CORRELATION BETWEEN BIOMASS CONCENTRATION AND EXTENT OF SULPHATE REDUCTION IN AN ACIDOGENIC REACTOR

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Abstract

Two stage high rate anaerobic treatment systems comprising of an acidogenic reactor (or equalisation/ buffer tank) followed by a methanogenic reactor are becoming increasingly popular to treat high strength wastewater from industries. In these systems, sulphate present in the wastewater is reduced to sulphide either partially or completely in the acidogenic reactor and completely in the methanogenic reactor. The effect of fermentation products on the extent of sulphate reduction in the acidogenic reactor was investigated in a continuously-fed, well mixed laboratory-scale 3 L fermenter operating at a temperature of 35^{o}C and pH of 6. The feed was based on either glucose or molasses as the carbon source. It was observed that as the carbohydrate concentration in feed was increased sulphate reduction was suppressed. It was confirmed that volatile organic acids like acetic, propionic and butyric acids, hydrogen and residual glucose did not cause inhibition of sulphate reduction. However, biomass concentration correlated negatively with extent of sulphate reduction. This correlation was expressed mathematically and the same expression with the same parameters adequately predicted the effect of biomass concentration on extent of sulphate removal for both steady state and transient data irrespective of glucose or molasses feed. It was seen from the best fit of this expression that a biomass concentration of 3300 mg-COD.L^{-1} would completely repress sulphate reduction in the acidogenic reactor. Even when sulphate removal was suppressed the presence of sulphate reducing bacteria (SRB) in the reactor was confirmed through Fluorescent In Situ Hybridisation (FISH) visualisation. Moreover, the numbers of SRB seemed to increase with carbohydrate concentration in feed. It was speculated the suppression of sulphate removal might be due to a switch in function of SRB from sulphate reducing to fermentation or acetogenesis.

Keywords*: sulphate reducing bacteria, anaerobic digestion, inhibition, sulphate reduction, acidogenic reactor, high rate anaerobic treatment*

1. INTRODUCTION

Two stage high rate anaerobic treatment systems are becoming increasingly popular to treat high strength wastewater from industries. In this system, wastewater flowing into the first stage, which serves as an equalisation or buffer tank, is partially acidified to volatile organic acids, primarily acetic, propionic and butyric acids. Hence this stage is also referred to as the acidogenic reactor. The partially acidified wastewater is then pumped into the second stage where the organic carbon is mineralised to methane and carbon dioxide. The second stage is referred to as the methanogenic reactor. If sulphate is present in the wastewater it is reduced to sulphide by sulphate reducing bacteria (SRB) under anaerobic conditions. The extent of sulphate reduction can be affected by several operational conditions such as hydraulic

and solid retention times (Mizuno et al., 1994; Mizuno et al., 1998b), pH (Reis et al., 1988; Reis et al., 1991; Visser, 1995), temperature (Westermann and Ahring, 1987; Abdollahi and Nedwell, 1979;Omil et al., 1997; Rebac et al., 1996), dissolved oxygen (Marschall et al., 1993, Dilling and Cypionka, 1990, Frund and Cohen, 1992; Lens et al., 1995) and redox potential (Delgado et al., 1999; Mossey, 1985). Additionally in the acidogenic reactor, high concentration of the weak acid fermentation products, most importantly acetate, might also cause acute toxicity. Ghose and Wiken (1955) undertook the first study and showed that acetic acid and other weak acids can inhibit sulphate reducing bacteria. The toxicity tests were undertaken by deliberately spiking known amounts of acetate, propionate and butyrate into reactors fed with lactate and sulphate. Since pH was not controlled in their experiments a precise cause of the toxicity could not be determined. Based on serial batch experiments, Reis et al., (1990) found that acetate inhibited sulphate reduction and it followed a non-competitive inhibition model with an inhibition constant (K_I) of 54 $mg.L^{-1}$. This finding has been widely accepted and quoted by other investigators (Nedwell and Reynolds, 1996; Okabe et al., 1995; Reis et al., 1992; Reis et al., 1991; and Hickey and Goodwin, 1991). Bhattacharya et al., (1996) speculated that sulphate reducers might be inhibited by high concentration of methanogens in an acetate-fed methanogenic reactor. Han and Levenspiel, (1988) have suggested that very high concentration of biomass can act as an inhibitor in fermenters.

In this paper results from laboratory scale investigations that were carried out to study the effect of the products of fermentation on sulphate reduction in an acidogenic reactor of a two-stage high rate anaerobic wastewater treatment system are reported. The fermentation products considered included volatile organic acids like acetic, propionic and butyric acids, hydrogen and biomass. Experiments were conducted using continuously-fed, well-mixed fermenters operated at constant temperature, pH and redox potential rather than batch tests, to minimise the acclimation of microorganisms to new environments. This also enabled manipulation of the concentration of inhibitor while keeping other factors constant.

2. METHODS

2.1. Apparatus

The acidogenic reactor was a continuously-fed, stirred, cylindrical, plexiglas vessel with a maximum volume of 3000 mL and an operating volume of 2300 mL. The reactor contents were stirred by a stainless steel, six-bladed impeller and the stirring motor provided variable speeds ranging from 20 - 2000 rpm. Stirring speed was kept constant at 750 rpm. The reactor was covered with aluminium foil to prevent light penetration that might promote growth of phototrophs. A Shimaden Temperature Controller series SR 22-2P-000 was used to control the temperature of the acidogenic reactor at 35^{o}C. The heating element was a 185 W Helios Cartridge Heating Element, which was inserted through a stainless steel thermowell attached to the reactor lid. The pH of the acidogenic reactor was maintained by automatic addition of 1 N NaOH solution. The pH probe was interfaced into a 486 DX-2, 50 MHz computer, and pH set point was controlled from this computer. The pH probe was cleaned and re-calibrated once a week. Masterflex variable-speed peristaltic pumps were used to pump feed, effluent and caustic. Gas production was measured on-line by a displacement gas meter interfaced to a computer.

2.2. Feed

The feed was based on either glucose or molasses as the carbon source. Nitrogen and phosphorous were supplied in the form of NH_4Cl and KH_2PO_4 respectively in appropriate amounts to meet a ratio of TOC:N:P in the feed of 100:10:2 (by weight). Trace amounts of metals such as Fe, Ni, Mn, Cu, Zn, Mo and B were also added. Sulphate in the form of sodium sulphate was also added to the feed. The feed was anaerobically stored in a 20 L polypropylene plastic tank (Nalgene®) in a refrigerator to keep its temperature below 4^{o}C.

2.3. Analysis

Liquid samples were collected and analysed for insoluble COD (taken as biomass-COD), glucose, acetate, propionate, butyrate, total sulphate and sulphide. Glucose, acetate, propionate and butyrate were determined using a High Performance Liquid Chromatograph (HPLC) equipped with Bio-Rad Aminex HPX-87H (300 x 7.8 mm) column with a micro-guard cation cartridge H^+ (30 x 4.6 mm) supplied with an on-line 2 μm filters, Waters M-45 pump, Waters PE 200 RI detector, Waters WISP 710B auto sampler injector, and Waters 740 data integrator. The instrument was operated at a column temperature of 65^{o}C, using 0.6 $mL.min^{-1}$ of 0.008 N H_2SO_4 eluent and an injection volume of 30 μL. The instrument was calibrated by injecting a standard mixture and programming integrator to perform automatic external standard calibration.

Calibration was undertaken every 10 to 15 samples being analysed. Prior to measurement, samples from the reactor were centrifuged for 15 minutes, and then filtered through a 0.22 μm cellulose acetate filter (Millipore®). Filter, was flushed with high purity water first as it might have contained some organic acids. The samples were then stored in 1.5 mL chromatography vials prior to measurement.

Sulphate was measured using Ion Chromatography (IC). Prior to measurements, samples were purged with O_2 free-N_2 to get rid of dissolved H_2S and then were diluted 3 to 4 times with milli-Q water to give reading below 20 $mg.L^{-1}$, centrifuged for fifteen minutes and then filtered through a 0.22 μm filter (Millipore®). For both HPLC and IC analysis samples from molasses-fed experiments, filtrate was passed through a previously activated solid separation kit (Waters®, No. 1510) to remove colour.

Sulphide was measured using methylene blue technique (Merck Spectroquant® No. 14779) (APHA, 1992). Since hydrogen sulphide may escape into air, a known amount of liquid samples were taken anaerobically using a syringe. These samples were then immediately injected into a known amount of zinc acetate solution in order to trap sulphide as zinc sulphide, which has low solubility in water. To minimise the loss of H_2S the tip of the syringe was submerged below the surface of zinc acetate solution during application.

Both insoluble (taken to be biomass-COD) and soluble COD was analysed by digesting samples with $K_2Cr_2O_7$ and then measuring the Cr^{3+} photometrically. Merck Spectroquant no. 14541 was used for the test, which was capable of measuring COD concentration from 100 $mg.L^{-1}$ to 1500 $mg.L^{-1}$. 3 mL of sample was added into the test tubes containing reagent, the samples were then digested at 148°C for 2 hours. The COD of the sample was then measured using A Merck SQ 180 photometer after cooling the samples down to below 35°C. Samples which had COD concentrations above 1500 $mg.L^{-1}$ were diluted to give final concentrations in between 500 and 1000 $mg.L^{-1}$ before measurement.

Gas samples were analysed for methane, hydrogen, carbon dioxide and hydrogen sulphide. Gas composition (H_2, CH_4 and CO_2) was determined using a Perkin Elmer Autosystem Gas Chromatograph equipped with a haysep Q column at a column oven temperature of 40°C, detector temperature of 100°C and injector temperature of 75°C. A standard gas mixture of 0.773% hydrogen, 46.2% CH_4 and balanced CO_2 was used initially for calibration. The calibration was performed daily before injecting gas samples.

Hydrogen sulphide in gas was measured by dispersing a known amount of the gas into a known amount of zinc acetate solution by continuous shaking for two minutes to ensure all sulphide in the gas phase has been transferred into in the solution. ZnS solution was stored in a test tube supplied with rubber stopper. Following this a similar procedure as for measuring liquid sulphide was then used.

During steady state, populations of SRB, archaea, beta proteolytic and gamma proteolytic bacteria were visualised using *Fluorescence In-situ Hybridisation* (FISH) methods following sonification to break up flocs (Hugenholtz and Blackall, 2000). This method also yielded the approximate fraction of each group of bacteria visualised to the total bacterial population. This information along with the total biomass-COD and assuming that all microorganisms have the same empirical formula of $C_5H_7O_2NS_{0.1}$ (Speece, 1996), concentration of each group of bacteria, in terms of biomass-COD, was calculated.

2.4. Experiments

The reactor was operated at 4 and 6 hour retention times and was fed glucose at different concentrations, while sulphate concentration was kept constant at around 123 $mg.L^{-1}$. Five experiments were done at an HRT of 4 hours with glucose concentrations of 400; 1,000; 2,500; 5,000 and 10,000 $mg.L^{-1}$. Four experiments were done at a HRT of 6 hours with glucose concentrations of 1,000; 6,000; 10,000 and 20,000 $mg.L^{-1}$. The operational conditions were changed only after the reactor had been operating for at least four to eight retention times after steady state was reached at the previous operational condition. In addition to these steady state experiments, transient experiments were also undertaken by stepping down glucose concentration in the feed from 6,000 to 1,000 $mg.L^{-1}$ and from 20,000 to 1,000 $mg.L^{-1}$. Four experiments were also done with a molasses-based feed. Three experiments at a molasses concentration of 10,000 mg $COD.L^{-1}$ with sulphate concentration of 153 $mg.L^{-1}$, 188 $mg.L^{-1}$ and 359 $mg.L^{-1}$ and a fourth experiment at a molasses concentration of 20,000 mg $COD.L^{-1}$ and sulphate concentration of 317 $mg.L^{-1}$ were done.

3. RESULTS AND DISCUSSION

As can be seen from Table 1, on gradually stepping up the glucose concentration from 400 $mg.L^{-1}$ to 20,000 $mg.L^{-1}$ and keeping the reactor unperturbed at each glucose concentration for at least 8 residence times, the sulphate concentration in effluent increased as glucose

concentration in feed increased and consequently the extent of sulphate removal decreased. It was seen that for concentration of glucose in feed above 2,500 mg.L^{-1} the extent of sulphate reduction was dependent on the concentration of glucose. For glucose concentrations below 2,500 mgL^{-1} all the sulphate fed was reduced resulting in zero concentration of sulphate in reactor effluent. At a glucose concentration of 10,000 mg.L^{-1}, sulphate-S increased to about 12 mg.L^{-1} for both HRTs, effluent sulphate concentration increased further to 26.5 mg.L^{-1} at glucose concentrations of 20,000 mg.L^{-1}. Sulphate concentrations in effluent appeared to be independent of HRT as seen from the overlap of sulphate concentration data for glucose concentrations of 1,000 and 10,000 mg.L^{-1} at both HRTs. For molasses feed at an influent concentration of 10,000 mg COD.L^{-1} the extent of sulphate removal varied between 47 and 76%. The above observations indicated that sulphate reduction in acidogenic reactor was inhibited at high organic carbon concentration in feed. It was observed that in all experimental runs, residual glucose concentrations were undetected suggesting that inhibition of sulphate removal was not due to accumulation of glucose but may have been due to accumulation of fermentation products.

Initially the effect of volatile organic acids on sulphate removal was investigated. Table 1 lists the concentrations of acetic, propionic and butyric acids measured during the various experiments. Propionic acid concentration did not correlate with extent of sulphate removal. Propionic acid initially increased with glucose concentration in feed, increasing to 356 mg.L^{-1} at glucose concentration of 5,000 mg.L^{-1} but it then decreased as glucose concentration was further increased. For molasses-fed experiments propionate concentrations tended to be very high, however the concentration (905 mg.L^{-1}) at which the sulphate removal was only 47% was much higher than the concentration (138 mg.L^{-1}) at which sulphate removal was 35% in the glucose-fed experiments. Hence it was concluded propionic acid accumulation did not have an inhibitory effect on sulphate removal. It would appear from butyric acid concentration data for glucose-fed experiments that butyric acid accumulation could have inhibited sulphate removal (except for 6,000 mg.L^{-1} glucose data). This would therefore seem to confirm observations by Ghose and Wiken (1955) that butyric acid was toxic to SRB. However, sulphate removal was also inhibited in molasses-fed experiments where butyric acid concentrations remained below 324 mg.L^{-1} which was much lower than butyric acid concentrations measured in some glucose-fed experiments. Thus butyric acid accumulation may have been the cause of reduction of sulphate removal. By considering data only from glucose-fed experiments inhibition of sulphate removal appeared to increase with acetic acid accumulation as proposed by Reis et al. (1990). Sulphate removal rate was only 35% at acetic acid concentration of 1,860 mg.L^{-1}. However, even though acetic acid concentration was higher in molasses-fed experiments (around 2,500 mg.L^{-1}) sulphate removal rate was higher between 47 and 76%. Hence, this was further investigation.

Separate experiments were conducted to verify further the toxicity of acetic acid on SRB. The reactor was operated at 6 hours hydraulic retention time, pH of 6.0 and was continuously fed 1,000 mg.L^{-1} of glucose and 41 mg-S.L^{-1} of sulphate. Sodium acetate to a final concentration of 6,000 mg-acetate.L^{-1} was then added into the feed tank and the reactor was then left at this operational condition for 72 hours. After that acetate concentration in the feed tank was increased to 10,000 mg.L^{-1} and was left at this operational condition for another 48 hours. Complete sulphate removal was achieved prior to sodium acetate addition. Figure 1 shows the expected (calculated from feed rate, operating volume of reactor and acetate concentration in feed) and measured acetic acid concentration, and sulphate concentration in the effluent from the reactor for the duration of the study. The nominal acetic acid concentration produced in the reactor for the operating conditions used in this experiment was 178 mg.L^{-1} which was much lower than acetic acid added externally into the reactor. The plots for the expected and measured concentrations overlap, indicating that acetic acid was not consumed in the reactor. Sulphate removal continued to be 100% (indicated by sulphate concentrations below detection limit in the effluent) as acetic acid built up to 6,000 mg.L^{-1} in the reactor (Figure1). Sulphate concentration of 4 mg-S.L^{-1} was detected after about 40 hours of operation and it rose quickly to 12 mg-S.L^{-1} when acetate in feed tank was increased to 10,000 mg.L^{-1}. However, sulphate concentration then dropped back to 4 mg-S.L^{-1} as acetic acid built up to 10,000 mg.L^{-1} in the reactor. Acetic acid concentrations above 6,000 mg.L^{-1} may have decreased sulphate removal temporarily to 92.5%, however further increase in acetic acid concentration (up to 10,000 mgL^{-1}) did not decrease extent of sulphate removal any further. The sudden increase in sulphate concentration in reactor which occurred shortly after acetic acid concentration in the feed was increased to 10,000 mg.L^{-1}, might be due to the shock load. These experiments showed that acetic acid

concentrations of up to 2,530 mg.L^{-1} at a pH of 6.0 in experiments presented in Table 1 would not have suppressed sulphate removal.

For the glucose-fed experiments the gas phase consisted of mainly hydrogen and carbon dioxide, where hydrogen concentration increased with glucose concentration in the feed reaching as high as 55% when glucose concentration in feed was 20,000 mg.L^{-1}. The carbon dioxide concentration varied between 30 and 36% for all experiments. SRB are known to utilise hydrogen as substrate and the possibility of hydrogen having caused inhibition of sulphate removal for the experiments presented in Table 1 was rejected because even though gas phase hydrogen concentrations were low for molasses-fed experiments sulphate removal was still suppressed. Moreover, gas phase hydrogen concentration 52% caused extent of sulphate removal to decrease to 87% in one experiment and at the same time extent of sulphate removal decreased to 50% when hydrogen concentration was 3.4%. Therefore, it was concluded that accumulation of hydrogen did not suppress sulphate reduction.

Bhattacharya et al., (1996) had found that at high acetate/SO_4 ratio in feed, sulphate reduction was inhibited in a methanogenic system. It was suggested that the inhibition might have been due to relatively high concentration of methanogens as compared to SRB, therefore, the relative advantage of SRB was diminished in such a system. Uberoi and Bhattacharya, (1995) found that SRB could only outcompete methanogens at low COD/SO_4 ratios. In studies presented here it was observed that methane concentrations decreased at high glucose concentrations, showing that the increase in glucose concentrations in the feed did not enhance methane production. In the separate experiments carried out to investigate acetic acid toxicity even though the acetate/SO_4 was high in the feed methanogenic activity in the reactor was not enhanced consequently sulphate reduction was not suppressed. This suggested that inhibition of sulphate reducers in these experiments was not due to increased methanogenic activity.

However, the extent of sulphate removal could be correlated to biomass concentration in reactor. Biomass concentration calculated as suspended matter in the reactor and expressed in mg COD.L^{-1}, increased with feed-glucose concentration (Table 1). The extent of sulphate removal data from glucose and molasses-fed experiments presented in Table 1 was plotted against biomass concentration and is shown in Figure 2. There was a negative correlation between sulphate removal and biomass concentrations, i.e., as biomass concentration increased extent of sulphate removal decreased. This suggested that the inhibition of sulphate reduction might have been the effect of high concentration of biomass. The correlation between fraction of sulphate removal (η) and biomass concentration (X) was expressed as follows:

$$\eta = \left(1 - \frac{X}{X_f}\right)^n$$

where X_f = maximum biomass concentration above which sulphate utilisation was completely inhibited (mg-COD.L^{-1}) and n = exponent. The above expression was taken from Han and Levenspiel (1988). The values of X_f (=3300 mg-COD.L^{-1}) and n (= 0.3662) were determined from the best fit of the above equation to the steady state data from the glucose-fed experiments only. It can be seen from Figure 2 that this expression adequately predicted data from molasses-fed experiments also.

The effect of biomass concentration on extent of sulphate reduction was also investigated for transient conditions. For both glucose-fed and molasses-fed experiments the biomass concentration in reactor was measured every two to eight hours after a step change in feed glucose (or molasses) concentration and/or a step change in feed sulphate concentration. For glucose-fed experiments the glucose concentration in feed was stepped down from 6,000 mg.L^{-1} to 1,000 mg.L^{-1} and from 10,000 mg.L^{-1} to 1,000 mg.L^{-1}. It was observed that as soon as the glucose concentration in feed was stepped down, the biomass concentration also decreased and concomitantly the extent of sulphate reduction increased. Once the biomass concentrations attained a steady value the extent of sulphate reduction also reached a constant value. When biomass concentrations were below critical value of about 600 mg-COD.L^{-1}, complete sulphate reduction was accomplished. Figure 3 shows the typical response of the extent of sulphate reduction to biomass concentration under transient conditions when the glucose concentration in feed was stepped down from 6,000 mg.L^{-1} to 1,000 mg.L^{-1}. A similar response was seen after stepping down glucose from 10,000 mg.L^{-1} to 1,000 mg.L^{-1} and is not shown here. The biomass concentration data and the corresponding extent of sulphate reduction for all measurements made during these experiments are shown in Figure 4. For molasses-fed experiments two step changes were made. In the first sulphate was stepped up from 62 to 120 mg-S.L^{-1} and in the second the organic concentration in feed was stepped up from 10,000 to 20,000 mg COD.L^{-1} and

simultaneously sulphate was also stepped up from 51 to 106 mg-S.L^{-1}. The biomass concentration data and the corresponding extent of sulphate reduction are summarised in Figure 4. It was seen that on increasing sulphate concentration in feed even though biomass concentration increased it did not adversely affect extent of sulphate reduction. However, when the organic concentration was doubled the biomass concentration increased and extent of sulphate reduction decreased. It was likely that in the sulphate step change only the concentration of sulphate reducing bacteria increased and this did not inhibit sulphate reduction. In the second step change the concentration of fermentative bacteria would have increased which appeared to inhibit sulphate reduction. It can be seen from Figure 4 that the correlation expression that fit the steady state data adequately predicts the effect of biomass on extent of sulphate removal for the transient data from both the glucose and molasses-fed experiments.

The presence of sulphate reducing bacteria at all operational conditions for the glucose-fed experiments was confirmed from FISH observations. The relative numbers of sulphate reducing bacteria were 5%, 6%, 13%, 4% and 3% of the total population for 400, 1,000, 2,500, 5,000 and 10,000 mg.L^{-1} of glucose in feed at HRT of 4 hours. If the measured biomass concentrations were assumed to be proportional to the total population then it appears that the numbers of sulphate reducing bacteria have increased with increase in glucose concentration. This was contrary to expectation. As the sulphate concentration was kept constant, it would be expected that the absolute concentration of SRB would also remain constant, if all the sulphate were consumed in the reactor and with the accumulation of sulphate the population of SRB would decrease. Therefore, it appeared that the inhibition of sulphate reduction was not due to SRB being washed out but they might prefer substrates other than sulphate at high carbon concentration. The shift in the function of SRB to that of acetogens has been previously reported in Widdel, (1988). Moreover, in the transient experiments the extent of sulphate reduction appears to have increased soon after the glucose in the feed was stepped down (Figure 3). If SRB were washed out when sulphate removal was suppressed, a more delayed response would have been observed.

4. CONCLUSIONS

It was observed that high carbohydrate concentration in feed inhibited sulphate reduction in a continuously-fed, well mixed acidogenic reactor operating at a temperature of 35^{o}C and pH of 6. It was seen that volatile organic acids like propionic and butyric acids, hydrogen and residual glucose could not have caused inhibition of sulphate reduction. In separate experiments it was confirmed that high concentrations of acetic acid would not also have caused the suppression of sulphate removal. An obvious correlation between biomass concentration and extent of sulphate reduction was observed in the system at both steady state and transient conditions. This correlation was expressed mathematically and the same expression with the same parameters adequately predicted the effect of biomass concentration on extent of sulphate removal for both steady state and transient data irrespective of glucose or molasses feed. It was seen from the best fit of this expression that a biomass concentration of 3300 mg-COD.L^{-1} would completely repress sulphate reduction in the acidogenic reactor. Even when sulphate removal was suppressed the presence of SRB in the reactor was confirmed through FISH examination. Moreover, the numbers of SRB seemed to increase with carbohydrate concentration in feed. It was speculated the suppression of sulphate removal might be due to a switch in function of SRB from sulphate reducing to fermentation or acetogenesis.

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Table 1. Steady state volatile organic acid concentrations and gas phase composition for glucose-fed and molasses-fed experiments

Reactor operational conditions			Effluent concentrations					Gas composition		Sulphate removal (%)
HRT (h)	Feed glucose (mg.L^{-1}) or molasses (mg COD. L^{-1})	Feed sulphate (mg-S.L^{-1})	Acetate (mg.L^{-1})	Propionate (mg.L^{-1})	Butyrate (mg.L^{-1})	Sulphate (mg-S.L^{-1})	Biomass (mg COD.L^{-1})	Hydrogen %	Methane %	
4	400 (glucose)	41	135	11	49	nd	99	0.07	1.4	100
4	1,000 (glucose)	41	265	32	153	nd	222	0.6	4.2	100
6	1,000 (glucose)	41	178	37	233	nd	269	no gas	no gas	100
4	2,500 (glucose)	41	660	114	365	nd	507	3.7	21.4	100
4	5,000 (glucose)	41	776	356	1097	5.4	1140	52	4.5	87
6	6,000 (glucose)	41	975	255	123	6.0	1398	48	10	85
4	10,000 (glucose)	41	1264	158	2833	12	2410	47	7.3	71
6	10,000 (glucose)	41	1108	100	2427	12.3	1870	50	6	70
6	20,000 (glucose)	41	1861	138	4319	26.5	3130	55	1	35
4	10,000 (molasses)	63	2529	961	324	15	2006	14.6	24	76
4	10,000 (molasses)	120	2525	905	312	64	2242	8.3	31	47
4	10,000 (molasses)	70	2516	864	272	35	2089	3.4	50	50

nd = not detected

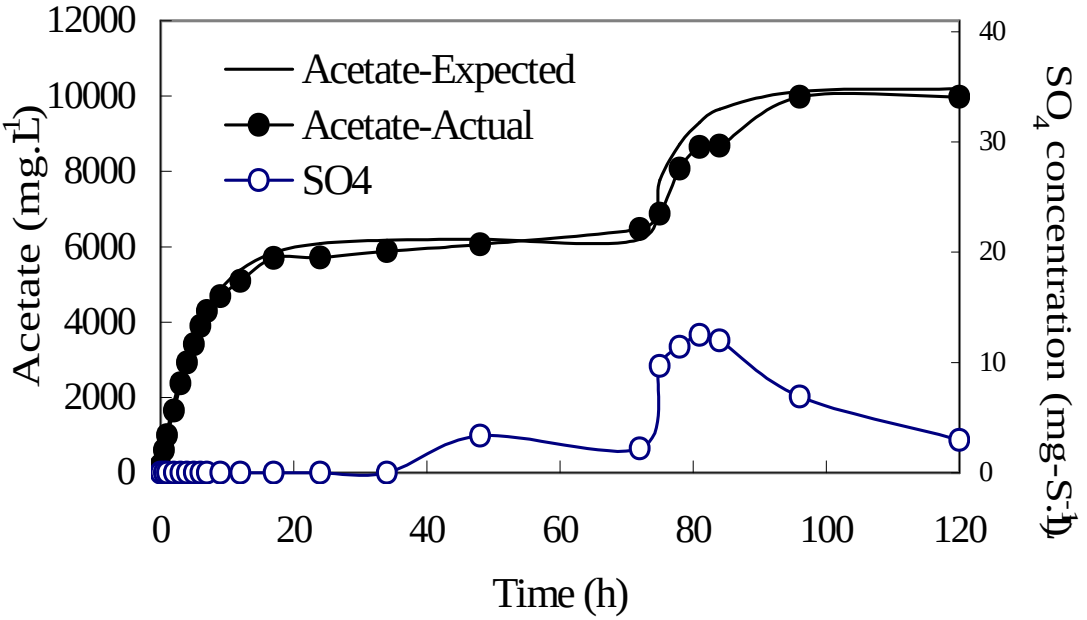

Figure 1. Effect of acetate concentration on sulphate reduction: time course presentation of expected and observed acetate concentrations and sulphate concentration.

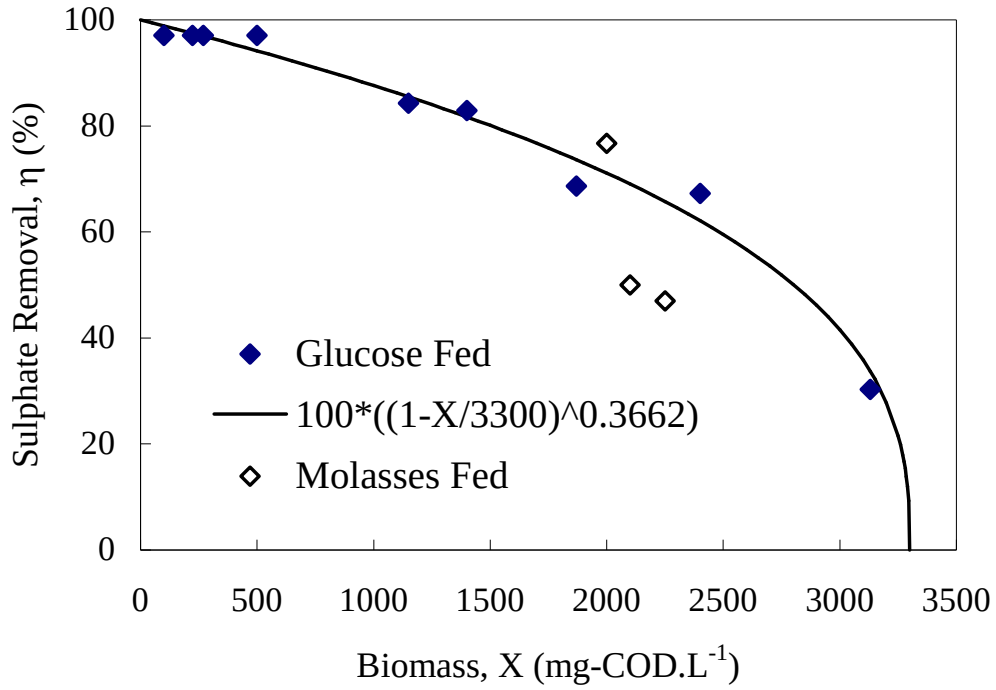

Figure 2. Effect of biomass concentration on extent of sulphate removal at steady state conditions

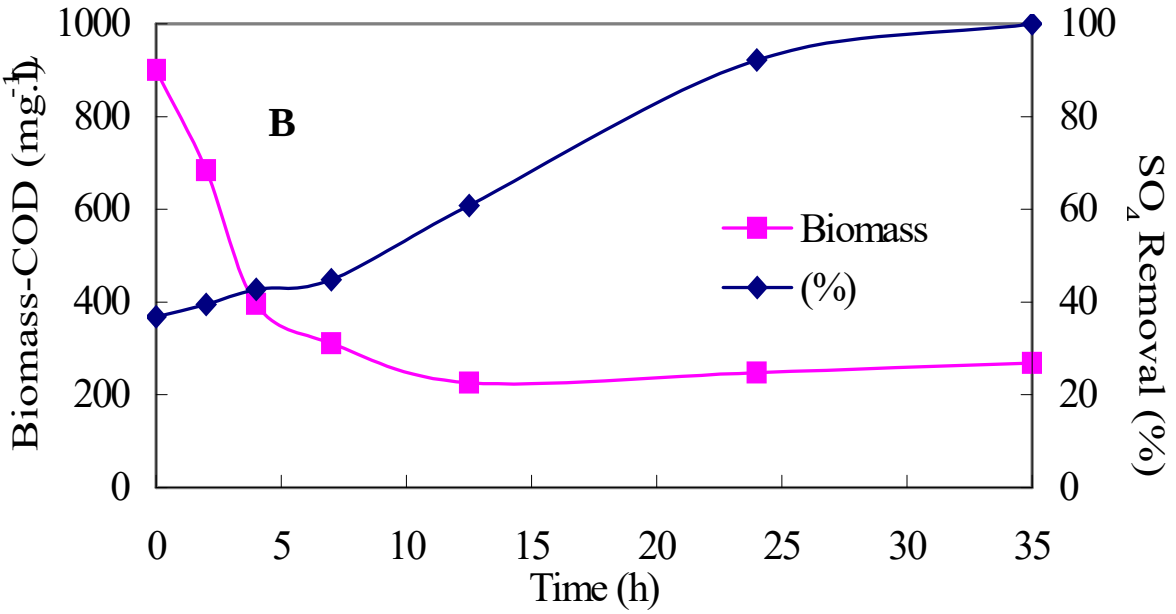

Figure 3. Effect of biomass concentration on extent of sulphate reduction after stepping down glucose concentration in feed from 6000 to 1000 mg.L^{-1}

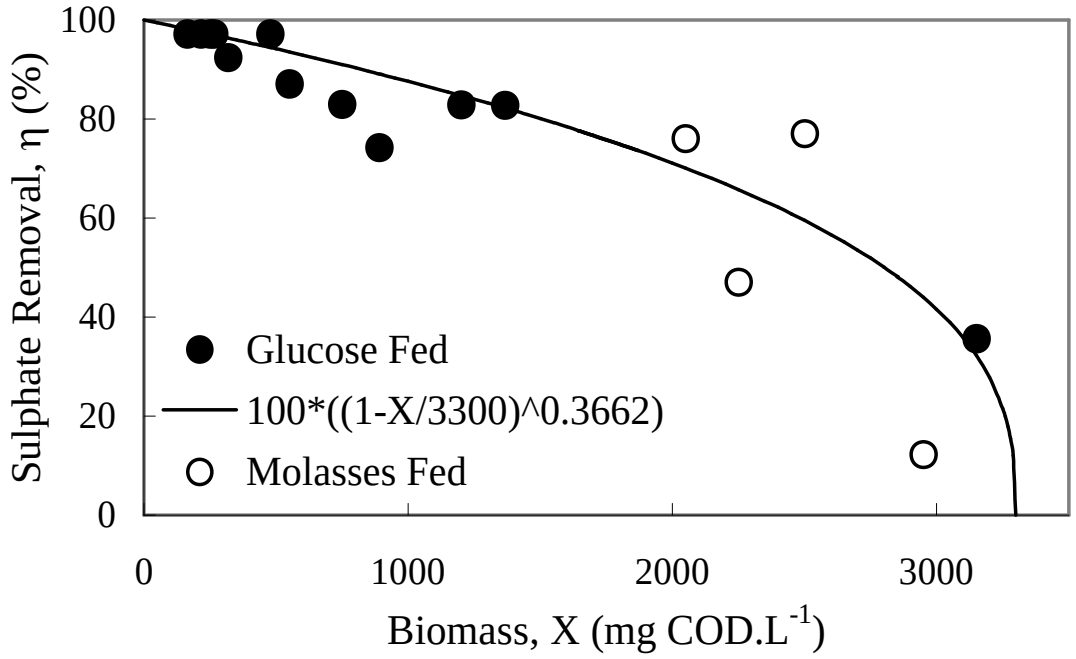

Figure 4. Effect of biomass concentration on extent of sulphate reduction during transient conditions.