0273-2289/95/5152—0735$06.40

Biotreatment of Produced Water for Removal of Sulfides, Organics, and Toxicity

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ABSTRACT

Water coproduced with petroleum may contain sulfides and organic constituents that give the water an aquatic toxicity preventing surface discharge. A simulated sour produced water and actual field samples of produced water were successfully biotreated with mixed cultures of *Thiobacillus denitrificans* and floc-forming heterotrophs. Complete removal of benzene, toluene, phenol, acetic acid, sulfides, and Microtox toxicity was achieved. These results indicate that a reactor system as simple in concept as a specialized activated sludge system can be used to treat produced water with these mixed contaminants, allowing surface discharge of the water for reuse.

Index Entries: Toxicity; sulfide; hydrogen sulfide; *Thiobacillus denitrificans*; benzene; toluene; phenol.

INTRODUCTION

Soluble sulfides (H_2S, HS^-, S^{-2}) are often found to contaminate water coproduced with petroleum especially in water-flooded reservoirs. Water that contains sulfides is said to be "sour." The source of these sulfides is generally the reduction of sulfates by sulfate-reducing bacteria (SRB). These bacteria are strict anaerobes that utilize a limited number of organic compounds as a source of carbon and energy, such as pyruvate, lactate,

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and ethanol. These compounds are the end products of the metabolism of fermentative heterotrophs and are readily available in a consortium of bacteria in an anaerobic environment. Therefore, SRB are ubiquitous to virtually any anaerobic environment conducive to microbial growth (*1*).

The toxicity and corrosivity of sulfides dictate stringent controls of their release into the environment and contact with iron and steel, as in tanks, pipelines, valves, and pumps. Sulfide production by SRB is directly or indirectly responsible for major damage each year owing to corrosion. The control of sulfide contamination may be approached in two ways. First, sulfide production may be reduced by inhibiting the growth of SRB. For example, water used in flooding operating may be treated with a biocide to control SRB growth in the injection well, reservoir, and piping. Since SRB are strict anaerobes, aeration of flooding water can also serve to inhibit sulfide production. These methods are of limited effectiveness, however, because SRB are generally found attached to a solid surface entrapped in polysaccharide gels produced by "slime-forming" bacteria. Within these gels, the SRB find themselves in a relatively protected environment that biocides and oxygen do not effectively penetrate (*2–4*).

If sulfide production cannot be prevented, sour water may be treated by a number of physiochemical methods. One of the more common methods is to strip water under acidic conditions with steam, flue gas, or fuel gas in a packed or plate-type column. In the case of steam or flue gases, the overhead vapors are condensed, and the noncondensables (including H_2S) are incinerated. In the case of methane stripping, the noncondensables are typically sent to an amine system and the methane recycled. Hydrogen sulfide recovered from the methane stripping gas is generally incinerated. Each of these processes converts a water pollution problem into an air pollution problem in that the combustion of H_2S produces sulfur dioxide, a regulated pollutant (*5*).

Sulfide may also be oxidized to thiosulfates by air oxidation at 190°F. However, elevated pressures of 50–100 psig (pounds/square-in. gage) are required, and thiosulfates have considerable COD and BOD (*5*).

Small amounts of sulfides can be precipitated as insoluble sulfides with copper (II) or zinc (II) salts. However, if these precipitates are exposed to acidic conditions, H_2S may be released. Therefore, these precipitates are considered to be hazardous wastes.

We have previously reported that the chemoautotroph and facultative anaerobe *Thiobacillus denitrificans* strain F can be used to treat sour water for sulfide removal with oxidation to sulfate. Strain F is a sulfide-resistant strain of *T. denitrificans* isolated by Sublette and Woolsey (*6*). Flocculated *T. denitrificans* strain F was used in an aerobic, bench-scale (3.5-L) bubble column at 30°C and pH 7.0 to treat water containing up to 25 m*M* sulfide. Complete removal of sulfide and oxidation to sulfate was observed over 9 mo of continuous operation (*7*). *T. denitrificans* strain F has also been used

in a continuous, anoxic reactor (2 L) scaled to the dimensions of an oil-field sour water retention pond to treat a simulated sour oil-field brine. Feed to the reactor consisted of a nutrient-amended simulated brine containing all of the inorganic components of an actual sour brine in a water-flooded field in Wyoming, where field tests were anticipated. Nitrate served as the terminal electron acceptor. The influent sulfide concentration was 3.1 m*M*, the pH was 8.0–8.8, and the maximum temperature was 40°C. Some 80% of the sulfide was oxidized to sulfate and the remainder to elemental sulfur during 2 mo of continuous operation (*8*).

In certain semiarid regions of the US, surface discharge of produced water is preferred over reinjection if the water has a reasonable low total dissolved solids (TDS) concentration, since this water can be used for agricultural and ranching purposes. Sour water coproduced with petroleum may contain organic constituents as well as sulfides, which contribute to the aquatic toxicity of the water, preventing surface discharge of the produced water. We report here an investigation of the biotreatment of sour water for simultaneous removal of both sulfides and certain target organics, namely benzene, toluene, phenol, and acetic acid. A simulated sour water containing these components was first treated followed by bench-scale treatment of an actual field-produced water sample from Sussex Field in Wyoming.

MATERIALS AND METHODS

Organism and Stock Cultures

T. denitrificans (ATCC 23642) was originally obtained from the American Type Culture Collection (Rockville, MD). A sulfide-resistant strain (strain F) was isolated by enrichment as previously described (*6*). Stock cultures were grown anoxically at 30°C in 10-mL culture tubes in thiosulfate medium as described (*9*). In this medium, thiosulfate is the energy source, nitrate the terminal electron acceptor, bicarbonate the carbon source, and ammonium ion the source of reduced nitrogen. The medium also contains a phosphate buffer and sources of Mg^{2+}, Ca^{2+}, Fe^{3+}, Mn^{2+}, and trace elements.

T. denitrificans was flocculated by aerobic coculture with floc-forming heterotrophs from a refinery activated sludge system as previously described (*10*). It is important to note that this procedure results in a mixed culture of the autotrophic sulfide-oxidized and various floc-forming heterotrophs, which, in the absence of any other carbon source, derive organic carbon and energy from waste products of *T. denitrificans* and/or products of cell lysis. These flocs have excellent settling properties, giving 80% compression in 80 s.

Table 1
Results of Analysis
of Sussex Field Sour Water

Component	mg/L
SO_4^{-2}	1000
Ca^{+2}	175
Fe^{+3}	2
Mg^{+2}	24
K^+	95
Na^+	1200
Cl^-	1000
Acetic acid	400
Phenol	5
Benzene	5
Toluene	2
Total sulfide	70

Biotreatment of Simulated Sour Water

Results of an analysis of the major components of sour produced water from Sussex Field in Wyoming are given in Table 1. From this analysis, a nutrient-amended simulated sour water medium was formulated as given in Table 2. This medium contained all of the standard components of thiosulfate medium (less thiosulfate and nitrate) plus all components of the Sussex Field sour water given in Table 1, except sulfide. As will be detailed below, sulfide was always fed to process cultures separately to avoid air oxidation and volatilization as H_2S.

Flocculated *T. denitrificans* strain F (2.4 g/L) was suspended in the medium shown in Table 2 without the organics in a B. Braun Biostat M fermenter (culture volume 1.5 L). The culture was agitated at 200 rpm and aerated with 95% air + 5% CO_2 at 0.35 L/min. The purpose of the CO_2 supplement was to ensure that the autotroph did not become carbon-limited. The outlet gas from the reactor was bubbled into a reservoir of 0.3 wt% zinc acetate where any fugitive H_2S from the reactor would be trapped as ZnS. The pH was controlled at 7.0 by automated addition of 6*N* NaOH as needed.

A daily feeding procedure was initiated in which 325 mL of mixed liquor from the reactor was removed and centrifuged at 5000*g* and 25°C. The biomass pellet was resuspended in 25 mL of the supernatant and returned to the reactor. The remaining supernatant was retained for analysis. At this time, 280 mL of the nutrient-amended simulated sour water medium containing all of the organics was added to the culture. After addition of the sour water medium, an Na_2S feed was initiated consisting of an approx 39 m*M* solution (pH 7.0–7.3) at 0.15 mL/min via a syringe pump (Harvard Model 975 Compact Infusion Pump). The Na_2S feed was

Table 2
Nutrient-Amended Simulated Sour Water Medium

Component	g/L*
Na_2HPO_4	1.2
KH_2PO_4	1.8
$MgSO_4$-$7H_2O$	1.25
NH_4Cl	0.5
$CaCl_2$	0.47
$MnSO_4$	0.02
$FeCl_3$	0.033
$NaHCO_3$	1.0
NaCl	1.05
Na_2SO_4	0.77
Sodium acetate	0.56
Phenol	5.0 mg
Benzene	5.0 mg
Toluene	2.0 mg
Trace metal solution (*9*)	15.0 mL
Mineral water	50.0 mL
pH 7.0, 30°C	

*Unless otherwise indicated.

introduced below the liquid level in the reactor through a stainless-steel tube to prevent stripping of sulfides as H_2S. A total of 20 mL of Na_2S was used with each daily feeding; therefore, approx 2.2 h were required to complete the sulfide feeding. The effective sulfide concentration in the feed (sour water medium + sulfide stock) averaged 2.6 m*M*, and the effective sour water residence time was 5 d. A schematic diagram of the reactor system is shown in Fig. 1.

This feeding procedure was repeated for 10 d. At the end of this time, the feeding procedure was changed in that a net 750 mL of culture mixed liquor (biomass returned to reactor) was removed daily, and replaced with 700 mL of sour water medium and 50 mL of 39 m*M* Na_2S (at 0.15 or 0.078 mL/min). The effective sour water residence time then was 2 d. After 4 d at this feed condition, the feeding procedure was again changed reducing the net daily removal of mixed liquid to 535 mL. Again the biomass was returned to the reactor, and the medium was replaced with 500 mL of sour water medium and 35 mL of 39 m*M* Na_2S (at 0.15 mL/min). This last fed-batch feeding procedure was maintained for an additional 17 d (effective sour water residence time of 3 d).

At the conclusion of the fed-batch experiment described above, a continuous feeding procedure was initiated. A total of 750 mL of mixed liquid was removed from the reactor daily, and the biomass recovered by centrifugation. The biomass was resuspended in 50 mL of supernatant and returned to the reactor. At this time, two continuous feeds were initiated:

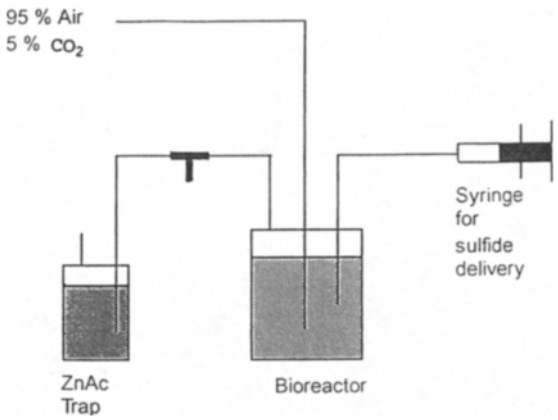

Fig. 1. Schematic diagram of the fed-batch reactor system.

(1) sour water medium at 27.5 mL/h and (2) approx 39 m*M* Na_2S at 1.68 mL/h. The medium was pumped by a B. Braum FE 211 positive displacement pump. A total of 660 mL was delivered every 24 h. The Na_2S feed was delivered via the syringe pump. A total of 40 mL of Na_2S feed was pumped into the reactor during every 24-h period. The medium and sulfide feed were mixed in a tee just prior to entering the reactor through a stainless-steel tube that extended below the liquid surface in the reactor. The agitation rate was 200 rpm. The culture was maintained at 30°C and pH 7.0, and was aerated with 95% air + 5% CO_2 at 0.35 L/min.

This feeding procedure was repeated daily for 9 d. On the 10th d, the feed rate was increased in that 1050 mL (net) of mixed liquor were removed daily from the culture (with biomass returned to the reactor) and replaced by 1000 mL of the sour water medium fed at 41.7 mL/h and 53.3 mL of approx 39 m*M* Na_2S fed at 2.4 mL/h. This feeding procedure was continued for another 8 d.

Biotreatment of Actual Field Water

Samples of produced water from Sussex Field were shipped in plastic containers by overnight service from the field. Samples were stored sealed at room temperature until used. Analysis of field water samples showed that benzene, toluene, phenol, sulfide, and most of the acetic acid (all but 18 mg/L) had been lost during sampling, transport, and storage. All of these components (except sulfide) were replenished, and the water supplemented with nutrients for *T. denitrificans* prior to use. The composition of the nutrient-amended field water medium is given in Table 3.

Flocculated *T. denitrificans* strain F was initially suspended in 1.5 L of nutrient-amended field water medium (Table 3) without organics in the B. Braun Biostat M. The culture was agitated at 200 rpm, and aerated with 95% air and 5% CO_2 at 0.35 L/min. The outlet gas was diverted to a zinc acetate trap as described above. Temperature and pH were maintained at 30°C and 7.0, respectively. A daily feeding procedure was initiated in which 325 mL of mixed liquor were removed and centrifuged at 5000*g* and

Table 3
Nutrient-Amended Field Water Medium

Component	g/L*
Na_2HPO_4	1.2
KH_2PO_4	1.8
$MgSO_4$-$7H_2O$	0.4
NH_4Cl	0.5
$CaCl_2$	0.03
$MnSO_4$	0.02
$FeCl_3$	0.02
$NaHCO_3$	1.0
Sodium acetate	0.56
Phenol	5.0 mg
Benzene	5.0 mg
Toluene	2.0 mg
Trace metal solution (9)	15.0 mL
Mineral water	60.0 mL
Sussex field water	925 mL
pH 7.0, 30°C	

*Unless otherwise indicated.

25°C. The biomass was resuspended in 25 mL of the supernatant and returned to the culture. The remaining supernatant was retained for analysis. At this time, a feed of 270 mL of the nutrient-amended field water medium containing all the organics was started. This 270 mL was pumped to the reactor over a period of 24 h (11.3 mL/h) by a B. Braun Biostat FE211 pump. Simultaneously, an Na_2S feed was initiated consisting of an approx 30 m*M* solution (pH adjusted to 7.0–7.3) at 1.7 mL/h via the syringe pump. The medium and sulfide feed were mixed in a tee just prior to entering the reactor. The feed was introduced below the liquid level in the reactor through a stainless-steel tube to prevent air stripping of sulfides as H_2S. A total of 30 mL of Na_2S was used with each daily feeding; therefore, approx 18 h were required to complete the sulfide feeding. The effective sulfide concentration in the feed (nutrient-amended field water medium + sulfide stock) averaged 3.06 m*M*. This feeding procedure was continued for 40 d.

Analytical

Sulfide feed solutions were prepared by dilution of an Na_2S stock solution that was standardized by titration with 0.01*M* $Pb(ClO_4)_2$ using an Orion Research Model 9416 sulfide/silver electrode and an Orion Research Model 701a pH/mV meter to detect the end point. Sulfide in culture medium samples was determined by the methylene blue method in samples pre-

served for analysis in 0.3 wt% zinc acetate (*8*). Hydrogen sulfide in the reactor outlet gas was monitored by means of GasTech (Yokohama, Japan) chromophoric analyzer tubes. Cumulative amounts of H_2S in the reactor outlet gases could be determined by analysis for ZnS in the zinc acetate traps. Elemental sulfur was determined by reaction with sodium cyanide to form thiocynate, which was quantitated by reaction with Fe^{3+} (*9*). Sulfate was determined turbidometrically following precipitation with $BaCl_2$ (*11*).

Mixed-liquor suspended solids (MLSS) was determined by filtering fixed volumes of culture mixed liquor through tared glass-fiber filters (Whatman GF/C) (*11*).

Benzene, toluene, and acetic acid in culture medium samples were determined by gas chromatography (GC) using an HP 5980 gas chromatograph with a column of 4% Carbowax 20 M on 80/100 Carbopak B-DA and flame-ionization detector. The carrier gas was He at 24 mL/min. The injector, column and detector over temperatures were 175, 200, and 200°C, respectively. Phenol was determined by high-performance liquid chromatography (HPLC) using an HP 1090 HPLC with a Hypersil ODS (5-μ) column, 100 × 4.5 mm and UV detector. Benzene and toluene could also be determined in this manner. Toxicity of produced water or reactor samples was determined in duplicate assays by Microtox^R. This test incorporates the bioluminescent bacterium *Photobacterium phosphoreum*, which is exposed to various concentrations of sample at 15°C. After incubation periods of 5 and 15 min, light outputs are measured and compared to a control value. Five and 15-min EC_{50} can then be calculated. The EC_{50} is the concentration at which a 50% effect (in this case, reduction in light output) is observed.

RESULTS AND DISCUSSION

Biotreatment of Simulated Sour Water

During the first period of fed-batch feeding of the flocculated *T. denitrificans* strain F reactor, the effective residence time was 5 d (300 mL/d, 1.5 L culture). During this time, no H_2S was detected in the outlet gas either directly or as ZnS in the zinc acetate trap. No sulfide was detected in the culture medium, and no elemental sulfur was found. The average effective sulfide and sulfate concentrations in the feed were 2.6 and 13.5 m*M*, respectively. The steady-state sulfate concentration in the culture medium was 16.7 m*M*. This is a reasonably good sulfur mass balance considering the inherent experimentally error in determining sulfates turbidometrically at these concentrations. No organics were detected in any culture medium samples throughout this period of operation. Note that culture medium samples were taken roughly 24 h following fed-batch feeding.

During the next brief period of fed-batch operation, the residence time was reduced to 2 d. At this feed condition, H_2S (200–300 ppmv) could be detected in the reactor outlet gas soon after delivery of Na_2S was initiated. Benzene and toluene were detected in the culture medium on the 4th d of operation at this feed condition. It appears that *T. denitrificans* was somewhat inhibited by the organics when added to the reactor at these concentrations and in this manner. Likewise the mixed heterotrophs were also somewhat sensitive to this feed condition, despite the previous 10 d of acclimation at the 5-d residence time. One interesting observation during this period is the fact that benzene and toluene were detected in the culture medium 24 h after the previous fed-batch addition of sour water medium. Therefore, these compounds were not being stripped entirely by air sparging, and their absence from earlier samples indicates at least some biodegradation of benzene and toluene.

When the effective residence time was reduced to 3 d, the performance of the reactor improved with respect to removal of sulfide and organics. For the first 10 d of operation at this feed condition, trace amounts of H_2S (<5 ppmv) were detected in the reactor outlet gas on four occasions, and trace amounts of benzene, toluene, and acetic acid were detected in the culture medium in three samples. For the last 7 d of operation, however, complete removal of sulfides and organics was observed. No elemental sulfur was observed. Stoichiometric conversion of sulfide to sulfate was again observed within experimental error. The MLSS concentration remained relatively constant during this time at 2.3 ± 0.1 g/L.

When the reactor was operated with a continuous feed of nutrient-amended sour water medium at a residence times of 2.1 d, the performance of the reactor was greatly improved relative to fed-batch operation at this same effective residence time. No elemental sulfur or sulfide was detected in the outlet gas during 9 d of operation. On day 6, trace amounts of benzene, toluene, phenol, and an unidentified compound were detected in the culture medium. However, at all other times at this feed condition, no organics were found. When the residence time was reduced to 1.4 d, the reactor continued to operate (8 d) satisfactorily with complete removal of sulfides, and only trace amounts of benzene and phenol were found on one day.

Biotreatment of Actual Field Water

Figure 2 shows the sulfate concentration in the culture medium during continuous feeding of nutrient-amended field water medium at a residence time of 5 d in comparison to the sulfate concentration in the medium. Time zero corresponds to the initiation of feeding. As seen in Fig. 2, the sulfate concentration in the feed averaged 17.1 m*M*. The average effective sulfide concentration in the feed was 3.06 m*M* (Fig. 3). The sulfate concentration in the reactor at the end of each feeding cycle reached steady

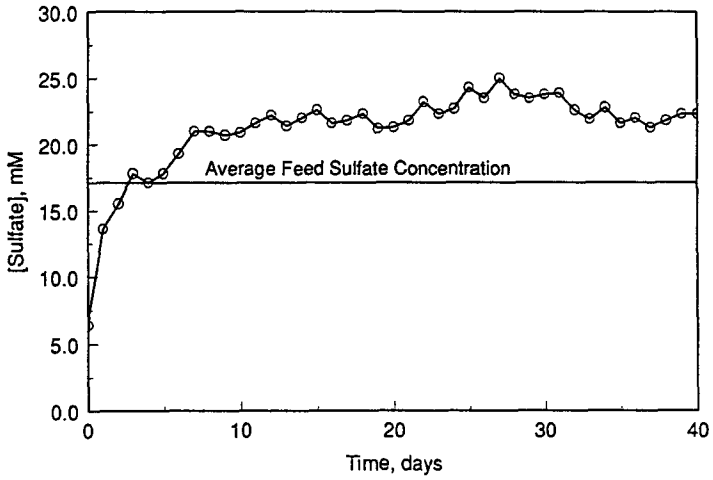

Fig. 2. Sulfate concentration in the culture medium during continuous feeding of nutrient-amended field water medium.

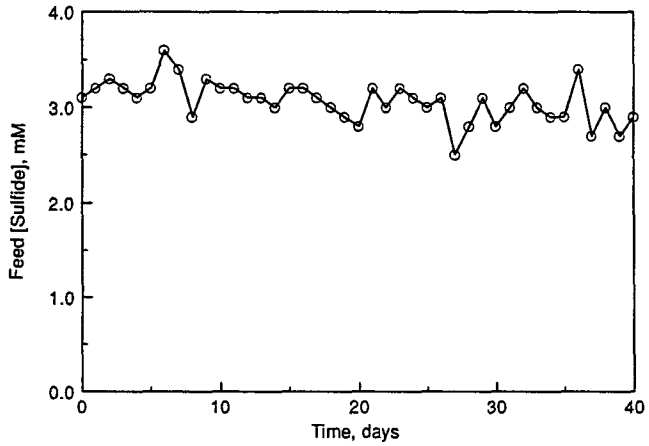

Fig. 3. Effective sulfide concentration in the feed of nutrient-amended field water medium to the continuous reactor system.

state after about 10 d. The average sulfate concentration in the reactor, after steady state, was 22.4 m*M*. Therefore, the sulfide-to-sulfate ratio at steady state was 1.11. Given the limitations of the sulfate turbidimetric method, this indicates that all the sulfide was converted to sulfate. No H_2S was observed in the outlet gas either directly or as ZnS in the zinc acetate trap. Figure 4 shows the mixed-liquor suspended solids (MLSS) concentration in the culture during the experiment. The MLSS concentration was seen to decline with time, indicating that either *T. denitrificans* or the heterotrophs, or both were not receiving enough of a limiting nutrient to sustain net growth. Presumably the MLSS concentration would have reached a steady state with time in which the total biomass in the reactor could just be maintained by the nutrients the reactor received.

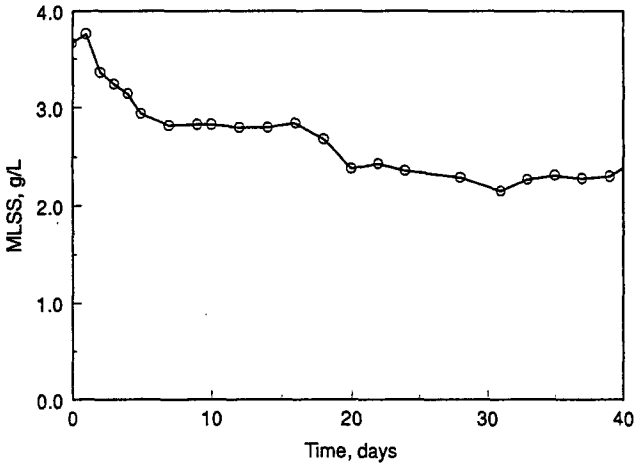

Fig. 4. MLSS concentration in the culture medium during continuous feeding of nutrient-amended field water medium.

Table 4
Results of Microtox^R Analyses of Sour Water Biotreatment Samples

Sample no.	Sample	Microtox^R 5-min EC_{50}, % vol	Microtox^R 15-min EC_{50}, % vol
1	Produced water 3-23-93	4.2	3.2
2	Produced water 3-23-93 + 70 ppm sulfide	6.2	4.2
3	Produced water 3-23-93 + organics and nutrients	1.9	1.9
4	Influent to bioreactor: Produced water 3-23-93 + organics + 70 ppm sulfide	3.9	3.7
5	Effluent from bioreactor day 30	>90.0	>90.0
6	Effluent from bioreactor day 37	>90.0	>90.0
7	Effluent from bioreactor day 37 + 70 ppm sulfide	25.7	16.0

Microtox^R toxicity tests were conducted on samples taken from the bioreactor after 30 and 37 d of continuous operation. The field water was also tested for toxicity before treatment with and without added organics and with and without added sulfide. Results are given in Table 4. The produced water was shown to be toxic before biotreatment with and without added organics and/or sulfide. However, effluent samples from the bioreactor after steady state was established were not toxic. Therefore, not only had biotreatment removed the sulfide and target organics, but other components contributing to toxicity had also been removed. None of the target organics were present in the produced water as received.

CONCLUSION

Produced water from a petroleum production site has been successfully biotreated for removal of sulfide, benzene, toluene, phenol, acetic acid, and unidentified components that gave Microtox^R toxicity using a flocculated culture of *T. denitrificans* strain F and mixed heterotrophs. Complete removal of all target compounds indicates that a reactor system as simple in concept as an activated sludge system can be used to treat produced water with mixed contaminants without emission of H_2S and associated odor problems.

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