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**Development of a Biological
Treatment System for Hanford
Groundwater Remediation:
FY 1989 Status Report**

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DEVELOPMENT OF A BIOLOGICAL TREATMENT SYSTEM
FOR HANFORD GROUNDWATER REMEDIATION:
FY 1989 STATUS REPORT

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SUMMARY

The primary objective of the biological treatment program is to develop and demonstrate a biological process for Hanford groundwater remediation that is capable of nitrate (NO_3^-) and organic contaminant destruction. Biotenitrification using facultative anaerobic microorganisms is a promising technology for the simultaneous removal of NO_3^- and organics from contaminated aqueous streams. During FY 1988, microbial consortium from the Hanford groundwater was shown to degrade both NO_3^- and carbon tetrachloride (CCl_4). A pilot-scale treatment system was subsequently designed and constructed based on the results of laboratory- and bench-scale testing. The pilot-scale system demonstrated continuous degradation of NO_3^- and CCl_4 in a simulated groundwater.

This report summarizes the results of biological groundwater treatment studies performed during FY 1989 at the pilot-, laboratory-, and bench-scales. Pilot-scale tests were conducted using a simulated Hanford groundwater with a continuous stirred-tank bioreactor (CSTR) and a fluidized-bed bioreactor that was added to the pilot-scale treatment system in FY 1989. The CSTR system was operated continuously for 5 months with a NO_3^- -bearing simulated groundwater influent, and then for 2 months with both a NO_3^- - and CCl_4 -bearing influent. Using acetate as the primary carbon source for microbial growth, an NO_3^- removal efficiency of greater than 99% was maintained at an influent flowrate of 6 L/hr (8-hour residence time) and influent NO_3^- concentration of 400 mg/L. Similarly, CCl_4 removal efficiencies averaged greater than 99% with an influent concentration of 200 $\mu\text{g/L}$. Less than 8% of the CCl_4 removal resulted from volatilization or stripping.

Laboratory tests focused on the degradation of CCl_4 and on the microbial toxicity from CCl_4 , hexavalent chromium (Cr^{+6}), and cyanide (CN^-). Based on the results of laboratory testing, the rate of degradation of CCl_4 does not appear to be affected by the concentration of CCl_4 between 0.1 and 10 mg/L. In addition, short-term toxicity tests with both active and starved bacteria indicate that the concentration of CCl_4 that causes a significant mortality (25% reduction in the viability, EC_{25}) is quite high (>50 mg/L) relative to Hanford groundwater concentrations (<5 mg/L). Also, biotdenitrification of Hanford groundwaters would not be greatly inhibited by the presence of CN^- or

Cr⁺⁶. Results of laboratory experiments indicate that CN⁻ and Cr⁺⁶ concentrations below 10 mg/L and 4 mg/L, respectively, do not inhibit denitrification. Concentrations of CN⁻ and Cr⁺⁶ detected in Hanford groundwaters have not exceeded 1 mg/L.

Bench-scale tests were conducted to determine CCl₄ degradation rates and confirm denitrification kinetics obtained in FY 1988. Results of previous tests linked NO₃⁻ degradation to acetate and volatile suspended solids (VSS) concentrations. During FY 1989, specific denitrification rates ranged from 8 to 81 mg NO₃⁻-N/g VSS-hr, compared to rates of 16 to 27 mg NO₃⁻-N/g VSS-hr obtained in FY 1988. [Nitrate-nitrogen (NO₃⁻-N) is the concentration of nitrogen (N) present as NO₃⁻.] Carbon tetrachloride degradation rates ranged from 9.7 to 31 µg/g VSS-hr and also appeared to be linked to the metabolism of acetate, the substrate. During endogenous decay, in the absence of acetate, no removal or degradation of CCl₄ was detected.

The fluidized-bed bioreactor using the Hanford groundwater microbial consortium supported on anthracite coal particles was also effective at reducing NO₃⁻ concentrations to levels below the drinking water standard (44 mg/L). Under steady-state operating conditions, the fluidized-bed bioreactor obtained volumetric denitrification rates 10 to 20 times greater than those obtained in the CSTR. This is attributed to the tenfold increase in microorganism density in the reactor resulting from the use of a support medium.

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1.0 INTRODUCTION

Liquid wastes containing radioactive, hazardous, and regulated chemicals have been generated throughout the 40 years of operations on the Hanford Site. Some of these wastes were discharged to the soil column and many of the waste components, including nitrate (NO_3^-) and carbon tetrachloride (CCl_4), have been detected in the Hanford groundwater. Current U.S. Department of Energy (DOE) policy prohibits the disposal of contaminated liquids directly to the environment, and remediation of existing contaminated groundwaters may be required. Bionitrification using facultative anaerobic microorganisms is a promising technology for the simultaneous removal of NO_3^- and organics from contaminated aqueous streams.

Biological denitrification is defined as the dissimilatory reduction of NO_3^- to gaseous dinitrogen (N_2) and/or nitrous oxide (N_2O) by facultative anaerobic microorganisms. Prokaryotes (bacteria) are unique among microorganisms in that they can metabolize nitrates and thus exhibit the physiological mechanism of denitrification. The extent of this ability is widely distributed among the various groups of naturally occurring bacteria. Denitrifying bacteria are aerobes, preferring to use oxygen as the terminal electron acceptor in enzyme-driven metabolism. However, when their supply of oxygen is reduced, denitrifying bacteria can function anaerobically, using NO_3^- as the terminal electron acceptor in the metabolic electron-transport chain. The co-metabolic degradation of chlorinated organics such as CCl_4 by facultative anaerobes has been observed (Bouwer and McCarty 1983); however, the mechanism for this type of reaction is less understood than that for denitrification. In a co-metabolic process the contaminant is likely degraded fortuitously by non-specific enzymes and is not used by the microorganism as a growth substrate.

The primary objective of the biological treatment program at Pacific Northwest Laboratory (PNL)^(a) is to develop and demonstrate a biological process for Hanford groundwater remediation that is capable of NO_3^- and organic contaminant destruction. During FY 1988, laboratory microorganism tests and bench-scale kinetics tests were performed with support from the

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Northwest Hazardous Waste RD&D Center (NW Center). A consortium of microorganisms obtained from Hanford groundwater was shown to degrade both NO_3^- and CCl_4 with acetate as the electron donor. Denitrification rates were determined from the results of bench-scale kinetics tests using the Hanford microbial consortium and a simulated Hanford groundwater. A pilot-scale treatment system was designed and constructed based on the results of laboratory- and bench-scale testing. A detailed description of this developmental work is presented in an FY 1988 status report (Koegler, Brouns, Heath, and Hicks 1989).

The results of this work provided information for the U1/U2 Groundwater Biological Treatment project. This project is currently directed at a biological treatment demonstration using contaminated groundwater from below the U1 and U2 cribs in the 200 West area of the Hanford Site. The U1 and U2 cribs received aqueous and solvent wastes containing NO_3^- and uranium (U). Analysis of groundwater samples from monitoring wells in the crib area indicate both NO_3^- and CCl_4 contamination, with elevated concentrations of U and technetium (Tc). Because of the difficulty in treating for these radioactive components in situ, a pump and treat system was proposed for U and Tc removal. Westinghouse Hanford Company (WHC) is currently evaluating the use of supported liquid membranes (SLMs) for U and Tc removal (Hodgson 1989). In conjunction with that effort, the pilot-scale biological treatment system was developed for demonstration of denitrification and CCl_4 degradation downstream of a U and Tc removal process.

This report summarizes the results of biological groundwater treatment studies performed during FY 1989 with the pilot-scale treatment system. Pilot-scale tests were conducted using a simulated Hanford groundwater with a continuous stirred-tank bioreactor (CSTR) and a fluidized-bed bioreactor (FBR) that was recently added to the pilot-scale treatment system. In addition, laboratory- and bench-scale studies continued in FY 1989 in support of a site-wide environmental restoration application. Laboratory tests focused on the degradation of CCl_4 and on the microbial toxicity from CCl_4 , chromium (Cr^{+6}), and cyanide (CN^-). Bench-scale tests were conducted to determine CCl_4 degradation rates and confirm denitrification kinetics.

This work was performed by researchers and engineers at PNL, the University of Washington, and Washington State University. Support during FY 1989 was provided by the NW Center and the DOE's Hazardous Waste Remedial Actions Program (HAZWRAP).

2.0 CONCLUSIONS

The overall conclusion based on the results of pilot-, laboratory-, and bench-scale testing of the biological groundwater treatment system is that simultaneous degradation of NO_3^- and CCl_4 in groundwater is technically feasible. The following are specific conclusions based on pilot-scale testing.

- A CSTR, inoculated with a microbial consortium from the Hanford groundwater, was effective at reducing NO_3^- concentrations in simulated groundwater from 400 mg/L to below the drinking water standard of 44 mg/L. A NO_3^- removal efficiency of greater than 99% was maintained during steady-state operation at a residence time of 8 hours over 5 months of operation. The specific denitrification rate averaged 10 mg NO_3^- -N/g volatile suspended solids (VSS)-hr. Nitrate-nitrogen (NO_3^- -N) is the concentration of nitrogen (N) present as NO_3^- .
- Continuous degradation of CCl_4 was demonstrated during a 2.5-month test period. A CCl_4 removal efficiency of greater than 99% was maintained, with less than 8% of the CCl_4 removal resulting from volatilization or air-stripping.

Carbon tetrachloride degradation was confirmed with a noninoculated control test. Under steady-state conditions in the absence of microorganisms, greater than 95% of the CCl_4 introduced to the CSTR was recovered in the reactor effluent and off-gas.

- An FBR using the Hanford groundwater microbial consortium supported on anthracite coal particles was also effective at reducing NO_3^- concentrations to levels below the drinking water standard. Under steady-state operating conditions, the FBR obtained volumetric denitrification rates 10 to 20 times greater than those obtained in the CSTR. The greater rates are directly attributed to the order-of-magnitude increase in the density of microorganisms in the reactor resulting from the use of an attachment medium. Specific denitrification rates ranged from 2 to 3 mg NO_3^- -N/g VSS-hr.

Additional conclusions based on the results of developmental work conducted during FY 1989, including laboratory- and bench-scale studies, are summarized below.

- Based on tabulation of Hanford groundwater monitoring data, continued evaluation of biodenitrification and CCl_4 degradation for groundwater treatment is justified. Nitrate and/or CCl_4 concentrations exceeding the drinking water standards were detected in samples from 72 groundwater monitoring wells in the 100 and 200 Areas of the Hanford Site.

- Based on the results of laboratory testing, the rate of CCl_4 degradation does not appear to be affected by the concentration of CCl_4 between 0.1 and 10 mg/L. Carbon tetrachloride degradation at the 100 mg/L level could not be confirmed. Additional CCl_4 degradation studies will be conducted using radiolabeled CCl_4 to investigate the mechanism for degradation.
- The concentration of CCl_4 that causes a significant mortality (25% reduction in the viability, EC_{25}) in the Hanford groundwater consortium was found to be greater than 50 mg/L. Short-term toxicity tests with both active and starved bacteria produced EC_{25} values of 300 and 55 mg/L, respectively. The concentration of CCl_4 causing a significant mortality is quite high relative to Hanford groundwater concentrations of less than 5 mg/L.
- Carbon tetrachloride degradation rates ranged from 9.7 to 31 $\mu\text{g/g VSS-hr}$ based on bench-scale kinetics tests. In addition, CCl_4 degradation appears to be linked to the metabolism of acetate. During endogenous decay, in the absence of acetate, no removal or degradation of CCl_4 was detected.
- Biotenitrification of Hanford groundwaters would not be greatly inhibited due to the presence of CN^- and Cr^{+6} . Results of laboratory experiments indicate that CN^- concentrations below 10 mg/L do not inhibit denitrification. At a Cr^{+6} concentration of 0.1 mg/L, denitrification was not inhibited; however, higher concentrations appeared to delay the onset of denitrification in the batch tests.

3.0 PILOT-SCALE TREATMENT SYSTEM

A pilot-scale, suspended-growth biological treatment system was designed, constructed, and installed in FY 1988 for use in evaluating and demonstrating the applicability of biodenitrification to Hanford groundwater remediation (Koegler et al. 1988). The pilot-scale process, using a CSTR, was operated in FY 1989 with a microbial consortium obtained from Hanford groundwater samples. Pilot-scale tests using a simulated Hanford groundwater demonstrated the degradation of both NO_3^- and CCl_4 . A noninoculated control experiment was also conducted to verify the CCl_4 material balance.

In addition to the CSTR, a pilot-scale FBR was designed and constructed for testing in FY 1989 in collaboration with staff at the Oak Ridge National Laboratory. The FBR was installed adjacent to the suspended-growth system to share the same support equipment and data acquisition system. The reactor was inoculated with the Hanford groundwater consortium and was operated with simulated groundwater for approximately 6 weeks in late FY 1989. Denitrification studies were conducted at two different residence times for comparison to the suspended-growth process testing.

3.1 COMPARISON OF SUSPENDED-GROWTH AND FIXED-FILM PROCESSES

Several types of bioreactors can be used to denitrify NO_3^- -bearing wastewaters. Sutton (1987) defines two classes of bioreactors used in industrial biological treatment systems. These are suspended-growth reactors, where the biomass is freely suspended as individual organisms or clusters, and fixed-film reactors, in which the organisms grow as a film on a solid medium.

A suspended-growth reactor is usually a batch reactor or CSTR. To achieve effective denitrification rates in a CSTR, some means of concentrating or recycling the biomass is necessary because of the continuous loss of cells in the effluent. Figure 3.1 shows a typical continuous-flow, suspended-growth reactor system. The suspended-growth reactor system used in the present study is described in section 3.2.

In a fixed-film bioreactor, a biofilm grows on the surface of the packing or support medium, which serves to concentrate the biomass to a greater extent than is possible in a suspended-growth system. Fluidized-bed, packed-bed.

FIGURE 3.1. Stirred-Tank Reactor Treatment System

trickling filter. and rotating biological contactor (RBC) are types of reactors considered to be fixed-film reactors. Figure 3.2 shows a typical FBR system where activated carbon, sand, or coal particles are used as the support medium. The FBR used in the present study is described in section 3.4.

Suspended-growth and fixed-film bioreactors have both been used in bio-denitrification processes. Biological denitrification systems have been successfully employed by several DOE contractors for treating nitrate-containing wastes. Both stirred-tank reactors (batch and continuous) and FBRs have been used (Franke, Clark, and Strohecker 1974; Napier, 1976). Fluidized-bed bio-denitrification of wastewaters, as demonstrated at Oak Ridge National Laboratory (Walker et al., 1981), was subsequently implemented at the Portsmouth Gaseous Diffusion Plant. Further process development was done in support of a large-scale biodenitrification process constructed at the Feed Materials Production Center at Fernald, Ohio (Walker, Helfrich, and Donaldson 1987, 1989). In general, fixed-film reactors are better suited to treating large volumes of wastewater with NO_3^- concentrations below inhibitory levels. Because they achieve higher biomass concentrations, fixed-film reactors require lower residence times and correspondingly smaller reactor sizes.

FIGURE 3.2. Fluidized-Bed Reactor Treatment System

The primary distinguishing performance feature between suspended-growth and fixed-film reactors is the volumetric reaction rate (amount of reactant consumed per unit time per unit volume of reactor). In general, a fixed-film reactor exhibits at least an order-of-magnitude greater degradation rate on a per volume basis, primarily because the fixed-film can support much higher concentrations of microorganisms. The volumetric reaction rate is essentially proportional to the concentration of microorganisms. For large flowrates, this tenfold (or more) difference in denitrification rates means that the physical size of the reactor vessel(s) is much smaller for a fixed-film reactor compared to a suspended-growth reactor. However, if the volume of waste is so small that maintaining a higher flowrate is less important, then the need for higher reaction rates becomes less critical.

In addition to the difference in microorganism concentration between the suspended-growth and fixed-film systems, specific differences exist between the CSTR and FBR. Higher reactant concentrations are generally maintained in the entry region of an FBR, which helps promote higher reaction rates in the

lower bed. By contrast, the feed stream entering a CSTR is immediately diluted to near-effluent conditions, limiting the concentration gradient between the bulk fluid and the cell walls of the microorganisms. This concentration gradient is the driving force for mass transfer. However, mass transfer of nongaseous components is not usually limiting in biological treatment processes. In addition, if recycling of the FBR effluent is required to maintain a sufficient superficial velocity through the column, the reactor behaves very similarly to the CSTR. The feed stream becomes diluted by the recycle stream, reducing the concentration gradient in the reactor.

If the concentration of various components in the waste stream that affect biological activity is high, a CSTR configuration may be preferred because of its ability to immediately dilute the feed to noninhibitory levels. In an FBR, inhibition of biological activity would be expected, especially in the entrance region, greatly diminishing the overall (global) kinetics and thus the efficiency of an FBR. With effluent recycle, the FBR behaves like a CSTR, diluting the feed to noninhibitory levels. Therefore, inhibition could be controlled.

Other secondary factors (i.e., stability, process control, sludge wasting, mixing) are sometimes significant on a case-by-case basis, but may not be uniformly applicable. The use of FBRs in a variety of biotechnology applications has recently been reviewed (Schugerl 1989). The use of CSTRs in the bioengineering industry establishes these reactors as a proven technology. The use of FBRs is relatively new to biodenitrification; however, FBRs have been quite successful in more traditional engineering applications. Both processes are relatively stable during pulse inputs or upsets, but for different reasons. The CSTR masks pulses via dilution, whereas the FBR can tolerate pulses via retention of a large inoculum of immobilized culture. Experience has shown that the pulse is frequently absorbed by the culture and does not show up at the effluent.

The CSTR has a relatively sluggish response (hence a natural stability) because of dilution and long liquid residence time, alleviating some performance constraints for process control components. While an FBR has a quicker response, its larger cell population can withstand small process pulses stemming from imperfect process control. There is little or no evidence that

the fast response of an FBR is a detriment to successful operation of the system. However, depending on the recycle ratio, the FBR can produce a less homogeneous effluent over time.

Both types of bioreactor produce excess biological sludge. Since the microbiology and biochemistry are identical for the same application, the quantity of sludge is similar for both processes. Although the procedure for wasting excess sludge differs, it is relatively straightforward in both cases. Whether clarification of the effluent is required will depend on what is to be done with the effluent, not on which process is selected.

A CSTR requires mechanical agitation, whereas an FBR will produce its own internal agitation but requires pumping against the hydrostatic head of the column. The agitation in an FBR is more likely to achieve turbulence since there is a limitation on the shear rate imposed by agitators in CSTRs to avoid wholesale cell rupture. Improved mass transfer is often associated with the turbulent nature of the flow in an FBR. However, detailed mass transfer calculations may or may not reveal a significant difference in many cases.

The volumetric reaction rate is essentially proportional to the concentration of microorganisms since mass transfer from the bulk solution is usually not limiting. The biofilm in a fluidized-bed bioreactor also generally exhibits kinetics comparable to well-stirred suspensions--that is, the internal or heterogeneous kinetics are not diffusion-limited in a properly operated fluidized bed. Still, experience has shown that film thickness can be a factor: thin films give higher reaction rates than bulky thick films. This suggests that at some point the global kinetics can be influenced by internal diffusion limitations. A biofilm loading of 5% to 10% VSS on coal was found to be satisfactory (Walker et al. 1981). The stress on the particles as they overflow the column and pass through vibrating screens or centrifugal pumps in large-scale systems acts to shear off excess biomass and maintain thin films.

3.2 DESCRIPTION OF PILOT-SCALE EQUIPMENT

The pilot-scale biodenitrification system consists of two groundwater feed tanks; a CSTR; an FBR; a biomass settling tank or clarifier; and pressure, temperature, off-gas, and pH monitoring and control equipment as shown in the process flowsheet (Figure 3.3). Figure 3.4 shows a photograph of the process equipment prior to installation of the FBR. The FBR was added to the

FIGURE 3.3. Process Flow Diagram for the Bionitrification Pilot Plant

FIGURE 3.4. Biotenitrification Pilot Plant

biodenitrification system during FY 1989 and is described in more detail in Section 3.4. The FBR shares the feed system and process control and data acquisition equipment with the CSTR.

3.2.1 Feed System

Two 1800-L fiberglass-reinforced plastic feed tanks are used to supply NO_3^- -bearing waste water or simulated groundwater to the bioreactors. With a nominal design feed flow rate of 240 L/d, each feed tank is capable of supplying groundwater to the bioreactors for 7 days. Automatic flow controllers and level indicators are incorporated in the feed system to ensure an uninterrupted supply of feed to the bioreactor. A concentrated acetic acid solution is also fed to the bioreactor via a low-flow metering pump from a 5-L polypropylene container. Carbon tetrachloride is added directly to the bioreactor via a syringe pump for the CCl_4 degradation tests.

3.2.2 Bioreactor

The 50-L (working volume) anaerobic bioreactor is a cylindrical, 304L stainless steel vessel 61 cm tall and 36 cm in diameter (see center of Figure 3.4). To ensure adequate agitation, the bioreactor is equipped with four internal baffles and two flat-blade impellers driven by a 0.1 kw (1/8-hp), variable-speed motor. Thermocouples and a submersible pH electrode are included for monitoring and automatic control of the reactor pH and temperature.

3.2.3 Clarifier

The reactor effluent, which contains suspended biomass, is fed to a settling vessel (clarifier) to recover the biomass for recycle and to clarify the denitrified simulated groundwater, as shown in Figure 3.3. The clarifier consists of both aeration and settling zones. Air is introduced through porous ceramic diffusion stones into the aeration zone of the clarifier to shift the microorganisms to aerobic metabolism and consume any residual acetate. In addition, biomass settling is enhanced in aerobic environments where the bacteria tend to agglomerate. A recycle pump returns concentrated biomass to the bioreactor.

3.3 STIRRED-TANK BIOREACTOR TESTING

The pilot-scale CSTR was operated in a continuous mode to obtain both denitrification and CCl_4 degradation data and provide information on system operability. A control test in which the groundwater and nutrient feed streams were maintained, but biomass was eliminated, was performed to complete a CCl_4 material balance. The control test was conducted to determine whether biological degradation mechanisms were responsible for the observed disappearance of CCl_4 .

The composition of the simulated groundwater, listed in Table 3.1, was based on analysis of groundwater from the U1 and U2 crib area. The carbon-to-nitrogen (C/N) weight ratio was maintained at 2.0 through the addition of a 1 M acetic acid solution at 1.2 L/d. The sludge recycle from the clarifier to the bioreactor was maintained at a ratio (recycle flow/feed flow) of 0.4. The bioreactor temperature was controlled at $30^\circ\text{C} \pm 1^\circ\text{C}$.

Samples of the reactor feed, effluent recycle, and product streams were obtained periodically (2/week minimum) and analyzed for biomass, NO_3^- , and acetate concentrations. Biomass concentrations were measured by the VSS technique described in Standard Methods for the Examination of Water and water (APHA 1985). Nitrate concentrations were determined spectrophotometrically every day using a standard cadmium reduction method available from Hach

TABLE 3.1. Composition of Simulated Hanford Groundwater

<u>Component</u>	<u>Concentration, mg/L</u>
Na^+	482
Ca^{2+}	2.21
Mg^{2+}	14.0
K^+	13.8
Si^{4+}	45.0
CO_3^{2-}	90.6
SO_4^{2-}	4.04
NO_3^-	400
PO_4^{4-}	109
Cl^-	405
F^-	19.9

Chemical Company (Loveland, Colorado). These NO_3^- analyses were confirmed twice weekly using ion exchange chromatography with conductivity detection. Acetate concentration was measured using either gas chromatography with a flame ionization detector (FID) or ion exclusion chromatography with conductivity detection.

3.3.1 Denitrification Tests

The bioreactor was initially inoculated with the Hanford groundwater consortium using 4 L of laboratory culture. The process was operated in a batch mode through several denitrification cycles. then fed continuously with simulated groundwater at 1.0 L/h (2-day residence time). The feed flow rate was gradually increased to 6.0 L/h (8-hour residence time). The pilot-scale system was operated over a 160-day period to obtain steady-state denitrification data.

Figure 3.5 shows the NO_3^- -N concentration in the reactor effluent over the 160-day test period. Except for a major process upset at day 60 and several minor upsets, the bioreactor successfully produced an effluent with a NO_3^- -N level below the 10 mg/L drinking water standard. The upset at day 60 was caused by failure of a solenoid valve on the pH control system. Approximately 5 L of sodium hydroxide solution drained to the reactor, resulting in a rapid increase in the pH from 7.9 to greater than 11.5. By adjusting the pH and continuing the feed flow, the system recovered within approximately 5 days.

Reactor effluent VSS concentrations are shown in Figure 3.6. The VSS concentration increased from less than 100 mg/L at a 2-day residence time to greater than 1000 mg/L at an 8-hour residence time. Fluctuations in VSS concentrations were caused primarily by upsets to the sludge recycle system. The specific denitrification rate averaged 10 mg NO_3^- -N/g VSS-hr during steady-state operation with a low NO_3^- -N (<1 mg/L) effluent concentration.

3.3.2 Denitrification and Carbon Tetrachloride Dearadation Tests

Carbon tetrachloride degradation was tested by injecting 3.5 wt% CCl_4 in methanol directly into the bioreactor using a low-flow syringe pump. The quantity of CCl_4 injected simulated approximately 200 $\mu\text{g/L}$ in the groundwater. All liquid influent and effluent streams in the process were sampled and monitored during the test using gas chromatography with electron capture detector (ECD) and FID with standard purge and trap techniques. The

FIGURE 3.5. Nitrate Concentration in the Bioreactor Effluent During the 160-day Test

FIGURE 3.6. Volatile Suspended Solids Concentrations in the Bioreactor Effluent During the 160-day Test

bioreactor off-gas flowrate and CCl_4 concentration were also monitored during the test. Except for the addition of CCl_4 , process conditions were maintained identically to those used during the denitrification test (8-hour liquid residence time, recycle ratio of 0.4, and 30°C). The effluent NO_3^- -N and VSS concentrations are shown in Figure 3.7 for the 75-day CCl_4 test. Minor upsets caused by interruptions to the sludge recycle pump resulted in low VSS concentrations several times during the test period (18, 52, 59, and 73 days into the test).

Steady state was achieved between days 60 and 70 of the CCl_4 test, as determined by steady flow rates and NO_3^- -N and VSS concentrations. During this period, the microorganisms successfully degraded ~93% of the CCl_4 within the bioreactor. Most of the remaining CCl_4 was air-stripped in the clarifier, for an overall removal efficiency of 99.5%. The CCl_4 concentrations in the bioreactor and clarifier effluent for the 75-day test are shown in Figure 3.8,

At the conclusion of the CCl_4 test, a control experiment was conducted in which the simulated groundwater and CCl_4 feeds were maintained, but the biomass was flushed from the bioreactor. All process influent and effluent

FIGURE 3.7. Volatile Suspended Solids and Nitrate Concentrations in the Bioreactor Effluent During the 75-day CCl_4 Test

FIGURE 3.8. Concentration of CCl_4 in the Bioreactor and Clarifier Effluent During the 75-day CCl_4 Test

streams were monitored for CCl_4 as previously described. The test approached steady state at 32 hours (four liquid residence times), as indicated by effluent concentrations (Figure 3.9). As shown in Figure 3.10, greater than 99% of the CCl_4 was accounted for in this test. The results from this control test help confirm that biological degradation was responsible for the disappearance of CCl_4 in earlier tests.

3.4 DESCRIPTION OF FLUIDIZED-BED PROCESS EQUIPMENT

A pilot-scale, fluidized-bed bioreactor was constructed of glass pipe, with major features as shown in Figure 3.11. The reactor consists of a 1.5-L fluidized-bed section where most of the denitrification occurs, and a deentrainment section (expansion zone) equipped with an overflow clarifier. The overall liquid volume of the FBR is 15.5 L. The fluidized-bed section consists of a 3.8-cm inside diameter (ID) glass pipe that is 120-cm tall. The reactor is equipped with sampling ports at the top and middle of the bed. Anthracite coal, 65-80 mesh (.175-.208 mm), provides the support medium for microorganism attachment. A screen at the bottom of the bioreactor bed prevented particles from falling back into the feed line. A conical shaped entrance region will be used for future testing of the FBR.

FIGURE 3.9. Carbon Tetrachloride Mass Flow Rates During a Noninoculated Control Test

FIGURE 3.10. Material Balance for CCl₄ Based on Steady-State Testing and a Noninoculated Control Test

FIGURE 3.11. Schematic of the Fluidized-Bed Bioreactor

The fluidized-bed system, shown in Figure 3.12, was instrumented with pressure, temperature, off-gas, pH, and flow monitoring equipment. Type-K thermocouples were used to monitor temperatures in the feed stream, the bed of particles (reaction section), the expansion section, and the effluent stream. The feed and effluent pH were also monitored. An acetic acid solution was pumped directly into the groundwater feed stream immediately before entering the bioreactor. The pH of the acetic acid solution was adjusted to ensure that the feed pH was greater than 6.5 and a C/N ratio of 2.0 was maintained. External heaters connected to a temperature control unit were used to preheat the groundwater feed and maintain an average temperature of 30°C in the fluidized-bed. The off-gas, which contained nitrogen (N₂) and carbon dioxide (CO₂), was vented to the atmosphere. The off-gas flowrate was measured with a wet-test meter.

Samples of effluent and internal process streams were collected periodically to determine VSS, NO₃⁻-N, and acetate concentrations. Because of the volatility of coal, VSS measurements were made using two bed samples of equal volume. One sample was sonicated and rinsed several times to remove all attached bacteria. The true VSS of the biomass was calculated as the difference between the VSS of the column sample and the VSS of the sonicated split sample. Five sampling ports allowed sampling of the groundwater feed, the bioreactor effluent, the recycle stream, and the two locations in the fluidized-bed.

3.5 FLUIDIZED-BED BIOREACTOR TESTING

The FBR was inoculated with approximately 2 L of the Hanford groundwater consortium (8 g VSS) and 0.3 L (270 g) of anthracite coal. To promote initial growth of the microorganisms on the coal particles, the system was operated in a batch mode using total recycle. Following this start-up period, the fluidized-bed system was operated continuously to obtain denitrification rates.

To maintain fluidization of the particles, a superficial velocity of 0.5 to 1.0 cm/s through the column was required. Therefore, at a feed flow rate of 6.0 L/h, a recycle flow rate of approximately 26 L/h was used to maintain a sufficient superficial velocity. During start-up of the bioreactor, clumping of bacteria and coal in the reaction section of the column resulted in repeated loss of fluidization and high levels of effluent NO₃. The clumping

FIGURE 3.12. Fluidized-Bed Biodenitrification Process

was attributed to large films of biomass on the coal particles. Recycling the coal through a vibrating screen or centrifugal pump would reduce the biofilm thickness; however, the design of the bioreactor did not permit particle recycle. A gas sparge controlled by a solenoid valve and timer was added to the bioreactor base. A nitrogen gas (20 psig) sparge for 10 seconds every hour dramatically improved the bioreactor operation.

The FBR operated at steady state, as determined by steady flow rates and NO_3^- -N and VSS concentrations, at a 6.0 L/h feed flow rate (2.6-hour residence time). Effluent NO_3^- -N concentrations were less than 1.0 mg/L while VSS concentrations averaged 17.4 g/L. After 12 days, the feed flow rate was increased to 12 L/h (1.3-hour residence time) and operated for another 7 days. An initial increase in the reactor effluent NO_3^- -N concentration was observed; however, within 24 hours the NO_3^- -N concentration had returned to less than 1.0 mg/L. At the higher feed flow rate, the VSS concentration increased to an average of 22.6 g/L. During the 2.6- and 1.3-hour residence time tests, specific denitrification rates averaged 2.0 and 3.0 mg NO_3^- -N/g VSS-h, respectively. Volumetric denitrification rates averaged 34 and 69 mg NO_3^- -N/g VSS-h, compared to 11 mg NO_3^- -N/g VSS-h for the CSTR.

The CSTR and FBR have both demonstrated continuous denitrification of a simulated Hanford groundwater at the pilot-scale. Through increased concentration of microorganisms, the FBR has the potential to reduce the reactor volume required for a full-scale groundwater treatment process. However, the operational limits of the reactors have not yet been identified because both reactors were tested at very low effluent NO_3^- -N concentrations (<1 mg/L). At these low concentrations, the reactors are operating under NO_3^- -limiting conditions. Additional tests will be conducted at higher feed flow rates (lower residence times) to determine the operational limits of both reactors. The pilot-scale, fluidized-bed treatment system will be modified to permit recycle of particles. Operational tests will be conducted to ensure that the system can be used for the pilot-scale groundwater treatment demonstration in FY 1990. A recommendation for a full-scale groundwater treatment system will be based on both technical and economic considerations, with emphasis placed on operational reliability and regulatory compliance.

4.0 SUPPORT ACTIVITIES FOR BIOLOGICAL TREATMENT DEVELOPMENT

In support of site-wide environmental restoration at Hanford, several work activities were conducted during FY 1989 to aid in developing biological treatment processes. These activities supported both pilot-scale process development for the U1/U2 groundwater and evaluation of the applicability of biological treatment to other Hanford Site groundwaters.

Hanford groundwater contamination was assessed to identify research needs and direct laboratory studies. Based on this assessment, laboratory studies were conducted to evaluate the effect of CCl_4 concentration on CCl_4 degradation rate and microbial toxicity. The toxicity of cyanide (CN^-) and hexavalent chromium (Cr^{+6}) was also evaluated in laboratory tests. In addition, bench-scale denitrification and CCl_4 degradation tests were conducted to identify and confirm reaction rates. These activities, which were conducted by staff from the University of Washington, Washington State University, and PNL, are described in the following sections.

4.1 HANFORD GROUNDWATER ASSESSMENT

A survey was conducted in FY 1989 to identify all areas of groundwater on the Hanford Site containing chemicals that may be amenable to biological treatment. Current data (March 18, 1989) from the Hanford groundwater monitoring results were accessed. A preliminary screening of approximately 400 monitoring wells identified 90 wells with either NO_3^- or organic contaminants at concentrations above drinking water standards. Results from full chemical analyses for each of these 90 wells over the past 2 years was then obtained from the groundwater monitoring data base. Based on this tabulation, 61 wells in the 200 Areas of the Hanford Site and 11 wells in the 100 Area contain concentrations of NO_3^- and/or biodegradable organics above drinking water standards.

Biologically treatable groundwaters in the 100 Area of the Hanford Site primarily contained NO_3^- . Average NO_3^- concentrations from 11 monitoring wells over the past 2 years ranged from 59 to 460 ppm. The drinking water standard for NO_3^- is 44 ppm. In addition to elevated concentrations of NO_3^- , other inorganic contaminants were also detected, which may affect the feasibility of biological treatment. Gross alpha and beta measurements indicated elevated concentrations of radioactive compounds in several of these wells.

Chromium concentrations in the 60 to 600 ppb range were also measured in 7 of the 11 wells with NO_3^- contamination. The drinking water standard for chromium is 50 ppb.

Monitoring wells in the 200 Areas of the Hanford Site were also identified as potentially amenable to biological treatment. In the 200 East Area, 15 monitoring wells were identified with average (2 year) NO_3^- levels ranging from 63 to 350 ppm. Nitrate- and organics-containing groundwater was identified in the 200 West Area. Forty-six wells were identified with NO_3^- or CCl_4 concentrations exceeding the drinking water standards of 44 ppm and 5 ppb, respectively. Average CCl_4 concentrations ranged from 11 to 4900 ppb in 41 of these wells. Twenty-nine of the 46 monitoring wells had both NO_3^- and CCl_4 concentrations exceeding the drinking water standards. Several wells were also identified with CN^- concentrations in the 30 to 70 ppb range.

Based on tabulation of groundwater monitoring data, continued evaluation of biodenitrification and CCl_4 degradation for groundwater treatment was justified. Laboratory-scale testing was suggested to evaluate the effect of CCl_4 , CN^- , and Cr^{+6} concentrations on microbial activity. In addition, bench-scale tests were warranted to confirm denitrification rates and determine reaction rates for CCl_4 degradation.

4.2 LABORATORY-SCALE MICROORGANISM TESTING

Laboratory testing in FY 1988 focused on confirmation of denitrification and CCl_4 degradation by the Hanford groundwater microbial consortium. Tests conducted in FY 1989 were directed primarily at the following: CCl_4 degradation as a function of concentration, toxicity of CCl_4 to microbial metabolism, and toxicity of Cr^{+6} and CN^- to microbial denitrification.

4.2.1 Carbon Tetrachloride Degradation

Laboratory tests were conducted to evaluate the effect of CCl_4 concentration on CCl_4 degradation. Four concentrations of CCl_4 (0.1, 1.0, 10.0, and 100.0 ppm) were used in 20-ml head-space vials with Teflon®-lined septa.

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Three inoculated replicates and one noninoculated control were used for each concentration of CCl_4 at each sampling period (10 sampling periods). The vials were inoculated with a 0.1% defined media (Shelton and Tiedje 1984), acetate, NO_3^- , CCl_4 diluted in ethanol, and 0.1 mL of the Hanford groundwater consortium grown overnight under aerobic conditions.

The concentrations of CCl_4 in the incubated samples were analyzed directly from the 20-mL head-space vials using a Hewlett-Packard 19395A head-space analyzer coupled with a Hewlett-Packard 5880 gas chromatograph (Hewlett-Packard Company, Houston, Texas). The head-space analyzer/gas chromatograph/electron capture detection system was calibrated using CCl_4 standards prepared in the same salt media as the samples, and run under identical instrument conditions.

The results of the time course experiments are shown in Figure 4.1. This graph shows the change in the ratio of CCl_4 concentration in the test sample to the experimental control concentration, as a function of time. From these data we can conclude that there was degradation in the samples, and that in general the rate of CCl_4 degradation was not significantly different between the 0.1, 1.0, and 10 mg/L samples. Data from the 100 mg/L samples were not plotted because there was no detectable difference between the experimental samples and the controls over the first four days of the experiment.

Although the experiment was set up to run 28 days, it was terminated at 12 days. By day 8 the remaining concentration of CCl_4 was either too low to detect, as was the case with the 0.1 mg/L samples, or the difference between the samples and the controls was difficult to distinguish. This result may be due in part to experimental design. Carbon tetrachloride will diffuse through materials such as Teflon and silicone, both of which are used in the septa that seal the head-space vials. Thus, the CCl_4 appears to diffuse out of the head-space vial through the septum seal as the sample incubation proceeds. In the case of the 100 mg/L samples, the diffusive loss of CCl_4 appears to happen at a much greater rate than the microbial decomposition. Although decomposition may occur at this concentration, it could not be detected when measured against the diffusive losses.

Another problem with the experimental design is that the solution concentration of CCl_4 will change when the solution is placed in a head-space vial. Because of its high vapor pressure, CCl_4 is inclined to volatilize rapidly.

FIGURE 4.1. Time Course Plots for the Degradation of CCl_4 in Simulated Hanford Groundwater

In a closed system which exists in a head-space vial, the concentration at equilibrium in the head-space may be much greater than it would be in the aqueous solution. As the material in solution degrades, the system will react to maintain equilibrium, and more material will dissolve into the media. Because the medium is a salt solution, the partitioning of the CCl_4 to the head-space is even greater than for pure water, possibly by a factor of two or more. Therefore, after equilibration the solution concentration to which the microbes were exposed was probably about 200 times less than the prepared concentrations.

Because of problems with CCl_4 volatilization, the results of these tests were evaluated based on the ratio of CCl_4 concentration in the test vials to the concentration in the controls. This analytical method minimizes errors resulting from CCl_4 volatilization and confirms that CCl_4 degradation occurred during the test period.

4.2.2 Carbon Tetrachloride Toxicity

Short-term toxicity tests were conducted to determine the effective concentration of CCl_4 that would cause a significant mortality in the bacterial population of the Hanford consortium. Because results from the CCl_4 degradation tests were inconclusive at the 100 ppm concentration, these short-term tests were conducted to identify the maximum CCl_4 concentration that would still support microbial activity. In addition, a comparison was made between CCl_4 exposure to stationary-phase cells and aged (72-90 day) cells. Stationary-phase cells were obtained from a nutrient-limited environment immediately after a rapid growth phase. Stationary-phase cells are representative of microorganisms in a CSTR operating at a long residence time (sludge age >30 days) and with very low residual NO_3^- and substrate. The aged cells were obtained 72 to 90 days after the rapid or exponential growth phase. These bacteria are representative of groundwater microflora in a starvation-survival state.

Toxicity tests were conducted by exposing stationary-phase and aged cells to varying concentrations of CCl_4 over a 1-hour test period. The effective concentration of CCl_4 causing a significant mortality was defined as that concentration of toxicant that caused a 25% decrease in the survival of the population (EC_{25}). Population was measured as colony-forming units (CFUs) using a standard spread plate technique (APHA 1985). The Hanford groundwater consortium was grown in the simulated groundwater as described in Section 3.3. Stationary-phase cells were obtained after 48 hours of growth and aged cells were obtained after 72 to 90 days. Cell samples were placed in a 0.85% saline solution (Rosen and Heppel 1973; Stewart 1974) and blended to disperse agglomerated cells. The blended dilutions (1.1 mL) were inoculated into 2.2-mL vials containing CCl_4 in distilled water at concentrations ranging from 0.0 to 500 ppm. To reduce CCl_4 volatilization, the vials were tightly sealed using caps lined with aluminum foil, leaving no head space. The vials were mixed, and the cells were exposed to the CCl_4 for 1 hour at 22°C . Appropriate controls were run for each experiment.

After exposure, 0.5- to 1.0-mL cell samples were diluted in 0.1% peptone solution for enumeration. Most samples were run in duplicate or triplicate. The percentage of survival was calculated from the difference between CFUs of the 0 ppm control and the test samples.

The observed decrease in percent of surviving bacterial population with the increase in CCl_4 concentration is shown in Figures 4.2 and 4.3. It should be noted that the effect of concentration on percent survival was more apparent at the lower CCl_4 concentrations (e.g., there is a greater decrease in percent survival from 10- to 20-ppm CCl_4 than from 110- to 120-ppm CCl_4). The EC_{25} was obtained at approximately 30 ppm CCl_4 for the aged culture and at approximately 165 ppm for the stationary-phase culture. The aged culture had a markedly lower survival rate than the stationary-phase culture. As Kong (1988) noted regarding the toxic effects of toluene and o-xylene on aged cells, the aged cells require less exposure time and lower concentrations to reach an EC_{25} .

Based on these results, stationary-phase cells are more resistant to the toxic effects of CCl_4 than are aged cells in a starvation-survival state. In addition, the concentration of CCl_4 causing a significant mortality was found to be quite high (~165 ppm) compared to measured Hanford groundwater CCl_4 levels of less than 5 ppm. Therefore, biodenitrification and CCl_4 degradation should not be inhibited at the concentrations of CCl_4 currently found in samples from Hanford groundwater monitoring wells.

4.2.3 Chromium and Cyanide Toxicity

The effect of CN^- and Cr^{+6} on biodenitrification was evaluated at the laboratory scale to determine whether concentrations detected in groundwater samples may inhibit or reduce biological activity. The effect on biodenitrification was determined by monitoring N_2O production over time using the acetylene inhibition method and head-space analyzer described previously (Kogler et al. 1989). Separate tests were conducted with CN^- at 0-, .1, 1.0-, 10-, and 100-ppm concentrations and Cr^{+6} at 0-, .11-, .35-, 4.1-, and 40-ppm concentrations in septum vials containing Shelton's media, acetate, NO_3^- , and an inoculum of the Hanford groundwater consortium.

The results from these experiments indicate that concentrations of CN^- up to 10 ppm do not inhibit denitrification by the consortia, although there may be a slight delay in the onset of denitrification at concentrations above 0.1 ppm (Figure 4.4). At 10 ppm there appears to be a stimulation of denitrification after an initial delay in comparison to the control, while at 100 ppm neither growth nor denitrification were observed within the time frame of this experiment. A potential explanation for this increase may be that the consortium is metabolizing the CN^- . Hexavalent Cr^{+6} was also shown to inhibit

FIGURE 4.7. Carbon Tetrachloride Toxicity Using Aged Consortium

FIGURE 4.3. Carbon Tetrachloride Toxicity on Stationary-Phase Consortium

FIGURE 4.4. Denitrification by the Hanford Groundwater Consortium in the Presence of Cyanide

denitrification. At concentrations greater than 0.11 ppm, the onset of denitrification was delayed. Denitrification was not observed at Cr⁺⁶ concentrations of 4.1 and 40 ppm within the time frame of this experiment (Figure 4.5). These experiments suggest that even at the maximum concentrations of CN⁻ and Cr⁺⁶ observed in the Hanford unconfined aquifer groundwater, denitrification would not be greatly inhibited, except possibly to accommodate a period of adaptation for Cr⁺⁶.

4.3 BENCH-SCALE DEGRADATION TESTING

Denitrification reaction rates were identified in FY 1988 based on bench-scale test results (Koegler et. al 1989) and were used for the design and operation of a pilot-scale bioreactor. However, results of pilot-scale tests in FY 1989 indicated much higher denitrification rates than anticipated based on the bench-scale test results. Additional bench-scale degradation testing was conducted in FY 1989 to further evaluate the denitrification reaction rate and to study CCl₄ degradation in terms of acetate and NO₃⁻ metabolism.

FIGURE 4.5. Denitrification by the Hanford Groundwater Consortium in the Presence of Hexavalent Chromium

The bench-scale bioreactor system, shown in Figure 4.6, consisted of two 4-L Erlenmeyer flasks equipped with feed and effluent ports and a gas-tight closure constructed of glass and Teflon. Magnetic stir plates and stir bars were used to agitate the reactor contents. Low-flow piston pumps were used to supply the reactors with simulated groundwater and acetic acid and to withdraw treated groundwater. The reactors were designed for operation in either fed-batch or continuous-flow modes. During fed-batch operation, a programmable timer was used to control the agitator, groundwater and acetate pumps, and effluent pump. At a predetermined time, the agitator was automatically shut off for approximately 30 minutes to permit sludge settling. The effluent pump was then used to withdraw a portion of the clarified contents. The agitator was then re-started and the feed pumps activated to fill the reactors with fresh feed.

4.3.1 Denitrification Tests

Initially, the fed-batch reactors were operated on a 5-day schedule. The feed consisted of simulated Hanford groundwater (described in Section 3.3), with 880 mg/L of NO_3^- -N and 5100 mg/L of acetate. Every 24 hours, 200 mL of

FIGURE 4.6. Schematic of the Bench-Scale Bioreactor Test System

simulated groundwater with acetate was fed to the reactors. At the end of the 5-day period, approximately 1 L of denitrified liquid was withdrawn from the reactors. Two batch kinetics tests were conducted by sampling the reactor contents over several hours immediately after feeding. Reactor samples were analyzed for NO_3^- -N, acetate, soluble chemical oxygen demand (COD), and VSS. Spectrophotometric methods available from Hach Chemical Company (Loveland, Colorado) were used for NO_3^- -N and COD measurements. Acetate concentrations were measured using gas chromatography with a volatile acids column (0.3% carbonex 20 ml 0.1% H_3PO_4 on 60/80 carboxpack C) and flame ionization detector (FID). Volatile suspended solids were measured according to procedures in Standard Methods for the Examination of Water and Wastewater (APHA 1985).

Results of the initial batch tests indicated that NO_3^- reduction was occurring rapidly after feeding. Nitrate concentrations in the reactor were reduced from approximately 40 mg/L NO_3^- -N to less than 1 mg/L over a 3-hour period. In one test, the concentration of NO_3^- -N dropped below detection.

limits in less than 40 minutes after feeding. These results indicate that during much of the 24-hour period between feeding, the consortium is starved for NO_3^- .

The fed-batch cycle time was reduced to 12 hours in an attempt to maintain NO_3^- and acetate concentrations at all times. Every 3 hours, 500 mL of simulated groundwater with acetate was fed to the reactors. The NO_3^- -N and acetate concentrations in the feed were reduced to approximately 100 mg/L and 400 mg/L, respectively. Four batch kinetics tests were conducted, two at low initial NO_3^- -N levels (10 mg/L) and two at higher initial NO_3^- -N levels (40 mg/L). Volatile suspended solids concentrations ranged from 1000 to 2200 mg/L during the tests, depending on the reactor used and total volume.

Specific denitrification rates from these batch kinetics tests ranged from 8 to 81 mg NO_3^- -N/g VSS-hr. Similar (order-of-magnitude) rates of 16 to 27 mg NO_3^- -N/g VSS-hr were obtained in 1988 using continuous-flow, non-recycle bioreactors (Koegler et al. 1989). The range of denitrification rates obtained in FY 1988 was explained by a second-order reaction rate expression linking NO_3^- degradation to both acetate and VSS concentrations. However, no significant correlation could be determined to explain the variability in denitrification rates from the FY 1989 batch tests. Linear regressions of the batch data using the second-order rate expression did yield reasonable correlation (R^2 of .95 to .99); however, high variability in the rate constant, k , and NO_3^- yield, Y_N , was obtained between tests.

Two sets of batch kinetic tests were conducted during a period of high influent feed concentrations, and four sets of tests were conducted when lower influent acetate and NO_3^- concentrations were used. A second-order expression based on the soluble COD or acetate level was used to evaluate the kinetic rate data because NO_3^- was not limiting during these runs:

$$dS/dt = K SX$$

Where: S = Soluble COD or acetate concentration, mg/L
 X = Volatile suspended solids concentration, mg/L

For the first two tests conducted at high influent concentrations, the rate constant (K) ranged from 0.004 to 0.02 L/mg VSS-day. The lower value is within the range of rate constants observed during FY 1988. For the tests using lower acetate levels, the rate constant generally ranged from 0.04 to

0.08 L/mg VSS-day. The lower kinetic rates appear to be related to the presence of much higher acetate concentrations (>300 mg/L), suggesting the possibility of substrate inhibition for the test runs. The higher kinetic rates relate better to the performance of the pilot-scale system.

Overall, rate constants obtained from these batch tests were larger than those obtained previously. Effluent NO_3^- levels at the pilot-scale were lower than those anticipated based on the previous kinetic constants; therefore, the higher values obtained from batch tests were expected. Lower values may have been a result of inhibition from unusually high salt concentrations in the FY 1988 continuous-flow tests.

Yield coefficients for NO_3^- metabolism were also calculated from the batch test data. Values of .10 to .27 mg NO_3^- -N/mg VSS were obtained, compared to a value of .22 mg NO_3^- -N/mg VSS obtained in FY 1988. Yields based on tests conducted at the higher acetate and NO_3^- concentrations were generally higher (.16 to .27 mg NO_3^- -N/mg VSS) than those obtained at low acetate and NO_3^- levels. Nitrate yields obtained from batch tests were not significantly different from those obtained previously.

4.3.2 Endogenous Decay and Biomass Yield

The endogenous decay rate and biomass yield were also obtained during fed-batch denitrification testing. Endogenous denitrification rates were measured during a batch test at approximately zero COD. A specific denitrification rate of .03 mg NO_3^- -N/mg VSS-day was obtained, which converts to approximately .066 mg VSS/mg VSS-day. This rate compares well to the endogenous decay coefficient, K_d , of .050 mg VSS/mg VSS-day obtained from bench-scale continuous-flow tests. Biomass yield coefficients were obtained from mass balances of VSS conducted during fed-batch operation. Yield, Y_B , values ranged from .09 to .30 mg VSS/mg acetate, compared to .064 mg VSS/mg acetate obtained in FY 1988. The wide range of values may be a result of microbial changes during fed-batch operation (i.e., enzyme activity, reproductive capacity) resulting from repeated feast and famine cycles.

4.3.3 Carbon Tetrachloride Removal

Carbon tetrachloride degradation was studied in conjunction with the denitrification testing. A low-flow syringe pump was used to inject CCl_4 (dissolved in methanol) directly into the reactor during fed-batch feeding.

The concentration of CCl_4 in the feed to the reactor was maintained at approximately 400 to 500 $\mu\text{g/L}$. Carbon tetrachloride concentrations were measured using gas chromatography (GC) with a mass spectrometer and purge and trap. Reactor samples were taken with Teflon-lined septum vials with zero head space. Gas samples were also taken from the head space of the reactor using a gas-tight syringe and Teflon-lined sampling valve. Gas samples were measured for CCl_4 by direct injection into the GC.

Carbon tetrachloride degradation was first studied during the endogenous decay test. Although the NO_3^- concentration dropped from 57 to 29 mg/L during the 19-hour test period, there was no apparent reduction in the CCl_4 concentration at approximately 280 $\mu\text{g/L}$. Results from this test indicate that CCl_4 degradation is linked to acetate metabolism. Additional CCl_4 degradation tests were conducted under continuous-feed conditions. Because of the relatively slow rate of CCl_4 degradation compared to denitrification, it was difficult to obtain a significant CCl_4 reduction before NO_3^- concentrations dropped below 1 mg/L . Therefore, continuous-feed tests were conducted at a flow rate of 1.06 mL/min and feed NO_3^- -N and acetate concentrations of approximately 1500 mg/L and 6700 mg/L , respectively. During two continuous-feed closed-reactor tests, the specific CCl_4 degradation rates ranged from 9.7 to 31 $\mu\text{g/g VSS-hr}$. The variability in specific degradation rates could not be explained by substrate level or substrate removal rates because of the limited data. However, the CCl_4 degradation rate compares well to the value of 22 $\mu\text{g/g VSS-hr}$ obtained during steady-state pilot-scale testing.

5.0 FUTURE WORK ACTIVITIES

A pilot-scale groundwater treatment demonstration with U1/U2 groundwater is scheduled for FY 1990 and 1991. The pilot-scale process will be upgraded to accommodate actual groundwater contaminated with NO_3^- , CCl_4 , and U. An SLM process will be incorporated in the pilot-scale system for removal of radionuclides prior to biological treatment. All activities conducted with contaminated groundwater will require permitting and approval from the Department of Energy (DOE), Washington State Department of Ecology (WDOE), and the Environmental Protection Agency (EPA). The pilot-scale system will be permitted as a treatment, storage, and disposal (TSD) facility as required by state and federal regulations.

Laboratory studies will continue in FY 1990 to evaluate the fate of heavy metals and radionuclides exposed to denitrifying bacteria. Chemical analyses will be performed early in FY 1990 to determine whether decreases in Cr^{+6} concentrations during laboratory toxicity tests resulted from adsorption to bacteria or chemical reduction to trivalent chromium (Cr^{+3}). In addition, laboratory-scale sorption studies will be conducted with U-bearing groundwater to determine if biosorption to denitrifying bacteria can be implemented as a treatment method.

In addition to laboratory studies with Cr^{+6} and U, CCl_4 studies will continue through FY 1990. The mechanism for CCl_4 degradation by the Hanford microbial consortium will be studied at Washington State University. Results from laboratory studies will continue to provide information needed for development of both above-ground and in situ biological treatment processes.

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