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Technological aspects of the microbial treatment of sulfide-rich wastewaters: A case study

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Abstract

Thiobacillus denitrificans has been shown to be an effective biocatalyst for the treatment of a variety of sulfide-laden waste streams including sour water, sour gases, and refinery spent-sulfidic caustics. The term 'sour' originated in the petroleum industry to describe a waste contaminated with hydrogen sulfide or salts of sulfide and bisulfide. The microbial treatment of sour waste streams resulting from the production or refining of natural gas and crude oil have been investigated in this laboratory for many years. The application of this technology to the treatment of sour wastes on a commercially useful scale has presented several technical barriers including substrate inhibition (sulfide), product inhibition (sulfate), the need for septic operation, biomass recycle and recovery, mixed waste issues, and the need for large-scale cultivation of the organism for process startup. The removal of these barriers through process improvements are discussed in terms of a case study of the full-scale treatment of sulfide-rich wastewater.

The ability of *T. denitrificans* to deodorize and detoxify an oil-field produced water containing sulfides was evaluated under full-scale field conditions at Amoco Production Co. Salt Creek Field in Midwest, WY. More than 800 m^3/d of produced water containing 100 mg/L sulfide and total dissolved solids of 4800 mg/L were successfully biotreated in an earthen pit (3000 m^3) over a six-month period. Complete removal of sulfides and elimination of associated odors were observed. The system could be upset by severe hydraulic disturbances; however, the system recovered rapidly when normal influent flow rates were restored.

Introduction

The path from laboratory to successful full-scale or field application of any chemical or biochemical process presents many technical barriers. A process based on chemical transformations by living microorganisms can be especially difficult technically given the commonly narrow range of environmental conditions that will maintain the viability of the process culture. If aseptic conditions are required another level of technical difficulty and cost are added. These costs can be justified if high-value products are produced, but the production of commodity chemicals or waste treatment must often be accomplished under septic conditions to be cost effective. For a waste treatment process which employs a specialized culture, operation under septic conditions is another technical barrier to successful full-scale application because of potential competition for nutrients between the process microorganisms and contaminants which become established in the process culture. This paper describes such a waste treatment process, the technical barriers to its use on a commercial scale, and a case study of its full-scale application.

Thiobacillus denitrificans has been shown to be an effective biocatalyst for the treatment of a variety of sulfide-laden waste streams including sour water, sour gases, and refinery spent-sulfidic caustics (Sublette &

Sylvester, 1986, 1987a, 1987b; Sublette, 1987; Sublette, 1990; Camp & Sublette, 1992; Lee & Sublette, 1993; Raterman et al., 1993; Sublette et al., 1994a; Cho et al., 1995; Rajganesh et al., 1995a; Rajganesh et al., 1995b; Rajganesh et al., 1995c). The term 'sour' originated in the petroleum industry to describe a waste (gas, liquid or solid) contaminated with hydrogen sulfide (H_2S) or salts of sulfide or bisulfide. The microbial treatment of sour waste streams resulting from production or refining of natural gas and crude oil have been investigated in this laboratory for many years. It is the treatment of sour water which will be addressed in this paper.

T. denitrificans is a strict chemolithotroph and facultative anaerobe first described in detail by Baalsrud and Baalsrud (1954). Sulfide, elemental sulfur, and thiosulfate may be used as energy sources with oxidation to sulfate.

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \quad (1)$$

Under anoxic conditions, nitrate may be used as a terminal electron acceptor with reduction to elemental nitrogen.

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

Work in this laboratory has 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 (Sublette & Sylvester, 1986, 1987a, 1987b; Sublette, 1987). When H_2S (1% H_2S, 5% CO_2, and balance N_2) was bubbled into cultures previously grown on thiosulfate, H_2S was metabolized with no apparent lag. At loadings of 4–5 mmol H_2S/h-g biomass, H_2S concentrations in the outlet gas could be reduced to undetectable levels with 1–2 s of gas-liquid contact time. Under sulfide-limiting conditions, concentrations of total sulfide (H_2S, HS^-, S^{2-}) in the culture media were $< 1\mu$M. Complete oxidation of H_2S to sulfate was observed.

We have also investigated the effect of H_2S loading on reactor performance (Sublette & Sylvester, 1986; Sublette, 1987). 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 sulfur and inhibitory levels of sulfide in the culture 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 (either aerobic or anoxic) were not exposed to the accumulated sulfide for more than 2–3 h. Maximum loading of the biomass, the specific feed rate at which H_2S breakthrough occurs, was estimated to be 5.4–7.6 mmol H_2S/h-g biomass under anoxic conditions and 15.1–20.9 mmol H_2S/h-g biomass under aerobic conditions.

Much of our early work on the oxidation of sulfides by *T. denitrificans* concerned the removal of H_2S from gas streams as indicated above. There were two reasons for this focus. First, there was a need for new technology in the petroleum industry for treatment of gas streams contaminated by H_2S. Secondly, the removal of H_2S from a gas stream presented fewer technical problems with respect to reactor design and operation. This simplified the study of the microbiology of the process. However, the application of *T. denitrificans* cultures to the treatment of sour water on a commercially-useful scale presented several new technical barriers in addition to those encountered in treating sour gas. All of these barriers and the process improvements required to overcome them are discussed below.

Substrate inhibition

Sulfide is toxic to *T. denitrificans* and, therefore, an inhibitory substrate. The practical implication is that reactor systems employed to treat any sulfide-laden wastes must be operated on a sulfide-limited basis so that the steady-state concentration of sulfide in the bulk liquid phase of the process culture is below inhibitory levels. Further, reactor systems must be well-mixed to avoid high gradients in sulfidic concentrations and, therefore, isolated pockets of inhibitory sulfide concentrations. Clearly any process for the removal of sulfides from sulfide-laden waste streams would be more resistant to upset if a sulfide-tolerant strain of *T. denitrificans* was utilized. A sulfide-tolerant strain (strain F) has been isolated by enrichment from culture of the wild type (Sublette and Woolsey, 1989). This culture was obtained by repeated exposure of *T. denitrificans* cultures to increasing concentrations of sulfide (as Na_2S). At each step only tolerant strains survived and grew. Eventually strain F was obtained which exhibited growth comparable to controls at sulfide concentrations of up to 2.5 mM. The wild type is inhibited by sulfide concentrations as low as 0.1–0.2 mM.

Product inhibition

Sulfate is the end product of the aerobic or anoxic (with nitrate) oxidation of sulfides by *T. denitrificans*. *T. denitrificans* cultures have been shown to be inhibited by sulfate concentrations in excess of 250 mm (Sublette, 1990). This is likely not an inherent inhibition by sulfate but an effect of increasing ionic strength. In any regard this product inhibition places an operating restriction on both batch and continuous reactors. In batch systems the accumulation of sulfate can determine the cycle time between batches. In a continuous system the sulfate concentration at steady state is determined by the dilution rate; therefore, at any given sulfide feed rate, the reactor volume or hydraulic throughput is controlled by the sulfate concentration to be maintained in the culture.

Septic operation

The autotrophic medium used to grow *T. denitrificans* will not support the growth of heterotrophs because there is no organic carbon source. However, if aseptic conditions are not maintained, heterotrophic contamination develops in the *T. denitrificans* cultures. Evidently *T. denitrificans* releases organic material into the medium in the normal course of growth, or through lysis of non-viable cells, which supports the growth of heterotrophs. To investigate the effect of heterotrophic contamination on the performance (in terms of sulfide oxidation) of a *T. denitrificans* continuous stirred-tank reactor (CSTR), an anoxic reactor which became contaminated was allowed to operate for an extended time (30 days) (Sublette & Sylvester, 1987c). The reactor was originally contaminated by two unidentified heterotrophic bacteria with distinctly different colony morphologies when grown on nutrient agar. After 145 hours of operation, the reactor was injected with suspensions of four different heterotrophic bacteria (*Pseudomonas* species) known to be nutritionally versatile. The total heterotroph concentration increased to about 10^8 cells/mL and leveled off. Apparently growth of the contaminants became limited by the availability of suitable carbon sources. The viable count of *T. denitrificans* at steady state was 5.0 x 10^9 cells/mL. The steady-state composition of the culture medium, and the outlet-gas condition, were indistinguishable from that of a pure culture of *T. denitrificans* operated under the same H_2S feed conditions. These results indicated that in the absence of an externally derived organic carbon source, the growth of heterotrophs in *T. denitrificans* cultures operated septically will be controlled by the availability of organic carbon derived from growth of the chemolithotroph. Therefore, a runaway competition for nutrients is avoided. These observations led to the efforts to immobilize *T. denitrificans* by co-culture with floc-forming bacteria.

Biomass recycle and biomass retention

If a sulfide-oxidizing *T. denitrificans* bioreactor is operated in a batch mode, the biomass must be economically separable from the bulk liquid phase (and sulfide oxidation product) at the end of the cycle. In continuous flow this separation must occur on a continuous basis in order to decouple the biomass and hydraulic retention times and allow for recycle of the biomass. This technical problem is critical to the successful operation of a continuous bioreactor treating sour water where hydraulic loadings can be quite high. The most economical method of separating biomass from a bulk liquid phase would be gravity settling. However, gravity settling requires that the biomass be flocculated to such a size as to allow for sedimentation in a reasonable length of time.

Many microorganisms exist co-immobilized in nature in associations often of benefit to all members of the population. Many species of bacteria produce extracellular biopolymers which adsorb and entrap other non-flocculating microbial cells, forming protected environments for the latter, and establishing beneficial cross-feeding. Such immobilized mixed populations are exploited in activated sludge systems, trickling filters, anaerobic digesters, and similar systems for the treatment of waste water.

T. denitrificans has been immobilized by co-culture with floc-forming heterotrophs obtained from activated sludge taken from the aerobic reactor of a refinery waste water treatment system (Ongcharit et al., 1989). *T. denitrificans* cells grown aerobically on thiosulfate and washed sludge were suspended together in fresh thiosulfate medium without nitrate. The culture was maintained in a fed-batch mode at pH 7.0 and 30 °C with a gas feed of 5% CO_2 in air. This medium was thiosulfate-limiting with respect to the growth of *T. denitrificans*. When thiosulfate was depleted, the agitation and aeration were terminated and the flocculated biomass was allowed to settle under gravity. The supernatant liquid was then removed and discarded. In this way the culture was enriched for *T. denitrificans* cells which had become physically associated with the floc. The volume then was made up with fresh medium, and aeration and agitation restarted. After 5–

6 cycles a gravity-settleable, sulfide-active floc was obtained. Immobilized *T. denitrificans* was shown to oxidize H_2S(g) in a CSTR with cell recycle at molar feed rates of up to 6.3 mmoles/hr (2.0 L culture volume) and total biomass concentrations of up to 13 g/L. During five months of continuous operation, the biomass exhibited excellent settling properties demonstrating the long-term stability of the relationship between *T. denitrificans* and the floc-forming heterotrophs. No external addition of organic carbon was required at any time (Ongcharit et al., 1990). At a biosolids concentration of 3 g/L, 70% compression of the biomass was observed in 10 minutes.

We have also used flocculated *T. denitrificans* to treat sour water (Lee & Sublette, 1993). Sour water containing up to 25 mM inorganic sulfide was successfully treated in an aerobic up-flow bubble column (3.5 L) containing 4.0 g/L of flocculated *T. denitrificans*. The sulfide-laden water was supplemented with mineral nutrients only. The sulfide-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 sulfide feed rate (mmol/h sulfide) was more important in determining the capacity of the reactor for sulfide oxidation than either the hydraulic retention time or the influent sulfide concentration (mmol/L). At a biomass concentration of about 4 g/L, the column could be operated at a molar sulfide feed rate of 12.7–15.4 mmol/h without upset. Therefore, the specific activity of the flocculated biomass for sulfide oxidation was 1.1 mmol/h-g.

In these systems it seems that the growth of the chemolithotroph *T. denitrificans* was balanced with the growth of the floc-forming heterotrophs through a commensal relationship in which the growth of the heterotrophs was limited by organic carbon derived from *T. denitrificans*. The result was an immobilization matrix which grew with the *T. denitrificans*. This development reduces the process of microbial H_2S oxidation to the level of technical simplicity of an activated sludge system.

Large-scale cultivation of T. denitrificans

Commercial-scale application of *T. denitrificans* for treatment of sulfide-laden wastewater or other waste streams requires large amounts of biomass for start-up of the process. Growth of *T. denitrificans* for biomass production on sulfide under sulfide-limiting conditions would be exceedingly tedious and potentially hazardous for operators. Fortunately, *T. denitrificans* can be cultivated readily on a nontoxic energy source (thiosulfate) which can readily be supplied at high concentrations in the culture medium allowing for maximum growth rates. Flocculation of the culture offers a simple mechanism for harvesting the biomass. However, before any large-scale applications of microbial oxidation of sulfides could be attempted, it was necessary to demonstrate that the organism could be cultivated economically on a large scale and have sufficient "shelf life' for storage and transport to an application site.

Large-scale cultivation of *T. denitrificans* strain F was demonstrated as follows (Hasan et al., 1994): *T. denitrificans* strain F was immobilized by aerobic coculture with floc-forming heterotrophs from a local refinery-activated sludge system in the thiosulfate medium (without nitrate) as described above. When a sulfide-active, gravity-settleable floc was obtained, this culture was used to inoculate 189 L of thiosulfide mineral salts medium in a jacketed stainless-steel, stirred-tank reactor. The culture was again maintained at 30 °C and the pH was monitored and maintained at 7.0 ± 0.05. The culture was aerated with line air from an in-house compressor at 85–142 std L/min. The reactor also received a gas feed of pure CO_2 (carbon source) from a compressed gas tank at a rate of about 5% of the aeration rate. The culture was agitated by means of a single 15-cm, six-bladed, disk-type impeller at 30-50 rpm. When thiosulfate was depleted (2–3 d), the contents of this reactor were used to inoculate a 3.8-m^3 reactor made from a stainless-steel milk-holding tank. The tank was horizontal and semicylindrical, 170 cm deep and 660 cm long on the inside. The tank was jacketed with cooling/heating coils running lengthwise in the jacket annular space. A 2-hp variable-speed DC motor and gearbox were mounted on a platform that bridged the center of the vessel. The motor drove a paddle-type stirrer that was 81 cm in diameter and 12 cm wide. The agitation rate was 50 rpm. On either side of the stirrer platform were stainless-steel lids that completely closed the top of the vessel. The tank was modified by fitting with stainless-steel baffles (each 1/10 of the major or minor dimensions of the tank) and a sparger. The sparger was fabricated from 2.54-cm stainless-steel tubing in a U-shape fed with air at the bottom of the U through a 2.54-cm stainless steel tube that extended through the wall of the vessel at the center and bottom. The sparger

was centered under the stirrer with the branches of the U equal in length to the stirrer diameter. The U branches had equally spaced 0.318-cm holes drilled on the bottom such that the total hole area on each branch was two times the cross-sectional area of the tube.

Air was fed to the reactor using both a ring compressor and line air from an in-house compressor. About 850 L/min of air were supplied by the blower to the sparging system described above. Air from the blower was cooled with an after-cooler or heat-exchanger using house water at 15 °C. Line air was introduced into the reactor at each end with two supplemental spargers, which consisted of 1.27-cm stainless-steel tubes bent at one end to produce a 30.5-cm section that was perforated with 0.318-cm holes. An additional 425-567 L/min of air could be provided to the reactor in this manner.

Temperature control in the 3.8-m^3 tank was achieved by circulating water from a refrigerated, recirculator through the jacket coils. Some heating could also be obtained as needed by reducing the cooling water flow rate to the blower after-cooler, thereby increasing the temperature of the air. The temperature was maintained at 30 $\pm$ 1 °C and the pH was maintained at 7.0 $\pm$ 05 by addition of 85% H_3PO_4 or 50% NaOH as needed.

Each batch of *T. denitrificans* biomass was produced as follows: The 3.8-m^3 tank was filled with tap water and agitated with the stirrer. Components of thiosulfate mineral salts medium were then added and allowed to dissolve one at a time. The smaller-volume cultures described above used CO_2 as a source of carbon. At the 3.8-m^3 scale, this was prohibitively expensive; therefore, $NaHCO_3$ was used as the sole carbon source. The first inoculum used was produced in the 189-L stirred-tank reactor described above. Subsequent inocula consisted of a fraction of the biomass produced in the previous batch. Following inoculation, each batch was maintained under conditions described above until thiosulfate was depleted. The medium was thiosulfate-limiting. When the thiosulfate was completely utilized, the contents of the 3.8-m^3 tank were pumped to a 2.3-m^3 open-top conical-bottom tank (in two batches) to allow the flocculated biomass to settle under gravity for about 2 h. A concentrated suspension of biomass was then drawn from the bottom of the tank. On the average, 38-57 L of concentrated suspension were obtained. About 20% was used to inoculate the next batch; the remainder was stored at 4 °C in 227-L polypropylene barrels in a walk-in cold room.

Typically 48–72 h were required for each batch from the time of inoculation until thiosulfate was depleted. Harvesting of biomass required about 4 h on the average and typically controlled the turnaround time of the reactor, since the reactor could be replenished with fresh medium in less time than required to recover a concentrated inoculum from the previous batch. The average mixed liquor suspended solids (MLSS) concentration following thiosulfate depletion in each batch was 0.47 g/L (dry weight). The range was 0.33–0.58 g/L. Average recovery of flocculated biomass in the conical settling tank (2 h settling time) was 45% (range 31-55%). Increasing the settling time beyond 2 h did not significantly increase biomass recovery. In terms of dry weight of biomass, the average recovery was 0.21 g/L (range 0.15–0.26). As noted above, 20% of the biomass recovered in each batch was used to inoculate the next batch; therefore, the net yield per batch averaged 0.17 g/L or approx 640 g/batch on a dry-wt basis.

Sixty batches of flocculated *T. denitrificans* strain F biomass were produced in this manner. The average cost per batch in terms of the cost of nutrients and NaOH for pH control was $208. An average of 700 kW-h per batch were required for the blower-agitator, and recirculator. At $0.08/kWh, the estimated utilities costs/batch were $56. The cost/g dry wt of biomass was, therefore, $0.413/g.

T. denitrificans biomass remained highly active after long storage at 4 °C. Inoculation of thiosulfate mineral salts medium in lab-scale fermenters with biomass as old as 3 mo resulted in rapid growth of the organism and depletion of thiosulfate. The biomass produced as described above was used to inoculate a sour-water retention pond at a petroleum production site. This field test is the case study described subsequently in this paper.

Mixed waste treatment

Most, if not all, sulfide-laden wastewaters contain numerous other undesireable components in addition to sulfides. For example, sour water produced in petroleum production operations may contain organic constituents as well as sulfides. The biotreatment of these mixed waste streams requires not only removal of sulfides but also removal (or at least tolerance) of organics such as benzene, toluene, phenols, volatile carboxylic acids, and other constituents which contribute to aquatic toxicity. We have demonstrated that when flocculated *T. denitrificans* strain F cultures

are exposed to mixed aqueous wastes containing sulfides, the heterotrophs in the culture can be adapted by enrichment to degrade the organic components of actual oil field sour water while maintaining sulfide-oxidizing capability and excellent settling properties (Rajganesh et al., 1995a). Produced water from an oilfield site in Wyoming was successfully treated to remove sulfide (70 mg/L), benzene (5 mg/L), toluene (2 mg/L), phenolics (5 mg/L), volatile carboxylic acids (400 mg/L) and unidentified components that gave Microtox® toxicity using a flocculated, continuous culture of *T. denitrificans* strain F and mixed heterotrophs. Following a brief acclimation period the process culture exhibited excellent settling properties and complete removal of all target compounds and Microtox® toxicity. These results indicated that a reactor system as simple in concept as an activated sludge system can be used to treat mixed aqueous waste containing sulfides with removal of sulfides and biodegradable organics.

Case study

Microbial treatment of sour produced water

Water or brine co-produced with petroleum is often contaminated with soluble inorganic sulfides (H_2S, HS^-, S^{2-}) which result from the activities of sulfate-reducing bacteria or other sulfide-producing bacteria in the reservoir and well bore. These sulfides 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 sulfides as H_2S. However, this method of 'treatment' simply converts a potential water pollution problem into an air pollution and odor problem.

The ability of the *T. denitrificans* strain F to deodorize and detoxify an oil-field produced water containing sulfides was evaluated under simulated field conditions (Raterman et al., 1993). Strain F was used to remove inorganic sulfide from a synthetic sour brine containing 4000 mg/L total dissolved solids (TDS) and 100 mg/L sulfide. 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 22–40.5 °C and 7.5–8.8, respectively. The start-up biomass concentration was approximately 100 mg/L (by dry weight). No additional *T. denitrificans* strain F biomass was added to the reactor after start-up. At residence times of 0.3 days and greater inorganic sulfide was undetectable in the effluent. No H_2S was detected in the outlet gas or the zinc acetate trap. Approximately 80% of the sulfide feed was oxidized to sulfate and removed from the reactor in the liquid effluent. The remainder was partially oxidized to elemental sulfur which was retained in the reactor. These experiments demonstrated that oxidation of inorganic sulfides by *T. denitrificans* strain F represented a viable process concept for the treatment of sour water co-produced with oil and gas.

This process of microbial oxidation of sulfides in sour produced water was field tested at Amoco Production Co. Salt Creek Field in Midwest, WY. The Salt Creek Field is located 40 miles north of Casper, WY, and is one of the oldest oil fields in the region. Currently, it is operated as a mature water flood with approximately 1,000 production and 950 injection wells. Production averages 10,000 barrels (1590 m^3) of oil and 750,000 barrels (119,250 m^3) of water per day. Of the total water production nearly 95% is reinjected while the remainder is discharged to the surface. These discharges are termed 'beneficial' in that they represent an important source of fresh water for the support of local livestock and wildlife in this predominantly arid region.

Over time, produced fluids from the field have become progressively sour due to microbial reduction of sulfate introduced into the reservoir during water flooding. Because potential odor and toxicity issues are associated with sour water discharges, the merits of this practice are under active evaluation. If complete water reinjection is to be avoided, a cost-effective treatment option which allows the continued discharge of water while eliminating the odor and toxicity attributed to H_2S must be found.

Several remediation options exist for the control of soluble inorganic sulfides in water such as chemical oxidation, air stripping, precipitation, air oxidation, and biological treatment to name a few. For Salt Creek, the choice of a particular treatment option is severely restricted by the operating environment. Beyond obvious technical issues, the most significant restrictions are associated with stringent capital and operating expense limits. Of necessity these limits mandate the use of existing facilities (production pits) and manpower.

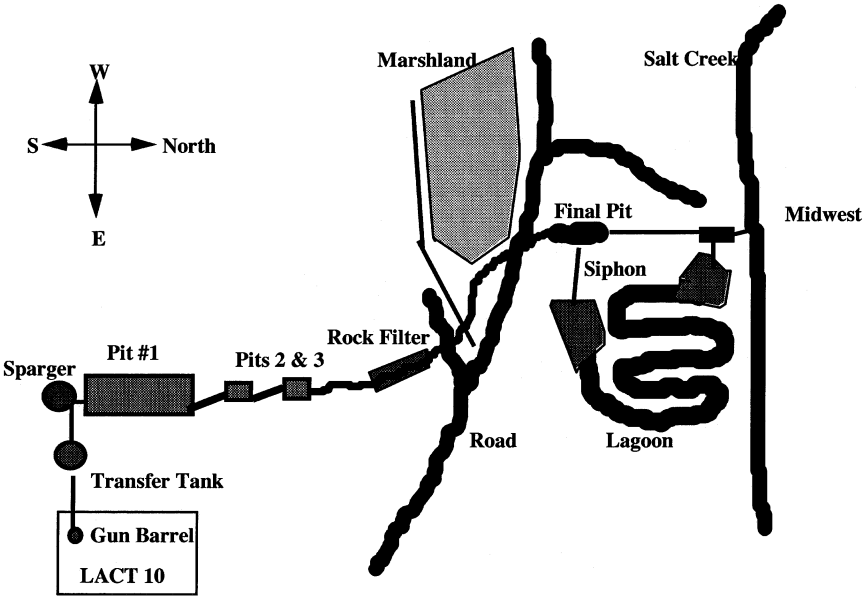

Figure 1. Amoco LACT 10 unit, Salt Creek Field

Of the accepted chemical or physical treatment options available, most can be rejected out-of-hand because of their high operating expenses, capital costs, or inability to effect immediate H_2S odor control in an unconfined production pit. From the remaining options only biological treatment appears to offer sufficient process flexibility to achieve effective control at low costs and under the imposed operating restrictions. The method chosen was bioaugmentation of existing pits and lagoons of the Salt Creek discharge system with a self-sustaining bacterial population capable of efficient sulfide oxidation at prevailing conditions, specifically, *Thiobacillus denitrificans* (strain F).

Site description

The LACT 10 unit of the Salt Creek Field is an oil/water separation facility wherein approximately 159 m^3/d oil and 15,580 m^3/d water are processed. Upon separation, water is diverted from the treatment train to a large transfer tank from which all but 795 m^3/d is routinely utilized for water flood reinjection. The 795 m^3/d overflow is shunted to the surface discharge system in order to maintain the associate pits, lagoons, and wetlands (Figure 1). On occasion, injection plant failures necessitate full discharge to the surface system. Thus, large flowrate variations are inherent to the system.

Water enters the surface discharge system at Pit 1 through a submerged 40.6-cm line. Pit 1 is an earthen lagoon which serves as an oil-skimming pond. Its approximate dimensions are 79 m x 33.5 m with a variable depth from 0.3 to 6.1 m. The estimated volume of the pit is 3021 m^3. From Pit 1 water flows to a series of smaller detention pits, over a cascade, through a meandering lagoon, and eventually to Salt Creek.

The average water temperature entering Pit 1 is 41.7 °C and the pH is slightly alkaline (7.8). The brine is relatively fresh with a Total Dissolved Solids (TDS) content of 4,800 mg/L. The sulfide concentration is 100 mg/L. Based on an average daily flowrate of 795 m^3, sulfide influx to the pit is about 80 kg/d. Dissolved oxygen (DO) levels within the pit are less than 1 mg/L and, therefore, the pit is largely anoxic.

Pilot design/operation

Because of capital limitations, Pit 1 was chosen as the primary treatment facility for the bioaugmentation pilot. Conceptually, the piloting program sought to establish the efficacy of 'seeding' Pit 1 with a self-sustaining population of *Thiobacillus denitrificans* strain F capable of sulfide oxidation under prevailing conditions of flowrate, temperature and sulfide flux. The facility was designed as a partially aerated basin to facilitate mixing and to take advantage of higher reaction rates associated with the aerobic biooxidation of sulfide. The collection and recycle of biomass from pit effluents was not attempted because of the extensive facilities modifications required. Carbon for cell

Table 1. Composition of Salt Creek Discharge Brine

Component	g/L
$CaCl_2 \cdot 2H_2O$	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	

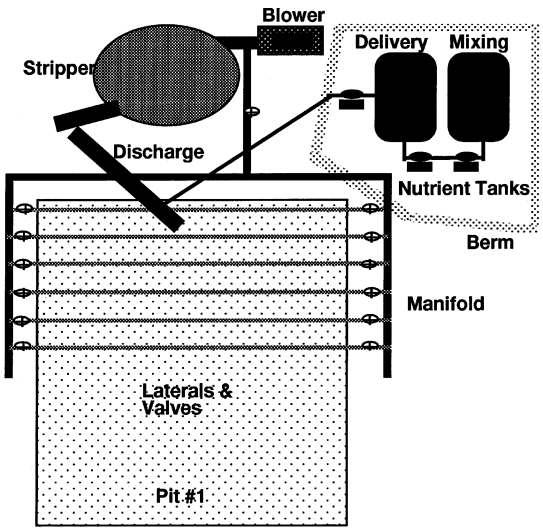

Figure 2. Facilities modifications to the LACT 10 unit.

growth was obtained from the field brine as carbonate (Table 1). Additional nutrients were limited to ammonia added as ammonium nitrate and phosphate as P_2O_5.

Construction

Two modifications to the existing LACT 10 facility were required to conduct the pilot; specifically, the means to add nutrients to the discharge water prior to its entering the pit and aeration of approximately one-fourth the pit volume (Figure 2).

Because of cost and availability, the primary nutrient (and potential electron acceptor source), ammonium nitrate, was obtained as a prilled solid in 18.2-kg bags. This necessitated the construction of a batch-mixing facility to deliver the nutrient in liquid form. The mixing facility consisted of two 2.84-m^3 fiberglass tanks each outfitted with a centrifugal pump for solids circulation. The first tank served as a primary mixing tank. The second tank served as a holding and delivery vessel. Nutrient solution was metered to the produced water discharge line by means of a variable stroke chemical metering pump. All materials used in the construction of the system were compatible with corrosive liquids. The entire mixing facility was enclosed within an earthen berm for spill containment. Drainage was provided to the pit.

To completely aerate the first 9.1 m of the pit, an air manifold was constructed and connected to an existing 28.3 std m^3/min blower (Sutorbilt 7 MF). The manifold was made from 12.7-cm PVC pipe and extended down either side of the pit. PVC laterals 7.6 cm in diameter were spaced at 1.52 m intervals on each leg of the manifold. The laterals were 18.3 m in length. Holes 0.64 cm in diameter, spaced on 0.305-m centers, were drilled in the last 9.1 m of each capped lateral. To provide aeration at depth, the laterals were weighted with several 6.1-m lengths of 1.27-cm iron rod. The approximate average depth of aeration was 1.52 m. A valve was placed on each lateral to control and balance air rates.

With the exception of the aeration manifold, all major capital items were obtained from field surplus equipment. Field personnel were utilized for construction at an estimated cost of material and labor of $5,000.

Pilot start-up

Prior to physically operating the pilot, a sufficient amount of biomass had to be cultured in order to adequately 'seed' the pit. About 18.2 kg (dry weight) of flocculated *T. denitrificans* strain F was produced as previously described above and shipped to the site in 2.08-m^3 drums.

Before inoculating the pit, a complete physical and chemical survey of the pit was conducted. Direct nutrient addition to the discharge was initiated approximately 36 hours before inoculation. The nutrient was added as a 40 wt% ammonium nitrate concentrate supplemented with 0.019 kg/L P_2O_5. Both chemicals were obtained from a commercial source and were made-up in the field produced water. Initial nutrient rates were based upon the calculated nitrate requirement (3.29 mg NO_3^-/mg S^{2-}) to achieve complete sulfide oxidation under anoxic conditions for an average daily influx of sulfide. At this rate biomass

requirements for nutrient nitrogen (0.084 mg NH_3/mg S^{2-}) and phosphorous (0.013 mg $PO_4{}^{3-}$/mg S^{2-}) were easily attained (Sublette & Sylvester, 1987a).

A method was devised to reduce the influx of sulfide to Pit 1 during start-up. Namely, the discharge flowrate was lowered from 795 to 159 m^3/d and the water was stripped with air upstream of Pit 1 to reduce the sulfide concentration from 100 mg/L to nearly 10 mg/L. In so doing, the potential to overload the small inoculum and thereby exceed its toxicity limit to sulfide was diminished significantly. The pit was inoculated by pouring the biomass concentrate directly into the pit near the discharge inlet. The deepest point in the pit was directly under the discharge inlet. The presence of adequate nutrients at the point of inoculation was ensured by the direct addition of fertilizers to the inoculation area.

Operation

The pilot was operated continuously for 170 days. The first three days post inoculation served as an acclimation period for the bacteria. The sulfide flux to the pit was kept low by air stripping during this period. On Day 9 the water discharge rate was returned to its daily average of 795 m^3. As a consequence, the sulfide influx to the pit increased although stripping continued. From Day 9 to Day 14 air was gradually diverted from the stripper to the pit aeration system. By Day 14, 100% of the blower capacity (26.9 m^3/min 10 psig) had been diverted to the pit. At this point the sulfide flux to the pit was approximately 92 kg/d. Flow and air rates remained constant thereafter, unless otherwise noted.

On Day 17 nutrient influx to the pit was reduced from its start-up level to a level based upon twice the calculated ammonia demand. This was done to determine if nitrate was required in lieu of oxygen for anoxic oxidation of sulfide. Further reductions in the nutrient rate were attempted throughout the program to assess the minimum nutrient requirement.

Several instances of operational upsets were of note. On three separate occasions (Days 43, 100, and 135) full discharge events occurred. These events were initiated by unforeseen injection plant failures of variable duration. The longest was on Day 135 and lasted for nearly two days. During these periods no attempt was made to adjust nutrient delivery rates in accordance with the flowrate. Hence, sulfide fluxes to the pit exceeded anticipated nutrient requirements. In a related instance (Day 34), water flow to the pit fell significantly below the daily average due to a controller failure. Because this event occurred while ambient temperatures were significantly below freezing, pit temperatures briefly dropped below stated optimums for bacterial activity. On numerous other occasions, pump, aerator, or pipe ruptures interrupted air or nutrient service to the pit. Typically, these events were of short duration and negligible effect.

Monitoring

During the first weeks of start-up and operation an intensive monitoring program to assess pilot performance was undertaken. Key variables included aqueous phase sulfide, nitrate, phosphate, ammonia, sulfate, and dissolved oxygen concentrations. Flowrate, pH, and temperature were also monitored. Sampling of the discharge system routinely occurred at three locations: (1) upstream of Pit 1, (2) from Pit 1 at the influent position and, (3) the effluent from Pit 1. Periodically, additional samples were obtained elsewhere within the pit and the discharge system. Nutrient delivery rates were estimated by gauging the nutrient holding tank daily.

Most water analyses were conducted by spectral methods with a Hach DR/2000 spectrophotometer. These included Nessler ammonia, nitrate by cadmium reduction, sulfate by barium precipitation, and reactive phosphate. All reagents were obtained ready-to-use from Hach Chemical Company (Loveland, CO) with a stated accuracy supplied by the manufacturer. Sulfide ion was determined by Sensidyne Gastec sulfide ion analyzer tubes. The stated accuracy was ± 2 ppm over a 0 to 100 mg/L range. The pH and temperature were evaluated with a Beckman Φ11 portable pH meter. A two point calibration procedure was performed routinely. Dissolved oxygen was measured with Chemetric (Calverton, VA) test kits for low (0-1 mg/L) and high (0-10 mg/L) ranges. All tests were conducted in the field within minutes to hours of sampling.

Ambient air quality above the pit surface was monitored periodically with an Industrial Scientific HMX 271 personal air monitor. The detection limit of the instrument is less than 1 ppmv. The stated accuracy is ± 2 ppmv. The instrument was calibrated daily with a known H_2S/N_2 gas mixture standard.

Water flowrates were estimated by timing the water discharge into a container and averaging several determinations. The method proved effective for rates below 1590 m^3/d. For rates above this value, estimates were calculated on the basis of the difference between

Table 2. Bacterial Enumerations of Sulfide Oxidizers by MPN Method

Day	Bacteria/mL	NO_3 - Utilization
3	1.5×10^7	Yes
17	9.5×10^7	Yes
48	7.5×10^7	Yes
50	2.5×10^7	Yes

daily injection plant volumes and monthly total water production averages. Estimates based on this method are subject to significant error.

Water samples for bacterial enumerations were obtained at preinoculation (Day 0), post inoculation (Day 4), Day 17, 48, and 50 (Table 2). Enumerations were based on the most probable number (MPN) method using thiosulfate as the sole energy source (Rodina, 1972). Hence, MPN determinations were specific to sulfur-oxidizing bacteria.

Daily monitoring occurred from Day 0 to Day 35 and from Day 49 to Day 56. Otherwise, pilot data were collected by field personnel as time allowed. Only routine analyses (sulfide, nutrient rate, and flowrate) were attempted at these times, although periodic ammonia, pH and temperature data were also collected.

Results and discussion

Prior to inoculation and nutrient addition, a background survey of Pit 1 and the associated discharge system was conducted to determine sulfide losses under prepilot conditions. In addition to temperature and pH, specific analytes included sulfide, dissolved oxygen, ammonia, and nitrate. At the time of the survey the air stripper was operating and the flowrate to the discharge system was 556.5 m^3/d. Pit 1 was partially aerated.

On the basis of survey data, it was reasonably established that prior to inoculation no naturally significant reduction of sulfide occurred within the pit at residence times on the order of days. Specifically, upstream of the air stripper water conditions were 100 mg/L, sulfide, 41.7 °C, and pH 7.3. After the stripper, water entered Pit 1 at 28 mg/L sulfide, 32.2 °C, and pH 7.8. Pit 1 effluent was 28 mg/L, 27.2 °C, and pH 8.4. A significant loss of sulfide was associated with stripping but little, if any, with passive volatilization and oxidation within Pit 1. The latter was

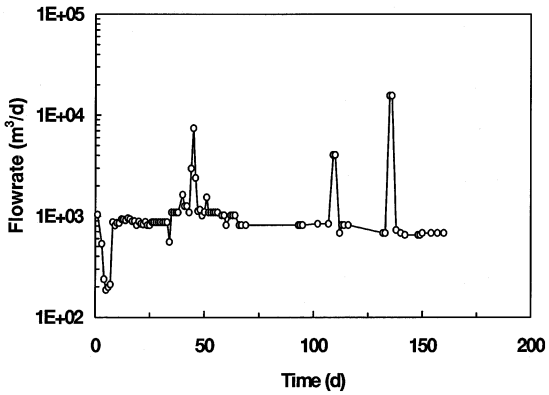

Figure 3. Hydraulic flowrate to Pit 1.

in spite of a calculated five-day hydraulic residence time. The absence of sulfide losses within the pit were potentially attributed to (1) a hydraulic short-circuit which significantly reduced fluid residence times, or (2) a shift to higher pH values and concomitantly a propensity for dissolved hydrogen sulfide to remain in solution as bisulfide ion. The temperature survey at the pit periphery did to indicate a hydraulic short-circuit since cold spots or stagnant regions were not identified. The pH data, however, did confirm a shift to higher pH values and presumably higher sulfide solubilities. Higher pH values were likely a consequence of contact between the discharge water and the alkaline soils of the pit.

Initial dissolved oxygen (DO) levels within the pit were uniformly less than 1 mg/L with minimal aeration. Ammonia and nitrate concentrations were also very low prior to nutrient addition. Hence, the need for aeration and nutrient addition were confirmed.

As was mentioned previously, sulfide influx to the pit was limited by air stripping prior to and immediately after (Day 4–Day 16) inoculation with *T. denitrificans*. During this acclimation period the sulfide influx to the pit was gradually increased from 0 to the typical daily average of 80–100 kg/d. The sulfide concentration in the pit influent averaged 100 mg/L. The corresponding sulfide concentrations over this time span never exceeded 5 mg/L at the inlet end of the pit. Moreover, no sulfide was detected in Pit 1 effluents. Hence, it appeared that the bacteria were capable of oxidizing average sulfide loads to the pit within a matter of days post inoculation and the cessation of stripping.

After acclimation, pit effluent sulfide concentrations remained zero but for three hydraulic upset events (Figure 3). On a cumulative basis this perfor-

mance represented a treatment efficiency approaching 99% of the total sulfide discharged to the surface system. (In the absence of upsets this treatment efficiency approached 100%.) Under normal operating conditions, the oxidation of sulfide to sulfate appeared complete. For instance, during one three-day period the average concentration of sulfide entering the pit was 110 mg/L, while the net sulfate concentration exiting the pit was 340 mg/L (well below inhibitory levels). This value agrees well with the expected mass ratio of sulfate to sulfide of 3 to 1, if oxidation is complete.

With respect to fugitive emissions, no H_2S could be detected immediately above the pit surface under normal operations. This was a consequence of the ability of the bacteria to instantaneously oxidize sulfides as they entered the pit; evidence of which was demonstrated by near inlet samples whose sulfide content rarely exceeded 5 mg/L. In terms of treatment efficiency, this performance represented a minimum of 95% sulfide removal within the first few feet of the aerated zone of the treatment system.

Significant discharges of sulfide from the pit occurred as upset events on Days 34, 46, 110, and 136 (Figure 4). They could be attributed to two causes. The first (Day 34) was unique in that low flowrates to the pit and low ambient temperatures (<-17.8 °C) combined to cause water temperatures within the pit to drop below 15.6 °C. At this temperature biological sulfide oxidation was significantly inhibited to the extent that an upset was observed. The upset was quickly remedied, however, by raising the flowrate to the pit to maintain water temperatures closer to the optimum of 30 °C. The second cause for pilot upsets was largely uncontrollable. On Days 46, 110 and 136 sulfide influx to the pit significantly exceeded the daily average, i.e., >500 vs. 90 kg/d. These occurrences were caused by injection plant failures which necessitated full discharge to the pit. Each was characterized by a large sulfide flux, rapid temperature increase, and inadequate nutrient supply imposed by the unanticipated large hydraulic throughput. The pilot response to these failures was encouraging in that approximately 30% of the total sulfide was at least partially oxidized. Partial oxidation was observed as an accumulation of elemental sulfur on the banks of the pit and the absence of sulfate ion in pit effluents. In terms of sulfide removal, recovery from these events was evidenced in a matter of hours of the flow returning to normal. However, complete stoichiometric oxidation of sulfide to sulfate (mass of sulfate = 3 × mass sulfide) required several days for recovery. The observation that *T. denitrificans* resorted to a partial oxidation of sulfide under stress was in keeping with laboratory observations of similar events (Sublette and Sylvester, 1987a; Sublette, 1987). Moreover, the production of elemental sulfur served as a convenient visual indicator of a stressed treatment system.

Because the addition of nitrate as an alternate electron acceptor represented the largest chemical demand for the pilot, the requirement for nitrate was assessed within days of inoculation. This was accomplished by gradually reducing the nutrient rate to the pit over a period of several days to a lower limit based upon the calculated ammonia requirement (0.084 kg NH_3/kg S^{2-}). By Day 14 the nitrate influx to the pit dropped below the calculated nitrate demand (3.29 kg NO_3/kg S^{2-} times the sulfide mass flowrate in kg/d) and, thereafter, always remained below it. At no time could a pilot upset be attributed to this condition. Therefore, it was presumed that the biological oxidation of sulfide occurred in large measure aerobically. This conclusion was based upon the fact that oxidation of sulfide by denitrifying mechanisms is not instituted in *T. denitrificans* until O_2 levels fall below 1 mg/L.

Ammonia influx to the pilot remained for the most part above the calculated demand. Overall, it would appear that the calculated ammonia demand was sufficient to sustain the bacterial population in the pit with an adequate margin of safety.

Generally, the pH of the influent to Pit 1 ranged from 7.0 to 8.0 in the absence of air stripping. After microbial treatment, however, the pH of the discharge water was more alkaline (8.0–8.6) with no discernible difference from entrance to exit. The relatively low pH of the upstream fluids was consistent with the amount of dissolved H_2S in the water. Although complete oxidation of sulfide was indicated, the excess acidity associated with sulfuric acid production was apparently removed by contact with the alkaline soils of the pit. At no time did the pH within the treatment system appear to affect sulfide oxidation by *T. denitrificans*.

Under normal flow conditions the pit temperature averaged about 23.9 °C with a maximum day-to-day fluctuation of about 5.6 °C. Fluctuations were attributed to changes in flowrate or heat input to the pit and variable ambient temperatures. (Over the piloting period ambient temperatures ranged from −23.3 to 23.9 °C). During full discharge events, large temperature increases were realized within the pit in a matter of hours. On one such occasion the observed change

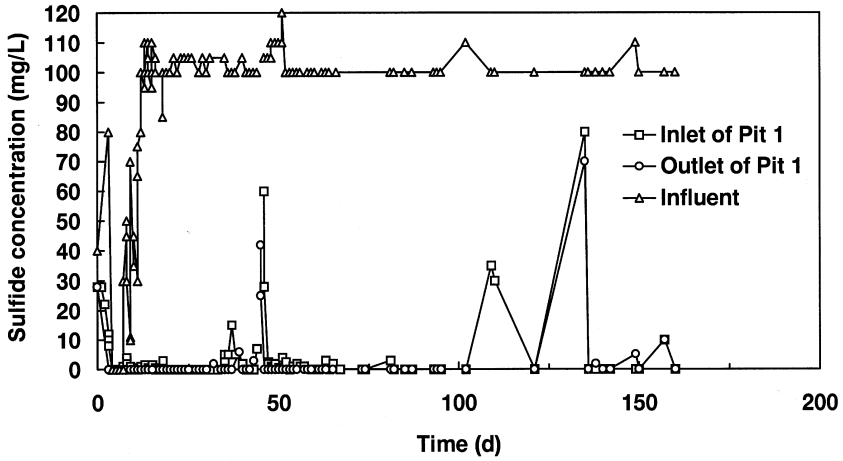

Figure 4. Sulfide concentration in the influent to Pit 1 and in the inlet and effluent ends of Pit 1.

was −1.7 °C. Fortunately, such temperature swings had no discernible lasting effect on pilot performance.

Bacterial enumerations conducted were specific to bacteria capable of utilizing reduced sulfur as their sole energy source. Enumerations were conducted before pit inoculation, 15 minutes and two days afterwards, and on Days 17, 48, and 50. All water samples for bacterial counts were obtained adjacent to the discharge point to Pit 1. The background bacterial count was 4.5×10^3/mL, most of which were identified as aerobic organisms incapable of utilizing nitrate for anaerobic sulfide oxidation. Immediately after inoculation the bacterial count increased to 4.5×10^7/mL. These bacteria were capable of using nitrate and thus indicated that viable *T. denitrificans* had been introduced to the pit. Subsequent bacterial enumerations were on the order of 10^7/mL and the bacteria were always capable of nitrate utilization. This suggested that a steady state population of *T. denitrificans* had been established. Whether indigenous organisms were significantly represented in this population is unknown.

Finally, the primary expenses associated with pilot operations were nutrient costs and energy costs for aeration. With respect to nutrient utilization, a minimum demand based upon ammonia requirements appears to offer a reasonable lower limit. Using ammonium nitrate as the ammonia source at a cost of \$0.22/kg, the estimated chemical treatment cost for the LACT 10 discharge is \$0.0088/m^3. The rated horsepower of the blower at 1640 RPM and 10 psig backpressure is 58. The calculated energy cost per day at \$0.03/kW-hr is \$31.10. For an average daily flow of 795 m^3, the energy cost per treated m^3 is \$0.039. The total treatment cost is thus approximately \$0.048/m^3 or about \$38/d. Contrary to operating expenses, maintenance costs were rather high. This was attributed to repeated materials failures during cold weather and in the presence of corrosive nutrient solution.

A more detailed description of this field test is given by Sublette et al. (1994b).

Conclusions

This case study has demonstrated the successful application of a bioaugmentation technology for the treatment of sulfide-laden produced water to control fugitive air emissions of H_2S. Specifically, the following was demonstrated:

1. *Thiobacillus denitrificans* strain F was successfully introduced into the existing discharge system of the LACT 10 unit of the Salt Creek Field, WY.
2. Sulfide removal efficiencies approaching 99% could be attained for routine discharges of 795 m^3/d containing 100 mg/L sulfide.
3. Aerobic sulfide oxidation to sulfate was almost instantaneous and, therefore, no odorous emissions of H_2S were detected emanating from the discharge under normal conditions.
4. As designed and operated, the system was capable of processing about 1590 m^3/d or 160 kg S/d.
5. Pilot recovery from transient full discharge upset events (15582 m^3/d) was on the order of hours. During such events partial oxidation of sulfide to elemental sulfur was observed indicating that

severe sulfide gradients and localized inhibitory sulfide concentrations existed in the pit.

6. Hydrocarbons in the pit, clearly visible floating on the surface, had no effect on sulfide oxidation by *T. denitrificans*.
7. Minimum nutrient requirements were based upon bacterial reduced nitrogen (NH_3) needs. No clear requirement for nitrate as an alternate electron acceptor for anoxic oxidation of sulfide was demonstrated.
8. A steady state population of *T. denitrificans* was established at about 10^7/mL without provisions for biomass recycling. The flocculated, sulfide-active biomass appears to have settled to the lowest point in the pit so that no significant amounts of biomass were carried out with effluent. This population was stable even in the event of full hydraulic discharges.
9. Estimated treatment costs for normal discharges were less than 5 cents/m^3.

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