Biodenitrification of Hanford Groundwater and Process Effluents: FY 1988 Status Report

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September 1989

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REPORT

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SUMMARY

Laboratory screening tests were performed to select microorganisms for their ability to destroy nitrates and carbon tetrachloride in Hanford groundwaters. The microorganisms were subsequently tested in bench-scale experiments to determine the fundamental kinetic constants for denitrification with acetate. The microorganisms were also used as the inoculum for the pilot-scale bioreactor system, which was designed, constructed, and operated in FY 1988. The bench-scale denitrification kinetic data were analyzed using two rate models. The best fit was obtained using a first-order expression. The kinetic constants determined in the bench-scale experiments were later used to set operating parameters for the pilot-scale bioreactor test. The pilot-scale bioreactor system consisted of a 50-L continuous-stirred tank bioreactor, a 280-L clarifier, associated feed components, and a data acquisition and control system. The pilot plant was designed as a module for ease of installation and to facilitate relocation for on-site demonstration testing. The pilot-scale bioreactor was installed and operated in FY 1988, but steady-state operating data are not yet available. Preliminary denitrification data show destruction of nitrate to concentrations less than drinking water standards. A preliminary engineering evaluation was also completed in FY 1988 that examined the engineering feasibility of biodenitrification for the UO_3 Plant process condensate through microorganism laboratory testing, development of flowsheets, and equipment size and cost estimates.

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We would like to thank Dr. H. David Stensel from the University of Washington's Department of Civil Engineering for his support in this project. Dr. Stensel was responsible for the bench-scale kinetics tests that were conducted to determine denitrification reaction rates with the Hanford groundwater consortium.

CONTENTS

SUMMARY	iii
ACKNOWLEDGMENTS	v
INTRODUCTION	1
RESULTS AND CONCLUSIONS	3
DESCRIPTION OF BIODENITRIFICATION	5
MATHEMATICAL MODEL	6
BIOREACTORS	8
MICROORGANISM TESTS	11
OBJECTIVES OF MICROORGANISM TESTS	11
MICROORGANISM TEST RESULTS	11
CARBON TETRACHLORIDE TESTS	13
CARBON TETRACHLORIDE TEST CONCLUSIONS	15
BENCH-SCALE KINETIC TESTS	17
OBJECTIVES OF KINETIC TESTS	17
DESCRIPTION OF KINETIC TESTS	17
RESULTS OF KINETIC TESTS	18
PILOT-SCALE BIOREACTOR TESTS	23
OBJECTIVES OF PILOT-SCALE TESTS	23
Task 1, Bioreactor and Pilot-Plant Design	23
Task 2, Pilot-Plant Fabrication and Installation	24
Task 3, Pilot-Plant Testing	26
DESCRIPTION OF PILOT-SCALE EQUIPMENT	26
Feed Tanks	28
Bioreactor	29

Clarifier	30
pH-Control/Adjust System	30
Process Control	31
Data Acquisition	31
PILOT-SCALE TEST METHODOLOGY	32
FY 1988 Tests Completed	33
Problems/Resolution	33
UO ₃ PLANT CONDENSATE TESTS	35
LABORATORY DENITRIFICATION RESULTS	35
BIODENITRIFICATION PROCESS CONCEPTUAL DESIGN	39
COST ESTIMATE	44
UO ₃ STUDY CONCLUSIONS	45
REFERENCES	47

FIGURES

1	Suspended Growth Bioreactor	8
2	Fluidized Bed Bioreactor	9
3	Denitrification in a Simulated Waste Stream Inoculated with Denitrifying Culture Obtained from Hanford Groundwaters	12
4	Flow Diagram of Method Used for Monitoring $^{14}\text{CO}_2$ in Samples . . .	14
5	CCl_4 Degradation in Samples Incubated with Hanford Denitrifying Cultures	15
6	Substrate Removal Efficiency as a Function of Biomass Concentration and Residence Time	19
7	Substrate Yield as a Function of Residence Time	20
8	Flow Diagram of Bionitrification Pilot-Plant	24
9	Bioreactor and Process Control Instrumentation	27
10	Bioreactor Feed Tanks	28
11	Clarifier	29
12	Test 1: Laboratory Denitrification Rate in Moles of Nitrogen Produced per Hour from a 10-mL Sample	36
13	Test 2: Laboratory Denitrification Rates in Moles of Nitrate Destroyed per Hour from a 10-mL Sample	38
14	Test 2: Percent Nitrate Destroyed per Hour	39
15	Flow Diagram for UO_3 Process Condensate Continuous Bionitrification	40
16	Flow Diagram for UO_3 Process Condensate Batch Bionitrification	40

TABLES

1	Cultures Tested for Denitrification	12
2	Environmental Parameters Influencing Biotenitrification	13
3	Steady-State Operating Conditions	18
4	Calculated Kinetic Constants	21
5	Biotenitrification Pilot-Plant Flowsheet	25
6	Biological Denitrification of Process Condensate for a 10-mL Sample Diluted 10:1 with Water	37
7	Flowsheet for UO ₃ Process Condensate Continuous Biotenitrification	42
8	Flowsheet for UO ₃ Process Condensate Batch Biotenitrification	43

INTRODUCTION

This report summarizes the results of biodenitrification studies performed at the laboratory, bench, and pilot scales by the Pacific Northwest Laboratory (PNL)^(a) in FY 1988. The studies were conducted under the Environmental Restoration Remedial Actions Program and the Northwest Hazardous Waste RDDT&E Center. The purpose of the biodenitrification program is to determine the applicability of biological denitrification and organic contaminant destruction to groundwaters and process effluents. This technology will be transferred to the Hanford operating contractor and to other U.S. Department of Energy (DOE) facilities.

Liquid wastes have been generated by 40 years of Hanford Site operations. Previously, some of these wastes, which contain radioactive, hazardous, and regulated chemicals (including nitrates and organics) have been discharged to the soil column. Current DOE policy prohibits the disposal of contaminated liquids directly to the environment. Biodenitrification using facultative anaerobic microorganisms is one promising technology for nitrate removal from the contaminated aqueous streams.

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RESULTS AND CONCLUSIONS

The biodenitrification project was successful in developing and testing microorganisms and processes for treating nitrates in Hanford groundwater and the UO₃ Plant process condensate. The following are specific project accomplishments for FY 1988:

- Microorganisms selected from a variety of soils and sludges were tested for their capacity to destroy nitrates. The more effective microorganisms were further tested for their ability to degrade CCl₄ under denitrifying conditions. Carbon tetrachloride destruction was confirmed by radio-labeled CCl₄ tests. A culture of these microorganisms was used as the inoculum for the bench-scale kinetic tests and for subsequent pilot-scale demonstration tests.
- Bench-scale tests run with a series of 5-L continuous flow bioreactors determined the kinetic constants for nitrate destruction using acetate as the carbon source (substrate). The rate constants k and K_d were calculated, together with the biomass and nitrate yields. These constants were used to determine pilot plant operating parameters, including the feed rate and the optimum acetate/nitrate feed ratio.
- A 50-L continuous-flow, stirred-tank bioreactor system was designed and constructed. The bioreactor was operated with simulated U1/U2 crib groundwater feed and demonstrated denitrification at a feed rate of ~2 L/h. The bioreactor is suitable for obtaining engineering and process information to determine the relevance of bioremediation to specific Hanford applications. The unit is skid-mounted for easy transport to a remote site for future on-site demonstrations.
- A laboratory and technical study to determine the feasibility of biological denitrification of UO₃ Plant process effluents indicated that biodenitrification is a viable option. Flowsheets and equipment estimates were prepared from the laboratory microorganism feasibility test results.

The biological tests completed at the bench and pilot scales demonstrated the denitrification of simulated U1/U2 groundwater. Further tests will be completed in FY 1989, also with simulants, to confirm CCl₄ degradation at the pilot scale. Tests with actual U1/U2 groundwater, either on site or at the 324 Building, will be required to confirm operating characteristics. The pilot-scale bioreactor equipment is suitable for either low-nitrate groundwater or high-nitrate process effluent testing. The bioreactor

can be used for additional tests in FY 1989 and FY 1990 in its present location at the 324 Building, or at a remote location.

DESCRIPTION OF BIODENITRIFICATION

Biological denitrification is defined as the dissimilatory reduction of nitrate (NO_3^-) to gaseous dinitrogen (N_2) and/or nitrous oxide (N_2O) by facultative anaerobic microorganisms. Prokaryotes (bacteria) are the only organisms that have the physiological property of denitrification; this property is widely distributed among the various groups of bacteria. Denitrifying bacteria are basically aerobes and prefer to use oxygen as their terminal electron acceptor. However, in the absence of oxygen, these bacteria possess the capacity to use nitrate as the terminal electron acceptor in an electron transport chain.

Numerous environmental factors are known to affect biodenitrification. Of particular importance is oxygen. Generally, denitrification is repressed in the presence of oxygen. However, denitrification has been observed at oxygen concentrations approaching 5% (Knowles 1982). The level of oxygen that can be tolerated appears to be dependent upon the taxonomic group involved in the process. Other factors include, but are not limited to, temperature, pH, the carbon/nitrogen (C/N) mole ratio, the need for cofactors such as phosphorus, or the presence of inhibitors such as heavy metals.

The overall general equation for biological denitrification as described by McCarty et al. (1969) is:

For each nitrate ion converted, a hydroxyl ion is produced. The hydroxyl ion generation causes the pH to increase in the bioreactor as denitrification proceeds and may require neutralization to maintain the environment in the bioreactor within pH limits of 6.5 to 8.5. Walker et al. (1981) give the approximate chemical composition of biomass as $\text{C}_5\text{H}_7\text{O}_2\text{N}$. Measurements indicated that 0.065 moles of biomass ($\text{C}_5\text{H}_7\text{O}_2\text{N}$), 0.76 moles of CO_2 , and 0.47 moles of N_2 were produced, and 1.08 moles of methanol or 0.54 moles of ethanol were consumed for each mole of nitrate converted. The biodenitrification reaction is also exothermic with a heat of reaction of -138.36 kcal

per mole of methanol (Knowles 1982). The temperature increase caused by the reaction may need to be considered in the design and operation of a bioreactor.

MATHEMATICAL MODEL

Mathematical models of biological systems are used for the scale-up of laboratory or bench-scale processes. Microbial growth and metabolism have traditionally been modelled using the Monod expression which relates cell or biomass growth rate to substrate and biomass concentrations (Monod 1949). The model's simplicity and ease of application have resulted in its extensive use in both fermentation and wastewater treatment systems to describe substrate utilization and microbial growth. The rate expression for substrate utilization is:

$$-dS/dt = (k S X)/(K_s + S) \quad (2)$$

where S = substrate concentration, mg/L

X = biomass concentration, mg/L

K_s = Monod constant or concentration intensity factor, mg/L

k = reaction rate constant, h^{-1}

t = time, h.

Studies of denitrifying bacteria have found that nitrate does not limit bacterial growth and metabolism until concentrations drop below regulated nitrate discharge levels (Sundstrom et al. 1979). Therefore, the carbon source becomes the limiting substrate for kinetic considerations. The growth rate is simply the rate of substrate utilization multiplied by a biomass yield coefficient. The rate of change of biomass concentration also includes a biomass decay term to account for cell death. The overall biomass growth rate expression is:

$$dX/dt = Y(-dS/dt) - K_d X = Y(k S X)/(K_s + S) - K_d X \quad (3)$$

where Y = biomass yield coefficient (dimensionless)

K_d = biomass decay constant, h^{-1} .

Other kinetic expressions have also been used successfully to describe microbial growth in wastewater systems. Zero-, first-, and second-order models are frequently used (Sundstrom et al. 1979, Benefield et al. 1980). The first-order model shown below, although simpler than the Monod expression, has been used to model substrate utilization.

$$-dS/dt = k S X \quad (4)$$

As with the Monod equation, a biomass growth rate expression includes yield and decay terms.

$$dX/dt = Y(k S X) - K_d X \quad (5)$$

Rate constants, yield coefficients, and decay constants are determined from batch and continuous growth data. Several models are used when analyzing the data to determine which expression best describes the microbial growth and metabolism. Substrate and biomass rate expressions are then used in overall material balances to design larger bioreactors for nitrate treatment systems. Reaction rates must be confirmed at each stage of development to ensure that the final treatment process will operate effectively. Residence time and reactor volume are regulated by the kinetics of the denitrifying bacteria and the composition of the process stream requiring treatment.

A solids residence time is also important in biological systems and is controlled in most processes by the recycle rate, settling characteristics of the biomass, and sludge wastage rate. The minimum solids residence time, or sludge age, required is calculated from growth kinetics but can be used as a process variable to control discharge concentrations. The ratio of carbon to nitrogen (C/N) in the bioreactor is a particularly important parameter for denitrification system design. Large C/N ratios will result in the discharge of effluent from the bioreactor containing very little nitrate, but the biological oxygen demand (BOD) may exceed acceptable limits due to excess

carbon. If low C/N ratios are maintained, low nitrate discharge levels may be difficult to attain. Optimum sludge age, temperature, pH, and C/N ratio must be determined from kinetic studies using laboratory or bench-scale continuous bioreactors.

BIOREACTORS

Several types of chemical reactors can be used to denitrify nitrate-containing waste waters. Sutton (1987) describes the two classes of bioreactors used for all industrial biological treatment as suspended-growth reactors, in which the biomass is suspended as free organisms, and fixed-film reactors, in which the organisms grow on a solid medium.

The suspended-growth reactor is usually a batch or continuous-stirred tank reactor. To achieve effective denitrification rates in a continuous-stirred tank reactor, some means of concentrating or recycling the biomass is necessary. A continuous-suspended growth reactor is shown in Figure 1.

In the fixed-film bioreactor, a biofilm develops at the surface of the packing media which serves to concentrate the bacteria or biomass higher than

FIGURE 1. Suspended Growth Bioreactor

is possible in a suspended-growth reactor. Activated charcoal or coal particles can be used in a fluidized bed process to create a special type of fixed-film reactor (Figure 2). The fluidized bed reactor is better suited for high volumes of waste water with low nitrate concentrations. It is less susceptible to plugging than the fixed bed design and allows high biomass concentrations with a fairly low residence time. However, process control is more difficult with the fluidized bed reactor than the stirred tank bioreactor because variations in feed concentration in the fluidized bed reactor may "shock" the system, killing some of the microorganisms. This usually results in detachment of the organisms from the support matrix, which leads to a washout condition. The stirred-tank bioreactor is less susceptible to upset due to variations in flow rate or feed composition because of the inherent dampening effect of the larger tank volume; it is more appropriate for low volumes with higher concentrations of nitrates. In all bioreactor systems, provisions must be made for feed composition, pH, and temperature control and for separation of the biomass produced by the reaction from the product effluent.

FIGURE 2. Fluidized Bed Bioreactor

Biological denitrification has been used by several DOE contractors for cleanup of nitrate-containing wastes. Both stirred tank reactors (batch and continuous) and fluidized bed reactors have been used at Oak Ridge National Laboratory (ORNL) and the Oak Ridge Y-12 Plant for denitrification of waste waters. Several continuous suspended-growth reactors were developed at the Oak Ridge Y-12 Plant to denitrify acidic process effluents containing 10% to 15% nitrate (Franke, Clark, and Strohecker 1974). A batch biodenitrification technique is routinely used to process nitrate waste water from the Oak Ridge Y-12 Plant. The batch biodenitrification facility consists of nine 500,000-gallon tanks and a water treatment plant.

Fluidized bed microbial denitrification of waste waters was demonstrated by ORNL (Walker et al. 1981) and subsequently employed on a larger scale at the Feed Materials Production Center (FMPC) at Fernald, Ohio (Hopper et al. 1987). The FMPC process uses four 52-ft-tall fluidized bed reactors, each 4 ft in diameter, expanding to 7 ft at the top. The reactors, scaled up from the 0.2-m ORNL pilot-plant bioreactors, also use anthracite coal particles as growth sites for the microorganisms and physical separation of the excess biomass from the aqueous raffinate.

FIGURE 2. Fluidized Bed Reactor

MICROORGANISM TESTS

OBJECTIVES OF MICROORGANISM TESTS

The objective of the microorganism evaluation study was to select denitrifying bacteria that had the ability to destroy nitrates present in process streams and/or groundwater. To reach this objective, two tasks were performed: 1) isolation of denitrifying organisms from a variety of environmental samples; and, 2) characterization of the isolated culture in terms of denitrifying ability and the effect of environmental conditions on denitrification.

The first task focused on selecting suitable denitrifying candidates. Denitrifiers were obtained from various environmental sources including sewage sludge, soil, ponds, and Hanford groundwater and were tested for their effectiveness in reducing the nitrate content in simulated process waste streams. Potential candidates were inoculated into 100-mL serum bottles containing the simulated waste stream. Nutrients, including acetate, were added and the head space in the bottles was replaced with N_2 . Acetylene was injected into the bottles to begin the incubation. Acetylene inhibits the conversion of N_2O to N_2 which allows the denitrification process to be assessed by monitoring the production of N_2O .

Once a suitable culture was selected, various environmental parameters were adjusted over a range of values to determine their effect on the rate and extent of biodenitrification. At least three different values for temperature and pH were tested. Also, different organic electron donors were examined to identify the optimal C/N ratio as well as the best carbon source.

MICROORGANISM TEST RESULTS

Nine cultures, obtained from a variety of environmental sources (Table 1), were tested for their denitrification ability.

Although all cultures could denitrify to some extent, the culture obtained from the Hanford groundwater was selected as the best candidate. This culture could remove approximately 96% of the nitrate present in test

TABLE 1. Cultures Tested for Denitrification

<u>Isolate</u>	<u>Source of Inoculum</u>
1	Drainage ditch
2	Farm pond
3	Columbia River
4	The Great Salt Lake
5	Pacific Ocean
6	Dead Sea
7	Greasewood Rhizosphere
8	Hanford Groundwater
9	Soil Sample from ALE Reserve

samples (Figure 3). In this experiment, nitrate destruction was determined by nitrate analysis of the sample at the conclusion of the experiment. In addition, because it is native to the Hanford environment it has the greatest

FIGURE 3. Denitrification in a Simulated Waste Stream Inoculated with Denitrifying Culture Obtained from Hanford Groundwaters

chance for adapting to the environmental conditions likely to be encountered under "real world" conditions (i.e., treatment of Hanford nitrate-contaminated groundwater).

As of this writing, the makeup of the Hanford groundwater consortium has not been determined. Efforts to identify and isolate microorganisms responsible for denitrification are planned for FY 1989 and will be reported later.

Following the selection of the Hanford bacteria as the denitrifying culture to be used in further studies, the influence of environmental conditions such as pH, temperature, and carbon source was examined (Table 2). These bacteria appear to favor a slightly alkaline environment and temperatures of approximately 29°C. Of the three carbon sources examined, the Hanford denitrifiers preferred acetate, although they could grow on methanol and, to some degree, ethanol.

CARBON TETRACHLORIDE TESTS

The objective of this study was to determine the ability of previously obtained denitrifying organisms to degrade carbon tetrachloride (CCl_4). The culture obtained from Hanford groundwaters was used in this study. The rationale for using this culture was that, because of its previous exposure to CCl_4 , CCl_4 degradation ability may already have been enriched. The culture was grown in a nutrient broth containing nitrate. Following growth in the nutrient broth, 0.1 mL was injected into the samples containing a minimal media amended with $^{14}\text{C-CCl}_4$. Carbon tetrachloride degradation was monitored using $^{14}\text{C-CCl}_4$ and analyzing for $^{14}\text{CO}_2$. A flow diagram describing the method is shown in Figure 4.

Results for CCl_4 degradation are shown in Figure 5. Approximately 13% of the added $^{14}\text{C-CCl}_4$ was degraded in the samples inoculated with the Hanford

TABLE 2. Environmental Parameters Influencing Biotenitrification

<u>Parameter</u>	<u>Range Examined</u>	<u>Optimum Value</u>
pH	7.95 to 11.58	8
Temperature (°C)	24, 29, 37	29
Carbon Source	acetate, methanol, ethanol	acetate

FIGURE 4. Flow Diagram of Method Used for Monitoring $^{14}\text{CO}_2$ in Samples

denitrifying culture. In uninoculated samples less than 2% was degraded or mineralized to nonorganic forms. While the data indicate that CCl_4 was degraded by the microorganisms, the percent degraded was less than expected. A mass balance determination of remaining $^{14}\text{C-CCl}_4$ indicated that approximately 50% of the $^{14}\text{C-CCl}_4$ could not be accounted for. Equipment failure in the initial stages of the experiment forced us to delay sampling by several days. One possibility is that, due to the increased incubation time, $^{14}\text{C-CCl}_4$ and/or $^{14}\text{C-CO}_2$ leaked from the culture tubes. A new experiment is planned to examine this possibility.

FIGURE 5. CCl₄ Degradation in Samples Incubated with Hanford Denitrifying Cultures. Data represent the mean of three replicates.

CARBON TETRACHLORIDE TEST CONCLUSIONS

The results of this experiment suggest that denitrifying populations indigenous to Hanford groundwaters have developed the ability to degrade CCl₄. The mechanism for degradation has not been determined; however, the results of other researchers indicate that CCl₄ is hydrolyzed to CO₂ by the bacteria (Bouwer and McCarty 1983).

FIGURE 5. Cell degradation in 24 hr. incubated with 100% oxygen. Cultures: Data represent the mean of three replicates.

CELL DEGRADATION TEST CONDITIONS

The results of this experiment suggest that the ability of cells to degrade independent of nutrient gradients has developed the ability to degrade. The mechanism for degradation has not been determined; however, the results of other research indicate that CCl₄ is hydrolyzed to CO₂ by the bacteria (Barnett and McCarty, 1971).

BENCH-SCALE KINETIC TESTS

OBJECTIVES OF KINETIC TESTS

The primary objectives of the bench-scale biodenitrification kinetic tests were to determine denitrification rate constants and to observe the effect of sludge age on nitrate degradation. The goal was to operate continuous flow bioreactors to obtain rate data for identification of an applicable kinetic model. Kinetic constants would then be calculated and used to determine operating conditions for the pilot-scale biodenitrification system.

DESCRIPTION OF KINETIC TESTS

Four continuous-flow non-recycle bioreactors (one 5-L and three 8-L) were operated at 5-, 8-, 12-, and 20-day residence times to obtain substrate utilization kinetics. Because the reactors were operated without solids recycle, the solids residence time through the reactor is equivalent to the hydraulic residence time. The reactors were operated for a minimum of 65 days to ensure that a steady state was achieved for each reactor during the test period. Steady-state was identified when the suspended solids, nitrate, and acetate concentrations stabilized to within $\pm 20\%$ of the mean value over a five- to ten-day period. The bioreactors used magnetic stirrers, peristaltic feed pumps, and stoppered covers during operation. The feed solution consisted of simulated groundwater (Grande Ronde Number 4) to which was added sodium nitrate to raise the concentration of nitrate to 1500 mg/L. Acetic acid was added as the carbon source. The bioreactors were inoculated with the culture of microorganisms indigenous to the Hanford groundwater that were obtained from laboratory testing.

To monitor reactor performance and obtain the necessary data for kinetic rate determination, samples were removed daily from each of the reactors and the feed stream. A minimum of three samples per week were analyzed for chemical oxygen demand (COD), acetate, nitrate and nitrite, total suspended solids (MLSS), and volatile suspended solids (MLVSS). Temperature, pH, and feed flowrate were also recorded daily.

RESULTS OF KINETIC TESTS

The steady-state concentrations obtained for each of the four continuous reactors are shown in Table 3. An equivalent nitrate concentration was calculated to account for nitrate partially reduced to nitrite. The equivalent concentration was used in all kinetic calculations. The microbial growth and substrate utilization kinetics were first assumed to follow the Monod model with acetate as the limiting substrate. Although a linear relationship was obtained, a negative intercept was calculated. Negative intercepts result in negative values for both the rate constant and the endogenous decay constant. This indicates that the Monod model does not adequately describe the reaction kinetics. Various kinetic expressions including first- and second-order

TABLE 3. Steady-State Operating Conditions

Reactor Residence Time (Days)	5	8	12	20
<u>Inlet Concentrations (mg/L)</u>				
COD	1310	1310	1310	1340
Acetate	1650	1650	1520	1500
Nitrate (NO_3^- -N)	329	329	344	374
<u>Outlet Concentrations (mg/L)</u>				
COD	556	361	351	369
Acetate	722	462	398	297
Nitrate (NO_3^- -N)	158	42	72	72
Nitrite (NO_2^- -N)	43	10	0	29
MLSS(a)	93	97	89	46
MLVSS(b)	44	55	48	37
<u>Calculated Values</u>				
Total Equivalent NO_3^- -N (mg/L)	184	48	72	89
C/N (mg/mg)	2.62	1.73	1.69	1.72
% Volatile Solids	47	57	54	80

(a) Biomass concentration - as-mixed liquor suspended solids (total biomass).

(b) Biomass concentration - as-mixed liquor volatile suspended solids (estimate of active biomass).

models were also evaluated with both acetate and nitrate as the limiting substrate. The first order expression shown in Equation (4), with acetate as the limiting substrate, most accurately described the bench-scale data. Equations (6) and (7) identify the nitrate and biomass balances that were used with Equation (1) to generate linear equations for determining the rate constant k , the biomass yield and decay constants Y_B and K_d , and the nitrate yield Y_N .

$$dN/dt = Y_N (-dS/dt) = Y_N (k \times S) \quad (6)$$

$$dX/dt = Y_B (k \times S) - K_d X \quad (7)$$

Material balance expressions were developed for acetate and biomass as shown in Equations (8) and (9), respectively. Linear regressions of the data using the material balance expressions are shown in Figures 6 and 7. Kinetic

FIGURE 6. Substrate Removal Efficiency ($S_o/S - 1$) as a Function of Biomass Concentration and Residence Time ($X \tau$) (substrate material balance used to calculate reaction rate constant k)

FIGURE 7. Substrate Yield $((S_0 - S)/X)$ as a Function of Residence Time (τ) (microorganism material balance used to calculate decay coefficient and biomass yield)

constants and coefficients k , Y_B , and K_d were obtained from the slopes and intercepts of these plots and are shown in Table 4. The nitrate yield (removal) coefficient was calculated from the inlet and outlet acetate and nitrate concentrations. The nitrate yield coefficient is inversely proportional to the C/N ratio.

$$S_0/S - 1 = k \times \tau \quad (8)$$

$$(S_0 - S)/X = K_d/Y_B \tau + 1/Y_B \quad (9)$$

where S_0 = acetate feed concentration, mg/L
 τ = residence time, days
 X = biomass concentration.

In addition to developing the kinetic expression adequate for modelling the denitrification reaction, estimates of the optimum C/N ratio and sludge

TABLE 4. Calculated Kinetic Constants

Constant	Value
k (mg MLVSS-day/L) ⁻¹	0.0051
K_d (day) ⁻¹	0.050
Y_B (mg MLVSS/mg acetate)	0.064
Y_N (mg (NO ₃ ⁻ -N)/mg acetate)	0.217

age were obtained. As shown in Table 3, the C/N ratio ranged from 1.69 to 2.62. The minimum C/N ratio was obtained at a sludge age of 12 days. The pilot-scale bioreactor should be operated at a sludge age of 8 days or greater to maximize nitrate metabolism and minimize the acetate required. The reactors were operated between 26°C and 35°C at a pH of 6.8 to 7.8. The optimum temperature and pH were not evaluated; however, results from other denitrification operations indicate that optimum denitrification occurs near 29°C and a pH of 7.5 (Knowles 1982).

Results of the bench-scale kinetic tests were used to set preliminary operating conditions for a pilot-scale bioreactor system. However, the presence of residual nitrites and low percentage of volatile suspended solids (<80%) in several of the bench-scale bioreactors indicates that culture conditions may not have been at an optimum for microbial growth and metabolism. The addition of more than 300 mg/L of NO₃⁻-N (2 g/L sodium nitrate) to the simulated groundwater may have elevated the total sodium concentration to an inhibitory level. Therefore, denitrification tests will be continued in FY 1989 along with bench-scale tests to confirm CCl₄ degradation.

TABLE 3. Calculated Kinetic Constants

Constant	Value
k (mg N/gSS-day) $^{-1}$	0.0021
k_d (day) $^{-1}$	0.080
Y_0 (mg N/gSS-mg acetate) $^{-1}$	0.084
Y_1 (mg N/gSS-mg acetate) $^{-1}$	0.217

age were obtained. As shown in Table 3, the C/N ratio ranged from 11.65 to 13.62. The minimum C/N ratio was obtained at a sludge age of 12 days. The pilot-scale digester should be operated at a sludge age of 8 days or greater to maximize nitrate reduction and minimize the acetate produced. The reactors were operated between 28°C and 35°C at a pH of 6.8 to 7.0. The optimum temperature and pH were not evaluated; however, results from other denitrification operations indicate that optimum denitrification occurs near 30°C and a pH of 7.5 (Knowles 1982).

Results of the bench-scale kinetic tests were used to set preliminary operating conditions for a pilot-scale bio reactor system. However, the range of residual nitrate and low percentage of volatile suspended solids (<30%) in several of the bench scale digesters indicated that denitrification may not have been at an optimum for microbial growth and nitrification. The addition of more than 300 mg/L of NO₃-N (2 g/L sodium nitrate) to the simulated groundwater may have elevated the total sodium concentration to an inhibitory level. Therefore, denitrification tests will be continued in FY-1983 along with bench-scale tests to confirm C/N concentration.

PILOT-SCALE BIOREACTOR TESTS

A pilot-scale bioreactor system was designed, constructed, and installed at the 324 Building in the 300 Area in FY 1988. The pilot plant was subsequently tested and operated with a microorganism culture developed in laboratory experiments. Although the bioreactor has not been operated long enough to reach steady state, and hence allow us to determine system capacities, denitrification to less than drinking water standards was demonstrated with a 220-ppm nitrate feed at a 1 L/h feed rate.

OBJECTIVES OF PILOT-SCALE TESTS

The primary objective of pilot-scale biotechnology testing is to demonstrate the feasibility of using denitrifying bacteria for the destruction of carbon tetrachloride at a scale large enough to develop engineering data for a full-scale system evaluation. This task is being accomplished through a two-year design and testing effort that began in FY 1988. Specific goals for FY 1988 were to design, construct, install, and begin operating a pilot-scale biotreatment process. These goals were successfully accomplished as described below.

Goals for FY 1989 and FY 1990 will be to determine operating conditions and kinetics associated with simultaneous denitrification and destruction of organics using the pilot-scale testing facility. The objective will be to provide sufficient baseline data to support the design of a full-scale biotreatment process that can be used to denitrify and remove various organics, including carbon tetrachloride, from contaminated Hanford groundwaters and process effluents.

The FY 1988 pilot-scale demonstration effort consisted of three major technical tasks: 1) bioreactor and pilot-plant design, 2) pilot-plant fabrication and installation, and 3) pilot-plant testing.

Task 1. Bioreactor and Pilot-Plant Design

A continuously operated, 50-L stirred tank reactor was selected as a design basis for the pilot-scale process. This reactor configuration has the inherent advantage of being more immune to process upsets (changes in feed

composition or other operating conditions) than plug flow configurations. Another advantage is that the mathematical models needed to describe the global kinetics associated with stirred tank reactors are readily available. These models account for the heterogeneous heat and mass transfer processes that modify the intrinsic reaction kinetics, as determined by bench-scale testing. By choosing a conventional reactor configuration, a major modelling effort to develop full-scale design equations is avoided. The process flow diagram shown in Figure 8 and the flowsheet shown in Table 5 were developed. The bioreactor and clarifier designs and the process instrumentation were developed with the assistance of PNL staff experienced in the design and operation of continuous fermentation systems.

Task 2, Pilot-Plant Fabrication and Installation

Under this task, the pilot-plant equipment was procured and installed. Off-site vendors fabricated and assembled the bioreactor and associated equipment and instruments on a skid-mounted module. A skid-mounted module was selected to facilitate installation and ease final disposal of the equipment. The pilot-plant module was installed in the EDL-101 facility located in the 324 Building.

FIGURE 8. Flow Diagram of Biotenitrification Pilot-Plant

TABLE 5. Biotenitrification Pilot-Plant Flowsheet

BASIS:										
Bioreactor Temperature	T	29	°C							
Acetate rate constant	k	0.00512	L/day • mg MLVSS							
Decay Constant	Kd	0.0503	1/day							
Biomass Yield Coefficient	Yb	0.0641	mg MLVSS/mg Acetate							
Nitrate Destruction Coefficient	Yn	.961	mg NO ₃ /mg Acetate							
Feed Nitrate Concentration	CNO	400	mg/L							
Effluent Nitrate Concentration	CNE	44	mg/L							
Feed Acetate Concentration	CAc	551	mg/L							
Bioreactor Volume	VBR	50	L							
Feed Flowrate	Qf	240	L/day							
Residence Time	Tau	0.21	day							
Settled Biomass Concentration	Xu	3000	mg/L							
Product Biomass Concentration	Xp	1.0	mg/L							
Sludge Age	Theta	112	day							
Stream #	1	2	3	4	5	6	7	8	9	10
Description	Groundwater	Acetic Acid	Feed	NaOH	Effluent	Product	Sludge	Recycle	Waste	Off Gas
MASS FLOWS										
Water (g/day)	239904	2350	242254	846	684751	243288	441805	441592	213	
Nitrate (g/day)	96.0		96.0		29.6	10.7	18.9	18.9	.213	
Acetic Acid (g/day)		134	134							
NaOH (g/day)				34.5						
Biomass (g/day)					1293	.243	1287	1286	.619	
N ₂ (std L/h)										.603
CO ₂ (std L/h)										.969
H ₂ O Vapor (std L/h)										.089
DENSITY (g/mL)	1.00	1.02	1.00	1.02	1.01	1.00	1.03	1.03	1.03	
TOTAL FLOW (L/day)	240	2.44	242	.863	672	243	429	429	.206	39.9
CONCENTRATIONS										
Nitrate (mg/L)	400		396		44.0	44.0	44.0	44.0	44.0	
Acetic Acid (g/L)		55.2	.555		.180					
NaOH (g/L)				40						
Biomass (mg/L)					1925	1.00	3000	3000	3000	
N ₂ (vol %)										36.3
CO ₂ (vol %)										58.3
H ₂ O Vapor (vol %)										5.38

Task 3, Pilot-Plant Testing

Under this task, all testing procedures and methodologies were established and a detailed two-year test plan was developed. The test plan underwent peer review as well as review by the line manager of PNL's Process Development Section, a safety engineer, a quality assurance engineer, and the building manager. Batch start-up of the pilot-plant was successfully accomplished, demonstrating the removal of nitrates from a simulated groundwater. The specific goals of the overall pilot-plant testing task are to:

- Determine the operability of the pilot-scale equipment through shakedown tests with water and then with the mixed biological culture used in microorganism tests.
- Operate the bioreactor in a batch mode to cultivate sufficient quantities of biomass for continuous operation.
- Demonstrate continuous biological denitrification of a simulated nitrate-bearing groundwater through extended operation (Phase I, FY 1988/89).
- Demonstrate continuous biodegradation of simulated nitrate- and CCl_4 -contaminated groundwater through extended operation (Phase II, FY 1989).
- Demonstrate biological treatment of actual groundwater and waste waters (Phase III, FY 1989 or FY 1990).

Test data that will be obtained in FY 1989 include denitrification and organic destruction rates, and identification of optimal bioreactor temperatures, flowrates, and pH.

DESCRIPTION OF PILOT-SCALE EQUIPMENT

The pilot-scale biodenitrification system consists of two waste-water feed tanks; a continuous stirred-tank bioreactor; a biomass settling tank or clarifier; and pressure, temperature, off-gas CO_2 , and pH monitoring and control equipment. Figures 9, 10, and 11 are photographs of the process equipment.

FIGURE 9. Bioreactor and Process Control Instrumentation

FIGURE 10. Bioreactor Feed Tanks

Feed Tanks

Two 490-gal fiberglass-reinforced plastic feed tanks are used to supply nitrate-bearing waste water or simulated groundwater to the bioreactor. The feed tanks are shown in Figure 11. With a design feed flow rate of 240 L/day, each feed tank is capable of supplying 7 days of groundwater to the bioreactor. Having two feed tanks enables the extended continuous operation of the process without requiring a downtime for restocking the feed solutions. Level controllers and indicators are incorporated in the feed tanks 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 2.5-gal polypropylene container.

FIGURE 11. Clarifier

Bioreactor

The 50-L bioreactor is a cylindrical 304L stainless steel vessel 2-ft tall and 14-in. in diameter. The bioreactor can be seen in the center of the process module photograph in Figure 9. To ensure adequate agitation, the bioreactor design incorporates four internal baffles and two flat-blade impellers driven by a 1/8 hp variable-speed motor. An acrylic sight glass is provided in the reactor wall for viewing the suspension culture. Thermocouples and a submersible pH electrode are included for monitoring. External heaters located on the sides of the vessel maintain internal operating temperatures. The reactor is designed for 1- to 2-g/L suspended solids (dry weight of microorganisms) and a residence time of ~5 h. To operate under

anaerobic or anoxic conditions, the reactor is sealed from the ambient air using neoprene gaskets on the vessel cover and an integral rotating seal for the impeller shaft.

Clarifier

The reactor effluent, containing suspended biomass, is fed to a settling vessel (clarifier) to recover the biomass for recycle and to clarify the denitrified simulated groundwater. Figure 11 is a photograph of the clarifier. The clarifier is constructed of 3/8-in. and 1/2-in. clear acrylic sheets and consists of both aeration and settling zones. Air is introduced through porous ceramic diffusion stones into a 13-L aeration zone of the clarifier. In this elevated oxygen environment, the microorganisms shift to aerobic metabolism and consume any residual acetate. In addition, biomass settling is enhanced in aerobic environments where the bacteria tend to agglomerate. The 280-L settling zone consists of a sloped base which funnels the settled biomass to a bottom exit port. A V-notched weir and exit port at the upper liquid level provides an adequate flow of denitrified and clarified effluent while retaining biomass within the clarifier. A recycle pump is incorporated for returning concentrated biomass to the bioreactor. The large volume of the clarifier will allow the system to be operated at flow rates greater than ten times the reactor design values.

pH-Control/Adjust System

To maintain an optimum pH for biomass growth and metabolism, the bioreactor is equipped with a pH-control system. During denitrification, the pH of the reactor contents increases due to the formation of hydroxide (OH^-) ions. Although the acetic acid feed solution may help neutralize excess OH^- , a pH-control system is present to maintain an ideal pH of between 7 and 8. Dilute sulfuric acid or sodium hydroxide can be introduced to neutralize the hydroxide or hydrogen ions, respectively. A pH-adjust tank containing the appropriate solution is mounted for gravity feed through a bellows valve to the bioreactor. The pH probe installed in the reactor is connected to both monitoring and control systems. Deviations from the set point pH open the pH adjust valve and result in neutralization of excess OH^- or H^+ . An oversized

spill container filled with calcium carbonate is located below the pH-adjust tank and valves to contain and neutralize acid or base in the event of a spill or leak.

Process Control

Operation of the biodenitrification process is conducted from four main control points: a feed flow controller, a recycle flow controller, and the bioreactor pH controller. The flow controllers are of the proportional-integral-derivative (PID) type and are located in the process instrument cabinet as shown in Figure 9. Flow rates are maintained by feedback from various flow monitoring devices. The bioreactor pH is maintained by an on-off controller built into a Chemtrix Model 45A pH meter. The pH set point is adjusted at the front panel of the pH meter. The bioreactor system is designed to run unattended during evenings and over weekends. Control valves are the "fail-close" type and will terminate feed flow if power is interrupted. In addition, a solenoid-operated "fail-open" valve will stop feed flow if the bioreactor high-level sensor is tripped.

Data Acquisition

All process temperatures, flows, bioreactor pH, and the off-gas CO₂ concentration are monitored and recorded electronically for real-time display and data analysis. The data acquisition system consists of a Hewlett Packard model 3497A data logger and a Macintosh® SE computer with an internal 20-megabyte hard disk. The sampling frequency, monitoring points, data display, and data recording are controlled by the computer using National Instruments Labview® software. Labview provides the operator with a graphically simulated instrument panel on the computer screen that allows the operator to visually monitor the process and specify the data display update and data-storing frequencies. No process control is performed from the

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® Labview is a registered trademark of National Instruments, Austin, Texas.

computer or the HP data logger as the controllers are "stand-alone." Data stored on the hard disk will be available for analysis using other software at the end of each experiment.

PILOT-SCALE TEST METHODOLOGY

The primary factors that affect denitrification kinetics include temperature, pH, hydraulic residence time, solids detention time, biomass concentration, and substrate concentrations. Small changes in any of these operating parameters can greatly influence effluent and biomass concentrations, settling characteristics, and reactor stability. Because of the inherently slow response of biological systems, the effect of changes to operating conditions can go undetected for long periods of time. To successfully demonstrate steady-state, continuous denitrification operating parameters such as temperature, pH, and residence time, substrate concentrations must be maintained at constant levels for extended periods. The Phase I tests will focus on the demonstration and operation of the pilot-scale bio-denitrification system for extended process times, with occasional minor modifications to the operating conditions. These experiments will be conducted to determine the operating range of the process.

The data acquisition system (DAS) will be used to record temperature and pH levels at 30-min intervals. In addition to temperature and pH measurements, the reactor contents will be sampled periodically to determine biomass, nitrate, acetate, and total organic carbon (TOC) or COD concentrations. Sampling ports are also incorporated at various locations in the piping between equipment for diagnostic purposes. Biomass concentrations will be measured as volatile suspended solids using standard analytical methods. Nitrate levels will be determined spectrophotometrically using a standard cadmium reduction method, and periodically confirmed using ion chromatography. Acetate levels will be measured using gas chromatography. An in-line off-gas analyzer will monitor CO₂ concentrations from the reactor exhaust to provide real-time determination of reactor performance. Periodic samples of the feed, the reactor effluent, the clarifier effluent, and the sludge recycle streams are required for analysis of concentrations and determination of denitrification efficiencies and reaction kinetics.

FY 1988 Tests Completed

Pilot-plant shakedown tests were conducted with water before actual operation with microorganisms to calibrate instruments, check for leaks, and test equipment operation. During the initial filling with water, the clarifier vessel failed at the slanted face along a glued seam. The clarifier was repaired by replacing the side with thicker (3/5" versus 3/8") material. Stiffening ribs were also added. During this time, extra structural support for the feed tanks was also attached to the module framework.

The bioreactor was charged with simulated U1/U2 groundwater and 4 L of a microorganism culture, and then started in batch mode. When the effluent nitrate level reached 44 ppm, continuous feed to the bioreactor was initiated. After several days operation at a feed rate of 1 to 2 L/h with no recycle, the reactor effluent nitrate concentration dropped to 4 ppm, well below the 44-ppm drinking water standard. As of this writing, the feed flow rate has been increased to 4 L/h, the clarifier is filling and recycle from the clarifier to increase the reactor biomass concentration has not yet been initiated. FY 1989 testing will bring the system to equilibrium at prototypic conditions and will determine reasonable recycle rates and other system parameters. Later tests will then demonstrate nitrate and CCl_4 degradation over an extended period.

Problems/Resolution

Most of the bioreactor operational problems seen so far have been equipment related. Modifications will be needed to the feed flow control system and reactor temperature controller. The feed flow meter (a simple tube-delta pressure arrangement) has an unacceptably high noise-to-signal ratio and will be replaced with another flowmeter. A proportional temperature control system will be installed on the bioreactor to better control temperature fluctuations. In addition, the off-gas flow and CO_2 monitoring equipment have yet to be tested. The off-gas flow rate and CO_2 level are expected to be important process control parameters.

Prior plant shutdown tests were conducted with water before actual operation with microorganisms to calibrate instruments, check for leaks, and test equipment operation. During the initial filling with water, the air-vent vessel fitted at the slanted face along a glass knee. The chlorine was removed by replacing the side with filter (50% versus 50% water). After filling this was also about. During this time, extra structural support for the tank was also attached to the whole frame work.

The reactor was charged with sterilized 10% ammonium and 4% of a microorganism culture, and then started in batch mode. When the nutrient level reached 40%, continuous feed to the reactor was initiated. After several days operation at a feed rate of 1 to 2 g/h with no recycle, the reactor effluent nitrate concentration dropped to 4 ppm, well below the 40 ppm drinking water standard. As of this writing, the feed flow rate has been increased to 4 g/h, the chlorine is fitting and recycle flow the chlorine to increase the reactor nitrate concentration and not yet been initiated. By 1988 testing will bring the system to equilibrium at process conditions and will determine reasonable recycle rates and other system parameters. Later tests will then demonstrate nitrate and EC10 degradation over an extended period.

Problems Encountered

Most of the reactor operational problems seen so far have been engineering related. Modifications will be needed to the feed flow control system and reaction temperature control. The feed flow meter is a simple differential pressure (differential) has an unacceptably high noise-to-signal ratio and will be replaced with another flowmeter. A proportional temperature control system will be installed on the nitrogen to better control temperature fluctuations. In addition, the off-gas flow and CO₂ monitoring equipment have yet to be tested. The off-gas flow rate and CO₂ level are expected to be important process control parameters.

UO₃ PLANT CONDENSATE TESTS

The UO₃ Plant process condensate contains the combined overheads streams from three uranyl nitrate (UNH) concentrators. The process condensate contains nitrate in concentrations of 10² to 10⁴ ppm with low levels of fluoride, uranium, and other metals. The present disposal process involves addition of phosphoric acid and neutralization to decrease the uranium soil mobility and subsequent disposal to the 216-U-17 crib. The objective of the denitrification study was to evaluate the use of biological technology for nitrate destruction in the process condensate stream.

LABORATORY DENITRIFICATION RESULTS

Three samples of the UO₃ Plant condensate were obtained during testing of a uranium treatment technology test conducted by Westinghouse Hanford Company (WHC) in February 1988. These samples were used in batch culture biodenitrification studies to determine if biodenitrification is a feasible mechanism for nitrate reduction and to determine the rate and extent of biological denitrification. Three condensates were used: condensate neutralized and treated for U removal (sample no. N-1), condensate neutralized but not treated for U removal (sample no. N-3); and condensate unneutralized and untreated (sample no. Pre-3).

Each plant process condensate was divided into two batches. One batch was amended with inorganic and organic nutrients (i.e., acetate); the second batch did not receive nutrients. The condensates were boiled under N₂ to remove O₂, transferred into an anaerobic chamber, and dispensed (10 mL) into 20-mL septa vials. Acetylene (1 mL) was injected into each vial to inhibit nitrous oxide reductase and allow for an accumulation of measurable N₂O. The vials were inoculated with denitrifying bacteria and incubated at room temperature. Denitrification was assayed by monitoring N₂O production using a gas chromatograph equipped with an electron capture detector. Similarly treated vials without inoculation served as controls to monitor abiotic denitrification.

Denitrification was observed in all the condensates amended with nutrients and inoculated with denitrifying bacteria (Figure 12), and was not observed in either the unamended or uninoculated samples. This observation suggests that biological denitrification of UO₃ Plant process condensate is feasible; however, nutrient addition will be required. Further studies are necessary to determine the optimum conditions (i.e., nutrient addition) for nitrate removal.

The extent and rate of denitrification varied between the three condensates (Table 6). Condensate N-1 exhibited a 42% reduction in NO₃⁻ while N-3 and Pre-3 exhibited a 3.2% and 5.6% reduction in nitrate, respectively. Based on the molar concentration of organic carbon (i.e., acetate) added to each vial (2.44×10^{-5} moles), approximately 3.05×10^{-5} moles of NO₃⁻ should have been removed from the vials (based on 0.8-mole organic carbon needed

FIGURE 12. Test 1: Laboratory Denitrification Rate in Moles of Nitrogen Produced per Hour from a 10-mL Sample

TABLE 6. Biological Denitrification of Process Condensate
for a 10-mL Sample Diluted 10:1 with Water

Condensate	Init. NO_3^- (moles)	Final NO_3^- (moles)	Consumed NO_3^- (moles)	Biomass Conc. (g/mL)	Rate Const. k (g NO_3^- per g biomass $\cdot$ h)
N-1	1.31×10^{-4}	7.58×10^{-5}	5.52×10^{-5}	3.50×10^{-4}	8.50×10^{-3}
N-3	3.02×10^{-4}	2.92×10^{-4}	1.00×10^{-5}	3.50×10^{-4}	4.60×10^{-3}
Pre-3	2.58×10^{-4}	2.44×10^{-4}	1.40×10^{-5}	2.58×10^{-4}	2.10×10^{-3}

for each mole NO_3^-). Rate constants calculated from the denitrification data varied from 2×10^{-3} to 9×10^{-3} g NO_3^- destroyed per g biomass per hour. Additional tests were performed to verify the N_2O test results.

A second laboratory batch culture experiment was performed to verify the denitrification rates. The results of the second experiment indicated that the denitrification rate is dependent on the biomass concentration and that the rate constant is independent of the solution type.

In the second experiment, the denitrification rate was measured by the decrease in nitrate in aqueous solution. Increased acetate concentration provided sufficient carbon source for complete denitrification and the biomass concentration of each sample was measured to give a better estimate of the denitrification rate constant. Figure 13 shows the denitrification rates in moles nitrate destroyed per hour, for waste condensate solutions N-1 (neutralized and treated for uranium removal), N-3 (neutralized), and PRE-3 (unneutralized).

As in the previous experiment, the N-1 solution showed a larger denitrification rate than the N-3 and Pre-3 solutions. This is clearly shown by the % nitrate removed with time (Figure 14).

The denitrification rate constant was calculated from the denitrification rate (Figure 12) and the measured biomass concentrations. For low nitrate concentrations, the following expression describes the reaction kinetics (first order with respect to the biomass concentration and zero order with respect to nitrate):

FIGURE 13. Test 2: Laboratory Denitrification Rates in Moles of Nitrate Destroyed per Hour from a 10-mL Sample

$$- dS/dt = k X \quad (10)$$

where S = substrate (nitrate) concentration (mg/L)

t = time (h)

k = rate constant (g nitrate/g biomass $\cdot$ h)

X = biomass concentration (mg/L).

The calculated denitrification rate constant k averaged 3.0×10^{-3} to 3.3×10^{-3} and did not vary significantly with the nitrate concentration or solution type (N-1, N-3, or PRE-3). The denitrification rate increase with time, evident in Figure 1, is due mainly to the gradual increase in biomass concentration with time and not a change in the rate constant.

The rate constants observed during this study were about a factor of ten below those commonly reported in the literature (Clark et al. 1975; Sundstrom and Klei 1979), but are based on a different biomass measurement technique.

FIGURE 14. Test 2: Percent Nitrate Destroyed per Hour

PNL used a simplified technique which may have given a higher than actual estimate of biomass. In addition, groundwater denitrification rate data were not yet available when this work was conducted. Therefore, the ORNL rate constants were used (Clark et al. 1975) in the flowsheet calculations instead of the measured values.

BIODENITRIFICATION PROCESS CONCEPTUAL DESIGN

Two flowsheets (Figures 15 and 16) were prepared and used to estimate flowrates and equipment sizes for nitrate destruction in the UO₃ Plant process condensate stream. Both flowsheets are based on a nitrate feed concentration of 1.6×10^4 ppm and a process condensate flowrate of 40 L/min. The flowsheets use calcium acetate as the carbon source (substrate) and produce an effluent nitrate concentration of 30 ppm (which is less than the drinking

FIGURE 15. Flow Diagram for UO_3 Process Condensate Continuous Bionitrification

FIGURE 16. Flow Diagram for UO_3 Process Condensate Batch Bionitrification

water quality standard of 42 ppm). Both processes also generate about 100 kg/day of biomass sludge that must be dried and disposed of as low-level solid waste.

The conventional process (shown in Figure 15 and Table 7) uses a continuous-stirred tank bioreactor with a separate clarifier to concentrate and recycle a portion of the biomass. Other configurations are also possible, including using a filter system in place of the clarifier, or using a fluidized-bed, fixed-film bioreactor in place of the stirred-tank bioreactor and clarifier. However, too little information is presently available to make a decision on the optimum configuration.

The bioreactor for this flowsheet is a 200,000 L (55,000 gal) vessel approximately 6 m (20 ft) in diameter and 7.5 m (25 ft) tall. The clarifier, based on an expected clarified biomass concentration of 10 g/L, will be approximately 4000 L (1000 gal) in volume. The bioreactor size is based on ORNL published denitrification rates (Clark et al. 1975) and the solids and hydrodynamic retention times calculated in the flowsheet. The proposed bioreactor is roughly twice the size of the 100,000-L Oak Ridge Y-12 denitrification facility bioreactors (Napier 1976) which process a higher nitrate concentration (15% nitrate), but at a lower flowrate (4 L/min). The Oak Ridge denitrification process produces a nitrate effluent of about 500 ppm and does not use sludge recycle.

A second, somewhat simpler batch process is shown in Figure 16. In the batch process one of two tanks becomes a bioreactor as the other tank is filled with process condensate. The tanks are sized to permit complete denitrification in one tank while the other tank is filling. For the 40 L/min process condensate rate, two 430,000-L (115,000-gal) tanks would be required using the same rate constants as for the conventional flowsheet (Table 8). This is based on an eight-day residence time, including one day for decanting and solution transfer. A 10% sludge heel is left in the tank at the end of each cycle to provide an inoculum for the next batch.

A separate solid/liquid separation device (clarifier or centrifuge) is required to further dry or dewater the biomass from either flowsheet before disposal. Determination of the settling/dewatering characteristics of the biomass sludge is beyond the scope of this study.

The advantage of the batch process is a decrease in complexity from the conventional (continuous) process. One vessel, several pumps, and at least

TABLE 7. Flowsheet for UO₃ Process Condensate Continuous Bionitrification

BASIS:										
Bioreactor Temperature	T	30	°C							
Settled Biomass Conc.	BMC	10	g/L							
Reactor Biomass Conc.	RBMC	3.34	g/L							
Rate Constant	k	0.055	1/h							
Denitration Rate	rd	4.39	g NO ₃ /L-day							
Bioreactor Working Vol	VBR	195000	L							
Nitrate Destruction Rate	NDR	855.47	kg NO ₃ /day							
Nitrate Concentration	CNO ₃	16	g/L							
Recycle Ratio	R	0.30								
Hydraulic Resid. Time	Tau	3.64	day							
Solids Detention Time	Theta	9.12	day							
Decay Constant	Kd	0.002	1/day							
Biomass Yield Coefficient	Y	0.12	g NO ₃ /g biomass							
Effluent Nitrate Concent.	ECNO ₃	0.03	g/L							
Stream #	1	2	3	4	5	6	7	8	9	10
Description	Condensate	Acetate	Feed	H ₂ SO ₄	Effluent	Product	Sludge	Recycle	Waste	Off Gas
MASS FLOWS										
Water (kg/day)	53567		53567	6090	76425.6	50847	26541	16471	10070	308
Nitrate (kg/day)	855		855		2.35	1.54	0.805	0.500	0.305	
Calcium Acetate (kg/day)		681	681							
H ₂ SO ₄ (kg/day)				677						
SO ₄ (kg/day)					842	553	289	179	110	
Biomass (kg/day)					268		268	167	102	
N ₂ (std L/h)										6.06
CO ₂ (std L/h)										9.80
H ₂ O Vapor (std L/h)										0.68
DENSITY (g/mL)	1.000	0.791	0.997	1.068	1.000	1.000	1.010	1.010	1.010	
TOTAL FLOW (L/day x 1000)	54.42	0.86	55.25	6.34	78.23	51.40	26.83	16.65	10.18	0.40
CONCENTRATIONS										
Nitrate (g/L)	16.00		16.00		0.030	0.030	0.030	0.030	0.030	
Calcium Acetate (g/L)		791.00	12.33							
H ₂ SO ₄ (g/L)				106.80						
SO ₄ (g/L)					10.77	10.77	10.77	10.77	10.77	
Biomass (g/L)					3.34		10.00	10.00	10.00	
N ₂ (vol %)										36.64
CO ₂ (vol %)										59.27
H ₂ O Vapor (vol %)										4.09

TABLE 8. Flowsheet for UO₃ Process Condensate Batch Biotenitrication

BASIS:							
Bioreactor Temperature	T	30	°C				
Initial Reactor Biomass Conc.	IRBC	1	g/L				
Final Reactor Biomass Conc.	FRBC	2.89	g/L				
Rate Constant	k	1.313	1/day				
Bioreactor Working Vol	VBR	425797	L/reactor				
Initial Nitrate Concentration	CNO ₃	16	g/L				
Reaction Time	RT	6.82	day				
Down Time	Td	1	day				
Decay Constant	Kd	0.002	1/day				
Biomass Yield Coefficient	Y	0.12	g NO ₃ /g biomass				
Effluent Nitrate Concent.	ECNO ₃	0.03	g/L				
Stream #	1	2	3	4	5	6	7
Description	Condensate	Acetate	H ₂ SO ₄	Effluent	Product	Sludge	Offgas
MASS FLOWS *							
Water (kg/day)	53549		6199	60613	47059	14010	313
Nitrate (kg/day)	871			1.85	1.43	0.42	
Calcium Acetate (kg/day)		693					
H ₂ SO ₄ (kg/day)			689				
SO ₄ (kg/day)				675	520	155	
Biomass (kg/day)				142		142	
N ₂ (std L/h)							6.17
CO ₂ (std L/h)							9.98
H ₂ O Vapor (std L/h)							0.69
DENSITY (g/mL)	1.000	0.791	1.068	1.000	1.000	1.010	
TOTAL FLOW (L/day x 1000)	54.42	0.88	6.45	61.75	47.58	14.16	0.40
CONCENTRATIONS							
Nitrate (g/L)	16.00			0.030	0.030	0.030	
Calcium Acetate (g/L)		791.00					
H ₂ SO ₄ (g/L)			106.80				
SO ₄ (g/L)				10.93	10.93	10.93	
Biomass (g/L)				2.89		10.00	
N ₂ (vol %)							36.64
CO ₂ (vol %)							59.27
H ₂ O Vapor (vol %)							4.09
* All stream flowrates are averaged over an 8 day reaction cycle							

two flow control loops are eliminated from the conventional process shown in Figure 15. The disadvantage of the batch process is that two large bioreactor tanks are now required instead of one smaller tank. If existing tanks are available, the batch process would likely be a less costly alternative.

COST ESTIMATE

Although no formal cost estimates were prepared for this process, a rough order-of-magnitude cost can be obtained by comparison with other industrial water treatment processes and, where available, with other DOE installations. A cost estimate for operating an anaerobic digester with sludge recycle is \$11 per 4000 liters, when processing 400,000 L/day (DeRenzo 1978). This cost includes 10-year amortized equipment costs with a \$1.3 million capital investment. The referenced facility is larger than the 60,000 L/day continuous system postulated for the UO_3 Plant, but the UO_3 Plant biodenitrification facility would have to be constructed for processing uranium-contaminated waste water, which would increase both the capital and operating costs over a "cold" facility.

Although no cost information is readily available for the Oak Ridge biological processes, installation of two of the four Fernald FMPC fluidized-bed reactors reportedly cost \$8 million with another \$9 million estimated for project completion (Hopper et al. 1987). The FMPC process is designed for a flowrate of 30 gpm with an inlet nitrate level of about 1500 ppm. Costs for a continuous UO_3 biodenitrification process would be less than for the Fernald facility because of the higher flowrate and lower nitrate feed concentrations at the FMPC. Based on this information, a rough estimate for a UO_3 Plant continuous denitrification facility would be between \$1 million and \$5 million. Depending upon the availability of existing tanks, the batch biodenitrification process could be implemented for considerably less than the continuous process.

Conversion of existing tanks would require installation of low-speed agitators, pH control, and, possibly, temperature controls for unheated

tanks. Depending on the modifications required, the batch process could be implemented with a fraction of the capital outlay required for the continuous process.

UO₃ STUDY CONCLUSIONS

The major conclusions of the UO₃ Plant study are that biodenitrification of the UO₃ process condensate stream is feasible and further evaluation of this technology is warranted. The results of laboratory tests indicated that denitrification occurred in all three condensate samples tested. The applicability of biological treatment to the UO₃ process condensate stream was evaluated by preparing a preliminary flowsheet and sizing equipment. A continuous biodenitrification system using a 200,000-L bioreactor would reduce the 40 L/min condensate stream nitrate level to less than drinking water concentrations. Two 430,000-L batch bioreactors would be required to achieve the same denitrification at the same inlet flow. Order of magnitude costs were also developed. For the continuous application, a capital cost of \$1 million to \$5 million was estimated. The cost to retrofit existing tanks for the batch process would be considerably less.

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