Pergamon

0043-1354(94)00177-4

Wat. Res. Vol. 29, No. 2, pp. 571–578, 1995

0043-1354/95 $7.00 + 0.00

SULFIDE PRODUCT INHIBITION OF *DESULFOVIBRIO DESULFURICANS* IN BATCH AND CONTINUOUS CULTURES

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(First received September 1993; accepted in revised form June 1994)

Abstract—Sulfide product inhibition kinetics for growth and activity of *Desulfovibrio desulfuricans* was investigated in batch and continuous cultures at pH = 7.0. A non-competitive inhibition model adequately described sulfide product inhibition kinetics. Inhibition coefficient (K_i) for maximum specific growth rate (μ_{max}^{inh}) was 251 mg l^{-1} S in a batch experiment. Cell yield determined in a chemostat was reduced in half by a sulfide concentration of about 250 mg l^{-1} S, which was very close to the K_i value for the batch growth. Maximum specific growth rate (μ_{max}^{inh}) and cell yield ($Y_{c/Lac}$) were strongly inhibited by high levels of sulfide concentrations, whereas specific lactate utilization rate increased with increasing sulfide concentrations. The results indicated an increase in the relative energy needed for maintenance to overcome sulfide inhibition and uncoupling growth from energy production. However, *D. desulfuricans* to some extent could recover from the shock of high sulfide concentrations. Stoichiometry for catabolic reactions (energy producing) did not change at high sulfide concentrations, while anabolic reactions (cellular synthesis) were strongly inhibited by high sulfide concentrations. These results suggested that separation of sulfide product inhibition into growth (cell yield) and activity (substrate utilization rate) was important to incorporate the sulfide product inhibition kinetics in a variety of applications.

Key words—Desulfovibrio desulfuricans, sulfide product inhibition, non-competitive inhibition kinetics, activity, cell yield, specific growth rate

NOMENCLATURE

D = dilution rate (t^{-1})
i = total sulfide concentration ($M_i l^{-3}$)
K_i = inhibition coefficient ($M_s l^{-3}$)
K_{Lac} = half-saturation coefficient for lactate ($M_s l^{-3}$)
m = maintenance coefficient ($M_s M_x^{-1} t^{-1}$)
S = effluent lactate concentration ($M_s l^{-3}$)
TS = total sulfide ($M_i l^{-3}$)
$Y_{c/Lac}^{intr}$ = intrinsic cell yield coefficient on lactate ($M_x M_s^{-1}$)
$Y_{c/Lac}$ = observed cell yield coefficient on lactate ($M_x M_s^{-1}$)
μ = specific growth rate (t^{-1})
μ_{max} = maximum specific growth rate in the absence of sulfide (t^{-1})
μ_{max}^{inh} = maximum specific growth rate in the presence of sulfide (t^{-1})

INTRODUCTION

Sulfate reducing bacteria (SRB) cause serious problems in sanitary sewer systems and industrial water systems because of production of highly toxic and corrosive hydrogen sulfide gas. The corrosion of concrete sewers occurs as a result of hydrogen sulfide production from sulfate-rich sewage by the activities of SRB (Mori *et al.*, 1992). Furthermore, problems are well known in the oil industry where SRB cause serious corrosion of installations, plugging of formations, and contamination of petroleum with H_2S (souring). In these industries, mathematical models have been developed for predicting and controlling SRB activity. A range of environmental factors affects the growth and activity of SRB and other bacteria in these systems, and a more quantitative knowledge of the significance of those environmental factors is needed for improving the mathematical models. One important factor is sulfide, a metabolic product from sulfate reduction, which has been reported inhibiting SRB growth and activity. Very high sulfide concentrations have, for example, been found throughout a petroleum formation with concentrations up to 1000 mg l^{-1} H_2S in the produced water (Subcasky, 1991), and may appear in anaerobic treatments of sulfate-rich wastewater (Hilton and Archer, 1988). Therefore, sulfide product inhibition may be expected to some extent in these industrial water systems.

Several studies of sulfide inhibition have been performed on anaerobic wastewater treatment,

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Table 1. Summary of the relevant literature on the effects of sulfide on growth and activity

Organisms	Concentration	pH	B/C[a]	Observation	References
Undefined mixed population SRB	400 mg l^{-1} S	7.0	B	Reduced sulfate reduction by 50%	Hilton and Oleskiewicz (1989)
Desulfotomaculum sp.	64–128 mg l^{-1} S	6.9–7.0	B	Ceased growth After removal of H_2S resumed growth.	Karhadkar *et al.* (1986)
Desulfotomaculum thermoacetoxidans	64 mg l^{-1} S	6.5	B	Ceased sulfate reduction.	Min and Zinder (1990)
Undefined mixed population SRB	448 mg l^{-1} S	7.15–7.33	B	Completely ceased sulfate reduction.	McCartney and Oleszkiewicz (1991)
Desulfovibrio desulfuricans	500 mg l^{-1} TS	7.0	C	Decreased cellular yield and cell size. Reduced lactate oxidation by 50%.	Okabe *et al.* (1992)
Desulfovibrio sp.	550 mg l^{-1} H_2S	6.2–6.6	B	Ceased sulfate reduction. H_2S is the most inhibitory form.	Reis *et al.* (1991)
Desulfovibrio sp.	547 mg l^{-1} H_2S	6.2–6.6	B	Completely inhibited growth. Reversible toxicity. H_2S is the most inhibitory form.	Reis *et al.* (1992)
Undefined mixed population SRB	500 mg l^{-1} H_2S	—	B	Completely inhibited growth.	Simada (1987)

[a]Refers to batch or continuous (chemostat) reactor.

especially on methane producing bacteria (Karhadkar *et al.*, 1986; Koster *et al.*, 1986; Hilton and Archer, 1988; Rinzema and Lettinga, 1988; Hilton and Oleskiewicz, 1989; Oleszkiewicz *et al.*, 1989; McCartney and Oleszkiewicz, 1991). The overall effect of sulfide on microbial sulfate reduction has been described qualitatively by several authors (Postgate, 1984; Klemps *et al.*, 1985; Shimada, 1987; Hilton and Oleskiewicz, 1989; Min and Zinder, 1990; McCartney and Oleszkiewicz, 1991; Reis *et al.*, 1991; Okabe *et al.*, 1992; Reis *et al.*, 1992). The relevant literature is summarized in Table 1. However, no quantitative SRB sulfide product inhibition datum has been reported. Most of the data listed in Table 1 are obtained from the batch experiment, in which pH, sulfide concentration, and limiting substrate cannot be maintained at the same levels over many generations. Therefore, batch culture data reported in the literature must be interpreted cautiously. Sulfide toxicity is strongly dependent on pH, because the chemical equilibrium of sulfide species is pH dependent. At pH 8 most of total sulfide (TS) is in the HS^- form, while at pH 6 most is in the H_2S form. Molecular hydrogen sulfide (H_2S) has been found to be the major toxic form of sulfide because H_2S can pass through the cell membrane (Speece, 1983; Reis *et al.*, 1991).

The distinction between growth and activity becomes very important when the environmental conditions become extreme and energy consumption shifts from growth to maintenance (Hunik *et al.*, 1990). Thus, it is speculated that under high sulfide conditions, the growth-associated H_2S production will decrease, whereas total H_2S production will remain relatively constant because the decrease in growth is counter balanced by increased nongrowth-associated H_2S production caused by increased maintenance energy requirement.

The goal of this paper is to describe quantitatively effects of sulfide on activity and growth of SRB, here exemplified by *Desulfovibrio desulfuricans*. The distinction between growth and activity was clearly made in this study; growth was defined as biomass (cell) production rate and activity was defined as substrate utilization rate (e.g. lactate).

THEORETICAL BACKGROUND

In this study, it is assumed that sulfide is a non-competitive inhibitor, which decreases maximum specific growth rate (μ_{max}) but does not alter half-saturation coefficient (K_{Lac}). Non-competitive inhibition kinetics for lactate-limited cultures can be described as follows (Aiba *et al.*, 1973)

$$\mu = \frac{\mu_{max} S K_i}{(K_{Lac} + S)(K_i + i)} \quad (1)$$

where i = sulfide concentration ($M_i l^{-3}$), K_i = inhibition coefficient ($M_i l^{-3}$), S = effluent lactate

concentration ($M_s l^{-3}$), and μ = specific growth rate (t^{-1}).

For $S \gg K_{Lac}$ (e.g. in a batch culture), equation (1) can be simplified and linearized by plotting $1/\mu_{max}^{inh}$ against i

$$\frac{1}{\mu_{max}^{inh}} = \frac{1}{\mu_{max}} + \frac{i}{\mu_{max} K_i} \quad (2)$$

where μ_{max}^{inh} = maximum specific growth rate in the presence of sulfide (t^{-1}). Thus, the x-interception of a best fit line gives $-K_i$. However, equation (2) cannot be applied to the case of $S \fallingdotseq K_{Lac}$ (i.e. in a chemostat culture).

μ_{max}^{inh} in a steady state chemostat at various sulfide concentrations can be determined based on a theoretical construct extrapolated from the measured lactate concentration (S) to what μ_{max}^{inh} would be based on the ratio of K_{Lac} to S as described rearranging equation (1)

$$\mu_{max}^{inh} = \frac{D(K_{Lac} + S)}{S}. \quad (3)$$

The value of K_{Lac} has been determined previously in a similar type of experiment (Okabe *et al.*, 1992). In this study, steady state S's were determined at a constant dilution rate (D) and various sulfide concentrations. In this way, the effect of sulfide concentration on μ_{max}^{inh} can be evaluated easily instead of conducting a series of experiments to determine dependence of S on D at various sulfide concentrations.

EXPERIMENTAL MATERIALS AND METHODS

Microorganisms

Desulfovibrio desulfuricans (ATCC 5575) was grown in Postgate medium G (Postgate, 1984) including Na-lactate (DL-lactic acid, SIGMA, L-1375) and Na_2SO_4. Trace elements and vitamins were added. The details of medium preparation and preculture of microorganisms have been described elsewhere previously (Okabe and Characklis, 1992).

Batch culture experiment

Sulfide inhibition kinetics were determined in batch experiments using 500 ml Erlenmeyer flasks. Flasks were equipped with butyl rubber stoppers fitted with an injection port and a gas exchange port. 400 ml of the pH adjusted (pH = 7.0). Postgate medium G (Postgate, 1984) containing 500 mg l^{-1} of lactate and 800 mg l^{-1} of sulfate was dispensed into each Erlenmeyer flask and autoclaved at 121°C for 20 min. Trace elements and vitamin solutions were aseptically added after cooling. $Na_2S \cdot 9H_2O$ solution was prepared separately in an air-tight bottle. After autoclaving, the pH of the sulfide solution was adjusted to 7.0 in the air-tight bottle using a sterile syringe and sterile 1.0 N HCl and NaOH solutions. Then appropriate volumes of the sulfide solution were added to the autoclaved culture medium to obtain the designed total sulfide concentrations (0, 70, 220, 350, 700 mg l^{-1} S). After addition of sulfide solution, the pH of the culture medium was remeasured and readjusted using sterile syringes. A sterile oxygen-free Na-dithionite solution was added to the sulfide-free medium to provide the required negative redox potential for growth (the final concentration of $Na_2S_2O_4$ was approximately 30 mg/l). *D. desulfuricans* taken from an actively growing chemostat culture was inoculated into each flask and incubated at 35°C. The inoculum size from the chemostat was adjusted to obtain an initial cell count of approximately 1×10^7 cells ml^{-1}.

Continuous culture experiment

The effect of sulfide on the activity and growth was determined in a lactate-limited chemostat operated at a constant dilution rate (D) of 0.20 h^{-1}. The chemostat was equipped with a butyl rubber wall growth scraper continuously rotated by an electric motor to prevent wall growth. Constant pH (±0.1 unit) and temperature (±0.5°C) were maintained using a pH control system with sterile 1.0 N HCl and NaOH solutions and thermoregulator, respectively. Various concentrations of sulfide solutions with pH roughly adjusted to 7.5, were separately prepared and autoclaved, then fed to the chemostat by a peristaltic pump and speed controller (Cole-Parmer, Chicago, Ill). A fine pH adjustment was conducted in the chemostat. Na-dithionite ($Na_2S_2O_4$) was added to the sulfide-free medium to provide the required negative redox potential. Redox potential was monitored continuously using a redox probe (Orion) with a saturated calomel reference electrode during the course of the experiments. The reported redox potential was standarized to a hydrogen redox couple. The details of chemostat set up protocol have been described elsewhere previously (Okabe and Characklis, 1992).

Analytical methods

At steady state, chemostat effluent samples were obtained for the following analyses: (1) total organic carbon (TOC); (2) soluble organic carbon (SOC); (3) total bacterial counts and cell size; (4) sulfate; (5) sulfide; (6) lactate; (7) acetate. The samples for SOC, lactate, and acetate were prepared by centrifugation at 20,000 *g* and 4°C for 20 min using stainless steel centrifuge tubes. The obtained supernatant was subjected to these analyses. The sample for sulfate analysis was fixed with 1% (w/v) ZnAc solution to remove sulfide species and then filtered by Sterile Acrodisc filter (pore size 0.2 μm, Gelman Science, No. 4192). Sulfate and acetate concentrations were measured using an ion chromatograph (DIONEX AI-450). Total dissolved sulfide concentration (TS, in mg l^{-1} S) in the liquid phase was measured using the methylene blue method described previously by Cline (1969). Total cell number and cell size were measured using an image analyzer (Cambridge/Olympus Quantment 10) by the epifluorescence method described previously by Hobbie *et al.* (1977). Samples (variable volumes) for cell counts were obtained directly from the chemostat, homogenized, stained with 0.02% acridine orange solution, and fixed on Nuclepore filters (0.2 μm pore size, 25 mm dia.). Cell number was reported as the mean of more than 20 measurements along the filter. Cellular carbon concentration was determined converting cell size determined by an epifluorescence technique to cellular carbon using various factors (Okabe and Characklis, 1992). The details of the rest of the chemical analytical methods have been described elsewhere previously (Okabe and Characklis, 1992).

RESULTS

Batch experiments

Maximum specific growth rate in the presence of sulfide (μ_{max}^{inh}) of *D. desulfuricans* was determined from the cell doubling times (t_d) at various total sulfide concentrations in batch cultures containing excess substrates (Fig. 1). The initial growth rates at various sulfide concentrations were determined by linear interpolation of the data in Fig. 1 because total sulfide concentration, substrate concentrations (i.e. lactate and sulfate), and the pH of culture medium changed

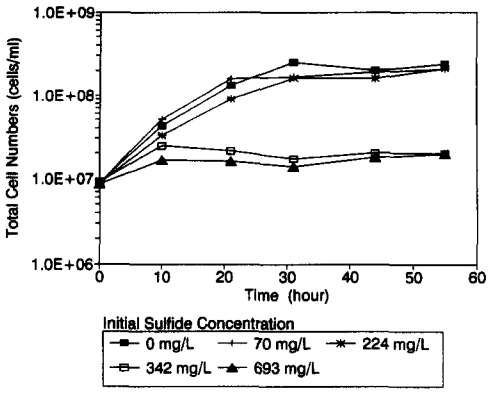

Fig. 1. Effect of sulfide on growth of *D. desulfuricans* in batch cultures: temperature = 35°C, pH = 7.0.

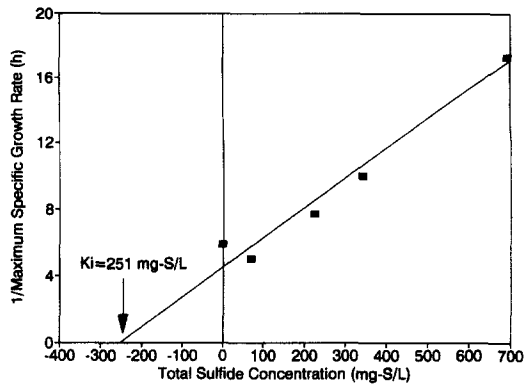

Fig. 2. Determination of K_i value in the batch culture. Theoretical plot of the reciprocal of maximum specific growth rate in the presence of sulfide ($1/\mu_{max}^{inh}$) as a function of sulfide concentration. K_i of 251 mg l^{-1} S was determined ($r^2 = 0.96$).

during the incubation. Cell numbers for initial sulfide concentrations of 0, 70, and 224 mg l^{-1} S leveled off after 30 h due to depletion of lactate. For data interpretation pH and total sulfide concentrations, doubling times, and corresponding maximum specific growth rates (μ_{max}^{inh}) are presented in Table 2. The maximum specific growth rates (μ_{max}^{inh}) determined in the batch cultures never exceeded 0.20 h^{-1} at each total sulfide concentration, which was significantly lower than values of continuous culture. The inhibition coefficient for maximum specific growth rate, K_i, was determined to be 251 mg l^{-1} S using the non-competitive inhibition model [equation (2)] as shown in Fig. 2. A high square of correlation coefficient (r^2) indicates that the non-competitive inhibition model adequately described sulfide inhibition.

Continuous experiments

Response to step changes in sulfide concentration. D. desulfuricans growing at a constant dilution rate (D) of 0.20 h^{-1} at 35°C and pH = 7.0 was continuously exposed to high total sulfide concentrations to examine the response to step changes in sulfide concentration in terms of growth and activity. Sulfide solutions were fed continuously into the reactor to maintain constant total sulfide concentrations during the experimental period. The initial total dissolved sulfide concentrations in both experiments were about 20 mg l^{-1} S. Addition of sulfide solutions was started at time zero. Cell concentration decreased about 25% after 8 h exposure to 133 ± 10 mg l^{-1} S, but recovered gradually to the original level after about 40 h [Fig. 3(a)]. The activity expressed as fractional lactate utilization $[(S_i - S)/S_i]$ was relatively constant during the experimental period as effluent lactate concentration increased slightly only after sulfide addition. Cell concentration decreased 64% by exposure to 212 ± 23 mg l^{-1} S. However, it recovered and approached a steady

Table 2. Results of sulfide effect on the maximum specific growth rate of *D. desulfuricans* in batch cultures. Initial lactate and sulfate concentration were 500 and 800 mg l^{-1} respectively

TS (mg l^{-1} S)		pH			
Initial	End	Initial	End	t_d (h)	μ_{max}^{inh} (h^{-1})
0	81	6.96	6.90	4.0	0.17
70	161	6.95	6.82	3.5	0.20
224	303	7.30	7.06	5.5	0.13
342	390	7.00	6.95	7.0	0.10
693	729	6.95	6.80	12.0	0.058

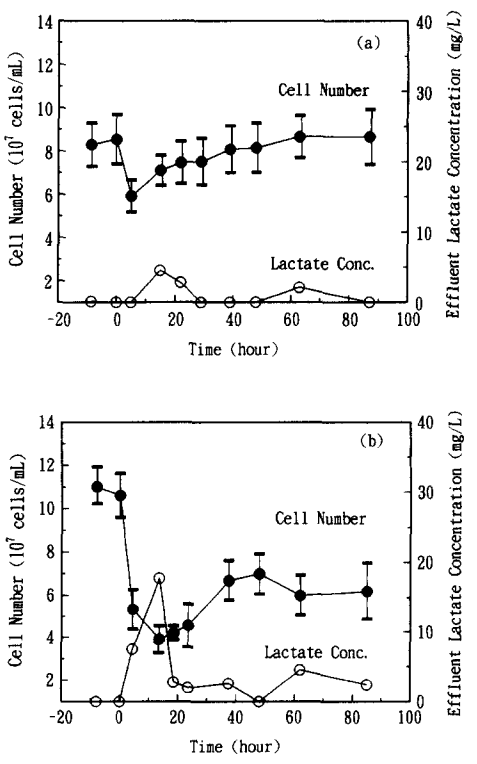

Fig. 3. Typical response of cell concentration and effluent lactate concentration to exposure to (a) 133 ± 10 mg l^{-1} S and (b) 212 ± 23 mg l^{-1} S: $D = 0.20$ h^{-1}, temperature = 35°C, pH = 7.0. Error bars represent the standard deviation of mean ($n = 2$).

state value, which was about 55% of the original cell concentration. A spike increase in effluent lactate concentration was observed after sulfide addition. Afterwards the effluent lactate concentration stabilized in the range of 0–5.0 mg l^{-1} even though the cell concentration was reduced by 45%. The culture approached steady state after about 40 h in both cases. Therefore, all samples in later experiments were taken at about 40 h after the sulfide concentration was changed. Standard deviations of duplicate measurements of all cell counts were within 20% of their respective mean values, which indicating that the accuracy of cell count is reliable. Redox potential was below −180 mV during both experiments.

Effect of sulfide on cell yield. Cell yield was determined in a chemostat at various sulfide concentrations (Fig. 4). The cell yield was maximum at 108 mg l^{-1} S, after that it decreased with increasing sulfide concentration. The cell yield was reduced in half at about 250 mg l^{-1} S (Fig. 4). The effluent lactate concentration, however, remained relatively constant with increasing total sulfide concentration up to 332 mg l^{-1} S and increased to only 22.2 mg l^{-1} at 437 mg l^{-1} S (Fig. 4), indicating that activity expressed as fractional lactate utilization $[(S_i - S)/S_i]$ was not affected in this range of sulfide concentration. These results suggested that specific lactate utilization rate increased when *D. desulfuricans* grew in high sulfide concentrations.

Effect of sulfide on growth rate. Maximum specific growth rates (μ_{max}^{inh}) were determined at various sulfide concentrations using equation (3) with the effluent lactate concentrations and K_{Lac} of 2.35 mg l^{-1} (Okabe and Characklis, 1992) (Table 3). The maximum specific growth rate in the presence of sulfide (μ_{max}^{inh}) decreased from 0.33 h^{-1} at low levels of TS to 0.21 h^{-1} at high levels of TS. μ_{max}^{inh} determined in the chemostat was higher than the one determined in the batch study at a corresponding sulfide concentration.

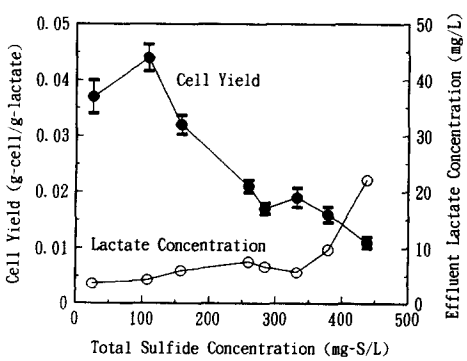

Fig. 4. Effect of sulfide concentration on cell yield and effluent lactate concentration: $D = 0.20\ h^{-1}$, temperature = 35°C. Error bars represent the standard deviation of mean ($n = 2$).

Table 3. The effect of sulfide on the maximum specific growth rate in a chemostat (mean ± SD)

TS (mg l^{-1} S)	Effluent lactate concentration	μ_{max}^{inh} (h^{-1})
26.1 ± 1.0	3.6 ± 0.2	0.33
108.2 ± 3.8	4.3 ± 0.1	0.31
157.9 ± 19.0	6.1 ± 0.3	0.26
259.1 ± 16.9	6.7 ± 0.8	0.24
284.4 ± 7.1	7.6 ± 0.2	0.25
332.0 ± 8.6	5.7 ± 0.1	0.26
378.5 ± 13.0	9.8 ± 0.1	0.22
437.5 ± 7.8	22.2 ± 0.4	0.21

Stoichiometry. Stoichiometry of sulfate reduction was determined at each sulfide concentration to elucidate whether the presence of sulfide results in an alteration of the metabolic pathway(s) for sulfate reduction. The stiochiometric ratios for catabolic (energy producing) reactions such as lactate oxidized to sulfate reduced, CO_2 produced, and acetate produced were independent of sulfide concentration (Table 4). The ranges of 0.42–0.51 for SO_4^{2-}/lactate, 0.81–0.94 for acetate/lactate, and 1.01–1.12 for CO_2/lactate approached the theoretical ratios of 0.47, 0.94, and 0.94, respectively (Okabe and Characklis, 1992). However, the stoichometric ratio for anabolic (cell synthesis) reactions, cell/lactate (i.e., cell yield), decreased with increasing total sulfide concentrations.

DISCUSSION

Effects of sulfide on cell yield and specific lactate utilization

It was very important to separate the effect of sulfide inhibition into an effect on cell yield (growth) and on lactate utilization rate (activity) for a correct interpretation of experimental data. Cell yield decreased dramatically from 0.036 (g cell)(g lactate)$^{-1}$ at low levels of TS to 0.011 (g cell)(g lactate)$^{-1}$ at 437 mg l^{-1} S, while fractional lactate utilization (shown as effluent lactate concentration in Fig. 4) decreased at TS of only 437 mg l^{-1} S. This decrease in cell yield may have been a result of a large part of the energy source being used for maintenance and uncoupling growth from energy production. Cell yield is known to be dependent on μ, the maintenance coefficient (m), and the intrinsic cell

Table 4. Steady state stoichiometry of the continuous culture of *D. desulfuricans* exposed to various total sulfide concentrations at a constant dilution rate of 0.20 h^{-1} (mean ± SD)

Total sulfide (mg l^{-1} S)	Stoichiometric ratio (M/M)			
	SO_4^{2-}/Lac	Ac/Lac.	CO_2/Lac.	Cell/Lac.
26.1 ± 1.0	0.42	0.85	1.03	0.148
108.2 ± 3.8	0.50	0.81	1.08	0.174
157.9 ± 19.0	0.49	0.84	1.05	0.128
259.1 ± 16.9	0.46	0.88	1.12	0.083
284.4 ± 7.1	0.51	0.91	1.01	0.067
332.0 ± 8.6	0.51	0.88	1.08	0.076
378.5 ± 13.0	0.48	0.83	1.09	0.065
437.5 ± 7.8	0.47	0.94	1.01	0.043

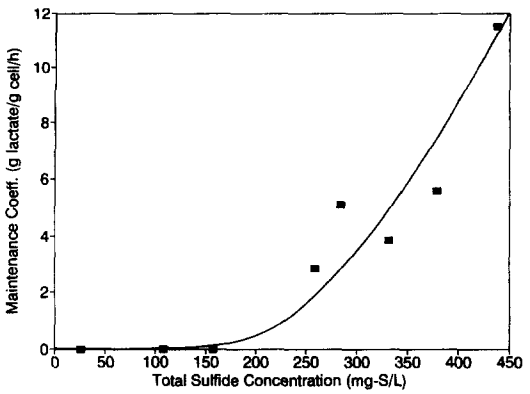

Fig. 5. Effect of sulfide concentration on maintenance coefficient (m). The maintenance coefficient was calculated based on equation (4) using $Y^{intr}_{c/Lac} = 0.03$ g cell (g lactate)$^{-1}$ (Okabe *et al.*, 1992).

yield ($Y^{intr}_{c/Lac}$) and is expressed as follows (Bulthuis, 1989; Characklis, 1990)

$$\frac{1}{Y_{c/Lac}} = \frac{m}{D} + \frac{1}{Y^{intr}_{c/Lac}}. \quad (4)$$

The maintenance energy was calculated using the observed cell yield ($Y_{c/lac}$) in this study and the intrinsic cell yield of $Y^{intr}_{c/Lac} = 0.03$ g cell (g lactate)$^{-1}$ measured in a similar type of experiment without sulfide inhibition (Okabe *et al.*, 1992) (Fig. 5). The calculated maintenance coefficient increased dramatically at total sulfide concentrations above 200 mg l^{-1} S.

A similar effect of sulfide has been observed in other types of bacteria. Mountfort and Asher (1979) reported that the biomass yield, the specific growth rate, and the intracellular levels of adenosine triphosphate (ATP) decreased with increasing sulfide level when *Methanosarcina barkeri* strain DM was grown in a batch system with methanol as carbon and energy source. Furthermore, they observed an increase in specific methane production rate. They concluded that the decrease in biomass yield resulted from an increase in the maintenance coefficient and uncoupling growth from energy production. Hobson and Millis (1990) also reported that the maintenance energy requirements for a mixed culture grown in a two-stage chemostat with phenolics increased with increasing sulfide concentration, possibly due to the need to repair damaged cell membranes.

Although data derived from batch culture experiments are often problematic, a degree of sulfide inhibition on cell yield in the chemostat agreed reasonably with the inhibition coefficient for μ^{inh}_{max} ($K_i = 251$ mg l^{-1} S) determined in the batch study, which is dependent on cell yield. The cell yield determined in the chemostat was reduced 50% at a sulfide concentration near 250 mg/l, even though K_i could not be determined in the chemostat study because equation (2) could not be used since the chemostat culture was lactate limited ($S \ll K_{Lac}$). This degree of sulfide inhibition on cell yield also agreed with the result of the recovery from sulfide shock which showed that the cell number was reduced to 55% of the original value by exposure to 212 ± 23 mg l^{-1} S [Fig. 3 (b)]. These results indicate that separation of sulfide inhibition into growth (cell yield) and activity (substrate utilization rate) is essential to interpret experimental data correctly.

Effect of sulfide on specific growth rate

The maximum specific growth rates in the presence of sulfide (μ^{inh}_{max}) determined in the batch culture were significantly lower than the continuous culture data at each corresponding sulfide concentration. In our previous studies (unpublished data), lower growth rates have been observed frequently in batch cultures, which is a normal observation for most SRB (Okabe and Characklis, 1992). This discrepancy of the values of μ^{inh}_{max} between the batch and the continuous experiment is attributed to several factors. Firstly, μ^{inh}_{max} for the continuous experiment was determined from the measured lactate concentration to what μ^{inh}_{max} would be based on the ratio of K_{Lac} to S [equation (3)], which is yield independent. In contrast, μ^{inh}_{max} for the batch experiment was determined by directly measuring the increase in cells, which is yield dependent. Specific growth rate (μ) is directly proportional to the lactate utilization rate unless maintenance energy requirement and intrinsic cell yield change [equation (4)]. If maintenance energy requirement increases due to sulfide inhibition, specific lactate utilization consequently increases. As a result of the increase in maintenance energy requirement, or in other words, the increase in specific lactate utilization, lactate concentration in the chemostat remained relatively constant and low, even though cell concentration decreases. Thus, specific growth rate determined in the chemostat under sulfide inhibition was higher than that observed in the batch experiment.

Secondly, the discrepancy is in part attributed to the selection of less susceptible microorganisms to high sulfide concentrations and the recovery from sulfide shock in the continuous culture as demonstrated in Fig. 3(a) and (b). Another contributing factor for the low cell yield could be that death and lysis rates may increase at high sulfide levels.

Finally, sulfide precipitates Fe in the batch experiment, so that Fe and other trace nutrients (not the carbon and energy source) may become the rate limiting factor. Moreover, pH, sulfide concentration, and limiting factor cannot be maintained at the same levels over many generations. If sulfide concentration changes as they grow, the pH shifts, or vice versa, another stress is imposed on the population which also requires more maintenance energy.

Effect of sulfide on stoichiometry

The stoichiometric ratios for catabolic reactions in this study did not change at high levels of TS. Thus, the same amount of energy (ATP) was generated

from substrate-level-phosphorylation and electron transport system (i.e. reduction of sulfate to sulfide). However, lower cell yields were observed in this study at high levels of sulfide, suggesting an increase in maintenance energy and uncoupling growth from energy production. Minor changes in the anabolic products would not have been detected in this study because of the precision of the analytical methods used in this study.

Effect of wall growth

Hill and Robinson (1975) reported that a possible wall growth in a chemostat culture exerts significant effects on the cell concentration and substrate utilization. Thus, the possible wall growth increases specific substrate utilization rate as a result of the decrease in suspended cell concentration. To evaluate the effect of wall growth on experimental results obtained in this study, the concentration of cells attached to the possible surfaces in the reactor system during an experimental period (about 2 weeks) was measured. The total numbers of attached cells were measured using the epifluorescence technique at the end of the experiment and found to be 2.4×10^4 cells cm^{-2}, which is approximately 10% of an average total suspended cell concentration for the reactor with surface area of 613 cm^2. Thus, the effect of wall growth was regarded as insignificant in this experiment.

Implication in sulfide inhibition model

The kinetics of *D. desulfuricans* growth on lactate as a function of sulfide concentration are presented in Fig. 6 based on experimentally determined rate coefficients (Okabe and Characklis, 1992; Okabe *et al.*, 1992) including the inhibition coefficient of 251 mg l^{-1} S determined in the batch experiment. These results suggest that under high sulfide concentrations such as in the petroleum formation [0–1000 mg l^{-1} H_2S (Subcasky, 1991)] and wastewater treatment systems (Hilton and Archer, 1988), sulfide inhibition kinetics should be considered in biological sulfide production models. Although other nutritional and physical conditions are suitable for SRB growth, SRB growth may be strongly inhibited by high sulfide concentrations. Furthermore, unless a change in specific sulfate reduction rate with increasing sulfide concentrations is taken into account, it is not possible to estimate the resulting sulfide production from growth rate data only. For instance, if SRB growth is monitored as change in total SRB cell count, it may underestimate H_2S production under inhibitory levels of sulfide concentration because the specific H_2S production rate may be elevated at higher sulfide levels even through bacterial growth rate and biomass production rate decreased by sulfide inhibition.

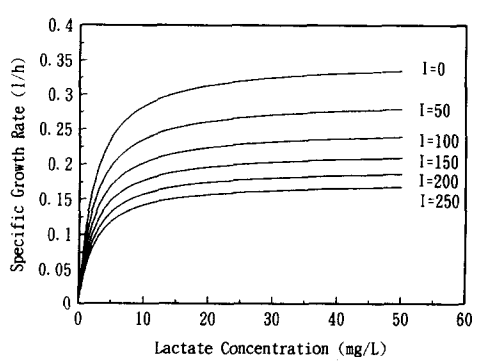

Fig. 6. Results of model simulation. Inhibition coefficient (K_i) was determined from experimental data: K_i = 251 mg l^{-1} S, $\mu_{max} = 0.34\ h^{-1}$, $K_{Lac} = 2.35$ mg l^{-1} (Okabe and Characklis, 1992; Okabe *et al.*, 1992).

CONCLUSIONS

Effects of sulfide on growth and activity of *D. desulfuricans* were quantitatively investigated in this paper. It can be concluded that separation of sulfide inhibition into cell yield (growth) and activity (lactate utilization rate) must be emphasized based on the following specific conclusions:

1. Cell yield and maximum specific growth rate decreased with increasing sulfide concentrations, whereas specific lactate utilization rate increased.
2. Inhibition coefficient (K_i) for maximum specific growth rate was 251 mg l^{-1} S in the batch experiment.
3. Cell yield determined in the chemostat was reduced in half by a sulfide concentration of approximately 250 mg l^{-1} S, which was very closed to the K_i value determined in the batch culture.
4. *D. desulfuricans* growing in the chemostat could recover from the shock of high sulfide concentrations.
5. A non-competitive inhibition model adequately described sulfide inhibition of *D. desulfuricans* in the batch experiment.

Acknowledgements—The authors gratefully acknowledge support from the cooperative agreement ECD-8907039 between Montana State University and the National Science Foundation and from the Center Industrial Associates.

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