Journal of Industrial Microbiology, 12 (1993) 21–28

Published by The Macmillan Press Ltd

Microbial treatment of a synthetic sour brine

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(Received 19 October 1992; revised 26 February 1993; accepted 3 March 1993)

Key words: Thiobacillus denitrificans; Sour water; Hydrogen sulphide; Petroleum production

SUMMARY

The ability of the chemoautotroph and facultative anaerobe *Thiobacillus denitrificans* to deodorize and detoxify an oil-field-produced water containing sulphides was evaluated under simulated field conditions. A sulphide-tolerant strain of *T. denitrificans* was used to remove inorganic sulphide from a synthetic sour brine containing 4000 mg L^{-1} total dissolved solids (TDS) and 100 mg L^{-1} sulphide. The sour brine was treated continuously in a rectangular plugflow reactor which approximated the scaled dimensions of an existing field detention pond. The head space of the reactor was purged with N_2 in order to capture H_2S off-gases in a zinc acetate trap. Brine was fed to the reactor continuously for 90 days at rates corresponding to residence times of 0.17–6 days. Temperature and pH ranged from 22 to 40.5 °C and 7.5 to 8.8, respectively. The start-up biomass concentration was approximately 100 mg L^{-1} (by dry weight). No additional *T. denitrificans* biomass was added to the reactor after start-up. At residence times of 0.3 days and greater inorganic sulphide was undetectable in the effluent. No H_2S was detected in the outlet gas or the zinc acetate trap. Approximately 80% of the sulphide feed was oxidized to sulphate and removed from the reactor in the liquid effluent. The remainder was partially oxidized to elemental sulphur which was retained in the reactor. It is suggested that oxidation of inorganic sulphides by *T. denitrificans* represents a viable process concept for the treatment of sour water co-produced with oil and gas.

INTRODUCTION

Water or brine co-produced with petroleum is often contaminated with soluble inorganic sulphides (H_2S, HS^-, S^{-2}) which result from the activities of sulphate-reducing bacteria or other sulphide-producing bacteria in the reservoir and well bore. These sulphides can make a significant contribution to the toxicity of these brines and must frequently be removed prior to surface discharge. A common method of treating these brines is to air strip the sulphides as H_2S. However, this method of 'treatment' simply converts a potential water pollution problem into an air pollution and odour problem.

If a sulphide-laden brine contains less than 2–3% total dissolved solids, as is the case at many oil production sites in Wyoming, the sour brine can potentially be treated microbially using a specialized sulphide-oxidizing bacterium such as *Thiobacillus denitrificans*. *T. denitrificans* is a strict autotroph and facultative anaerobe first described in detail by Baalsrud and Baalsrud [2]. Sulphide, elemental sulphur and thiosulphate may be used as energy sources with oxidation to sulphate. Under anoxic conditions, nitrate may be used as a terminal electron acceptor with reduction to elemental nitrogen (Eqn 1).

$$5HS^- + 8NO_3^- + 3H^+ \rightarrow 5SO_4^{-2} + 4N_2 + 4H_2O \quad (1)$$

Sublette and Sylvester [10–12] and Sublette [9] have demonstrated that *T. denitrificans* may be readily cultured under aerobic or anoxic conditions on H_2S (g) as an energy source at pH 7.0 and 30 °C. When H_2S (1% H_2S, 5% CO_2 and balance N_2) was bubbled into cultures previously grown on thiosulphate, H_2S was metabolized with no apparent lag. At loadings of 4–5 mmol H_2S h-g biomass^{-1}, H_2S concentrations in the outlet gas could be reduced to undectable levels with 1–2 s of gas–liquid contact time. Under sulphide-limiting conditions, concentrations of total sulphide in the culture media were less than 1 μM. Complete oxidation of H_2S to sulphate was observed.

Sublette and Sylvester [10] and Sublette [9] also investigated the effect of H_2S loading on reactor performance. In certain experiments the H_2S feed rate was increased in steps until H_2S breakthrough was obtained. At this point, the H_2S feed rate exceeded the rate at which the H_2S could be oxidized by the biomass. This upset condition was characterized by the accumulation of elemental sulphur and inhibitory levels of sulphide in the reactor medium. Nitrous oxide (N_2O) was also detected in the outlet gas and nitrite in the culture medium under anoxic conditions. This upset condition was reversible if the cultures were not exposed to the accumulated sulphide for more than 2–3 h. Maximum loading of the biomass, the specific feed rate at which H_2S breakthrough occurs, was estimated at 5.4–7.6 mmol H_2S h-g biomass^{-1} under anoxic conditions and 15.1–20.9 mmol H_2S h-g biomass^{-1} under aerobic conditions.

Sublette and Sylvester [12] also observed that heterotrophic contamination resulting from septic or non-aseptic operation of certain *T. denitrificans* cultures had a negligible effect on H_2S oxidation by the organism. The autotrophic

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medium used to grow *T. denitrificans* contained no organic materials to support heterotroph growth. Apparently organic carbon was obtained from waste products of *T. denitrificans* or cell lysis. Ongcharit et al. [3,4] have demonstrated that *T. denitrificans* may be flocculated by aerobic co-culture with floc-forming heterotrophs from an activated sludge system. An H_2S-active, gravity-settleable floc resulted which was used to scrub H_2S from a gas in a continuous stirred-tank reactor with biomass recycle.

Lee et al. [5] have also used flocculated *T. denitrificans* to treat sour water. Sour water containing up to 25 mM inorganic sulphide was successfully treated in an aerobic up-flow bubble column (3.5 L) containing 4.0 g L^{-1} of *T. denitrificans* immobilized by co-culture with floc-forming heterotrophs. The sulphide-laden water was supplemented with only mineral nutrients. The sulphide-active floc was shown to be stable for 9 months of continuous operation with no external organic carbon required to support the growth of the heterotrophs. The floc exhibited excellent settling properties throughout the experiment. Retention times in the reactor varied from 1.2 to 1.8 h. However, molar sulphide feed rate was more important in determining the capacity of the reactor for sulphide oxidation than either the hydraulic retention time or the influent sulphide concentration. At a biomass concentration of about 4 g L^{-1} the column could be operated at a molar sulphide feed rate of 12.7–15.4 mmol h^{-1} without upset.

Sublette and Woolsey [13] have isolated a sulphide-tolerant strain of *T. denitrificans* (strain F) by enrichment. Wild-type *T. denitrificans* is inhibited by sulphide concentrations of 0.1–0.2 mM. However, strain F is tolerant of sulphide concentrations in excess of 2.5 mM.

In this study, we have investigated the ability of *T. denitrificans* strain F to remove 100 mg L^{-1} of total inorganic sulphides (H_2S, HS^-, S^{-2}) from a simulated oil field brine treated continuously in a rectangular plugflow reactor, which aproximated the scaled dimensions of a field crude oil skimmer pond at the Amoco Production Co. Salt Creek Field site in Wyoming. An analysis of Salt Creek brine is given in Table 1. The brine composition and ultimate operating conditions of pH (7.0), temperature (105°F or 40.5 °C) and hydraulic residence time (0.3–6 days) were chosen to simulate field conditions. The reactor was operated anaerobically to reflect the anoxic conditions within the pond. The pond is anoxic due to the presence of a persistent hydrocarbon scum which prevents oxygen transfer. The effect of hydrocarbon on microbial oxidation of sulphide was therefore evaluated as well.

TABLE 1

Composition of Salt Creek discharge brine

Component	g L^{-1}
$CaCl_2 \cdot 2H_2O$	1.33
$MgSO_4 \cdot 7H_2O$	1.32
$MgCl_2 \cdot 6H_2O$	0.23
KCl	0.018
$Na_2CO_3 \cdot H_2O$	0.086
$NaHCO_3$	1.15
NaCl	1.82
Total dissolved solids (TDS)	4.81

pH = 8.5.

MATERIALS AND METHODS

Organism and culture

Strain F of *T. denitrificans* was isolated by enrichment from pure cultures of the wild-type (ATCC 23642) as previously described [13]. Stock cultures of *T. denitrificans* strain F were grown anoxically in a thiosulphate medium in 10-ml culture tubes at 30 °C [10]. In this medium thiosulphate (10 g L^{-1} as $Na_2S_2O_3$) is the energy source, nitrate (5 g L^{-1} as KNO_3) the terminal electron acceptor, bicarbonate (1.0 g L^{-1} as $NaHCO_3$) the carbon source and ammonium ion (0.5 g L^{-1} as NH_4Cl) the source of reduced nitrogen. The medium also contained a phosphate buffer (1.2 g L^{-1} Na_2HPO_4 and 1.8 g L^{-1} KH_2PO_4), $MgSO_4 \cdot 7H_2O$ (0.4 g L^{-1}), $CaCl_2$ (0.03 g L^{-1}), $FeCl_3$ (0.033 g L^{-1}), $MnSO_4$ (0.02 g L^{-1}) and trace elements.

A working culture of *T. denitrificans* strain F was prepared as follows. Strain F was first grown in standard thiosulphate medium (1.5 L) under anoxic conditions with nitrate as the terminal electron in a B. Braun Biostat M at pH 7.0 and 30 °C [10]. When the optical density at 460 nm reached about 1.0 (corresponding to about 10^9 cells ml^{-1}), the biomass was harvested by centrifugation at 5000 *g* and 25 °C and resuspended in the medium described in Table 2. This

TABLE 2

Thiosulphate medium for *T. denitrificans* supplemented with brine components[a]

Component	g L^{-1}[b]
Na_2HPO_4	1.20
KH_2PO_4	1.80
$MgSO_4 \cdot 7H_2O$	1.32
NH_4Cl	0.40
$CaCl_2$	0.02
$MgCl_2$	0.11
$MnSO_4$	0.02
$FeCl_3$	0.033
$NaHCO_3$	1.15
Na_2CO_3	0.073
NaCl	1.82
KNO_3	5.00
$Na_2S_2O_3$	10.00
KCl	0.018
Trace metal solution [10]	15.0 ml

pH = 7.0.

[a] The actual $CaCl_2$ concentration in the brine was not used during culture development to avoid excessive build up of a calcium phosphate precipitant.

[b] Unless otherwise indicated.

medium contained all of the components of standard thiosulphate medium plus components of the sour brine at the Amoco site referenced above. The strain F suspension was transferred back to the fermenter and incubated as before with pH control at 7.0 but with the temperature controlled at 22 °C (room temperature). The purpose of this change in culturing conditions was to acclimate the biomass to components of the simulated brine and a reduced temperature. Each day for the next 5 days one-half of the culture was removed and replaced with an equal volume of fresh medium (Table 2). Growth was monitored by optical density and thiosulphate concentration. At the end of this time the biomass was again harvested by centrifugation at 5000 *g* and 25 °C for 10 min and resuspended in the medium described in Table 3 (except with 0.4 g L^{-1} $MgSO_4 \cdot 7H_2O$) to a final biomass concentration of about 100 mg L^{-1} as determined by optical density (data not shown). This suspension was then used as the initial charge to the continuous reactor described below.

Operation of continuous reactor

A schematic diagram of the continuous reactor system used in this study is shown in Fig. 1A. The bioreactor consisted of a sealed, rectangular plexiglas vessel with inside dimensions 60 cm long, 25 cm wide and 5.5 cm high. The location of the inlets, outlets and sample ports on the bioreactor are shown in Fig. 1B. The liquid inlet was a 1/8-in stainless steel tube entering the reactor through a 1/8-in compression fitting. The inlet was flush with the inside wall of the vessel. A constant liquid-phase volume of 2 L was maintained by positioning the 1/8-in stainless steel outlet tube at a fixed height within the reactor. Liquid exited the vessel by overflow assisted by a N_2 positive pressure (see below). The gas space in the reactor was purged with N_2 at 2.5–3.5 L h^{-1} (by rotameter) or about one head space volume change per hour. Nitrogen exited the reactor at the liquid overflow.

Liquid feed to the reactor consisted of two components. One was a Na_2S sulphide stock solution delivered to the influent line by a Harvard Apparatus (South Natick, MA) Model 975 syringe pump fitted with two 50-ml syringes (to prevent air oxidation). The sulphide stock solution mixed with nutrient-amended brine in a tee. The nutrient-amended brine was delivered via a B. Braun FE 211 positive displacement pump (0.1 F pump head). The composition of the nutrient-amended brine was such that when blended

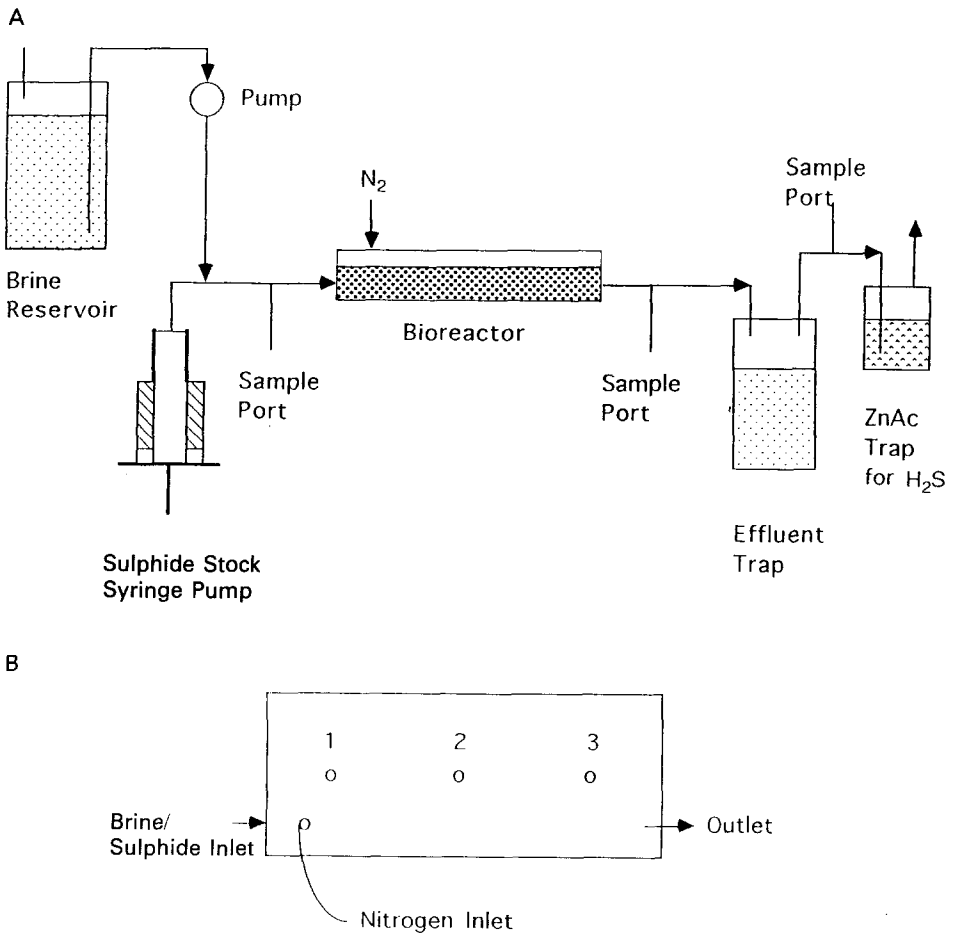

Fig. 1. (A) Schematic diagram of the continuous bioreactor system. (B) Bioreactor showing inlet, outlet and sample ports (1–3).Samples taken for pH were taken from port no. 3.

with the sulphide stock solution the resulting feed had the composition given by Table 3 plus 100 ppmw (parts per million on a weight basis) or 3.1 mM Na_2S. The sulphide stock concentrations and flow rates and brine flow rates used in this experiment are given in Table 4. The brine reservoir was a 4-L Erlenmeyer flask on a magnetic stirrer. Brine was withdrawn via a 1/8-in stainless steel tube and delivered to the reactor via 2-mm silicone tubing.

The gas/liquid effluent from the reactor was transported via 2-mm silicone tubing by the N_2 positive pressure in the reactor to a 4-L Erlenmeyer flask which served as an effluent trap and gas/liquid separator. The gas then flowed to a H_2S trap where the gas was sparged into 200 ml of 0.3 wt% zinc acetate. Here any H_2S was precipitated as ZnS.

Feed and effluent samples were taken by diverting flow through tees in the transmission lines (at location indicated in Fig. 1A) to tared sample containers containing 5–10 ml of 0.3 wt% zinc acetate. The purpose of the zinc acetate was to immediately precipitate sulphides and prevent losses due to volatilization of H_2S. Feed and effluent were taken daily at higher residence times and more frequently as residence times were decreased. Liquid samples could also be taken directly from sample ports shown in Fig. 1B. A daily sample (5 ml) was removed by syringe from the port shown in Fig. 1B for pH determination. Gas samples were taken at a tee located between the effluent trap and H_2S trap (Fig. 1A).

The reactor was operated at liquid residence times of 0.17–6 days by varying the liquid feed rate. However, the feed composition was always the same (Table 3 plus 3.1 mM Na_2S) except that: (1) the nitrate concentration was decreased near the end of the experiment, and (2) also near the end of the experiment, crude oil from an Amoco Wyoming site was injected into the feed line via a second syringe pump to give a final concentration in the feed of 500 ppmw. The initial temperature of operation was 22 °C (room temperature). Subsequently the bioreactor was submerged (to above the liquid level) in a controlled temperature water bath to allow the temperature to be increased in steps to 40.5 °C. Operating conditions are summarized in Table 4.

As noted above the initial charge to the reactor was 2.0 L of a suspension of *T. denitrificans* strain F (100 mg L^{-1}, dry weight). Liquid feed and the N_2 purge of the gas space were started immediately and corresponds to $t = 0$ in Table 4.

TABLE 3

Nutrient-amended synthetic brine[a]

Component	g L^{-1}[b]
Na_2HPO_4	0.20
$MgSO_4 \cdot 7H_2O$	1.32
NH_4Cl	0.40
$CaCl_2$	1.01
$MgCl_2$	0.11
$MnSO_4$	0.02
$FeCl_3$	0.033
$NaHCO_3$	1.15
Na_2CO_3	0.073
NaCl	1.82
KNO_3	5.00
KCl	0.018
Trace metal solution [9]	15.0 ml

pH = 7.0

[a] The phosphate concentration was reduced compared to standard thiosulphate medium to eliminate as much as possible any pH buffering except that provided by brine components.

[b] Unless otherwise indicated.

Operation of a control reactor

Prior to operating with *T. denitrificans* biomass present, the reactor was filled with 2 L of nutrient-amended synthetic brine and operated as a control without biomass with a liquid feed consisting of 12.3 ml h^{-1} nutrient-amended brine and 1.68 ml h^{-1} of 26.0 mM Na_2S giving a residence time of 6 days. The N_2 purge rate was 2.5–3.5 L h^{-1}. All other aspects of reactor operation and sampling were as described above.

Analytical

Sulphate was determined turbidimetrically by precipitation with $BaCl_2$ [1]. Elemental sulphur was determined by reaction with cyanide to produce thiocyanate which was quantitated as Fe $(SCN)_6^{-3}$ [8]. Sulphide stock solutions (1M) were prepared from $Na_2S \cdot 9H_2O$ and standardized by titration with 0.01 M $Pb(ClO_4)_2$ using a sulphide ion-specific electrode to detect the end point [10]. Feed and effluent samples taken for sulphide analysis were analysed by the methylene blue method as follows. Two reagents were required for colorimetric analysis of the precipitated sulphide, DMPD reagent and ferric reagent. The DMPD reagent was prepared by dissolving 1.0 g of *N,N*-dimethyl-*p*-phenylenediamine sulphate (Sigma Chemical Co., St Louis, MO), 1.0 g Zn $(CH_3COO)_2 \cdot 2H_2O$ and 50 ml of concentrated H_2SO_4 in distilled water and diluted to 1 L. Ferric reagent was prepared by dissolving 5.0 g $FeCl_3 \cdot 6H_2O$ in 20 ml of distilled water. Suspensions of ZnS (from feed and effluent samples, see above) were analysed by mixing 5.0 ml of the ZnS suspension (or a suitable dilution) with 4.9 ml of DMPD reagent followed by immediate addition of 0.1 ml of ferric reagent. The absorbance at 660 nm was then read after at least 10-min incubation at room temperature.

Nitrate was determined by the cadmium reduction method using gentisic acid in place of *N*-(1-naphthyl)-ethylenediamine in the colour development step [1]. Nitrite was determined by the diazotization method using chromatropic acid and sulphanilic acid [1]. Ammonium ion was determined by the Nessler's method without distillation. Thiosulphate was determined by titration with a standard I_2 solution and a starch indicator [6]. Counts of *T. denitrificans* were obtained by the most probable number method [7] using thiosulphate medium described elsewhere [10]. Hydrogen sulphide in the reactor outlet gas was estimated using chromaphoric analyser tubes (Gastec Corp., Yokohama, Japan).

TABLE 4

Operating conditions for continuous *T. denitrificans* reactor receiving a feed of nutrient-amended synthetic brine and sulphide[a]

Time (h)	Total liquid feed rate (ml h^{-1})[b]	Avg residence time (days)	T (°C)	Comments
0	14.0[c]	6.0	22.0	
624			23.0	
648			30.0	
696			33.0	
768			35.0	
816			37.0	
864			38.5	
912			40.5	
1080	19.6	4.3		
1128	25.8	3.2		
1176	57.4	1.5		
1248	114.7	0.73		
1272	135.4	0.62		
1320	245.2[d]	0.34		
1584	490.7	0.17		
1591	241.8	0.34		no sulphide feed
1609	245.2	0.34		sulphide feed restarted,
1848				incorporated 500 ppmw crude oil in feed
2136				experiment terminated

[a] Each new entry vertically represents a change to that condition.
[b] Volumetric flow rate of nutrient-amended brine plus sulphide feed.
[c] 12.3 ml min^{-1} brine and 1.68 ml min^{-1} of 26.0 mM Na_2S (pH 7.0–7.3).
[d] 241.8 ml min^{-1} brine and 3.36 ml min^{-1} of 228 mM Na_2S (pH 7.0–7.3).

RESULTS AND DISCUSSION

The reactor was first operated without biomass for about 10 days with a feed of nutrient-amended synthetic brine plus sulphide at a rate sufficient to give a 6-day residence time. Fig. 2 shows the influent and effluent sulphide concentrations during this time. As seen in Fig. 2, the feed sulphide concentration averaged about 3.0 mM. Sulphide was detected in the effluent within 2 days, with the concentration increasing to as much as 2.0 mM in about 6 days or one residence time. At about the same time that sulphide was detected in the liquid effluent, H_2S was detected in the gas outlet. The pH was seen to rise to 8.6–8.7 within 48 h and remain at this level, presumably due to volatilization of sulphide as H_2S. Fig. 3 shows the cumulative sulphide emissions obtained by analysing the accumulated ZnS in the zinc acetate trap. Since the reactor had not reached steady state when the control experiment was terminated, it was difficult to do a sulphur balance. However, it was clear from the control experiment that little removal of sulphide could be expected in the absence of biomass.

After the control experiment the reactor was emptied and refilled with nutrient-amended synthetic brine containing about 100 mg L^{-1} of *T. denitrificans* strain F. Liquid and

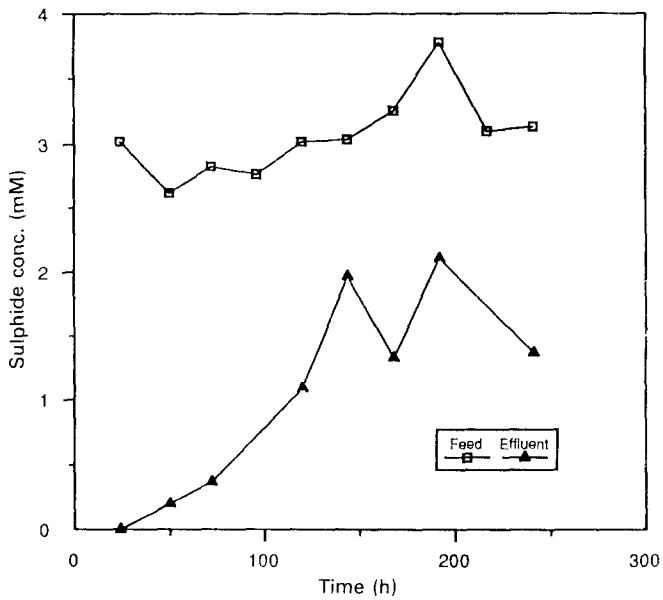

Fig. 2. Sulphide concentration in the feed and effluent of the continuous control reactor (without *T. denitrificans*) receiving a feed of nutrient-amended synthetic brine and sulphide.

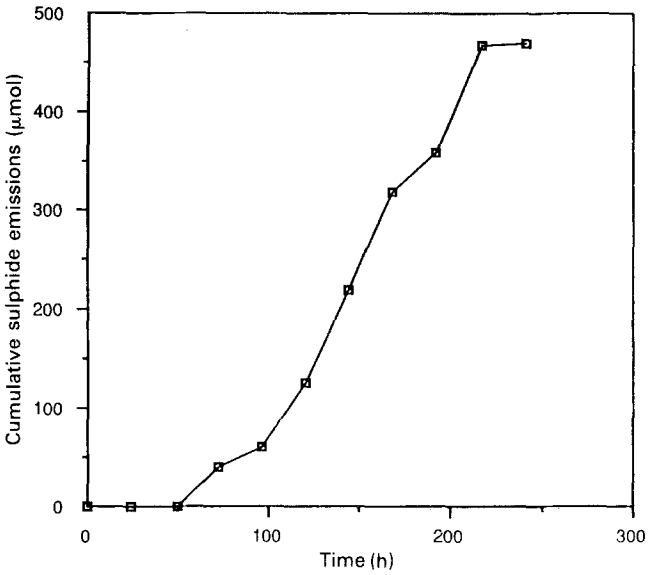

Fig. 3. Cumulative sulphide emissions from the continuous control reactor (without *T. denitrificans*) receiving a feed of nutrient-amended synthetic brine and sulphide.

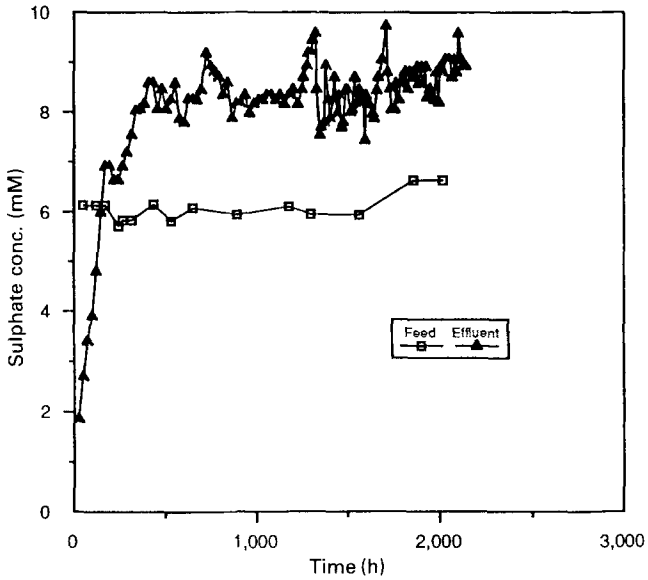

Fig. 5. Sulphate concentration in the feed and effluent of the continuous bioreactor (with *T. denitrificans*) receiving a feed of nutrient-amended synthetic brine and sulphide.

gas feeds were then initiated at the rates indicated in Table 4. Within 24 h, the reactor contents had clarified as the solids (calcium phosphate and cells) settled to the bottom of the reactor. Therefore, after 24 h no biosolids were lost in the effluent.

The performance of the reactor over the next 89 days is documented in Figs 4–7. As seen in Fig. 4, with one exception, no sulphide was detected in the liquid effluent throughout the entire experiment. The only exception was an effluent sample taken about 5 h after the residence time

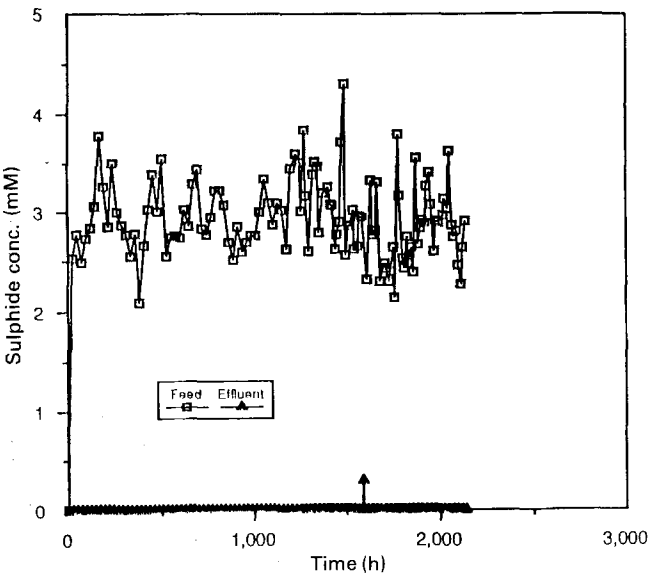

Fig. 4. Sulphide concentration in the feed and effluent of the continuous bioreactor (with *T. denitrificans*) receiving a feed of nutrient-amended synthetic brine and sulphide.

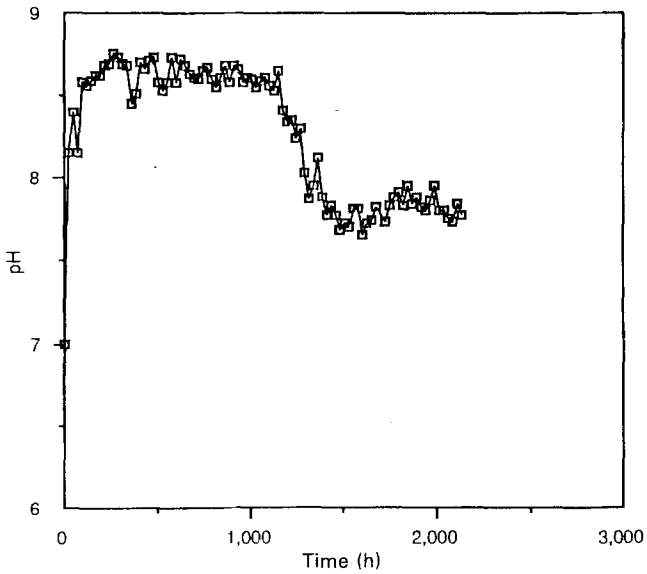

Fig. 6. pH of the culture medium in the continuous bioreactor (with *T. denitrificans*) receiving a feed of nutrient-amended synthetic brine and sulphide.

was briefly reduced to 0.17 days (see Table 4). In addition, no H_2S was detected in the reactor outlet gas either directly as H_2S or as ZnS in the zinc acetate trap. Sulphide could only be detected within the reactor near the inlet indicating that the bulk of sulphide removal occurred in this area. The sulphate concentrations in the feed and effluent are shown in Fig. 5. As indicated in Fig. 5, sulphate was a major product of sulphide oxidation by *T. denitrificans*. The sulphate concentration in the effluent average 8.5 mM versus about 6.0 mM in the feed. Since the average sulphide

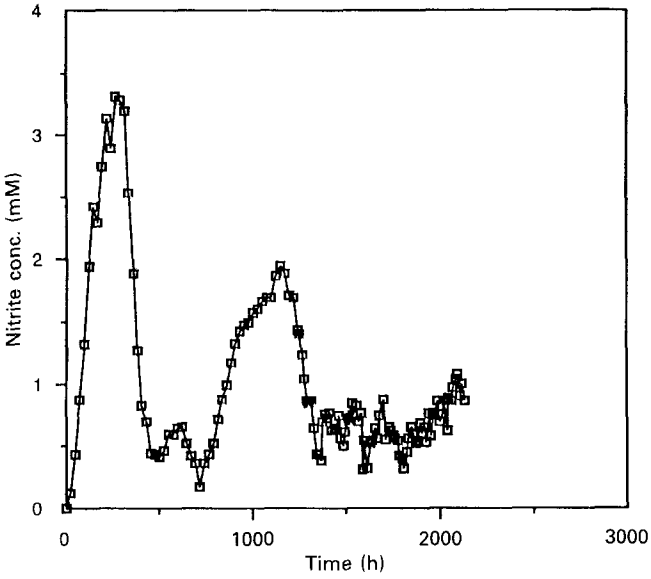

Fig. 7. Nitrite concentration in the effluent of the continuous bioreactor (with *T. denitrificans*) receiving a feed of nutrient-amended synthetic brine and sulphide.

concentration in the feed was about 3.0 mM, about 83% of the influent sulphide was converted to sulphate. The remaining sulphide was apparently oxidized to the level of elemental sulphur which accumulated at the bottom of the reactor. Elemental sulphur was detected in the reactor solids primarily near the reactor inlet. Microscopic examination showed copious amounts of gram-negative, short rods (like *T. denitrificans*) associated with particles of elemental sulphur. The association of the biomass with sulphur particles may have helped to create microenvironments for the cells which protected them from rather harsh conditions in the bulk liquid phase (see below). Since the sulphur was not uniformly distributed, it was difficult to close the sulphur balance. However, no other forms of sulphur were found in the reactor and no sulphide was detected in reactor effluents.

Fig. 6 shows the pH of the liquid phase in the reactor during the course of the experiment. During the first 44 days, when the residence time was 6 days, the pH was relatively constant at 8.6–8.7. The pH has previously been reported as inhibitory for the organism [2]. However, as noted above, throughout this period influent sulphide was completely oxidized to sulphate and elemental sulphur. Therefore, *T. denitrificans* remained viable and active during this time. As noted above, *T. denitrificans* cells were agglomerated with sulphur particles at the bottom of the reactor. The pH in the organisms' microenvironment could have been significantly less than that in the bulk liquid phase. As seen in Fig. 6, the pH declined as the liquid feed rate was increased (see Table 4). This could be due to better mixing in the reactor and greater availability of buffering capacity from medium components. Alternatively, Fig. 5 shows a surge in the effluent sulphate concentration as the liquid feed rate was increased. A greater fraction of sulphide oxidized completely to sulphate could have reduced the pH since this reaction is acid producing [10].

Fig. 7 shows the nitrite concentration in the reactor effluent. Nitrite results from the incomplete reduction of nitrate. As discussed in the Introduction, nitrite has been seen to accumulate in *T. denitrificans* reactors during upsets caused by excessive H_2S feed. Therefore, the appearance of nitrite in the reactor effluent is indicative of exposure of *T. denitrificans* cells to inhibitory sulphide concentrations in the inlet zone. In other words nitrite is a sign of stress. As shown in Fig. 7, the nitrite concentration increased after startup to a maximum of 3.3 mM then declined to 0.4–0.7 mM. The nitrite concentration decreased during the time the operating temperature was increased from 22 °C to 33 °C. However, then the temperature was increased further, the nitrite concentration again began to increase dramatically to about 1.0 mM then again declined as the liquid feed rate to the reactor was being increased (see Table 4). Between about 1400 and 1900 h of operation the nitrite concentration averaged about 0.5 mM ± 0.3 mM when the temperature was 40.5 °C and the residence time was 0.34 days. At about 1900 h the nitrite concentration again rose slightly when crude oil was introduced to the liquid feed (see below).

The NH_4^+ concentration in the reactor effluent averaged 5.7–6.1 mM during the entire course of the experiment versus 7.5 mM in the nutrient-amended brine. This decline indicated microbial activity in the reactor and utilization of NH_4^+ as a reduced nitrogen source. The nitrate concentration in the feed was maintained at 50 mM during the first 1500 h. The nitrate concentration was then reduced in steps, first to 25 mM for 24 h, then to 20 mM for 24 h and finally to 15 mM for the duration of the experiment. The effluent nitrate concentration then declined to about 10 mM ± 0.5 mM and remained at that level. The decrease in feed nitrate did not produce any change in reactor operation with respect to sulphide removal. The stoichiometry of nitrate utilization relative to sulphide oxidation was 1.7 moles nitrate per mole sulphide. This compares favourably to that reported by Sublette and Sylvester [10] for oxidation of H_2S by pure cultures of *T. denitrificans* under anoxic conditions.

Just prior to the introduction of crude oil in the liquid feed, the reactor contents were mixed and sampled. These samples were used to determine the concentration of *T. denitrificans* cells by the most probable number method. The result was 2.5×10^9 cells ml^{-1}. This compares to an estimated 8×10^8 cells ml^{-1} immediately after inoculation as determined by optical density (data not shown).

After the reactor was sampled, the reactor was operated at a residence time of 0.34 days (see Table 4) for another 168 h. Crude oil was then introduced into the feed as described above and the reactor operated under these conditions for another 288 h. The crude oil, as expected, eventually formed a layer over the entire reactor contents. However, as seen in Figs 4–5 the reactor continued to function normally with complete removal of sulphide from the feed and oxidation primarily to sulphate. Fig. 7 shows a gradual increase in nitrite concentration during this time, possibly indicating stress on the culture. However, by the

time the experiment was terminated the nitrite concentration in the effluent was starting to decline and generally remained below 1 mM while crude oil was present in the reactor.

CONCLUSION

It has been demonstrated that *T. denitrificans* strain F can be used to remove and oxidize inorganic sulphides (up to 100 mg L^{-1}) from a nutrient-amended synthetic brine in a continuous reactor scaled to simulate a sour water retention pond under anoxic conditions. Sulphides were immediately oxidized primarily to sulphate (83%); the remainder accumulated as elemental sulphur. No off-gassing of hydrogen sulphide was observed. Following initiation of sulphide feed and formation of elemental sulphur, the *T. denitrificans* biomass and sulphur agglomerated on the bottom of the reactor preventing significant loss of biomass in the effluent. The reactor operated with complete sulphide removal at residence times as low as 0.34 days, at pH values as high as 8.7, at temperatures as high as 40.5 °C and in the presence of crude oil. Oxidation of inorganic sulphides by *T. denitrificans* represents a viable process concept for treatment of sour water co-produced with oil and gas.

ACKNOWLEDGEMENTS

This work was funded by Amoco Production Co. (Tulsa, OK) and ABB Environmental Services (Portland, ME).

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