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# COMPETITION BETWEEN METHANOGENESIS AND SULFIDOGENESIS IN ANAEROBIC WASTEWATER TREATMENT

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## ABSTRACT

This study was conducted to investigate the methanogenic and sulfidogenic activities of biomass in a UASB reactor treating wastewater containing benzoate (680 mg $l^{-1}$) and sulfate (increased from 1080 to 2680 mg $l^{-1}$) at 37°C and 12 hours of hydraulic retention. Results showed that after 120 days of acclimation, sludge consistently removed 99.5% of benzoate regardless of increased sulfate concentrations. Sulfidogenesis gradually out-competed methanogenesis during the acclimation phase, as indicated by the increase of sulfate-reducing efficiency (up to 99%) accompanied by the decrease of methane production. Overall sulfate removal efficiency was limited after the reactor had reached its maximum sulfate reduction rate of 2.1 g S ($l$ $d^{-1}$). Further increasing sulfate concentration from 1080 mg $l^{-1}$ to 2680 mg $l^{-1}$ lowered the sulfate-reducing efficiency from 85% to 39%. Flow of available electrons toward sulfidogenesis increased with the decrease of benzoate concentration, and was only slightly affected by the sulfate concentration or the benzoate/$SO_4^{2-}$-S ratio. © 1998 Published by Elsevier Science Ltd. All rights reserved

## KEYWORDS

Anaerobic; benzoate; competition; cresol; methanogenesis; sulfidogenesis.

## INTRODUCTION

It has long been recognized that high concentrations of sulfate in wastewater could adversely affect the methane production in anaerobic treatment processes. One group of bacteria degrade substrate into bicarbonate and/or intermediates, such as acetate, in the process reducing sulfate to sulfide. On the other hand, another group of anaerobes syntrophically convert substrate into methane and bicarbonate. These two groups of bacteria, i.e. sulfidogens and methanogens, have many physiological similarities (Holt *et al.*, 1994); for example, most of them are strictly anaerobic, chemoheterotrophic and of similar optimum temperature and pH. Therefore, they are found co-existing in many anaerobic ecosystems.

A number of studies have been recently conducted on the anaerobic treatment of sulfate-rich wastewaters. However, it has been difficult to compare these published results meaningfully because experiments were

conducted under different conditions. There has been no agreement so far on what are the determining factors affecting the competition between sulfidogenesis and methanogenesis. Some reported that the competition between sulfidogenesis and methanogenesis was mainly dependent upon the ratio of COD (chemical oxygen demand) and sulfate in wastewater (Mizuno *et al.*, 1994; Uberoi and Bhattacharya, 1995; Li *et al.*, 1996); others attributed to factors such as substrate concentration (Isa *et al.*, 1986), organic loading rate (Yoda *et al.*, 1987) and even reactor design (Isa *et al.*, 1986). Yet, some believed that sulfidogens in general have a higher affinity to substrates than methanogens (Kristjansson *et al.*, 1982; Widdel, 1988), and thus would out-compete the latter for substrates, such as hydrogen, formate and acetate.

This study was conducted to investigate the competition between sulfidogenesis and methanogenesis in treating a medium-strength wastewater containing sulfate over a wide range of concentrations. Benzoate was chosen as the organic substrate because it is a key intermediate in the degradation of many aromatic chemicals (Knoll and Winter, 1989; Londry and Fedorak, 1993; Fang *et al.*, 1996). Anaerobic degradation of benzoate in wastewater has been recently studied under various conditions (Li *et al.*, 1995; Fang and Zhou, 1997). Therefore, results of this study could be compared with some of these published results of treating benzoate-containing wastewaters at the same temperature and in the same type of reactors.

## MATERIALS AND METHODS

### Experimental conditions

The experiment was conducted at 37°C for 251 days in a 2.8 l UASB (upflow anaerobic sludge blanket) reactor (Fang and Chui, 1993; Fang *et al.*, 1996). Wastewater containing benzoate of constant concentration and sulfate of increased concentrations was treated in the reactor with 12 hours of hydraulic retention time. The reactor was seeded with 1.5 l of mixed anaerobic sludge, including 0.5 l from the anaerobic sludge digester of a local sewage treatment plant, 0.5 l from a pilot UASB reactor treating carbohydrate-rich wastewater, and 0.5 l more of granulated sludge from another UASB reactor which had been treating wastewater containing benzoate (2520 mg $l^{-1}$) and sulfate (3000 mg $l^{-1}$) for over 3 months.

The experiment was divided into two phases. Phase 1 was the startup which allowed bacteria to become acclimated to benzoate and sulfate. The wastewater contained 1080 mg $l^{-1}$ of sulfate and 440 mg $l^{-1}$ of benzoate, plus sucrose as a co-substrate. The concentration of sucrose was initially 1000 mg $l^{-1}$ (Phase 1a, days 1-34); it was then lowered to 500 mg $l^{-1}$ in Phase 1b (days 35-153), and lastly completed removed from the wastewater in Phase 2. Wastewater in Phase 2 contained benzoate at a constant concentration of 680 mg $l^{-1}$ and an increased concentration of sulfate from 1080 mg $l^{-1}$ (Phase 2a, days 154-194), to 1580 mg $l^{-1}$ (Phase 2b, days 195-219) and eventually to 2680 mg $l^{-1}$ (Phase 2c, days 220-251), as illustrated in Figures 1a and 1b. Throughout the experiment, m- and o-cresols (100 mg $l^{-1}$ each) were also added to the wastewater with the initial intention to examine the toxic effect, if any, of the isomers.

### Analytical methods

The amount of biogas produced in each reactor was recorded daily using the water replacement method. The content of methane, $CO_2$, $N_2$ and $H_2S$ in the biogas was analyzed by a gas chromatograph (GC, Hewlett-Packard, Model 5890 Series II) equipped with a thermal conductivity detector and a 10 m stainless steel column packed with HayeSepQ (80/100 mesh). Helium was used as the carrier gas at a flow rate of 22 ml $min^{-1}$. The column was operated at a temperature program of 90°C for 1.2 minutes followed by 2 minutes at 110°C. The respective temperatures of injector and detector were 130°C and 200°C.

Sulfate concentration in the effluent was analyzed by an ion chromatograph (Shimadzu HPLC 10A) equipped with a CDD-6A conductivity detector and a Shim-Pack IC-A3 column. A solution containing 8.0 mM of 4-hydroxybenzoic acid and 3.2 mM of bis[2-hydroxyethyl]iminotris-[hydroxymethyl]methane was used as the mobile phase. The flow rate of the mobile phase was 1.0 ml $min^{-1}$, oven temperature was 40°C and detector temperature was 43°C. Sulfide concentration in the effluent was analyzed using the iodometric method in the *Standard Methods* (APHA 1989).

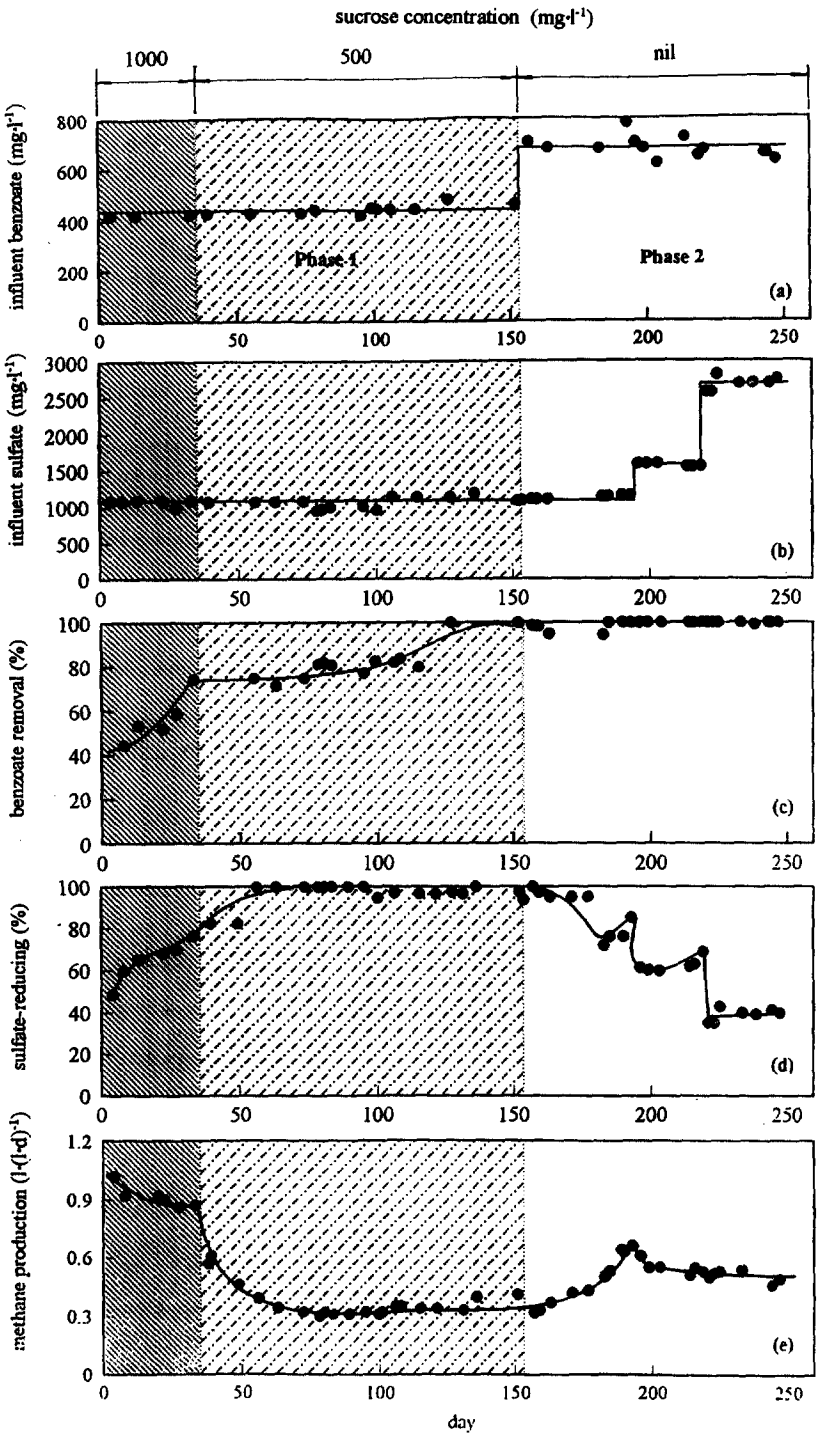



Figure 1. Concentrations of (a) benzoate and (b) sulfate in the wastewater, plus efficiencies of (c) benzoate removal and (d) sulfate-reducing, and (e) methane production rate of the reactor. (The wastewater contained 100 mg $l^{-1}$ each of m- and o-cresols, which are not shown, throughout the experiment.)

The concentrations of benzoate and fatty acids (from acetic to heptanoic acids) were determined by a second gas chromatograph (Hewlett Packard, Model 5890 Series II) equipped with a flame ionization detector and a 10m x 0.53mm HP-FFAP fused-silica capillary column. Samples were filtered through a 0.2 μm filter, acidified by phosphoric acid, and measured for free acids. The initial temperature of the column was 70°C for 4 minutes and then 140°C for 3 minutes, and finally 170°C for 4 minutes. The temperatures of injector and detector were both 200°C. Helium was used as the carrier gas at a flow rate of 25 ml $min^{-1}$. The column used in this study was unable to detect formic acid.

## RESULTS AND DISCUSSIONS

### Degradation of benzoate in the presence of sulfate

Anaerobic degradation of benzoate in the presence of sulfate is a complex process. Table 1 summarizes the key reactions and the bacteria involved. Benzoate is first either converted by syntrophic acetogenic bacteria into acetate and hydrogen (Reaction 1), or converted by sulfidogens incompletely into acetate (Reaction 2) or completely into bicarbonate (Reaction 3). In the subsequent degradation processes, acetate and hydrogen can be consumed by both methanogens and sulfidogens, producing methane by the former (Reactions 4 and 6) and sulfides by the latter (Reactions 5 and 7).

Sulfidogens are either in competition or in syntrophic association with other bacteria. For example, sulfidogens conducting Reactions (2) and (3) are in competition for benzoate with syntrophic acetogens conducting Reaction (1). On the other hand, sulfidogens conducting Reaction (2) producing acetate are in syntrophic association with methanogens conducting Reaction (4) and other sulfidogens conducting Reaction (5), both of which consume acetate.

Table 1. Reactions involved in converting benzoate into methane and sulfide

| Reaction | e - Donor | Bacteria[note-1] | Reactions[note-2] | $\Delta G^{o\prime}$ (kJ) |
|---|---|---|---|---|
| 1 | benzoate | SAB | $Be^- + 7H_2O \rightarrow 3Ac^- + HCO_3^- + 3H_2 + 3H^+$ | +89.7 |
| 2 | benzoate | SRB-i | $Be^- + 0.75SO_4^{-2} + 4H_2O \rightarrow 3Ac^- + 0.75HS^- + HCO_3^- + 2.25H^+$ | -24.3 |
| 3 | benzoate | SRB-c | $Be^- + 3.75SO_4^{-2} + 4H_2O \rightarrow 3.75HS^- + 7HCO_3^- + 2.25H^+$ | -165.8 |
| 4 | acetate | MPB-a | $Ac^- + H_2O \rightarrow CH_4 + HCO_3^-$ | -31.0 |
| 5 | acetate | SRB-c | $Ac^- + SO_4^{-2} \rightarrow HS^- + 2HCO_3^-$ | -47.6 |
| 6 | hydrogen | MPB-h | $H_2 + 0.25HCO_3^- + 0.25\,H^+ \rightarrow 0.25\,CH_4 + 0.753H_2O$ | -33.9 |
| 7 | hydrogen | SRB-c | $H_2 + 0.25SO_4^{-2} + 0.25\,H^+ \rightarrow 0.25HS^- + H_2O$ | -38.0 |

note-1: SAB: syntrophic acetogen; SRB-i: sulfidogen conducting incomplete degradation
SRB-c: sulfidogen conducting complete degradation; MPB-a: acetotrophic methanogen
MPB-h: hydrogenotrophic methanogen

note-2: $Be^-$: benzoate; $Ac^-$: acetate
$\Delta G^{o\prime}$ : change of standard Gibbs free energy per reaction at pH7

### Startup

Figure 1c illustrates that removal of benzoate steadily increased during the startup phase, when sucrose was used as co-substrate, from the initial 42% to 75% by the end of Phase 1a. During Phase 1b when sucrose concentration was reduced to 500mg $l^{-1}$, the removal efficiency further increased, although at a slower rate, eventually reaching 99.5% near the end of that phase. The increase of benzoate removal efficiency was because the sludge was becoming acclimated to the presence of benzoate and sulfate and gradually building up the population of sulfidogens and syntrophic acetogens. However, the increase of benzoate removal efficiency was accompanied by the increase of sulfate-reducing efficiency (Figure 1d) and the decrease of methane production rate (Figure 1e). The former was increased from 48% initially up to 99% near the end of Phase 1a. The latter was decreased from the initial 1.02 l-methane•$(l•d)^{-1}$ to 0.87 l-methane•$(l•d)^{-1}$ on day 35, and further lowered to an average level of 0.39 l-methane•$(l•d)^{-1}$ during days 56-153 (Figure 1e). This

indicated that, as the population of sulfidogens increased during startup, sulfidogenesis gradually outcompeted methanogenesis for benzoate.

Absence of sucrose co-substrate and increase of sulfate concentration

In Phase 2, co-substrate sucrose was completely absent from the wastewater while the benzoate concentration was increased to 680 mg $l^{-1}$. Throughout Phase 2, the benzoate removal efficiency remained consistently at 99.5% (Figure 1c) and the effluent contained little intermediate fatty acids, including acetate. During Phase 2a when sulfate concentration remained at 1080 mg $l^{-1}$, sulfate-reducing efficiency decreased from 99% to 72% (Figure 1d) while methane production increased reaching 0.60 $l\bullet(l\bullet d)^{-1}$ (Figure 1e) by day 183. This indicated that absence of co-substrate sucrose had an adverse effect on sulfidogenesis; but sulfidogenic activity recovered partially near the end of Phase 2a, reaching 85% by day 193. When sulfate was increased to 1580 mg $l^{-1}$ and 2680 mg $l^{-1}$, respectively in Phases 2b and 2c, sulfate-reducing efficiency progressively decreased to 77% in Phase 2b and 39% in Phase 2c (Figure 1d). The average sulfidogenic activities were 1.7, 2.0, and 2.1 g $S\bullet(l\bullet d)^{-1}$, respectively, in Phases 2a, 2b and 2c. It appears that the biomass in the reactor had a maximum sulfidogenic activity of about 2.1 g $S\bullet(l\bullet d)^{-1}$, and thus the overall sulfate-reducing efficiency decreased with the further increase of sulfate concentration in Phases 2b and 2c.

Removal of m- and o-cresols

Wastewater throughout the experiment contained 100 mg $l^{-1}$ each of m- and o-cresols. Removals of cresol isomers appeared to be affected by the presence of sucrose as co-substrate. At the end of Phase 1 when the wastewater contained 500 mg $l^{-1}$ of sucrose, an average of 11.2% of m-cresol and 13.5% of o-cresol were removed. The corresponding removal efficiencies became only 6.6% and 5.0%, respectively, in Phase 2 when sucrose was absent from the wastewater. On the other hand, the presence of cresol isomers, each at the concentration of 100 mg $l^{-1}$, did not appear to harm either methanogenesis or sulfidogenesis. The reactor was able to remove over 99.5% of benzoate and reduce 99% of sulfate over an extended period.

Electron flows

The parameter COD (APHA 1985) is a measurement of oxygen demand exerted by pollutants in wastewater. It may also be perceived as a measurement for the amount of electrons in the pollutants available for oxidation. Under anaerobic conditions, methanogens and sulfidogens compete for the available electrons to produce, respectively, methane and sulfide. According to the following equations, eight electrons are needed to produce one methane molecule or to reduce one sulfate ion to sulfide:

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$$
$$SO_4^{-2} + 4H_2O + 8e^- \rightarrow S^{-2} + 8OH^-$$

Thus, the degree of competition for electrons may be expressed as the percentage of electron flow (Isa *et al.* 1986) calculated from the quantities of methane produced and sulfate reduced. The percentage of electron-flow for sulfidogenesis equals to the number of moles of sulfate reduced divided by the total number of moles of sulfate reduced and methane produced.

Figure 2a illustrates that in Phase 2 of this study treating wastewater containing a constant 680 mg $l^{-1}$ of benzoate, the percentage of electrons flowing towards sulfidogenesis was only slightly dependent upon the sulfate concentration. When wastewater contained 1000 mg $l^{-1}$ of sulfate, 44% of available electrons were utilized for sulfidogenesis; the percentage increased to 47% when sulfate concentration was 1580 mg $l^{-1}$, and further up to 50% when sulfate concentration reached 2680 mg $l^{-1}$. Figure 2b illustrates the corresponding plot of electron flow for sulfidogenesis against the benzoate/$SO_4^{2-}$-S ratio.

Results of two similar UASB studies treating wastewater containing different concentrations of benzoate and sulfate at 37°C and 12 hours of hydraulic retention are also plotted in Figure 2 for comparison. Wastewater in one study (Fang and Zhou 1997) contained 1000 mg $l^{-1}$ of benzoate and sulfate ranging 1800-5600mg $l^{-1}$; the corresponding values in the other study (Fang *et al.* 1997) were 2520 mg $l^{-1}$ and 1000-7500 mg $l^{-1}$.

Figure 2 illustrates that the flow of available electrons toward sulfidogenesis increased with the decrease of benzoate concentration, and it was only slightly affected by the sulfate concentration or the benzoate/$SO_4^{2-}$-S ratio. In this study treating 680 mg $l^{-1}$ of benzoate, 44-50% of electron flow toward sulfidogenesis; the corresponding percentages were 30-35% in treating wastewater containing 1000 mg $l^{-1}$ of benzoate and only 9-12% in treating 2520 mg $l^{-1}$ of benzoate (Fang *et al.* 1997).

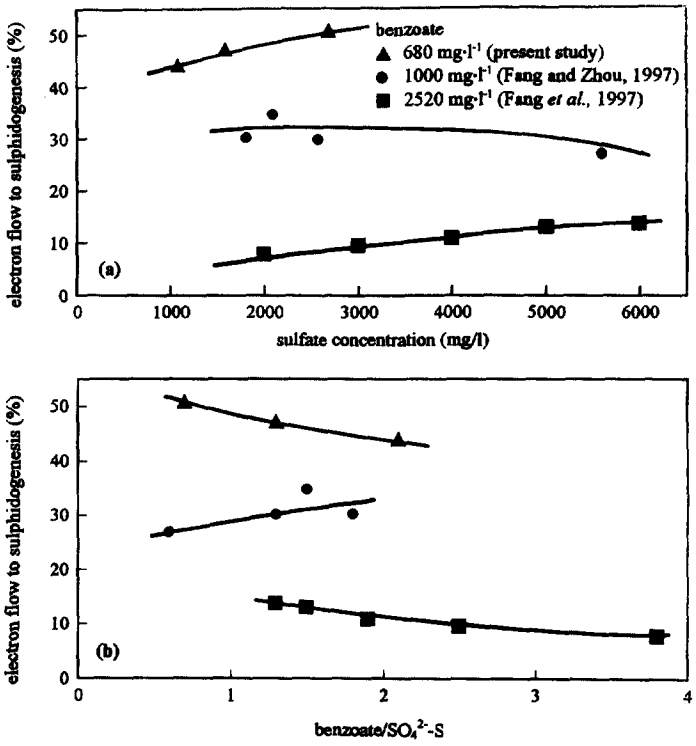
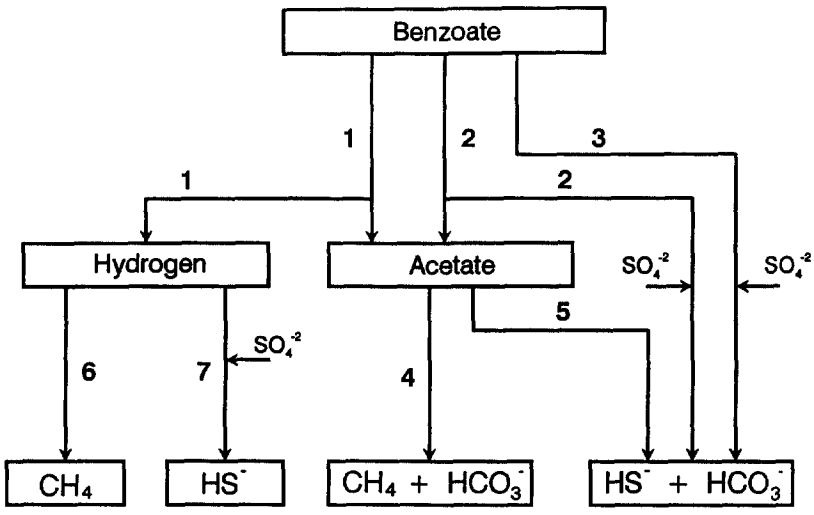



Figure 2. Percentage of electron flow for sulfidogenesis at three benzoate concentrations vs (a) sulfate concentrations, and (b) benzoate/$SO_4^{2-}$-S ratios.

This was probably because SRB in the reactors had reduced sulfate at a rate near its maximum capacity; a further increase of sulfate concentration would not divert additional electron flow toward sulfidogenesis. The sulfide reducing capacity in the reactor treating 1000 mg $l^{-1}$ of benzoate was 1.5-1.6 g S•(l•d)$^{-1}$ (Fang and Zhou 1997), comparable to the capacity of 1.7-2.1 g S•(l•d)$^{-1}$ for the reactor of this study. The capacity for the experiment treating 2520 mg $l^{-1}$ of benzoate was not available for comparison. On the other hand, SAB and MPB in these reactors were likely not yet reaching their maximal capacities. Thus, the increase of benzoate concentration provided extra electron donors for these bacteria, and the relative amount of electron flow toward sufidogenesis decreased accordingly.

Figure 3 illustrates the interactions and competitions between methanogenesis and sulfidogenesis of benzoate for the seven reactions summarized in Table 1. According to chemical thermodynamics, a reaction can proceed only if the $\Delta G$, change of Gibbs free energy, is negative. Reactions with higher negative value of $\Delta G$ have a higher tendency to proceed. The $\Delta G^{o'}$ listed in Table 1 are the $\Delta G$ values under standard conditions and at pH7. Thus, judging from the $\Delta G^{o'}$ differences, SRB are thermodynamically more favorable in competing for electron donors, particularly, in competing for benzoate. Reaction (3) conducted by SRB-c is highly favorable thermodynamically (-165.8 kJ) than Reaction (2) by SRB-i (-24.3 kJ), which is still considerably much more favorable than Reaction (1) by SAB (+89.7 kJ). However, results of this series of studies showed that the SRB-c activity was still limited.




Note: $H^+$ and $H_2O$ are not shown.

Figure 3. Interaction and competition between methanogenesis and sulfidogenesis.

This seems to echo the observation by Widdel (1988) that SRB-i often out-competes SRB-c for substrate, probably because SRB-c has less affinity to the substrate. Results of this study demonstrate that one cannot predict substrate utilization between competing reactions simply based on the relative magnitude of $\Delta G$ values.

## CONCLUSIONS

Based on results of this experimental study treating wastewater containing benzoate and sulfate in a UASB reactor, one may draw the following conclusions:

1. After 120 days of acclimation, sludge consistently removed 99.5% of benzoate regardless of the increase sulfate concentration.

2. During the acclimation phase, sulfidogenesis gradually out-competed methanogenesis, as indicated by the increase of sulfate-reducing efficiency (from initial 48% up to 99%) along with the decrease of methane production (from initial 1.02 to 0.39 l-methane•(l•d)$^{-1}$).

3. Overall sulfate removal efficiency was limited after the reactor had reached its maximum sulfate reduction rate of 2.1 g S•(l•d)$^{-1}$. Further increasing sulfate concentration from 1080 mg l$^{-1}$ to 2680 mg l$^{-1}$ lowered the sulfate-reducing efficiency from 85% to 39%.

4. Flow of available electrons toward sulfidogenesis increased with the decrease of benzoate concentration, and was only slightly affected by the sulfate concentration or the benzoate/$SO_4^{2-}$-S ratio.

5. Removal of m- and o-cresols (100 mg l$^{-1}$ each) was 11.2-13.5% in the presence of sucrose co-substrate (500 mg l$^{-1}$) but only 5.0-6.6% in the absence of sucrose. The presence of cresol isomers did not harm either methanogenesis or sulfidogenesis.

## ACKNOWLEDGEMENT

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