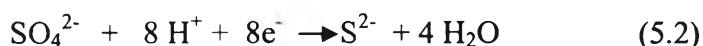
Sulfate reduces 96 g by receiving 8 moles of electrons to form S^{2-} 32 g (Equation 5.2). 2 moles of e^- equivalent to O_2 (COD) 16 g (Equation 5.3). That is

S^{2-} 32 g occurs from the reduction of SO_4^{2-} 96 g which is equivalent to 64 g COD. Thus, SO_4^{2-} reduces 3 mg equivalent to the reduction of COD 2 mg. ΔSO_4^{2-}-COD can be estimated by multiplying the amount of SO_4^{2-} reduced by the conversion factor of 2/3 (mg COD/mg SO_4^{2-}). The computation table to determine ΔSO_4^{2-}-COD is in Table G-9.
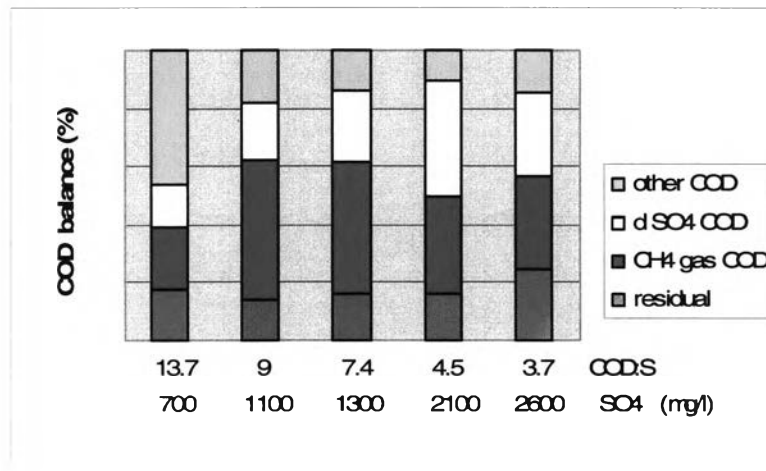
Figure 5.8 COD balance of methane, SO_4^{2-} and effluent at various ratios
of the COD:S. Computation table is shown in Table G-10.

Sulfate concentration affects the COD reduction by SRB or by MPB. Influent COD (100%) was converted to various forms, including the CH_4 gas-COD, reduced ΔSO_4^{2-}- COD, and COD remaining in the effluent at the end of the test. The loss was assumed to be biomass and any other gaseous COD. The percentages of CH_4gas-COD were influenced by the SO_4^{2-} content as shown in Figure 5.8. Notably, the percentages of CH_4 gas-COD dramatically increased with SO_4^{2-} concentration to some extent. CH_4gas-COD was increased when SO_4^{2-} was increased from 700 to 1,100 mg/l (COD:S from 13.7 to 9) but gradually decreased with the higher SO_4^{2-} concentration (COD:S from 9 to 3.7). When SO_4^{2-} concentration was higher than 1,100 mg/l (COD:S less than 9), the CH_4 gas-COD was decreased significantly. Within the range of COD:S from 13.7 to 4.5 in the feed solution, the COD reduction by MPB (CH_4 gas COD) was in the range 21 to 48% (Table 5.2).

The percentage of ΔSO_4^{2-}-COD was more significantly increased with SO_4^{2-} concentration from 700 to 2,100 mg/l (COD:S from 13.7 to 4.5). The COD reduction by SRB (ΔSO_4^{2-}-COD) increased from 14 to 40 % of the COD eliminated (Table 5.2).

Table 5.2 Recovery of COD and distribution of electrons to methanogenesis and sulfate reduction.

COD (mg/l)		(2) CH$_4$ gasCOD (mg/l)	SO$_4^{2-}$ (mg/l)		(3) Δ SO$_4^{2-}$COD (mg/l)	COD:S	Electron Flow to (%)		COD Balance (%)
Influent COD	(1) Effluent COD		Influent	Effluent			MPB	SRB	
3200 (100)	560 (17.5)	696 (21.77)	700	0	464 (14.59)	13.7	58.28	41.72	53.86
3200 (100)	445 (13.90)	1545 (48.27)	1100	118	637(19.91)	9	70.81	29.19	82.08
3200 (100)	526(16.43)	1446 (45.17)	1300	106	784(24.50)	7.4	64.84	35.19	86.10
3200 (100)	526 (16.43)	1057 (33.05)	2100	207	1280 (40.00)	4.5	45.24	54.76	89.48
3200 (100)	775 (24.21)	1032 (32.25)	2600	1167	929 (29.03)	3.7	52.64	47.36	85.49

CH$_4$gas-COD = recovered CH$_4$-COD in gas phase ; ΔSO$_4^{2-}$- COD = COD used for sulfate reduction ;
COD recovery = the sum of item (1) –(3).
Electron flow = electron flows distributed to MPB and to SRB.
Data in the parenthesis is in the unit of per cent.

However, when SO_4^{2-} reached 2,600 mg/l (COD:S 3.7) the ΔSO_4^{2-}-COD was significantly decreased to 29%. At COD:S 9 (SO_4^{2-} concentration of 1,100 mg/l) the COD reduction by MPB was 48.2 % and by SRB was 19.9% comparing to all recovered-COD.

A large fraction of COD eliminated was utilized for CH_4 production. So it can be deduced that MPB originating from a brewery wastewater treatment plant was a good competitor against SRB for glucose utilization. The optimum COD:S ratio for controlling the level of SO_4^{2-} reduction and promoting the methanogenesis was 9. Changing the ratio either way did not give good yield (CH_4). Also, having SO_4^{2-} present to some extent gives the highest performance for bioreactors.

The concept of "electron flow" was used to quantify the extent of competition between SRB and MPB in the reactors (Isa et al., 1986). Electron flow designated the ratio of COD scavenged by SRB or by MPB to the total COD removed.

Electron flow distributed to MPB = (CH_4gas-COD + CH_4aq-COD) /

$$(CH_4gas\text{-}COD + CH_4aq\text{-}COD + \Delta SO_4^{2-}\text{-}COD) \qquad (5.4)$$

Electron flow distributed to SRB = (ΔSO_4^{2-}-COD) /

$$(CH_4gas\text{-}COD + CH_4aq\text{-}COD + \Delta SO_4^{2-}\text{-}COD) \qquad (5.5)$$

Competition between SRB and MPB of acclimated seed sludge from a brewery wastewater treatment plant for glucose synthetic waste was observed via the percentage of electron flow. This study was tested in order to avoid substrate competition and suppress MPB growth. The result showed that the amount of electron flow apportioned to MPB changed with the amount of SO_4^{2-} imposed. At 700 mg SO_4^{2-}/l,   60% of electron flow was distributed to MPB (Figure 5.8 and Table 5.2). The higher percentage of electron flow to MPB was obtained when SO_4^{2-} increased to some extent. But at the higher SO_4^{2-} of 2,600 mg/l, only 52% of the electron flow was distributed to MPB. On the contrary, the amount of electron flow utilized by SRB increased with the amount of SO_4^{2-} in the feed solution.

MPB out-competed SRB for glucose when COD:S ratios were higher than 4.5 over the experimental period of 120 hour operation because a greater electron flow distributed to the MPB was demonstrated (Figure 5.9 and Table 5.2). At high COD:S ratios in this study, MPB predominated (Figure 5.9 and Table 5.2). This indicated

that a high COD:S ratio promotes methanogenesis. The most appropriate COD:S which promoted the highest methanogenesis was found to be 9. At this ratio, the recovered CH_4-COD in the gas phase (CH_4gas-COD) was highest (Figure 5.8).
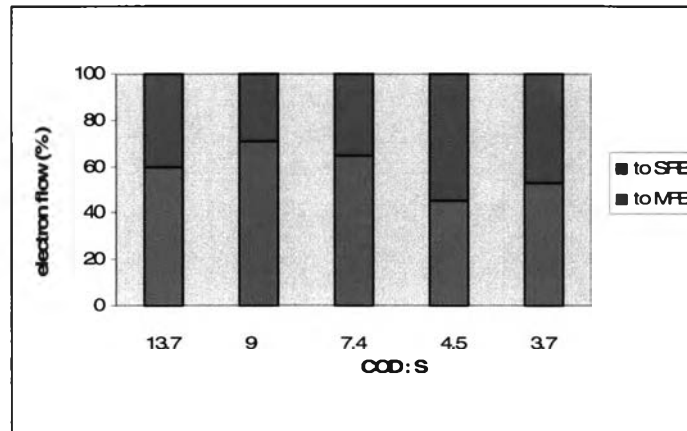
**Figure 5.9** Electron flow distribution to MPB and SRB as a function of COD:S.

The control of the amount of SO_4^{2-} was not only to prevent the inhibition of SO_4^{2-} reduction from hydrogen sulfide, but also to obtain the highest performance of the bioreactors. Choi and Rim (1991) studied the competition for SO_4^{2-} between SRB and methanogenic bacteria for acetate at different COD:S ratios and found that at high ratios (COD:S more than 8) the methanogenic bacteria predominated, while at lower ratios (COD:S less than 5) the SRB were more competitive. However Parkin et al. (1990) studied acetate and propionate as electron donor and reported that when COD:S was less than 10, both SO_4^{2-} reduction and CH_4 production shut down.

The performance of the reactor was investigated at this appropriate COD:S ratio of 9 (Table 5.2). Under this condition, it gave the highest system performance for COD removal (86%) and for CH_4 production while SO_4^{2-}reduction still occurred (89%).

In this study, the mass balances of COD over the experimental period of 120 hour operation indicated that when COD:S was less than 9, the higher the level of SO_4^{2-} the less CH_4 production occurred, because a greater electron flow was distributed to the SRB. Namely, in the experiment in which the highest SO_4^{2-} level was imposed, 47 % of the total COD removal was performed by SRB (Table 5.2). Harada et al. (1994), studied the interaction between SRB and MPB of sludge

originating from MSW by UASB reactors and investigated the electron flow. They found that the higher the level of SO_4^{2-} the less CH_4 was produced.

VFA's were investigated to explain the biomechanisms of the bacteria in this situation. The amount of VFA's at time 120 hour was recorded (Table G-11). The results that show a comparison among reactors containing different amounts of SO_4^{2-} in terms of VFA's is illustrated in Figure 5.10. The outcome of competition between sulfate reducers and fermentative, syntrophic, homoacetogenic and methanogenic bacteria are discussed. At limited amounts of SO_4^{2-}, at COD:S 9 (SO_4^{2-} 1,100 mg/l), there was competition among various types of SRB. The acetate-degrading SRB would be out-competed by the other types of SRB. Thus, MPB would out-compete the acetate degrading SRB for acetate degradation. SMA at this SO_4^{2-} concentration reached the maximum value (Figure 5.5). Thus, the system consisted of MPB and other types of SRB, such as propionate- and butyrate-degrading SRB, which were the syntrophic bacteria. The mechanism was mutualistic interaction. The amount of all VFA's, i.e. acetic, propionic, and butyric acid, was lower than the VFA's in the reactors that contained higher SO_4^{2-} (Figure 5.10). While increasing the amount of SO_4^{2-} to 2,100 and 2,600 mg/l (the COD:S ratios decreased to 4.5 and 3.7), there was sufficient SO_4^{2-} for all types of SRB. There was less competition among the acetate-degrading SRB and other types of SRB in reducing SO_4^{2-}. At these conditions, the acetate-degrading SRB competed with MPB and they out-competed MPB. A severe suppression of MPB was simply a consequence of SRB out-competing MPB. Methane was decreased and caused the decrease in the SMAs (Figure 5.5).
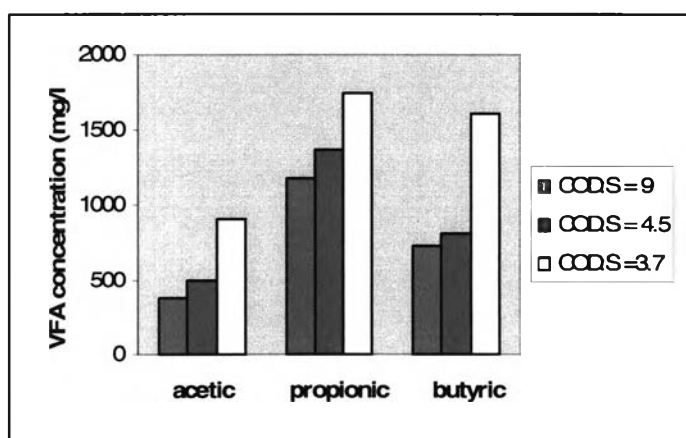
Figure 5.10 The acetic, propionic and butyric acid in the effluent of various reactors with various COD:S ratios.

Both SO_4^{2-} reduction and methanogenesis can be the final step in the anaerobic removal pathway of COD from wastewater. One type of the SRB as well as MPB are capable of using acetate as substrate while the other SRB are capable of using propionic and butyric as well. Moreover, in the presence of the optimum amount of SO_4^{2-}, SRB co-work and show a mutualistic interaction for organic substrate in wastewater.

As a result of the fundamental process information from this study, a sulfide detoxification and heavy metal remediation process at the optimum SO_4^{2-} concentrations has been developed. Development activities have included the test of microbial toxicity from SO_4^{2-} commingling with heavy metal(s) and the monitoring of critical process variables.

5.4 Conclusion

The optimum reactor conditions for simultaneous control of SO_4^{2-} and heavy metals in complex wastewater streams depend not only on the fundamental main parameters, such as temperature, pH, and nutrients in the system but they also depend on the conditions in the reactor that promote the organic reduction reaction, methanogenesis, and precipitation of the toxic substances in the system. The COD:S ratio is stated. Although SRB always compete with MPB for organic substrate as electron donors. However, under optimum conditions of the COD:S ratio with an appropriate bacterial consortium, the outcome of the mutualism between sulfate reducers and fermentative, syntrophic, homoacetic and methanogenic bacteria could be achieved. The COD:S ratios would promote reaction in COD removal efficiency, promote methanogenesis by increasing the use of COD by MPB and give the highest in the SMAs, suppress competition between SRB and MPB, and show mutual interaction between SRB and MPB for glucose synthetic waste, promote the level of SO_4^{2-} reduction by finding the optimum level of SO_4^{2-} reduction by SRB, and generate high pH to help promote metal sulfide precipitation. With the high performance bacterial assemblage from a brewery wastewater treatment plant, the most effective condition was found at COD:S of 9 (SO_4^{2-} concentration was 1,100 mg/l). With this relationship a mutualistic interaction between MPB and SRB was shown. The maximum SMA was 0.5815 (gCH$_4$gasCOD)/(gCOD removal-gMLVSS). The CH$_4$ produced under these conditions was 0196 ml(at STP) and the SO_4^{2-} reduced was 0.356 g per gram of

COD removal. At COD:S 9 the COD reduction by SRB was 20% and by MPB was 48%. This condition gave the highest system performance for COD removal (86%) and for CH_4 production while SO_4^{2-} reduction still occurred (89%). The final pH of the effluent was elevated but did not exceed a pH of 8, which was appropriate for metal sulfide precipitation. This result illustrated performance parameters for a reactor involved in simultaneous CH_4 production, sulfide detoxification and a heavy metal remediation process.

5.5 References

APHA, AWWA, and WPCF. 1992. Standard Methods for the Examination of Water and Waste Water, 19 ed. Washington, D.C.

Choi, E. and Rim, J. 1991. Competition and inhibition of sulfate reducers and methane producers in anaerobic treatment. Water Science and Technology 23, 1259-1264.

Ghigliazza, R., Lodi A., and Rovatti, M. 2000. Kinetic and process considerations on biological reduction of soluble and scarcely soluble sulfates. Resources Conservation & Recycling 29:181-194.

Harada, H., Uemura, S., and Momonoi, S. 1994. Interaction between sulfate-reducing bacteria and methane-producing bacteria in UASB reactors fed with low strength wastes containing different levels of sulfate. Water Research 28:355-367.

Isa, Z., Grusenmeyer S., and Verstrate, W. 1986a. Sulfate reduction relative to methane production in high-rate anaerobic digestion: Microbiological aspects. Applied and Environmental Microbiology 51: 580-587.

Isa, Z., Grusenmeyer, S., and Verstrate, W. 1986b. Sulfate reduction relative to methane production in high-rate anaerobic digestion: Technical aspects. Applied and Environmental Microbiology 51:572-579.

Kristjason, J. K., Schonheit, P., and Thauer, R. K. 1982. Different Ks values for hydrogen of methanogenic bacteria and sulfate reducing bacteria: An explanation for the apparent inhibition of methanogenesis by sulfate. Archives of Microbiology 131: 278-282.

Mizuno, O., Li, Y.Y., and Noike, T. 1998. Effects of sulfate concentration and sludge retention time on the interaction between methane production and sulfate reduction for butyrate. Water Science and Technology 30: 45-54.

Parkin, G. F., Lynch, N.A., Kuo W.C., Van Keuren, E. L., and Bhattacharya, S.K. 1990. Interaction between sulfate reducers and methanogens fed acetate and propionate. Research Journal WPCF 62: 780-788.

Percheron, G., Bernet, N., and Moletta, R. 1997. Start-up of anaerobic digestion of sulfate wastewater. Bioresource Technology 61:21-27.

Stefanie, J. W., Elferink, H.O., Pol, L.W., Visser, A., and Stams, A. 1994. Sulfate reduction in methanogenic bioreactors. FEMS Microbiology Reviews15:119-136.

Tchobaglous, G. and Burton, F.L. 1991. Revised from Metcalf & Eddy Inc. Wastewater Engineering Ttreatment, Disposal, and Reuse. pp. 1277-1279. Singapore: McGraw Hill Inc.

Visser, A., Gao, Y. and Lettinga, G. 1993. Effects of pH on methanogenesis and sulfate reduction in thermophilic (55^0C) UASB reactors. Bioresource Technology 44: 113-121.