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OPERATION OF A FLUIDIZED-BED BIOREACTOR FOR DENITRIFICATION*

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ABSTRACT

Denitrification, which is the biological conversion of nitrate ions to nitrogen gas and carbon dioxide, is an economically and environmentally sound treatment method for nitrate-containing industrial wastes.

Many commercial processes yield nitrate-containing wastewaters, which are currently discarded because traditional treatment methods are economically unacceptable. However, the anticipated discharge limits (10 to 20 ppm NO_3^-) being considered by many states will not allow the continued release of these wastewaters.

At the Oak Ridge National Laboratory, we have developed a fluidized-bed bioreactor process for denitrification of wastewaters with NO_3^- contents up to 10,000 ppm; streams with higher nitrate levels can be successfully treated after appropriate dilution. This program is primarily concerned with nitrate-containing streams generated by the nuclear fuel cycle but is also applicable to nitrate-containing wastewaters resulting from other sources. The basic concept of the process is that denitrification bacteria are allowed to grow and attach themselves to 30/60 mesh coal particles, thus forming a stable-bacterial-population bed through which nitrate-containing wastewater can be pumped. The bacteria also need a carbon source, such as ethanol, and micronutrients for successful growth.

We will report operating and scale-up data obtained from 5- and 10-cm-ID bioreactors as tall as 7 m under a variety of conditions.

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SUMMARY

Two denitrification fluidized-bed bioreactors of the same length (i.e., 5 m) but with different inside diameters (i.e., 5 and 10 cm) have been operated on feed ranging in nitrate concentration from 200 to 2000 g/m^3; thus far, good agreement has been obtained. Two 10-cm-ID bioreactors operating in series have also been tested; the results are in accordance with predicted results based on the performance of a 5-cm-ID bioreactor. The overall denitrification rate in the dual 10-cm-ID bioreactor system was found to be 23 kg $N(NO_3^-)$/day-m^3 using feed with a nitrate concentration of 1800 g/m^3. Data obtained in operating-temperature tests indicate that the maximum denitrification rate is achieved between 22 and 30°C. These data will form the basis of the design of our mobile pilot plant which consists of dual 20-cm-ID by 7.3-m-long bioreactors.

INTRODUCTION

Many commercial processes such as fertilizer production, paper manufacturing, and metal finishing, as well as the nuclear fuel cycle, yield nitrate-containing wastewaters, which are currently discharged because traditional recovery or disposal methods are economically unacceptable. Nitrate-containing wastewater causes stream eutrophication and is a health hazard. In addition, the anticipated discharge limit (i.e., 10 to 20 ppm NO_3) being considered by many states will not allow the continued release of these wastewaters.

At the Oak Ridge National Laboratory, we have developed a fluidized-bed bioreactor process for denitrification of nitrate wastewaters containing up to 10,000 ppm NO_3; higher levels can be successfully treated after appropriate dilution. This program is primarily concerned with nitrate wastewater streams resulting from the nuclear fuel cycle but is also applicable to nitrate-containing wastewaters arising from other sources. The basic concept of the process is that denitrification bacteria are allowed to grow and attach themselves to 30/60 mesh coal particles, thus forming a stable-bacterial-population bed through which nitrate-containing wastewater can be pumped. The bacteria also need a carbon source, such as ethanol, and micronutrients for successful growth.

We will report a scale-up comparison between 5- and 10-cm-ID by 5-m-tall bioreactor systems. Also, operating data will be presented for a 10-cm-ID bioreactor functioning as a single unit and two 10-cm-ID bioreactor units connected in series. The results from these tests form the basis of the design of our mobile pilot plant which consists of dual 20-cm-ID by 7.3-m-long bioreactors.

MATERIALS AND METHODS

Nitrate waste sources

It has been estimated that up to 2.5 million tons of dissolved nitrogen-bearing substances reach the surface waters of the United States annually.[1] The nitrogen waste discharged directly from industrial installations is estimated to be about 20% of the total, or 500,000 tons/year. Much of this nitrogen pollution is present as dissolved nitrates at high concentrations. The majority of industrial nitrate pollution comes from industrial sources such as metal finishing, fertilizer production, and paper manufacturing. Liquid effluents from the nuclear fuel cycle (Fig. 1) also contribute significantly ($\sim$ 5000 tons/year) to the total nitrate pollution problem.

Many aqueous nitrate waste streams are generated by the nuclear fuel cycle (see Fig. 2). 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, the reduction of nitrous oxides to nitrogen gas (chemically or biologically) appears to be the only acceptable long-range solution.

We recognize that attempts have been made to evade the liquid waste nitrate problem by volatilization into a gaseous effluent. This practice, although expedient in the short term, will probably not meet EPA restrictions in the future. Thus it appears that, in cases where nitrate cannot be economically recovered, biological denitrification is the preferred choice of processes because the end-product nitrogen gas, carbon dioxide, and biomass are ecologically acceptable; in addition, it is more economical than chemical reduction.

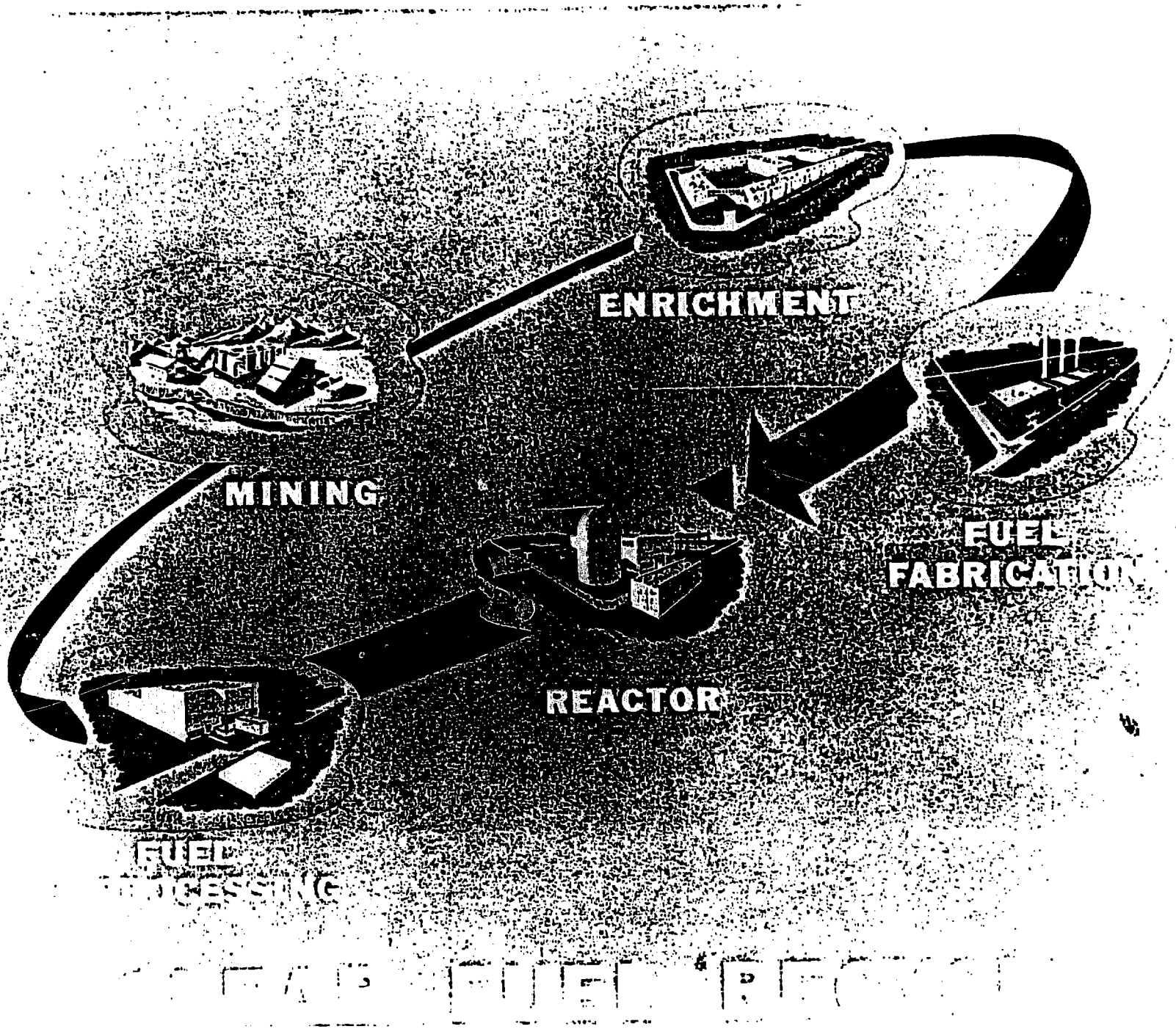


Fig. 1. Nuclear fuel recycle.

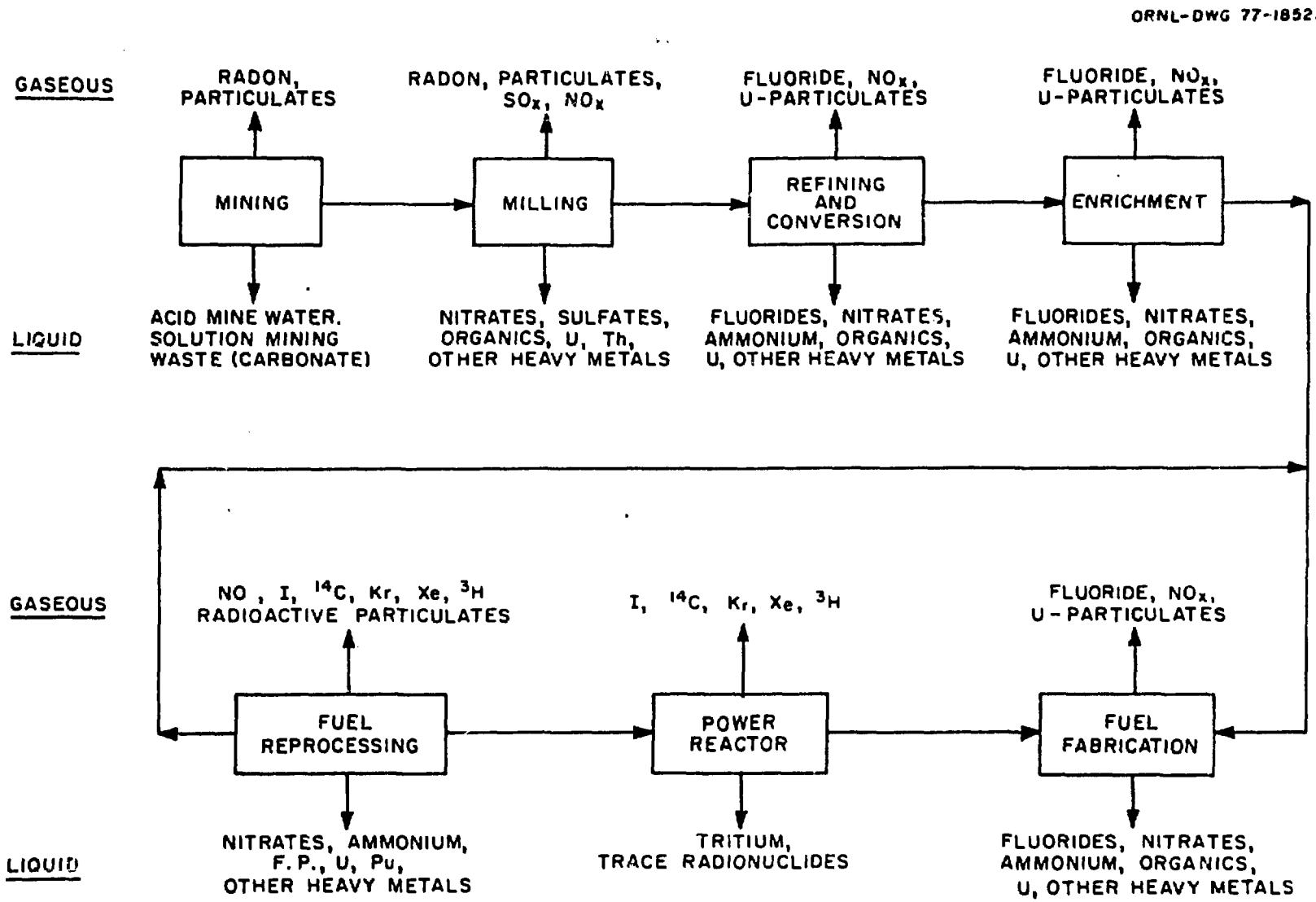


Fig. 2. Some effluent waste streams in the uranium fuel cycle.

Biological denitrification

Biological denitrification, as referred to in this paper, is the biological reduction of nitrate or nitrite to gaseous molecular nitrogen and carbon dioxide.[2] It commonly takes place in soil under anaerobic conditions by the various strains of facultative anaerobic bacteria, such as Pseudomonas denitrificans or Pseudomonas stutzeri, that are responsible for recycling nitrogen compounds to the atmospheric molecular nitrogen pool. The reaction requires a source of carbon, which has usually been provided in the form of various alcohols and acetates. The rate of denitrification is dependent on nitrate concentrations and the type of carbon substrate supplied as well as on 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:

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

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

The denitrification bacteria have been successfully allowed to grow and attach themselves to small solid supports (Fig. 3), thereby forming a stable-bacteria-population bed through which the nitrate wastewater can be processed.[3,4] Denitrification bacteria have been successfully attached to 30/60 mesh coal (density--1.5 g/cm^3) and sand (density--2.2 g/cm^3) particles. The diameters of such particles are 25 to 54 mm. Coal particles, which have a lower density than the sand granules, have been used for most of the tests

Fig. 3. Bacteria attached to a solid particle.

since they will fluidize at a lower liquid flow rate and hence provide a longer contact time for a given bioreactor length.

Feed solution

In the tests reported here, we selected a feed composition representing nitric acid wastewater that had been neutralized with ammonium hydroxide (Table 1). Ethanol was added as a carbon source at a carbon-to-nitrogen (C/N) ratio of 1.5. Ethanol was used rather than the less costly methanol because it could be easily determined in the presence of other soluble carbon compounds by the ethanol Calbiochem kit.[5] Sodium phosphate was added directly to the feed as it entered the column to eliminate phosphate precipitation problems at PO_4^{2-} concentrations of 5 to 7 ppm. The 20% nitrate feed concentration solution was diluted to the desired nitrate level with water before it was fed to the bioreactor.

Bacteria inoculum

The culture used for seeding the columns was taken from a stock of frozen bacteria that had been prepared for starting the Y-12 Plant denitrification reactors. These bacteria were grown in 55-gal drums, extracted with a centrifuge, and then stored at -80°C in small plastic containers inside a Revco freezer. The original seed culture for these drums was an ATCC culture of Pseudomonas stutzeri; however, since the drums were open to contamination, the bacteria are almost certainly a mixed population. The stability of the columns at various temperatures, nitrate concentrations, and organic carbon sources also indicates a mixed bacteria population.

Analytical methods

The analyses for nitrate, nitrite, and ethanol are performed using a Gilford 2400 spectrophotometer. The nitrate concentration is determined

Table 1. ORNL-DWG 78-5211

CONCENTRATED FEED SOLUTION
30% NITRATE

HNO_3	300 g/liter
NH_4OH	120 g/liter
ETOH	200 g/liter
$MgSO_4$	1 g/liter
Dow-Antifoam "A"	1 g/liter
Trace metal solution	0.1 g/liter

TRACE METAL SOLUTION MIX (g/liter)

H_3BO_3	1
$ZnSO_4 \cdot 7H_2O$	0.4
$NH_4MO_7O_{24} \cdot 4H_2O$	0.2
$MnSO_4 \cdot 7H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.45
$FeSO_4 \cdot 7H_2O$	0.25
KI	10
Fe-chelate	200

by measuring the absorbance at 220 nm[6] and then subtracting the reading at 275 nm from the 220-nm result to correct for interference by dissolved organics. Nitrite samples are combined with sulfanilic acid, EDTA, and naphthylamine hydrochloride to produce a red azo dye. The intensity of this dye is measured by absorbance at 520 nm. Ethanol is measured by using a commercial blood alcohol analysis kit from Calbiochem.[5] In this method, ethanol is enzymatically oxidized to acetaldehyde while nicotinamide-adenine dinucleotide (NAD) is reduced to NADH. The concentration of NADH is determined by measuring the absorbance at 340 nm. Three standards, whose concentrations span the range of the sample concentrations, are used for calibration in all of these methods. The concentration of total organic carbon (TOC) is measured with a Beckman 915-A carbon analyzer.

Biomass-on-coal determination

The biomass-covered particle is gently fluidized and washed with water to remove any nonattached biomass. The sample is air-dried for 24 hr at 105°C, cooled in air and then weighed. The dried sample is subsequently soaked in 4 M NaOH for 4 hr to remove the attached biomass. The cleaned particles are water-washed, dried at 105°C for 24 hr, cooled, and reweighed. The biomass is reported in terms of weight percent on a dry basis. The biomass is 58% carbon, based on the biomass formula $C_5H_7O_2N$.

Experimental equipment

Bioreactors. The 5- and 10-cm-ID pyrex glass bioreactors used in this series of experiments were similar in design. Each bioreactor consisted of a tapered bottom followed by five samplers and five cylinders alternately spaced, the top disengaging taper and the liquid gas separating section (Fig. 4). The sections are described in detail:

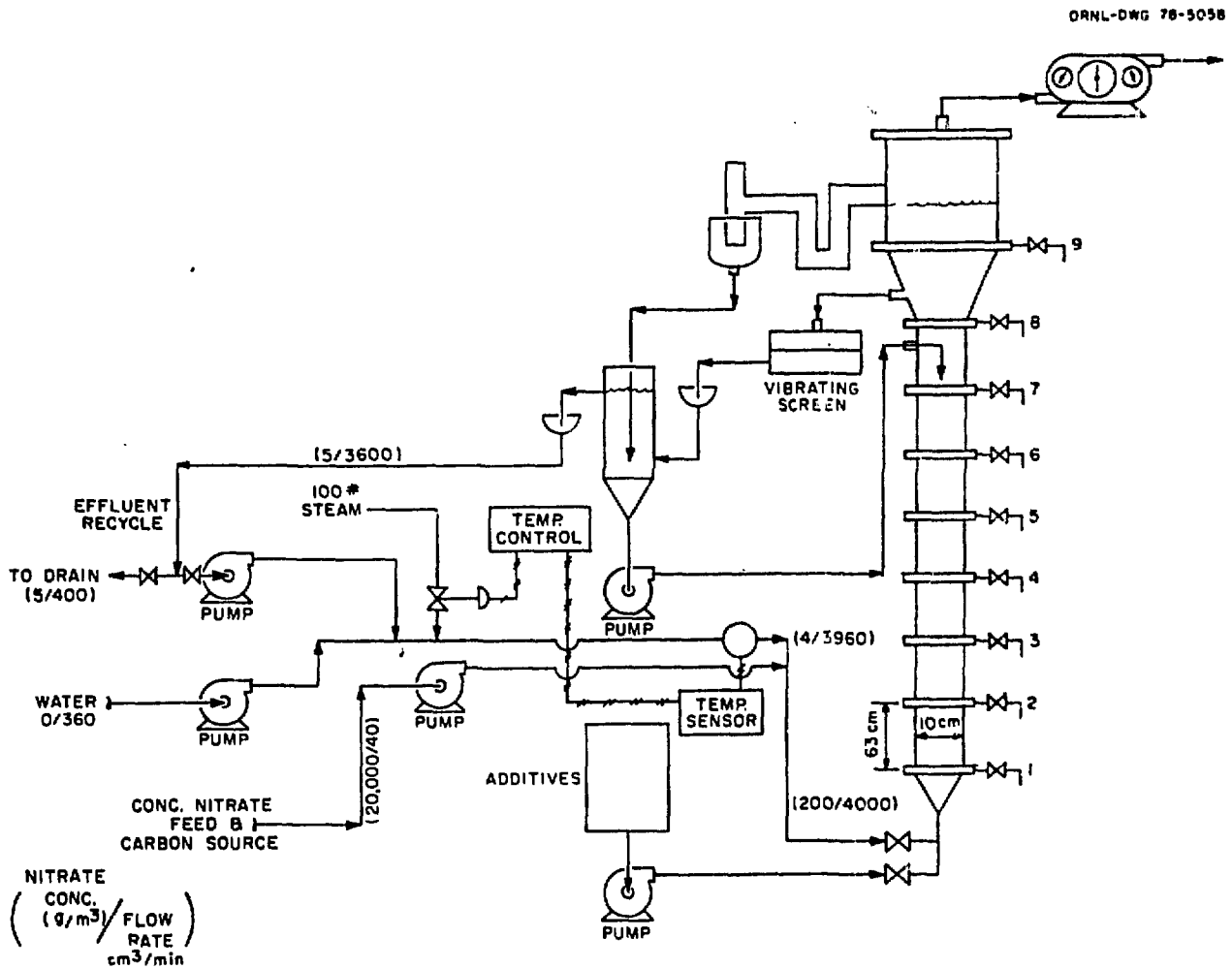


Fig. 4. Ten-cm-ID bioreactor system including pumps, temperature control, and recycle compatibility.

1. a tapered bottom 0.5 cm ID enlarging to 5 or 10 cm ID over a 15-cm length for flow distribution;
2. a 2-cm-thick plexiglass sampler for liquid and solid samples (see Fig. 5);
3. a 60-cm-long straight cylindrical section;
4. a top solid disengaging section tapering from either 5 to 10 cm or 10 to 15 cm ID and 60 cm long; and
5. a top liquid-gas disengaging section 10 or 15 cm ID and 60 m long.

The liquid fluidizing rates were 800 to 100′ ml/min for the 5-cm-ID bioreactor and 4000 ml/min for the 10-cm-ID bioreactor.

Two identical 10-cm-ID bioreactors piped in series were used in the dual bioreactor tests (Fig. 6). The liquid feed rate was 4000 ml/min. The inter-reactor feed pump rate was controlled so as to maintain a constant liquid level in the surge tank between the two bioreactors.

Pumps. The pumps used in these experiments were Milton Roy (Milton Roy Co., Flow Control Div., Ivyland, PA 18974) positive-displacement piston pumps, which are fabricated of 316 stainless steel.

Biomass control

The excess biomass generated in the denitrification reaction must be removed periodically for stable bioreactor operation. The amount of biomass to be removed is theoretically 0.1 g per gram of NO_3^- decomposed. Operating experience has indicated that a biomass loading of 5 to 10% dry weight is optimum. Overgrowth of a biomass has the following detrimental effects on the reactor operation:

1. The reactive surface area and, in turn, the denitrification rate are reduced.

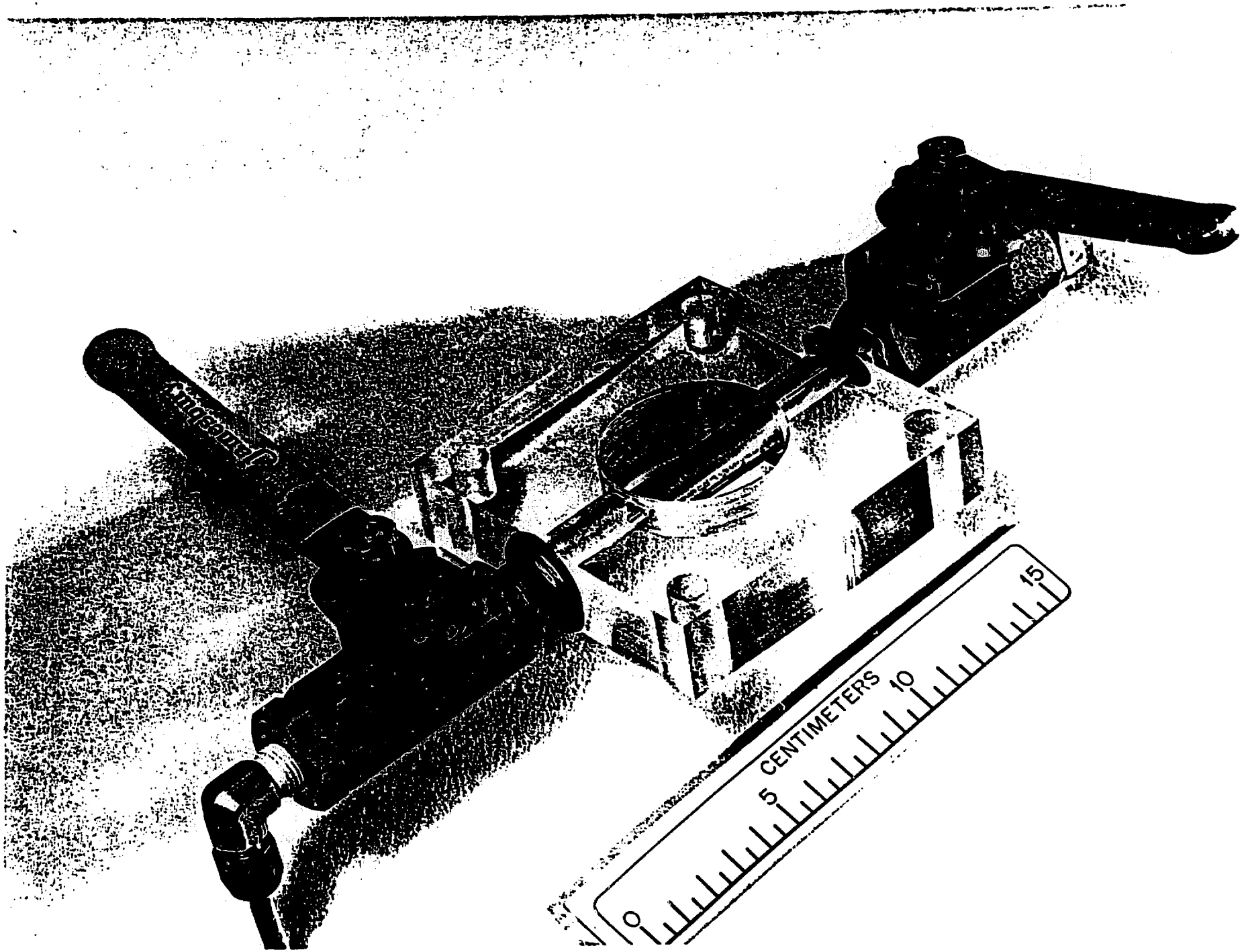


Fig. 5. Five-cm-ID bioreactor sampler section.

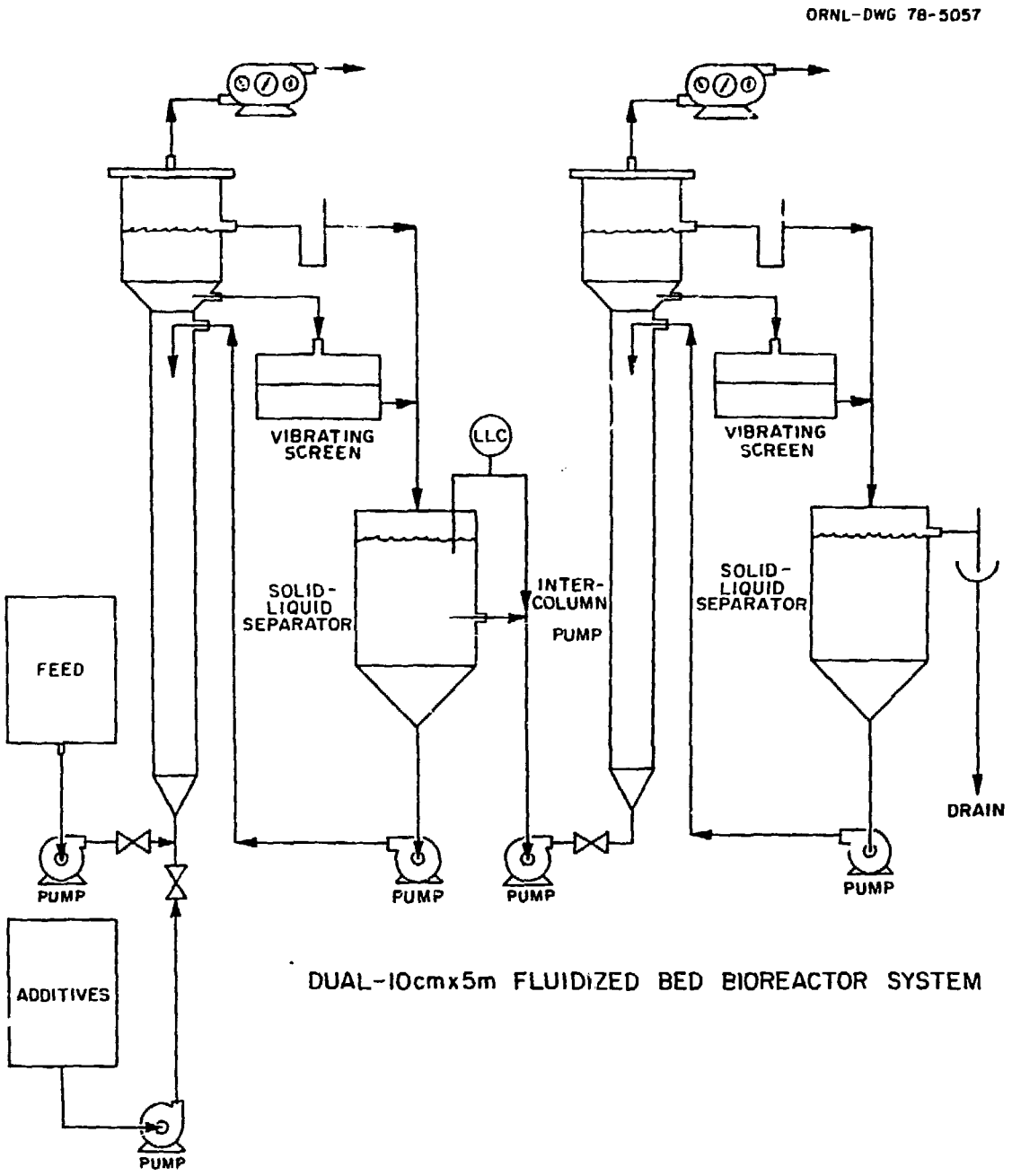


Fig. 6. Dual 10-cm x 5-m fluidized-bed bioreactor system.

2. Nitrogen gas that is formed during the denitrification reaction can be trapped, causing the particle to float and wash out of the bioreactor.
3. Particles tend to become sticky, and fluidization is difficult.

When the density of the overgrown biomass-covered particle decreases, the particle will migrate to the top of the bioreactor. The less-dense overgrown particle can be removed from the top of the bioreactor, mechanically cleaned, and returned to the top of the bioreactor. The clean particle will sink and begin to recover itself with biomass.

Our biomass control system consists of a Sweco vibrating screen equipped with a 30-mesh (0.50-mm-diam) screen. Liquid and solid are pumped from the top of the bioreactor, vibrated through the 30-mesh screen to remove most of the attached bacteria, and then returned to the bioreactor about 10 to 20 cm below the removal point (see Fig. 4). The unattached biomass is discharged with the bioreactor effluent for later disposal.

RESULTS

Operational theory

Many of the nitrate-containing industrial and DOE nuclear fuel cycle waste streams are very concentrated, some with nitrate contents as high as 20 or 30% (300,000 ppm NO_3), as compared with the maximum operating range of 10,000 g/m^3 NO_3 for biological denitrification. This makes it necessary to dilute the wastewater in some manner before it is fed to the bioreactor. It has been reported[7] that the denitrification bacteria in an unattached state exhibit a zero-order rate relationship to the nitrate substrate concentration. The operating rationale is to introduce feed with the highest possible nitrate concentration to the bioreactor but have a near-zero nitrate discharge in a reasonable length of bioreactor. The 5-cm-ID bioreactor has been operated with various inlet NO_3^- concentrations ranging from 200 to 7500 ppm. The series of tests indicated that denitrification rate [kg $N(NO_3)$/day-m^3 based on empty reactor volume], increased with increased nitrate feed concentration. The resulting data from a series of tests with different nitrate concentrations are shown in Fig. 7. The curve of nitrate concentration vs bioreactor length was constructed by superimposing the results of these denitrification tests (nitrate concentration at various bioreactor heights) "head to toe" to cover the NO_3^- range of 0 to 7500 ppm, producing a composite bioreactor length of 62 ft. The slope of the curve suggests a first-order overall reaction.

Comparison of operating performance of 5- and 10-cm-ID bioreactors

The 5- and 10-cm-ID bioreactors were operated under similar conditions of feed concentrations, pH, temperature, unit cross section, flow rate, and

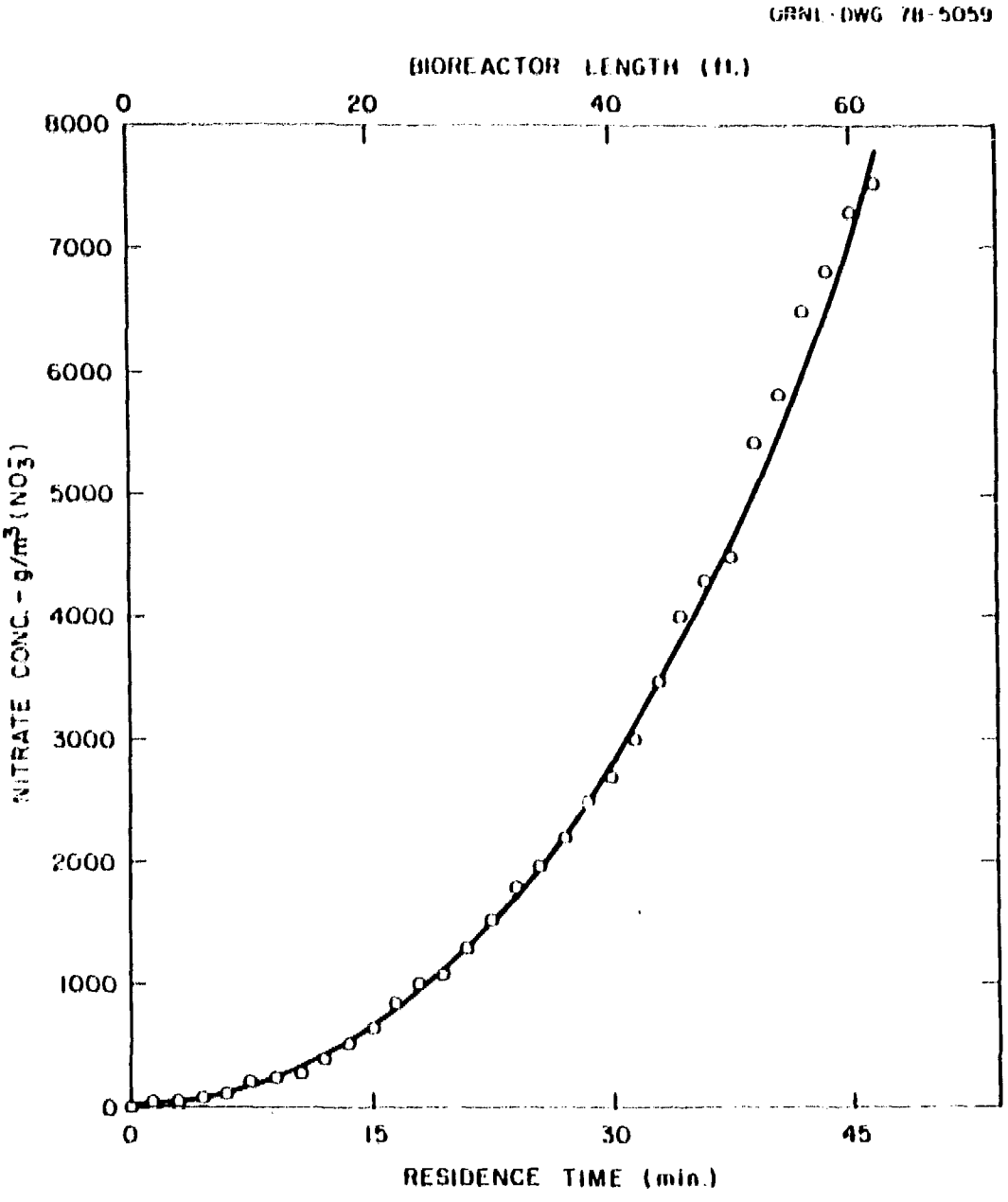


Fig. 7. Nitrate concentration vs bioreactor length for a number of tests compressed into one curve.

bacterial loading. Both units were operated with NO_3^- feed concentrations of 200, 500, 1000, 1500, and 2000 g/m^3. As shown in Table 2, the denitrification rates for the two units show no significant differences. Figure 8 shows the data for a typical set of tests using a feed with a NO_3^- concentration of 200 g/m^3. The 10-cm-ID unit was somewhat easier to operate because its larger diameter allowed gas bubbles to rise through the fluidized solids with less interference.

10-cm-ID dual bioreactors in series test

The two 10-cm-ID by 6-m-long bioreactors were operated in series to test the denitrification rates and the mechanical features of staged bioreactors.

The feed for the test contained ~ 2000 ppm NO_3^- with a C/N ratio of 1.5. The average ethanol carbon usage (C/N) ratio was 1.2. When the nitrate concentration profile along the length of each bioreactor in series was plotted along with 5-cm-ID data (nitrate vs bioreactor length, Fig. 7), good agreement of the data was obtained (see Fig. 9).

The fluidization pump for the second bioreactor operating with a liquid-level controller maintained a constant and correct flow of bioreactor 1 effluent for feed to bioreactor 2. The biomass control system, which used the Sweco filter to remove part of the attached biomass from the overloaded particle, controlled the biomass on the particles in the bioreactor to near the desired value of 8-10 wt % (dry basis).

Recycle mode of operation

If the cost or quantity of dilution water is limiting, it can be minimized by using effluent with a near-zero nitrate concentration to dilute the concentrated feed solution; this practice would also reduce the amount

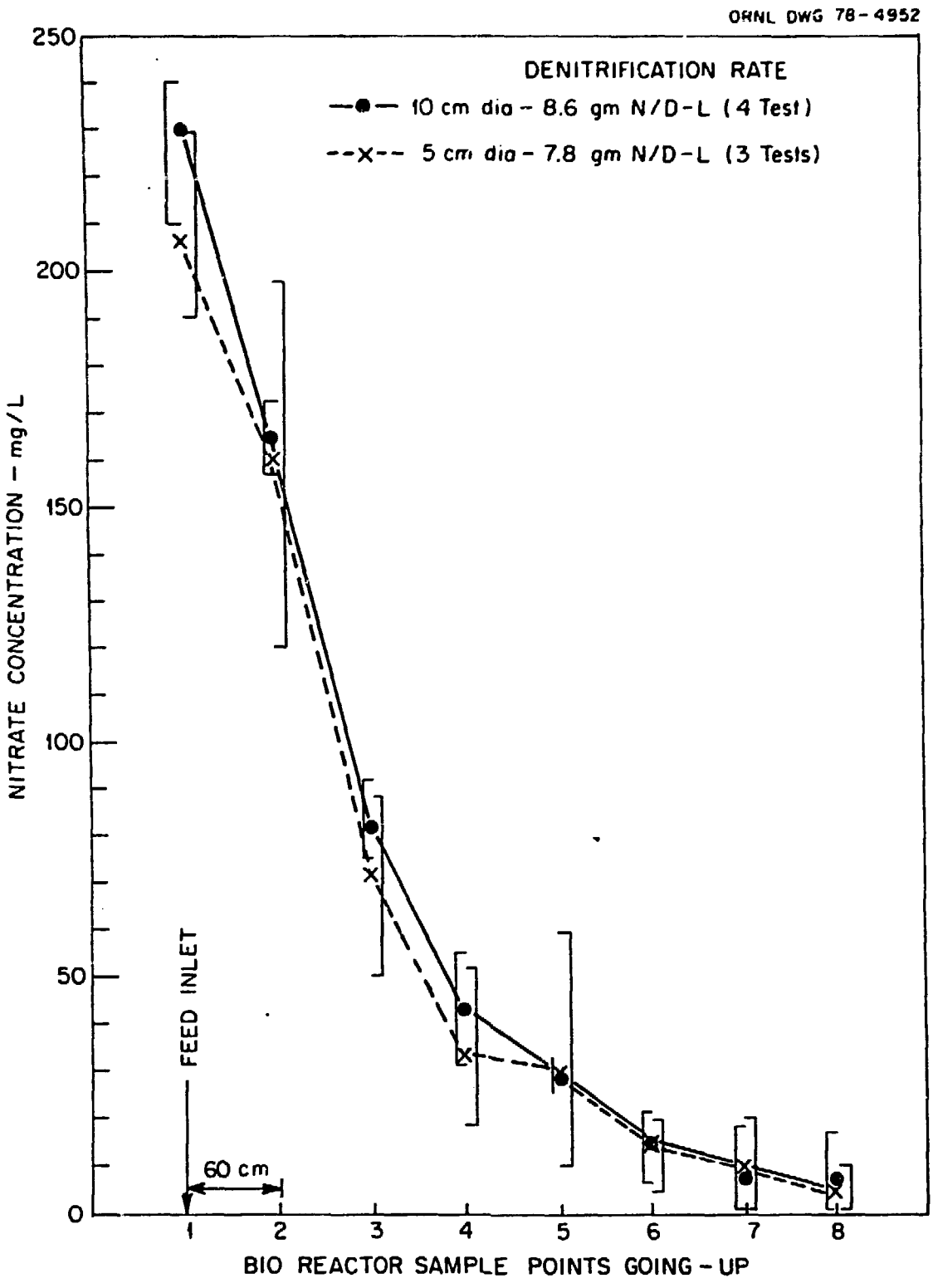


Fig. 8. Comparison of 5- and 10-cm-ID bioreactor tests starting at approximately 200 g/m^3 nitrate concentration.

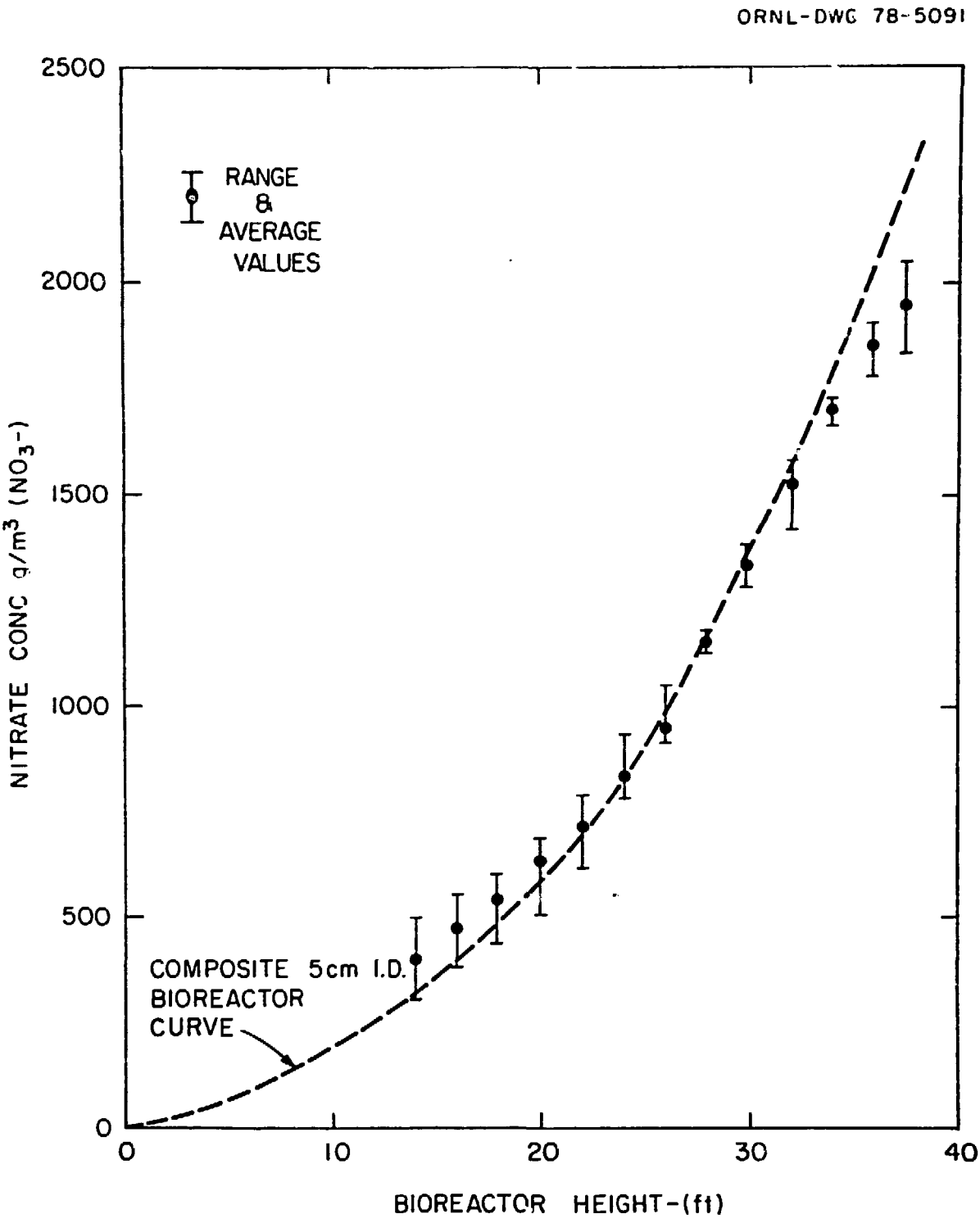


Fig. 9. Ten-cm-ID dual bioreactor tests; average and range data for four experiments and 5-cm-ID composite rate data, nitrate concentration vs bioreactor length.

Table 2. Comparison of denitrification rate for 5- and 10-cm-ID bioreactors

Feed/effluent concentration ($g\ NO_3^-/m^3$)		Denitrification rate [$g\ N(NO_3^-)$/day-liter]	
5-cm-ID unit	10-cm-ID unit	5-cm-ID unit	10-cm-ID unit
210/5	230/5	7.8	8.6
490/0	505/0	22.2	18.8
950/0	980/300	26.6	25.5
--	1500/185	--	50.3
2300/500		49	

of treated effluent to be discharged. The 10-cm-ID bioractor has been operated with a 9/1 recycle ratio and an inlet NO_3^- concentration of 200 g/m^3. Effluent solution was pumped to the bottom of the column at the rate of 3600 cm^3/min, where it was combined with fresh feed introduced at the rate of 400 cm^3/min before entering the column.

A typical nitrate concentration profile for the column is shown in Fig. 10. The denitrification rate and the average nitrate concentration were calculated for each 63-cm section of the column. These results are presented in Fig. 11. The average denitrification rate for the entire column was 6.1 kg $N(NO_3^-)/day\text{-}m^3$.

The chief disadvantage of this mode of operation is that any impurity in the feed solution will gradually accumulate in the column until its concentration reaches the level present in the concentrated feed. With every impurity, care must be taken to prevent buildup to a toxic level. The C/N ratio must also be monitored closely since any unused organic carbon is returned to the bottom of the column, where it remains and concentrates. In many instances, however, the extra care required by this mode of operation will be more than compensated for by the reduction in dilution-water requirements.

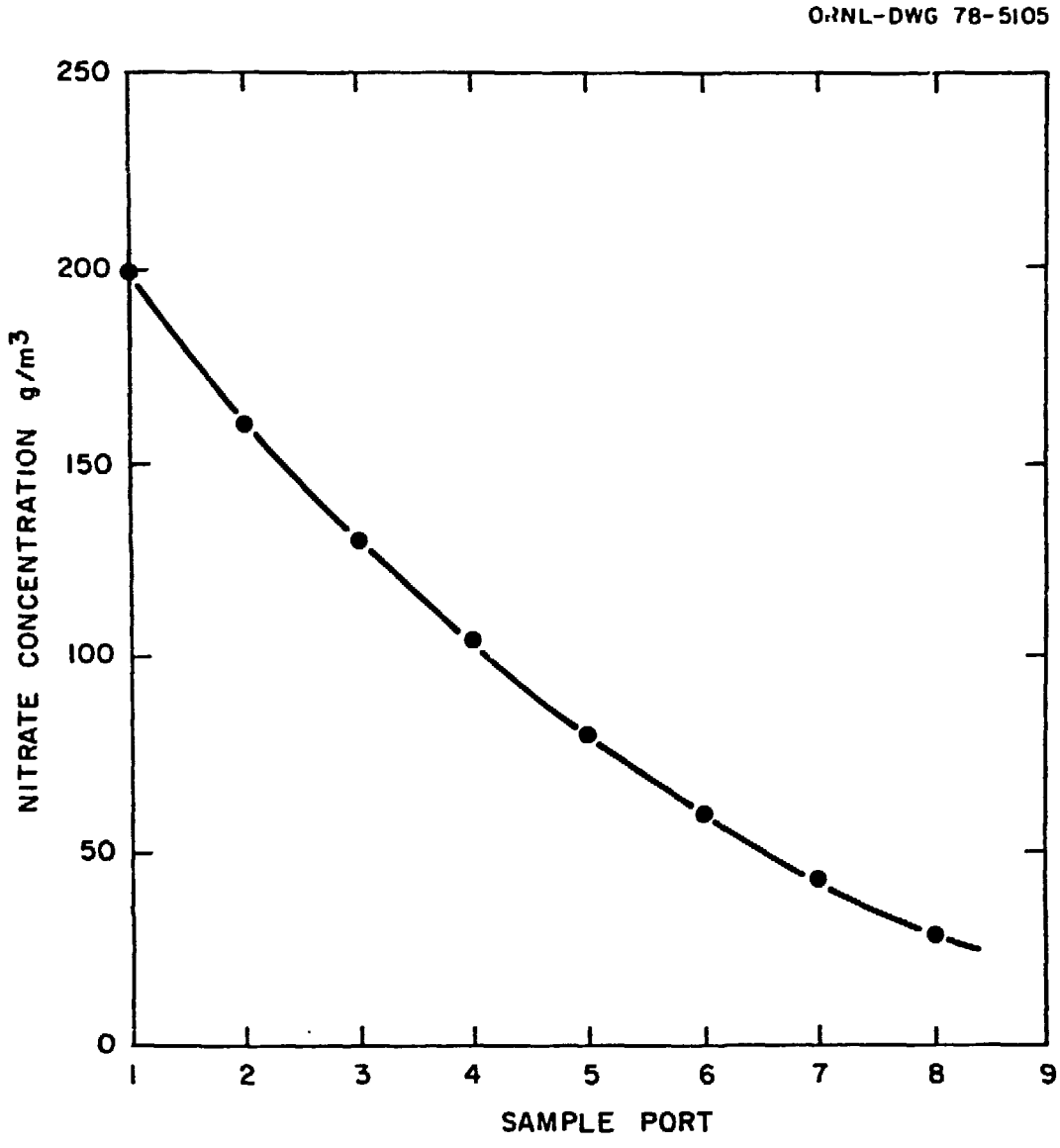


Fig. 10. Nitrate concentration vs column height for recycle mode operations.

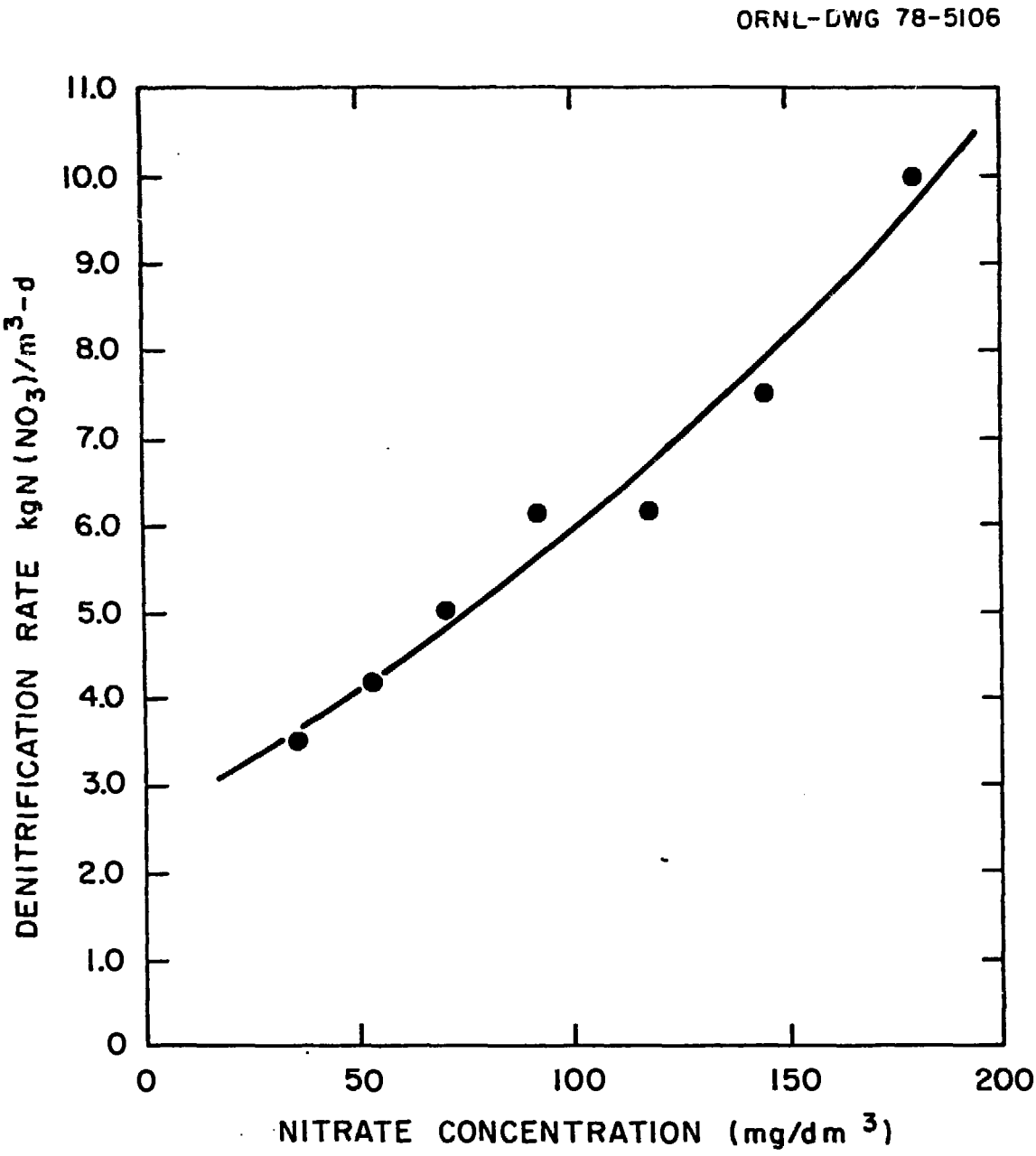


Fig. 11. Denitrification rate vs concentration for recycle mode operation.

Rate vs temperature

The effect of temperature on denitrification rate has been measured in the 10-cm-ID column. In the first set of experiments, a heat exchanger was used to control the inlet temperature to the column. The maximum temperature achievable with this design was 22°C. The inlet NO_3^- concentration was kept at 1000 kg/m^3 for this set of experiments. At the lower inlet temperatures, the combination of heat generated by the bacteria and convective heat flow into the column caused an increase in temperature along the length of the column. To minimize the effect of these temperature changes, the denitrification rate in the first 2-ft section of the column was used for comparing the rates at the various temperatures. The results of these experiments, summarized in Fig. 12, show that increasing the temperature has a dramatic effect on the denitrification rate.

A second set of rate-vs-temperature experiments was performed three months later. The inlet NO_3^- concentration for these tests was 500 mg/dm^3. A direct steam injection system was installed to control the inlet temperature. The denitrification rate was measured at 22, 27, and 32°C. At these temperatures, the heat generated by the bacteria was approximately balanced by the convective heat loss from the column; thus the temperature remained reasonably constant. No increase in rate was observed when the temperature was increased from 27°C to 32°C. The results obtained from this set of experiments are shown in Fig. 13, where the rates are plotted as a percentage of the rate at 20°C in order to remove the effects of the different nitrate concentrations in the two sets of experiments. A compilation of other published results is shown in Fig. 2 for comparison.[7] These data suggest that supplemental heating above 22°C will probably be uneconomical.

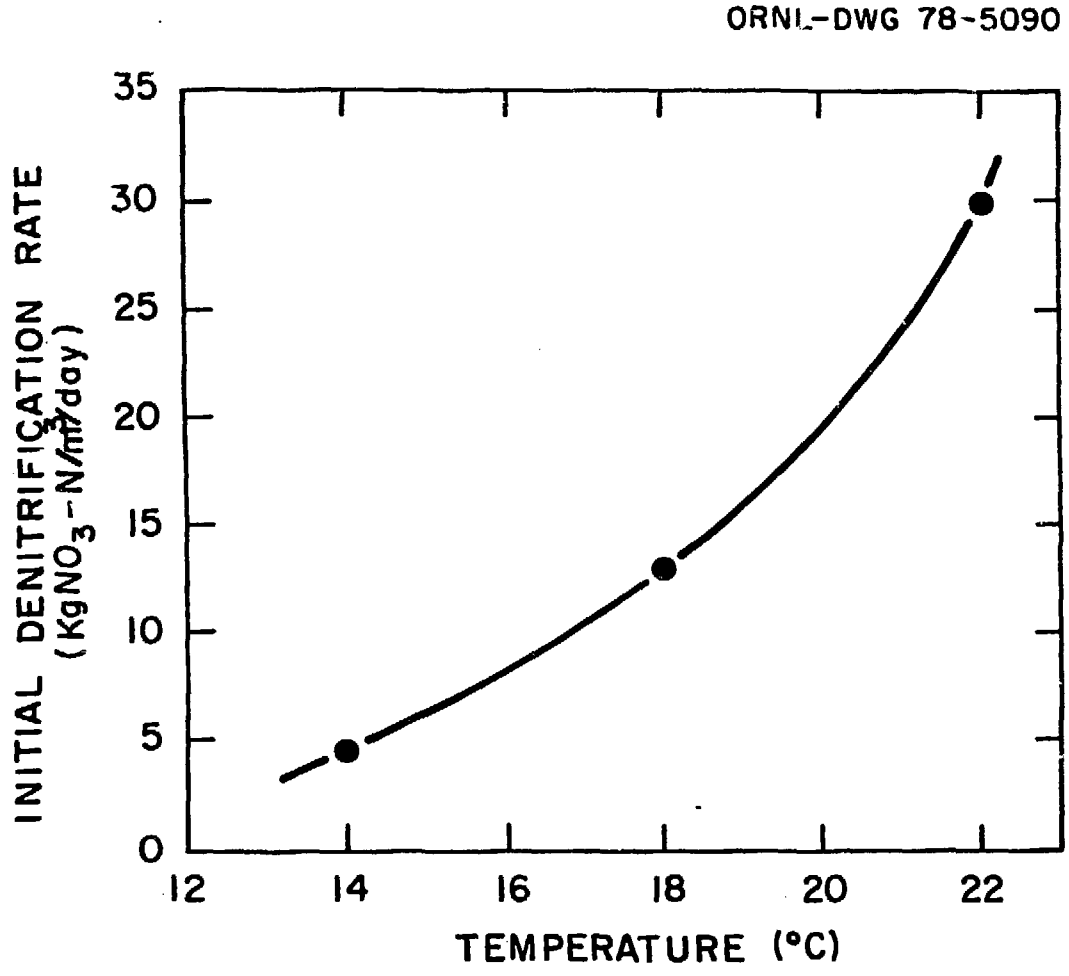


Fig. 12. Initial denitrification rate vs operating temperature.

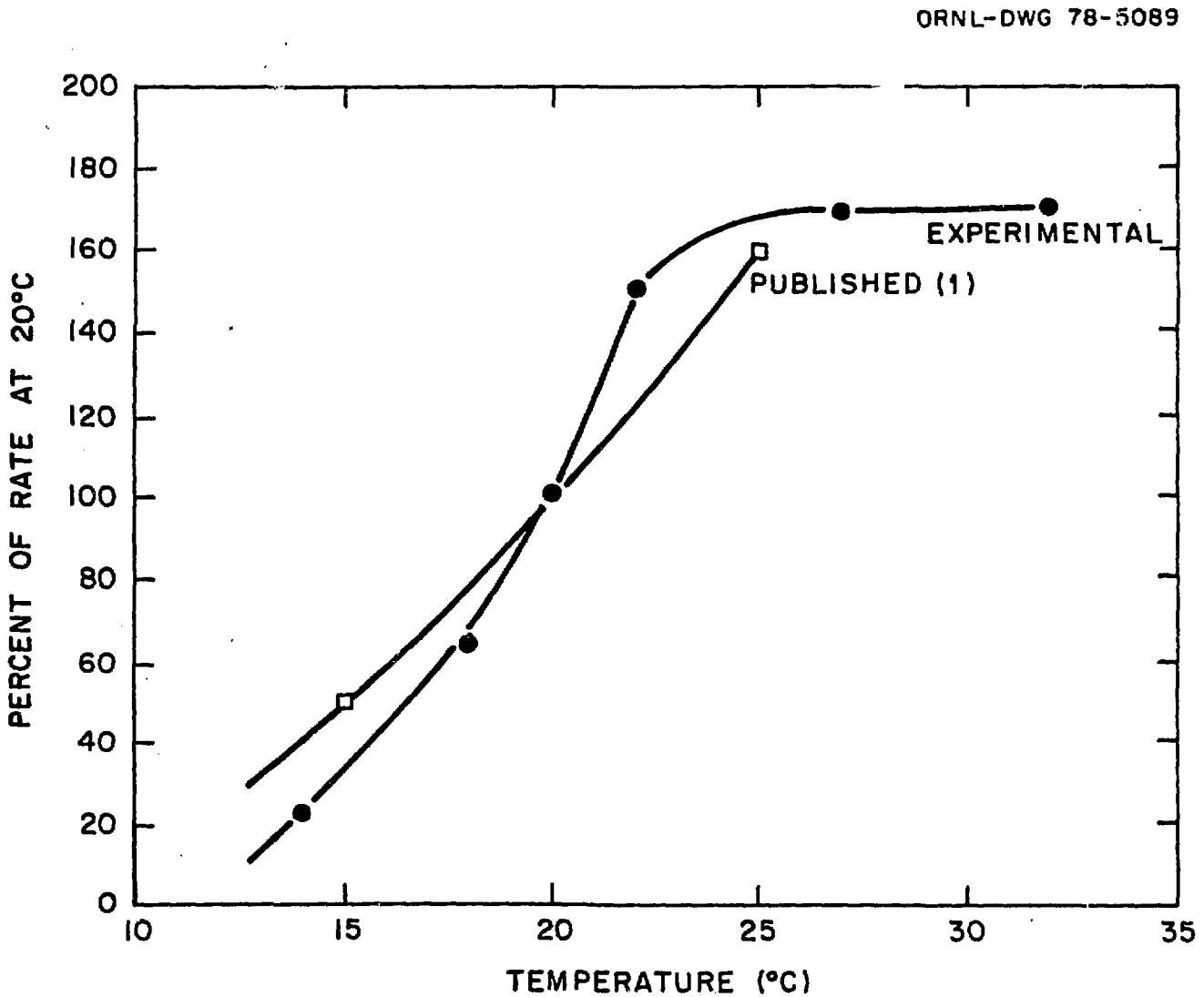


Fig. 13. Percent of denitrification rate at 20°C vs operating temperature.

Biomass control and distribution

The higher the nitrate concentration in the bioreactor the higher the denitrification rate and the faster the biomass buildup on the fluidizing particles. Our present mode of operation is to remove and partially clean the dense overgrown biomass covered particles which migrate to the top of the bioreactor, typically 5-10% (dry weight) reduced by one-half. The cleaned particles are returned to the bioreactor where their density causes them to fall through the fluidized bed to the lower zones of the bioreactor. The small part of the biomass growth that is not attached to particles is swept out of the bioreactor with the effluent. Soluble organic compounds, such as butyric acid, that are excreted by the biomass and dissolved carbon dioxide are discharged with the effluent along with excess ethanol used as carbon growth source. We attempted to control the carbon growth source utilization rate so that it matched the nitrate consumption; however, the varying biomass loading which in turn changes the carbon usage rate usually makes it necessary to provide a small excess.

The effluent will contain the following carbon sources: (1) biomass removed from particles; (2) biomass not attached to particles; (3) soluble organic biomass waste byproduct; (4) carbon dioxide reaction gas; and (5) excess organic feed nutrient. Figure 14 and Table 3 are a diagram and table showing typical levels of organic carbon and biomass distribution in a 10-cm-ID bioreactor. The carbon concentration of the effluent can be reduced to discharge levels using standard available sanitary sewage procedures. The removal of the filterable biomass is one possible solution, but since it is a minor component of the whole carbon discharge problem, we chose to treat the whole mixed effluent stream. We believe that if the bioreactor biomass was controlled at a 1 or 2% dry weight, the carbon discharge problem would be minimized.

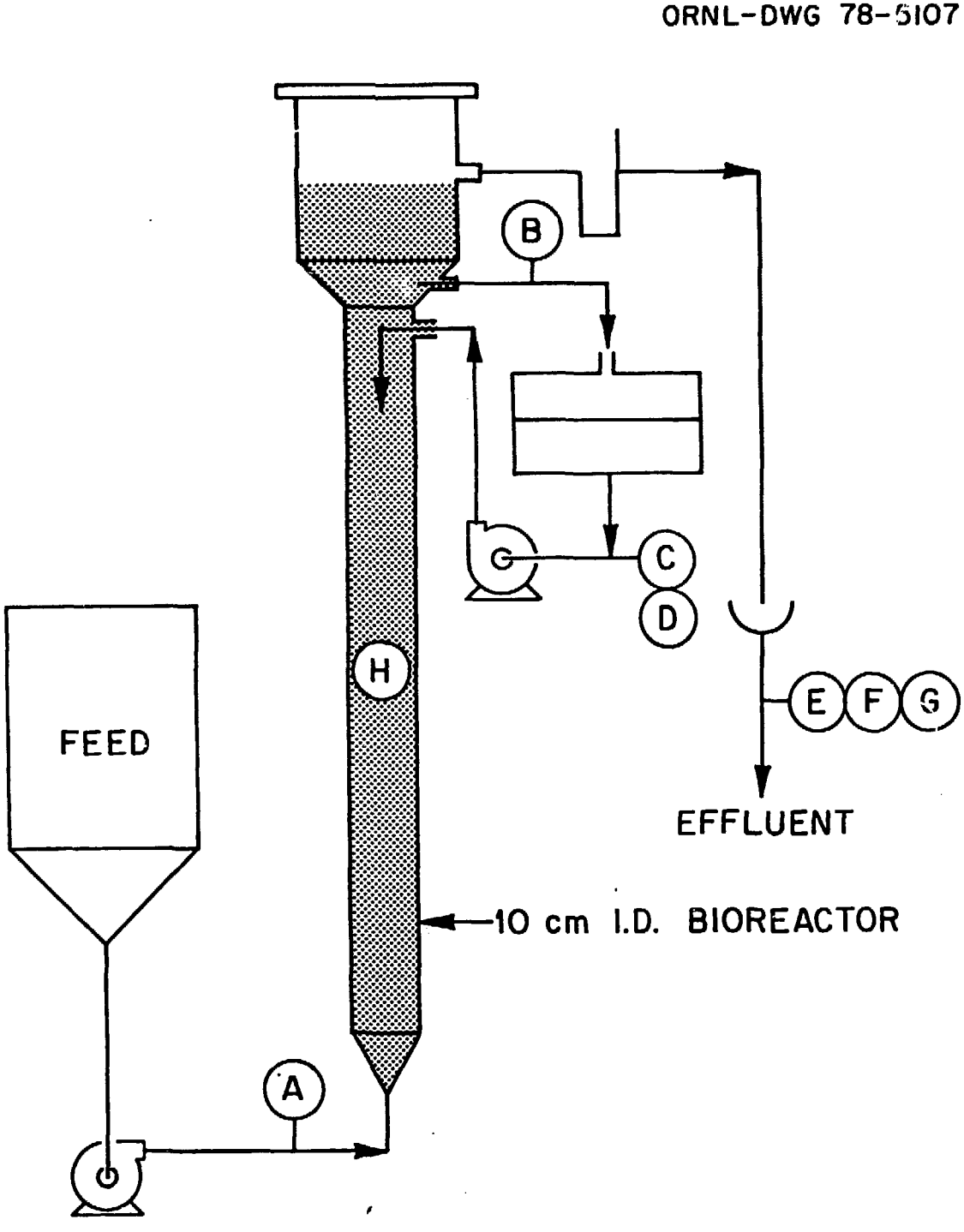


Fig. 14. Organic carbon distribution flowsheet.

Table 3. Typical organic carbon flowsheet balance

(500 g/m^3 NO_3 feed concentration test)

Stream	Flowsheet symbol (Fig. 14)	Carbon type	Carbon conc. (g/m^3)	Carbon quantity (g/day)
Feed	A	ETOH	200	1150
Effluent	D	Soluble*	100	620
	F	ETOH	30	180
	G	Biomass	50	330
				1130

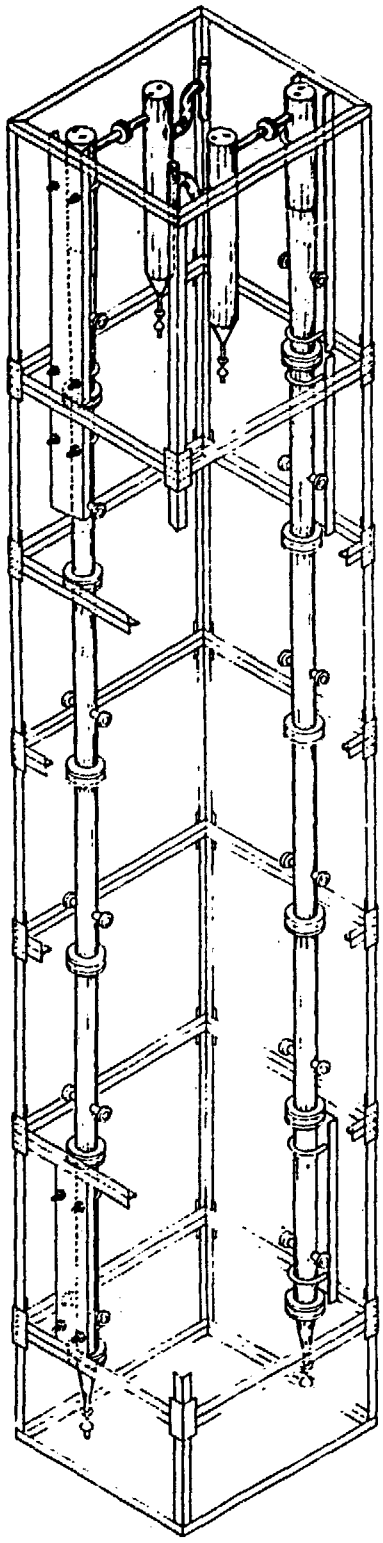
*(The soluble carbon in the effluent includes about 400 g/day of CO_2)

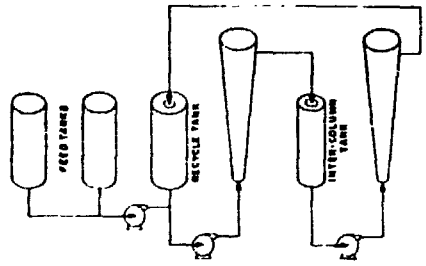
Bioreactor	H	10 cm ID x 6 m bioreactor contained 20 ℓ of settled solids - at 5% biomass loading = 1600 gm of carbon	
To Sweco screen	B	Attached biomass (~50% of bioreactor/day)	950
From Sweco screen	C	Unattached biomass	330

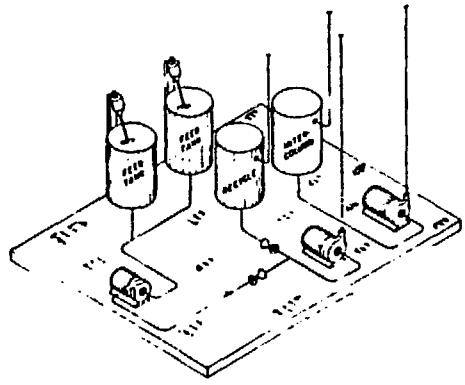
CONCLUSIONS

The purpose for the preceding development program was to design a mobile denitrification pilot plant that could be used in a number of DOE and commercial nuclear fuel cycle operations to test the applicability of this biological denitrification process to their individual nitrate wastewaters. The results indicate that the scale-up of the bioreactor from 5 cm ID to 10 cm ID is directly correlated and that the test with dual 10-cm-ID by 6-m-tall bioreactors corresponded satisfactorily with the 5-cm-ID bioreactor composite 0-7500 ppm NO_3^- concentration-vs-bioreactor length plot. Therefore, we have a basis for predicting the performance of our mobile pilot plant.

The mobile pilot plant will contain two 20-cm-ID by 7.3-m-long bioreactors operated in series (see Fig. 15 and Table 4). For near-zero NO_3^- effluent (5 to 10 ppm), the feed will have a maximum concentration of 4000 ppm and will be introduced at a flow rate of 16 liters/min. The temperature will be regulated between 22 and 30°C.







DEMONSTRATION PILOT PLANT FOR BIOLOGICAL WASTEWATER TREATMENT

Fig. 15. Conceptual design of demonstration pilot plant for biological wastewater treatment.

Table 4. Specifications of the mobile denitrification pilot plant

Bioreactors:	two operated in series (20 cm ID by 7.3 m long)	
Operating conditions:		
	Nitrate concentration:	Feed, 4000 g/m^3 max. Effluent, 5-1 g/m^3
	Flow rate:	16 liters/min (0.84 cm/sec)
	Temperature:	22-30°C
	pH:	Feed, 6.5-6.8 Effluent, 8.1-8.5

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