ROUGH DRAFT

MASTER

Conf-780316--7

BIOLOGICAL PROCESSES FOR ENVIRONMENTAL CONTROL OF EFFLUENT STREAMS IN THE NUCLEAR FUEL CYCLE*

S. E. Shumate II, C. W. Hancher, G. W. Strandberg
and C. D. Scott
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

For presentation at the "Waste Management and Fuel Cycles '78" Symposium, Tucson, Arizona, March 5-8, 1978.

---

*Research sponsored by the Division of Waste Management Operations, U.S. Department of Energy under contract W-7405-eng-26 with the Union Carbide Corporation.

BIOLOGICAL PROCESSES FOR ENVIRONMENTAL CONTROL OF EFFLUENT STREAMS IN THE NUCLEAR FUEL CYCLE

S. E. Shumate II, C. W. Hancher, G. W. Strandberg
and C. D. Scott
Oak Ridge National Laboratory
Oak Ridge, Tennessee 37830

INTRODUCTION

Many of the processing steps in the nuclear fuel cycle generate aqueous effluent streams bearing contaiminants that can, because of their chemical or radiological properties, pose an environmental hazard. Concentration of such contaminants must be reduced to acceptable levels before the streams can be discharged to the environment. Two classes of contaminants, nitrates and radioactive heavy metals, are addressed in this study. Specific techniques aimed at the removal of nitrates and radioactive heavy metals are being developed, tested and demonstrated.

REMOVAL OF NITRATES

It has been estimated that as much as 2.5 million tons of dissolved nitrogen - bearing substances reach the surface waters of the U.S. each year.[1] The nitrogen waste discharged directly from industrial installation is estimated to be about 20% of the total.[2] Much of this nitrogen pollution is is the form of dissolved nitrates at high concentrations. These high concentrations can contribute to eutrophication[3] and can also constitute a health hazard.[4] Consequently, regorous standards are being established for nitrate released in industrial effluents (e.g., the Department of Energy's Y-12 Plant at Oak Ridge, Tennessee is restricted to 45 g/m^3) and there are indications that these restrictions will become even more stringent. Although the majority of industrial nitrate pollution is attributed to fertilizer and paper manufacturers, liquid effluents from the nuclear fuel cycle contribute significantly to the total problem. Process steps in the uranium fuel cycle that generate nitrate wastes include milling, refining, and conversion, enrichment, fuel fabrication, and fuel reprocessing operations. Disposition of the nitrate (recovery, conversion, or discharge) will be governed by the economics of the process technologies which may be applied. In situations where nitrate recovery is not feasible, conversion of nitrogen oxides to molecular nitrogen (chemically or biochemically) appears to be the only acceptable long-range solution.

Biological Denitrification

Biological denitrification, as referred to in this paper, is the biological reduction of nitrate or nitrite to gaseous molecular nitrogen.[5] It commonly takes place in soil under anaerobic conditions by the various strains of facultative anaerobic bacteria which are responsible for recycling nitrogen compounds back to the atmospheric molecular nitrogen pool.[6] The reaction requires a carbon source which has been successfully supplied in the form of various alcohols and acetates. The rate of denitrification is dependent upon the type of carbon substrate supplied as well as upon other operating parameters such as the pH and temperature of the system. With ethanol as the carbon source, the reaction may be written in unbalanced form as:

$$3NO_3^- + 2C_2H_5OH \rightarrow CO_2 + N_2 + H_2O + OH^- + X\ C_5H_7O_2N$$

The coefficients on nitrate and ethanol reflect the observation that the ratio of carbon consumed to nitrogen (as nitrate) reacted is about 1.3 to 1.5 mole/mole. The composition of the biomass may be approximates as $C_5H_7O_2N$. The biomass yield is roughly 0.1 g/g of nitrate consumed.

Process Development Studies

The biological denitrification processes to date have been shown to be viable in a relatively narrow range of applications. Processes using low efficiency but simple and straightforward continuous stirred-tank bioreactors have been used effectively. These are very large, single-stage devices requiring many hours or days residence time to achieve adequate denitrification. Further, if the bacterial population is destroyed, as much as three weeks is required to reactivate the system. Thus, an expensive standby system would always be required.

Recent studies have indicated that efficient, multistage fluidized-bed systems could be quite effective in a number of industrial wastewater treatment applications.[7] At the Oak Ridge National Laboratory, high-rate denitrification processes are being developed which utilize denitrifying bacteria adhering to particles of anthracite coal. The particles, with adhering bacteria, are fluidized by flow of the aqueous stream being treated as it passes upward through a columnar bioreactor. Two types of column geometries have been studied: tapered (inverted, truncated cone) and cylindrical with a tapered top section. The tapered geometry permits operation over a greater range of flow rates than with a cylindrical geometry alone. One of the fluidized-bed bioreactors recently tested is shown in Fig. 1. The reactor consists of a cylindrical section of 5.1-cm diameter by 3.7 meters in length beneath a tapered 5.1-cm to 7.6-cm diameter by 0.6 meter long solids disengaging zone. Sampling ports were located at 0.6 meter intervals. After a sufficient population of denitrifying bacteria was established on a bed of (-30 +60) mesh anthracite coal, the reactor performance was tested using an ammonium nitrate feed with ethanol as a carbon source. The feed carbon/nitrate nitrogen ratio (C/N) was maintained between 1.8 and 2.0. The C/N utilized was about 1.5.

When operated at a nitrate feed concentration of 200 ± 50 mg/ℓ and a flow rate of 0.94 ℓ/min, the denitrification rate was 7.3 g $N(NO_3)$/day-liter. The concentration profiles of nitrate, nitrite, and carbon (as ethanol) are shown in Fig. 2.

Fluidized-bed systems studied thusfar have ranged in capacity from 2 to 24 liters. Feed nitrate concentrations ranging from 200 to 7500 g/m^3 have been treated, producing effluent concentrations in some cases of less than 1 g/m^3. Denitrification rates as high as 75 g/day-liter have been achieved. It has also been shown that irradiation rates of greater than 10^4 R/hr are required before denitrification rates are significantly reduced. Mobile pilot plant units are now being designed for process demonstration at both Department of Energy and commercial nuclear processing sites.

REMOVAL OF RADIOACTIVE HEAVY METALS

Many operations in Department of Energy and commercial nuclear processing facilities generate aqueous waste streams which contain trace quantities of dissolved heavy metals, including radionuclides. There are a number of physical and/or chemical methods for isolating heavy metals from aqueous streams including chemical precipitation, chemical oxidation or reduction, ion exchange, filtration or ultrafiltration, electrochemical treatment, and evaporative recovery. However, when the initial heavy metal concentration is in the range of 10-100 g/m^3 and a reduction to less than 1 g/m^3 must be achieved, such methods may be ineffective or noneconomic. In such situations, the adsorption of dissolved metal species by microorganisms offers a means of achieving a reduction in dissolved metal concentration to less than 1 g/m^3.

Biological Metal Removal

Studies of metal sorption by microorganisms in both mixed culture and monocultures have shown very high affinities for trace levels of dissolved metals. In some cases, solid phase (biomass) metal concentrations of 10 to 20 weight percent were attained.[8]

Batch and continuous culture methods are being used to study the growth of bacterial, yeast, fungal, and algal strains for utility in trace metal isolation processes. Equilibrium metal isolation tests were conducted using six strains of microorganisms and uranium as the dissolved metal element of interest. The cells were grown to the desired cell concentration, harvested, washed repeatedly with distilled water, and resuspended in solutions containing the desired concentrations of uranium. Flasks were pretreated with Siliclad® to prevent sorption of uranium by glass surfaces. Samples were periodically withdrawn and the cells were removed by centrifugation or by filtration in the case of the Penicillium chrysogenum. The supernatant (cell-free) fraction was then analyzed for uranium content. Controls were run in each case to assure that metals removed from solution were associated with biomass. The results of these preliminary tests are given in Table I. Metal distribution coefficients were calculated on the basis that metal removed from solution was sorbed onto biomass.

In designing a bioreactor/contactor system for the removal of dissolved heavy metals by sorption onto biomass, both equilibrium and kinetic data are important. The equilibrium information determines the amount of biomass required to effect the removal of a given amount of heavy metal; however, the kinetic information determines the contacting time required between the sorbent and the solution, thus affecting the size of the contacting equipment.

An experimental protocol was developed for studying the kinetics and equilibria of uranium accumulation by biomass. On the basis of preliminary screening tests, Saccharomyces cerevisiae and Pseudomonas aeruginosa were

Table I. Isolation of uranium from solution by microorganisms during resuspended contact. Initial uranium concentration was 20 mg/liter. Temperature was 25°C.

Microorganism	Removal (%)	Cell concentration (dry basis) (g/liter)	Metal distribution[a] coefficient
Pseudomonas aeruginosa (Bacterium)	92	1.2	9,600
Zoogloea ramigera (Bacterium)	72	1.0	2,730
Paecilomyces marquandii (Fungus)	94	1.2	13,100
Penicillium chrysogenum (Fungus)	97	3.1	10,300
Ashbya gossypii (Yeast)	73	7.0	390
Saccharomyces cerevisiae (Yeast)	95	1.3	15,000

[a] Metal distribution coefficient = $\frac{\text{g metal/g cells (dry)}}{\text{g metal/g solvent}}$.

chosen as the first microorganisms for study. The cells were grown to the desired cell concentration, harvested, washed three times with distilled water and resuspended in solution containing the desired concentration of uranium. The flasks were pretreated with Siliclad®. The flasks were agitated by shaking at a desired speed and were maintained at a constant temperature. At specified times, samples were withdrawn and the cells were removed by centrifugation.

The supernatant (cell-free) fraction was then analyzed for uranium content. The tests were run at 25 and 40°C. Initial uranium concentrations ranged from 4 to 85 g/m^3. The rate of uranium uptake was temperature dependent at the higher initial uranium concentrations (40 to 85 g/m^3), wich the rate being considerably higher at 40°C.

Figure 3 shows the removal of uranium from solution by washed, resuspended cells of *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* as a function of time at 40°C. Note that the removal of uranium by *Pseudomonas aeruginosa* was extremely rapid. The uranium concentration was reduced from 83 to less than 1 g/m^3 in 20 min. In this experiment, a distribution coefficient of 65,000 was achieved.

From a process utility viewpoint, the rate of approach to an equilibrium distribution between solution and cells appears quite promising. A bench-scale process sytem has been designed and fabricated to test this concept for the removal of radioactive heavy metals from aqueous process streams.

REFERENCES

1. L. Landner, Proceedings of the Conference on Nitrogen as a Water Pollutant, Vol. 1, LAWPR Specialized Conference, Copenhagen, Denmark (1975).

2. D. Jankins et al., Water Research 7, 265 (1973).

3. J. B. Lackey, Sewage Ind. Wastes 30(11), 1411 (1958).

4. C. E. Adams, Jr., Environmental Sci. Technol. 7(8), 696 (1973).

5. Glossary of Soil Science Terms, Soil Sci. Soc. Amer., Madison, Wisconsin, 34 (1973).

6. M. Alexander, Introduction to Soil Microbiology, John Wiley and Sons, New York, 1961.

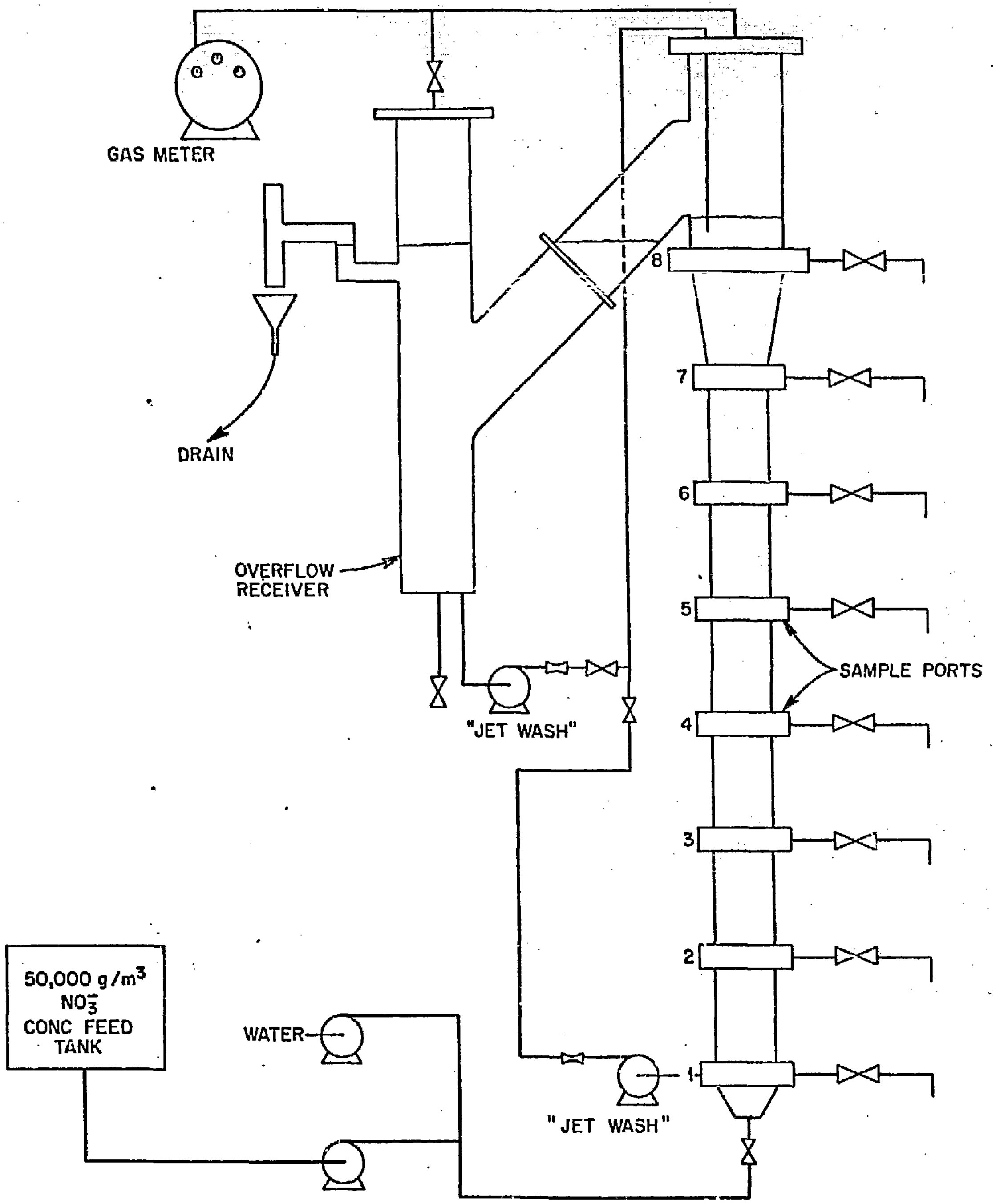
7. C. D. Scott, C. W. Hancher, and S. E. Shumate II, "A Tapered Fluidized-Bed as a Bioreactor," Proceedings of the 3rd International Conference on Enzyme Engineering, Plenum Publishing Company, New York, 1977.

8. R. D. Neufeld, J. Gutierrey, and R. A. Novak, Journal of the Water Pollution Control Federation 49, 489 (1977).

ORNL DWG. 77-1495

Figure 1. Fluidized-bed bioreactor system used in denitrification tests.

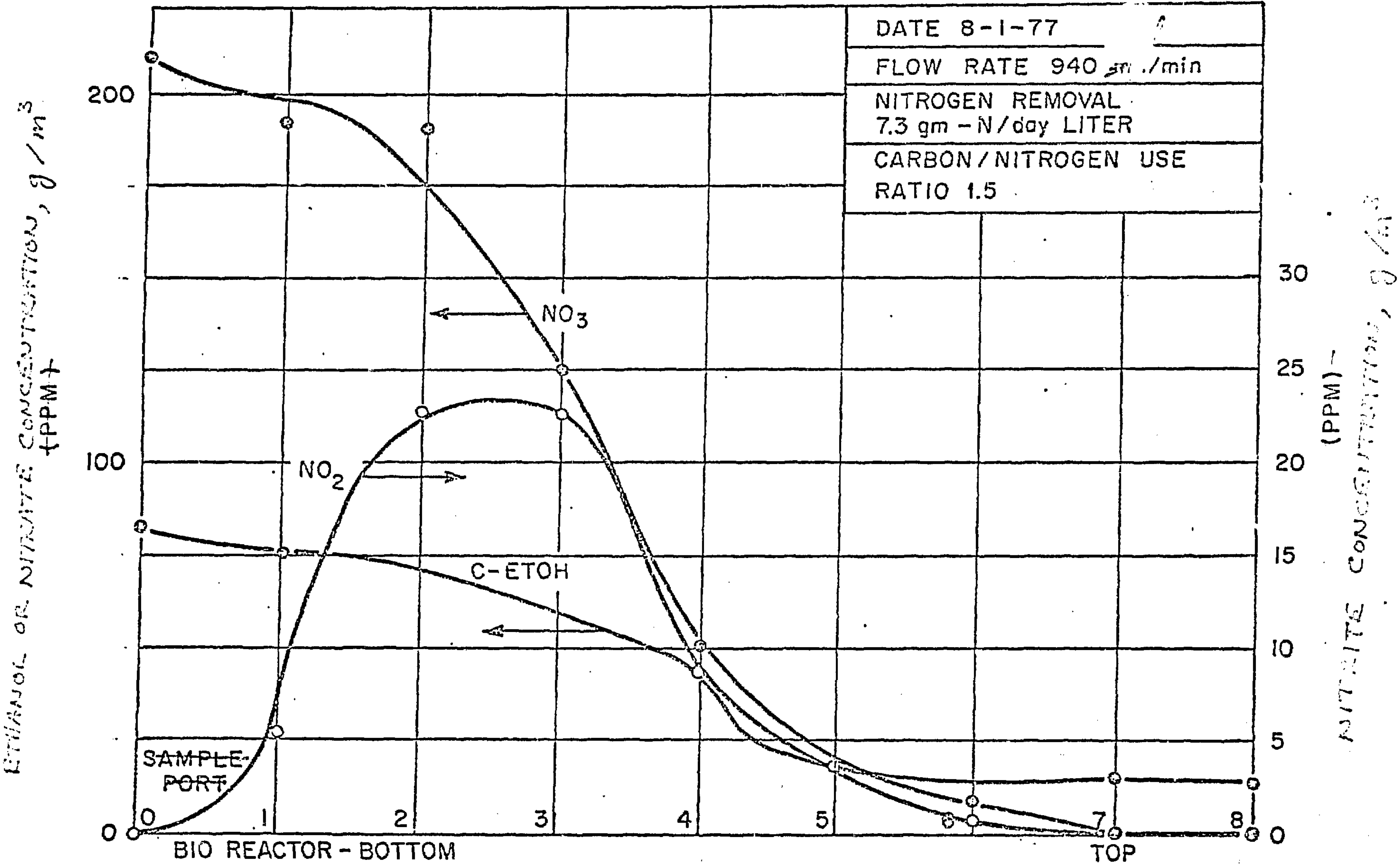
ORNL DWG 77-1495



ORNL DWG 77-1890R1
(with revisions)
see attached

Figure 2. Concentration profiles of nitrate, nitrite, and ethanol during denitrification test.

ORNL-DWG 77-1890R1



~~DENITRIFICATION TEST AT 213 g/m^3 NITRATE CONC.~~

SAMPLE PORT NUMBER

(attached drawing)

Figure 3. Removal of uranium from aqueous solution by washed resuspended cells of *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*

FROM [illegible] GLO[illegible]

[illegible] 1.5 mm Hg