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ANAEROBIC COLUMNAR DENITRIFICATION OF HIGH NITRATE WASTEWATER[1]

C. W. Francis and C. D. Malone[2]

ABSTRACT

Anaerobic columns were used to test the effectiveness of biological denitrification of nitrate solutions ranging in concentration from 1 to 10 kg NO_3/m^3. Several sources of nitrate $(CaCNO_3)_2$, $NaNO_3$ NH_4NO_3 and actual nitrate wastes from a UO_2 fuel fabrication plant) were evaluated as well as two packing media. The packing media were anthracite coal particles, whose effective diameter size ranged between 2 and 3 mm, and polypropylene Raschig rings 1.6 x 1.6 diameter. The anthracite coal proved to be the better packing media as excessive hydraulic short circuiting occurred in a 120 x 15 cm diameter glass column packed with the polypropylene rings after 40 days operation. With anthracite coal, floatation of the bed occurred at flow rates > 0.80 cm^3/s. Tapered columns packed with anthracite coal eliminated the floatation problem, even at flow rates as high as 5 cm^3/s. Under optimum operating conditions the anthracite coal behaved as a fluidized bed. Maximum denitrification rates were 1.0-1.4 g $NO_3/m^3/s$ based on initial bed volume. Denitrification kinetics indicated that rates of denitrification became substrate inhibited at nitrate concentrations > 6.5 kg NO_3/m^3. Anaerobic columns packed with

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anthracite coal appear to be an effective method of nitrate disposal for nitrate rich wastewater generated at UO_2 fuel fabrication plants and fuel reprocessing facilities.

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ANAEROBIC COLUMNAR DENITRIFICATION OF HIGH NITRATE WASTEWATER

In recent years, considerable effort has been directed toward the development of biological denitrification systems for removing nitrates from municipal and agricultural wastewater (Johnson and Schroepfer 1964; Smith et al. 1972; McCarty 1969). In general, these systems are either anaerobic packed beds or continuous flow stirred reactors. Nitrate concentrations in municipal and agricultural wastewater seldom exceed 250 g NO_3/m^3; thus, little research has been conducted on the removal of nitrates from wastewaters containing concentrations in excess of 1000 g NO_3/m^3.

A number of manufacturing operations, such as fertilizer and explosives production facilities, generate wastewater effluents containing concentrations of NO_3 > 1000 g/m^3. Our major concern is how to treat the large quantities of wastewater effluents containing high nitrate concentrations that are produced in nuclear fuel processing operations and uranium oxide fuel fabrication plants. Thus, the purpose of this paper is to present results of the biological denitrification of several sources of nitrate at influent concentrations exceeding 1000 g NO_3/m^3 in anaerobic columns using two packing media. Another paper, to be published later (Francis and Malone 1975a), will deal with denitrification at similar nitrate concentrations in continuous flow stirred reactors. A comprehensive review evaluating the advantages and disadvantages of each engineering design, anaerobic columns and continuous flow stirred reactors, for various nitrate enriched wastewater effluents associated with the nuclear fuel cycle has been published elsewhere (Francis and Callahan 1975).

THEORY AND METHOD OF EXPRESSING DENITRIFICATION RATES

Biological denitrification is the reduction by microorganisms of nitrate or nitrite to gaseous molecular nitrogen or oxides of nitrogen. Dissimilatory biological denitrification is nitrate reduction where nitrate serves as the terminal electron (hydrogen) acceptor in the oxidation of an organic substrate by a large number of facultative anaerobic bacteria. It should not be confused with assimilatory denitrification which is the reduction of nitrate or nitrite to ammonia for synthesis of amino acids, amino sugars and nucleotides in anabolic cell metabolism.

Biological denitrification can only occur under an anaerobic environment or at least a "reduced environment" where the redox potential is < 250 mV. Optimum denitrification occurs in the pH range from 7.5 to 8.5. Two substrates must be available, nitrate and an energy source, usually a hydrocarbon which also supplies carbon for additional cell synthesis.

Assuming an adequate carbon source is available, nitrate removal rate is dependent on nitrate concentration and microbial cell concentration. Anaerobic columnar units (conventionally called packed bed reactors) in which the influent nitrate stream is introduced at the bottom of the column encourage maximum microbial cell concentrations in denitrification reactors due to the large surface area provided by the packing matrix. As cell buildup in the column exceeds that washed from the reactor, the hydraulic residence time in the column decreases at constant influent flowrates. Continued cell buildup eventually plugs the column unless

preventative measures such as backwashing or flushing, etc. are taken. As denitrification progresses, the void spaces become filled with N_2 and CO_2 rather than substrate. Thus, any hydraulic residence time based on initial void space is a maximum value and the real hydraulic residence times after prolonged operation of a column are likely to be factors 5 to 10 lower. In the following work, denitrification rates were not considered valid unless at least 10 hydraulic residence times, based on initial void space of the packing material, had elapsed. Valid denitrification rates in anaerobic columns should include current hydraulic residence times as well as microbial concentrations. This was done in the continuous flow stirred reactor work (Francis and Malone 1975a), but for the columnar studies determinations of such parameters were considered impractical. For this reason, rates of denitrification are based on initial volume of packing medium in the following manner:

$$R = (\Delta NO_3)(W)/V$$

where

R = denitrification rate in mg $NO_3/m^3/s$,

ΔNO_3 = difference between influent and effluent NO_3 concentration in kg NO_3/m^3,

W = flow rate in cm^3/s, and

V = initial volume of packing medium in m^3.

MATERIALS AND METHODS

The packing media included anthracite coal particles and polypropylene Raschig rings. Both are available commercially. The anthracite coal was obtained from the Shamoking Filter Co., Inc.,

Shamokin, Pennsylvania and is "Filt-O-Cite No. 1.5" which has an effective particle diameter between 2 and 3 mm. The particles are angular, irregularly shaped and have an average particle density of approximately 1.5 kg/m^3. Initial bed porosity after placement in the column was 36%. The polypropylene Raschig rings, "Flexirings", were obtained from Koch Engineering Co., Wichita, Kansas. The rings are 1.6 x 1.6 cm diameter and have a geometric surface of 3.2 m^2/m^3 and a free space of 92%.

All reactors were seeded from a microbial stock culture obtained from soil. The original stock culture was obtained in the following manner. Approximately 10 g of a soil from an organic horizon of an Emory silt loam or the ORNL reservation were added to 8 dm^3 of $Ca(NO_3)_2$ solution containing approximately 1000 g NO_3/m^3 and 600 g CH_3OH g/m^3. Anaerobic conditions and occasional stirring every three to four days produced a healthy culture of denitrifiers after 10-20 days. Columnar units were seeded by recycling 80 dm^3 of $Ca(NO_3)_2$ solution containing approximately 1000 g NO_3/m^3, 600 g CH_3OH/m^3 and 2-3 dm^3 of an active culture of denitrifiers. Recycling continued until a microbial population was established on the packing media; subsequently, nitrate feed containing 0.6 g CH_3OH/g NO_3 was passed slowly (0.17 to 0.80 cm^3/s) through the column. Influent solutions were made with spring water and in addition to the nitrate and carbon substrate contained the following in mol/liter: 3.2×10^{-4} KH_2PO_4, 7.7×10^{-4} $MgSO_4$, 2.75×10^{-3} $FeCl_3 \cdot 6H_2O$, and 4.13×10^{-7} $NaMoO_4 \cdot 2H_2O$.

Nitrate measurements were made with an Orion Nitrate Specific Electrode Model 92-07 connected to a Corning Model 110, digital, expanded scale pH meter. Interferences by nitrite were eliminated

by complexing the nitrite with sulfanilamide in 0.01 $\underline{N}$ H_2SO_4 according to the procedure of Francis and Malone (1975b). Use of 0.01 $\underline{N}$ H_2SO_4 acts as an ionic strength adjustor and eliminates any interferences by HCO_3^- and CO_3^{-2}. Nitrite concentrations were determined colorimetrically according to Bremner (1965) and CH_3OH concentrations with a gas chromatograph.

Two columnar units were used: the first, a glass column 120 by 15 cm diameter, and the second, a tapered column made of "Plexiglas", as illustrated in Fig. 1.

RESULTS AND DISCUSSION

Anthracite Coal Packing - Glass Column

Nitrate Source - Solutions made from three nitrate salts ($Ca(NO_3)_2$, $NaNO_3$, and NH_4NO_3) and actual nitrate wastes from an UO_2 fuel fabrication plant were used as influent solutions in the columnar denitrification studies. As reported earlier (Auerbach et al. 1974), the use of $Ca(NO_3)_2$ results in the formation of $CaCO_3$ which causes high head losses (> 90 kPa) and eventually plugs the column. Calcium carbonate is the secondary reaction product formed from Ca and CO_2 produced by the denitrification reaction. Continuous flow stirred reactors appear to be a more realistic engineering design to denitrify nitrate wastes where the dominant complementary cations are Ca or Al (Francis and Callahan 1975).

With influent solutions made from $NaNO_3$ or NH_4NO_3 no insoluble carbonate compounds are formed which cause significant head losses. However, effluent pH values are considerably higher with $NaNO_3$ and NH_4NO_3 than with $Ca(NO_3)_2$ (Table 1). The major difference in the denitrification of solutions containing $NaNO_3$ and NH_4NO_3 is that rather high

Table 1. Influent and effluent pH values in columnar denitrification of [illegible] nitrate forms -- anthracite coal as packing [illegible]

Nitrate Form	pH		Number of Observations
	Influent	Effluent	
$Ca(NO_3)_2$	7.15 ± 0.09[1]	7.6[illegible] ± 0.2[illegible]	8
$NaNO_3$	7.46 ± 0.75	8.55 ± 0.[illegible]	20
NH_4NO_3	6.86 ± 0.09	8.25 ± 0.[illegible]	13
UO_2 Nitrate Wastes	6.93 ± 0.13	8.23 ± 0.[illegible]	10

[1]Standard deviation

concentrations of nitrite (as great as 1000 g NO_2/m^3) accumulate in the effluent of $NaNO_3$ feed solutions (Table 2). On the other hand, maximum concentrations of nitrite in the effluent of NH_4NO_3 solutions did not exceed 10 g NO_2/m^3 at any time during the study. A similar observation was made in the stirred reactor work. The addition of a small amount of ammonium (10-15 g/m^3) to $NaNO_3$ influent solutions did not lower the nitrite accumulation, indicating the accumulation of nitrite is due to the levels of Na rather than any possible ammonium nutrient deficiencies.

Denitrification rates with solutions containing NH_4NO_3 appear to be greater than those containing $NaNO_3$ (Table 2). For example, the average denitrification rate with influent solutions containing $NaNO_3$ was 245 mg $NO_3/m^3/s$ while influents containing NH_4NO_3 averaged 326 mg $NO_3/m^3/s$. This indicates that denitrification of influent solutions containing NH_4NO_3 is approximately 35% faster than for solutions containing $NaNO_3$. This interpretation may be misleading if denitrification proceeds according to first order kinetics because the average concentration of nitrate in NH_4NO_3 influents (11.8 kg NO_3/m^3) was nearly twice the average nitrate concentration in $NaNO_3$ influents (6.5 kg NO_3/m^3). In addition, influents containing NH_4NO_3 followed those containing $NaNO_3$ which introduces the possibility that a higher microbial population was present in the column for the NH_4NO_3 data. A more realistic comparison between denitrification rates can be made (if denitrification follows first order kinetics) by using Michaelis and Menten relationships in the form of Lineweaver-Burk plots based on nitrate substrate concentrations. This subject is addressed in

TABLE 2. Denitrification in anthracite coal packing

Run No.	NO_3 Influent kg/m³	NO_3 Effluent kg/m³	Flow Rate cm³/s	NO_2 Effluent g/m³	Denitrification Rate[1] mg NO_3/m³/s
			Influent containing $NaNO_3$		
1	4.43	1.15	0.86	970	153
2	5.76	2.00	0.92	1200	150
4	4.52	0.62	0.47	1020	107
5	5.54	1.23	0.23	164	106
8	8.19	1.42	0.85	1050	41
9	8.86	4.87	0.88	624	251
10	8.64	4.37	0.86	230	269
11	8.86	4.65	0.94	360	314
14	3.10	< 0.05	0.68	0.7	
15	3.94	0.59	1.13	624	266
16	3.80	0.17	0.99	296	288
17	1.24	< 0.05	0.95	< 33	
18	4.87	0.05	0.99	181	406
19	10.6	2.07	1.03	1250	585
			Influent containing NH_4NO_3		
20	3.76	< 0.05	1.09	< 0.7	
21	9.52	1.64	1.05	< 0.7	744
22	13.7	4.43	0.70	9.2	582
23	13.7	4.21	0.52	3.0	442
24	9.74	3.76	0.50	< 2.6	267
25	11.1	5.98	0.83	3.9	382
26	11.1	7.53	0.87	--	278
27	11.3	6.86	0.84	--	334
28	11.3	7.75	0.80	3.5	254
29	11.1	7.97	0.82	3.3	230
30	11.5	7.97	0.83	4.6	265
31	11.1	8.86	0.92	2.3	183
32	12.4	7.97	0.80	4.9	320
33	11.1	7.97	0.81	4.3	227
34	12.4	8.86	0.81	4.6	258
35	12.4	10.6	0.77	--	122
36	12.0	8.41	0.82	3.9	260
37	15.1	9.52	0.79	--	394

[1]Denitrification rate based on initial volume of packing medium, 11.12 dm³, in a 120 x 15 cm diameter glass column. For influents containing $NaNO_3$, rates were corrected for NO_2 accumulation.

another section. Denitrification rates determined in such a manner revealed little difference between maximum denitrification rate (R_{max}) using $NaNO_3$ influents (R_{max} = 307 mg $NO_3/m^3/s$) and those containing NH_4NO_3 (R_{max} = 351 mg $NO_3/m^3/s$. Thus, maximum denitrification rates calculated in this manner are approximately 14% higher for influents containing NH_4NO_3 than for influents containing $NaNO_3$. However, this may not even be statistically different. The major difference in the denitrification of solutions containing the two salts is not in the rate of denitrification but rather in the concentration of the intermediate product, nitrite.

Anthracite Coal Packing - Tapered Column

Columns packed with anthracite coal appear to be a highly effective method for denitrifying high nitrate wastes. However, in the 120 x 15 cm diameter column, floatation of the bed occurs at flow rates > 0.80 cm^3/s. Continued operation results in compaction of the bed against the top of the column which causes high head losses (> 50 kPa). Floating of the anthracite bed is caused by the low density (1.5 kg/m^3) of the anthracite, large microbial growth on coal surfaces, and high production of N_2. Use of the same anthracite coal in a tapered column alleviates compaction of the bed at the top of the column, even at flow rates of 5 cm^3/s, and under optimum operating conditions takes on many properties of a fluidized bed.

Denitrification kinetics indicate that rates of denitrification become nitrate substrate inhibited at nitrate concentrations > 6.5 kg NO_3/m^3. Thus, greater denitrification should be obtained in tapered columns than in conventional columns because greater quantities of

nitrate can be delivered to the unit at influent nitrate concentrations which do not inhibit denitrification, viz., a greater fraction of the microbial enzyme can be maintained in the active form at influent concentrations of 5.0 kg NO_3/m^3 and a flow rate of 5 cm^3/s than at an influent concentration of 31 kg NO_3/m^3 at 0.8 cm^3/s.

The column made of "Plexiglas" and tapered 0.23 rad deg outwardly (Fig. 1) was filled with 32.5 dm^3 of the same anthracite coal used in the previously described columnar studies. The column was seeded with an active denitrifying culture in the manner previously described, and an influent of NH_4NO_3 containing approximately 1 kg NO_3/m^3 with 0.6 kg CH_3OH/m^3 was fed to the unit for three weeks before the denitrification data presented in Fig. 2 and Table 3 were collected. Visual observations revealed that the microbial growth was predominantly located in the lower one-third of the column indicating that an equilibrium microbial biomass had not been attained over the entire bed. Yet, the denitrification rate of 511 mg $NO_3/m^3/s$ observed the first day of measurement (Table 3) was considerably higher than the average observed rate (332 mg $NO_3/m^3/s$) obtained with NH_4NO_3 in the 120 x 15 cm diameter column.

Influence of Nitrate and Carbon Sources, Bed Expansion and Time - Dentrification performance in the tapered column over a 41 day interval is illustrated in Fig. 2. On the fifth day, an influent containing 11.1 kg NO_3/m^3 as NH_4NO^3 and acetic acid (neutralized with NaOH) equivalent in terms of carbon to 0.6 g CH_3OH/g NO_3 was fed to the unit at 3.73 cm^3/s; influent pH was 7.35. The following day nitrate analyses of the influent and effluent, and the observation that there was no difference between the temperature of the two streams, indicated that denitrification had ceased.

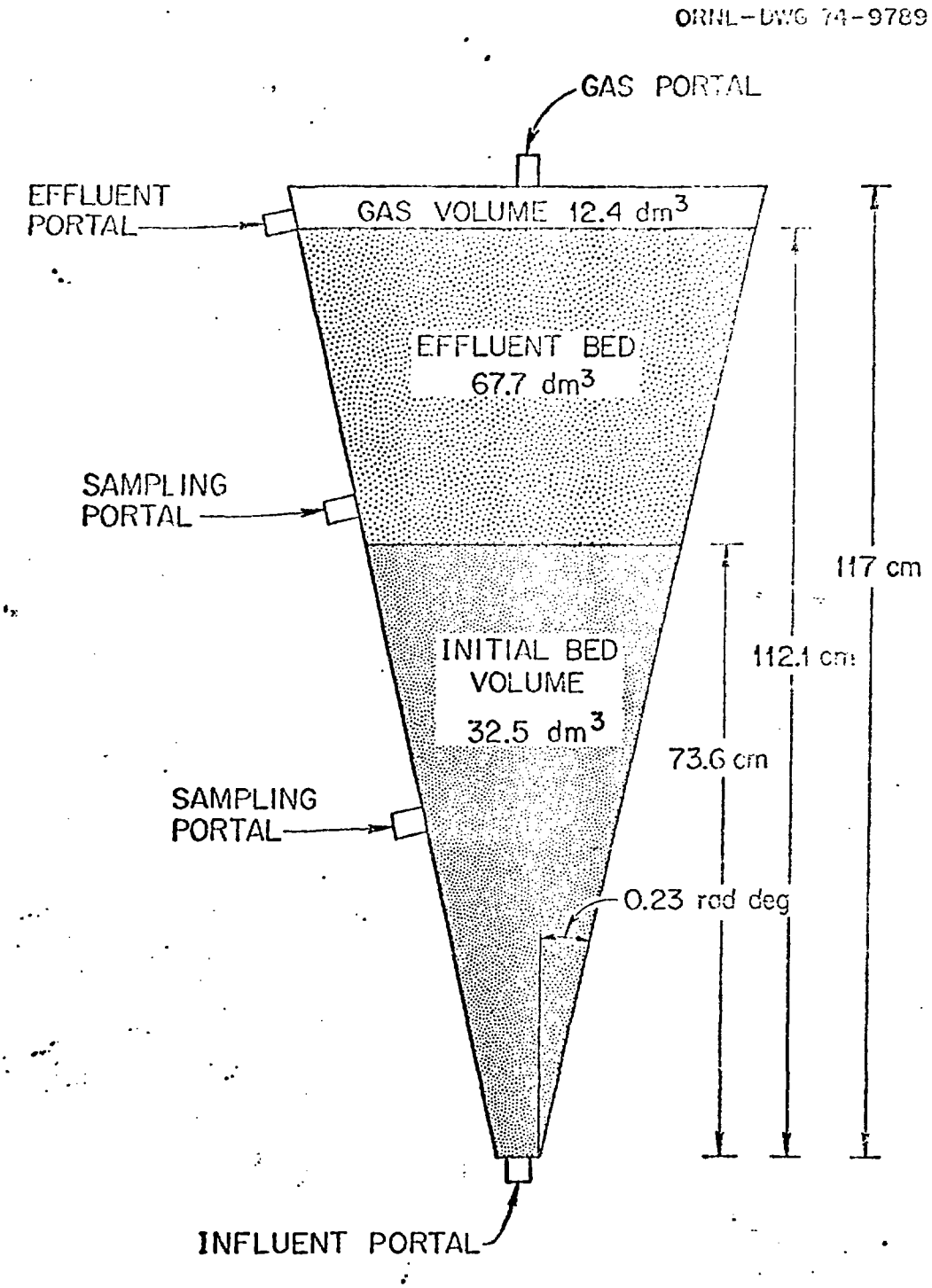
ORNL-DWG 74-9789
GAS PORTAL
EFFLUENT PORTAL
GAS VOLUME 12.4 dm3
EFFLUENT BED
67.7 dm3
SAMPLING PORTAL
INITIAL BED VOLUME
32.5 dm3
SAMPLING PORTAL
INFLUENT PORTAL
117 cm
112.1 cm
73.6 cm
0.23 rad deg

Fig. 1

Table 9. [illegible]trification in tapered column packed [illegible] anthracite coal

Run	Influent	Effluent	Flow Rate cm^3/s	Denitrification Rate[1] g $NO_3/m^3/s$
		Influent containing NH_4NO_3		
1	4.87	0.36	3.68	0.51
2	7.53	2.21	3.67	0.60
3	8.86	5.09	4.81	0.50
4	12.8	7.31	4.11	0.70
		Acetate used as carbon source		
8	22.1	15.5	1.22	0.25
9	22.1	15.5	1.25	0.26
		Influent containing $NaNO_3$		
10	23.0	19.5	1.33	0.14
11	20.4	17.7	1.33	0.11
12	17.7	15.9	1.33	0.07
		Influent containing NH_4NO_3		
		With bed expansion every 0.25 hr		
15	7.31	3.19	2.25	0.28
16	7.75	1.02	2.25	0.46
17	12.0	0.55	2.13	0.75
18	15.5	7.75	2.68	0.64
19	12.8	8.19	2.68	0.38
		With bed expansion every 2 hrs.		
22	10.2	4.21	2.07	0.38
24	7.53	2.66	2.42	0.36
25	15.5	2.97	1.55	0.60
		UO_2 fuel nitrate waste		
		with bed expansion every 2 hrs		
26	11.1	2.57	1.75	0.46
27	7.97	5.31	2.75	0.22
28	10.1	3.10	1.47	0.32
29	12.8	3.10	2.23	0.67
30	12.2	5.53	2.55	0.52
31	13.7	5.31	2.60	0.67
32	13.3	3.10	2.57	0.80
34	22.1	11.9	2.52	0.79
37	15.9	8.6	4.68	1.05
38	11.5	4.43	4.87	1.06
39	12.4	4.43	4.55	1.11
40	12.4	3.81	4.48	1.18
41	15.5	5.31	4.5	1.41

1/Denitrification rate based on initial volume of packing medium, 32.5 dm^3. Nitrite measurements were not routinely made; however, random effluent samples of NH_4NO_3 and UO_2 fuel nitrate wastes did not reveal any concentrations greater than 10 g NO_2/m^3.

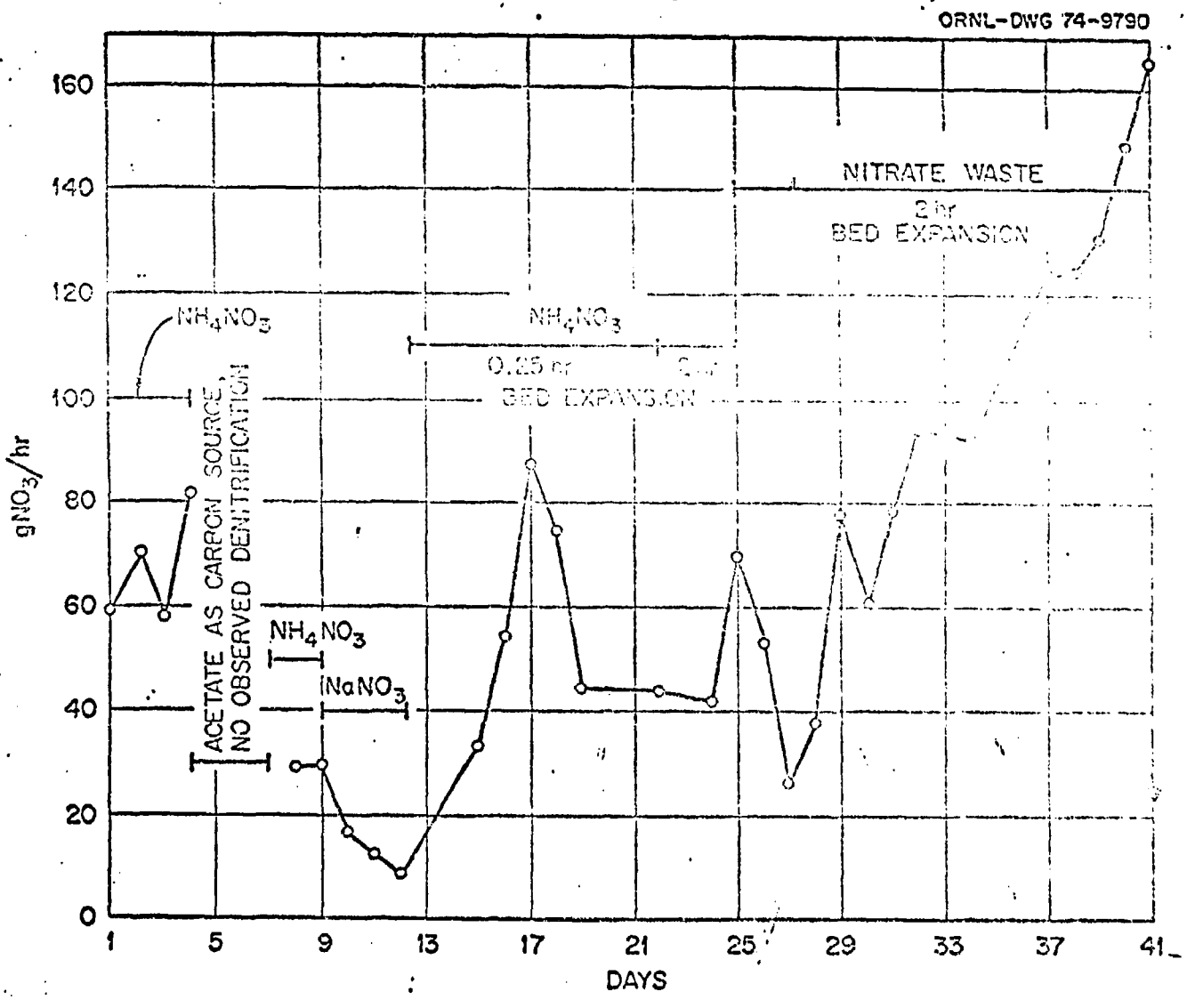
ORNL-DWG 74-9790
160
140
120
100
80
60
40
20
0
gNO3/hr
1
5
9
13
17
21
25
29
33
37
41
DAYS
NITRATE WASTE
2 hr
BED EXPANSION
NH4NO3
NH4NO3
0.25 hr
BED EXPANSION
ACETATE AS CARBON SOURCE,
NO OBSERVED DENITRIFICATION
NH4NO3
NaNO3

Fig. 2

To avoid loss of the established microbial population, methanol was replaced as the carbon substrate. Nitrate analyses on the eighth and ninth day verified that denitrification had resumed but at a much lower rate. However, the high influent nitrate concentrations may have been partially responsible for the low rates. Switching to $NaNO_3$ as a nitrate source reduced the denitrification 75% after three days. The reduction was due either to continued exposure to nitrate concentrations > 15.0 kg NO_3/m^3 or to some specific effect associated with $NaNO_3$.

Taking into consideration the large quantities of nitrate detected in the $NaNO_3$ effluents from the 120 x 15 cm diameter columns, the reduction in denitrification rate was likely due to the inability of the microorganisms to acclimatize quickly to $NaNO_3$. For example, a similar, but not as severe reduction in dentrification was noted on the substitution of UO_2 fuel nitrate wastes (predominantly NH_4NO_3 and HNO_3 neutralized to pH 6.4) for NH_4NO_3 on the 26th and 27th day. The difference between these nitrate sources was rather subtle, i.e., the ratio of nitrate to ammonium in the UO_2 nitrate wastes was 5 to 1 rather than 1:1 as in NH_4NO_3 and the influent concentration of calcium after dilution with spring water was about 600 g/m^3 compared to < 20 g/m^3 for NH_4NO_3 influent. Even so, acclimatization for approximately three days was required before similar denitrification rates were attained.

Through the "Plexiglas" walls of the column, one could observe that a large number of pores in the anthracite bed were filled with gas rather than nitrate solution indicating that the packing material was not being

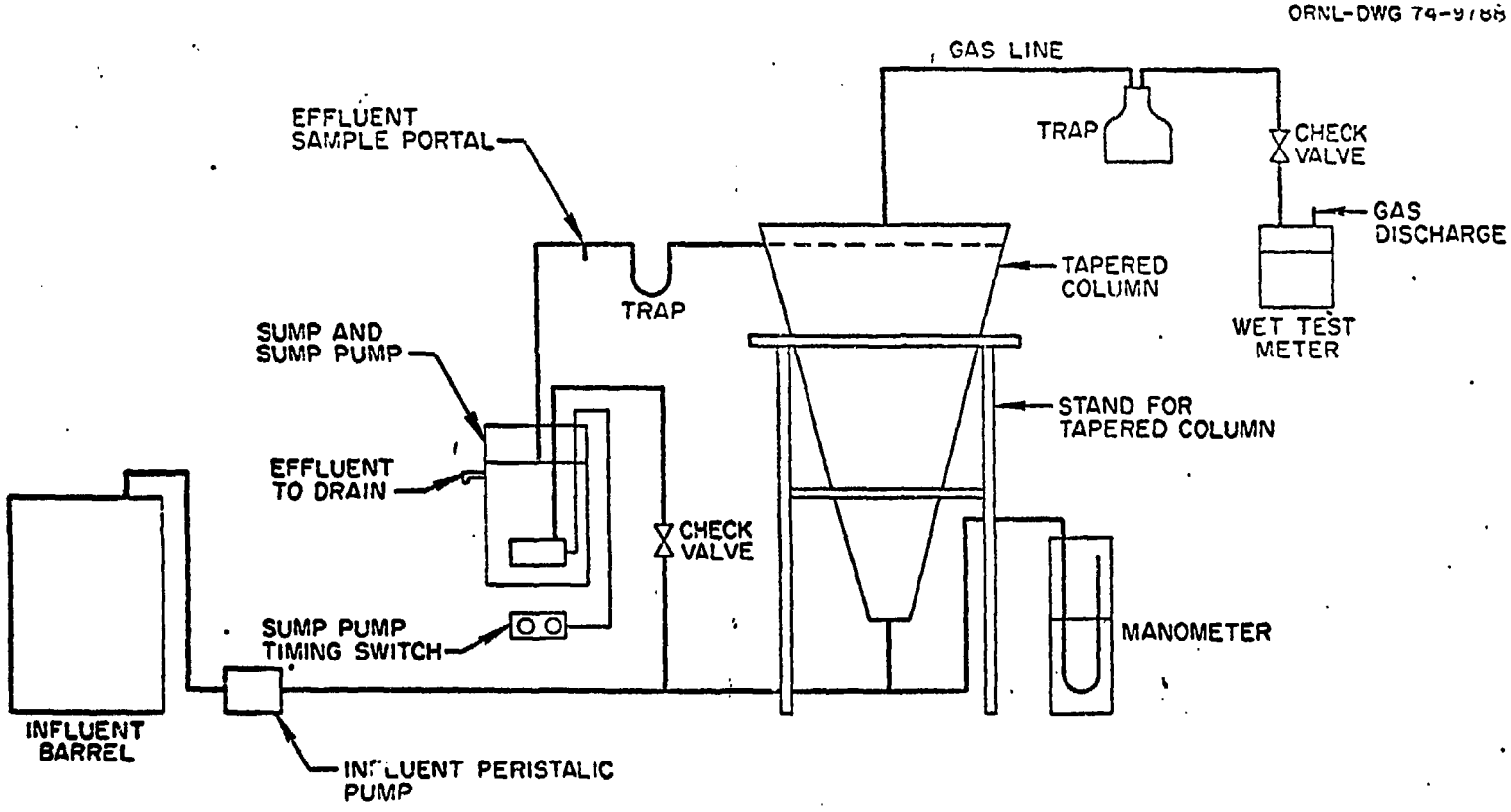
ORNL-DWG 74-9788
GAS LINE
TRAP
CHECK VALVE
GAS DISCHARGE
EFFLUENT SAMPLE PORTAL
TAPERED COLUMN
TRAP
WET TEST METER
SUMP AND SUMP PUMP
STAND FOR TAPERED COLUMN
EFFLUENT TO DRAIN
CHECK VALVE
SUMP PUMP TIMING SWITCH
MANOMETER
INFLUENT BARREL
INFLUENT PERISTALIC PUMP

Fig. 3

utilized efficiently. On the 15th day to maximize bed efficiency, the bed was expanded by pumping [illegible] approximately 5 dm^3 (flow rate 167 cm^3/s) of effluent from the tank every 0.25 hr. The manner in which this was conducted is illustrated in Fig. 3. This procedure agitated the packing medium so that the gaseous denitrification products were discharged, thus increasing the hydraulic residence time of fresh influent into the column. Bed expansion in this fashion was carried out every 0.25 hr until the 22nd day when visual observations and a sharp decline in rate of denitrification indicated that microorganisms were being washed from the column faster than they were being produced. On the 23rd day, bed expansion was changed to every 2 hr. Gaseous discharge during bed expansion was quite high; as great as 2 dm^3 or approximately 57 cm^3/s.

At the termination of the experiment, denitrification rates were established in excess of 1 g $NO_3/m^3/s$ (Table 3). These rates are 10-15 times higher than those reported in continuous flow stirred reactors (Francis and Callahan, 1975).

Gaseous Discharge - The primary gaseous products of denitrification are elemental nitrogen and carbon dioxide.

$$5CH_3OH + 6NO_3^- \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH$$

The gaseous discharge from the tapered column packed with anthracite coal and fed with UO_2 fuel fabrication wastes was approximately 92% N_2 and 8% CO_2 (Table 4). Considering the stoichiometric ratio of CO_2 to N_2 from the above equation, the concentration of CO_2 in the gaseous discharge was extremely low. However, the pH of the effluent was

Table 4. Denitrification gas analyses from tapered column packed with anthracite coal

Sample	Gas					
	N_2	CO_2	O_2	NO	Ar	MeOH
	% by volume					
1	95.42	4.54	0.008	0.01	0.03	0.002
2	90.36	9.42	0.0084	0.02	0.06	0.09
3	90.63	9.01	0.02	0.09	0.008	0.15
4	92.39	7.40	0.07	0.008	0.02	0.06
5	92.24	7.58	0.03	0.02	0.007	0.05
Average	92.21	7.59	0.027	0.03	0.025	0.07
S.D.	2.02	1.92	0.026	0.03	0.021	0.05

approximately 0.2 (Table 1); therefore the bulk of the carbon leaves the reactor in the form of HCO_3^- and CO_3^{-2}. Some carbon left the reactor in the form of microbial solids. Values for mixed liquor volatile suspended solids (MLVSS) in the effluents ranged from 100 to 900 g/m^3. Carbon was also retained inside the reactor as carbonates and microbial carbon.

Calcium and Phosphorus Retention - When anthracite coal is used for packing media, 99% of the calcium is removed from influent streams that contain < 1000 g/m^3 of calcium (Table 5). X-ray diffraction patterns of suspended solids in stirred reactors show that calcium is precipitated as $CaCO_3$. The same likely holds true for columnar denitrification. Elements other than calcium were also removed, for example, phosphorus, for which the mechanisms responsible remain unclear. X-ray patterns characteristic of any calcium phosphates were not detected in the suspended solids from the stirred reactors. Quite likely, a relationship exists similar to that of phosphorus adsorption in alkaline soils, i.e., phosphorus is either coprecipitated with or strongly occluded to $CaCO_3$.

Trace Element Retention - Reaction mechanisms governing the removal of the transition metals Zn, Ni, and Cu are unknown. Prolonged use of an anthracite bed for denitrification of influent streams containing appreciable quantities of these heavy metals will result in the accumulation of rather large quantities of these metals. It is also difficult to predict what effect the buildup of the metals will have on subsequent denitrification. Further research in this area is required. Cadmium, likely behaves like calcium because its ionic radius is nearly equal to

that of calcium; it probably precipitates as $CdCO_3$ or in mixtures of $CdCO_3 \cdot CaCO_3$.

Denitrification columns packed with anthracite coal might be useful in uranium recovery operations. For example, NH_4NO_3 solution containing 5-10 g/m^3 of uranium was lowered to < 0.5 g/m^3 in one pass through a column packed with 11.12 dm^3 of anthracite coal. The uranium likely coprecipitated on Ca and Mg carbonates or is converted to an insoluble phosphate mineral similar to that of apatite, which is known to selectively concentrate uranium. The chemistry of Th and Pu indicates these elements would behave in a similar manner. Thus, columns of this type may be useful in removing low levels of plutonium from waste streams containing nitrates.

Influence of Temperature - Denitrification is an exothermic reaction. The difference between influent (295° K) and effluent temperature (307° K) on the 39th day was 12 degrees. At a flow rate of 4.55 cm^3/s and assuming for water a density of 1 kg/m^3 with a specific heat of 4.48 J/g/°K, the rate of energy required to raise the influent to 307°K would be 228 J/s. Presumably, this heat is generated by the oxidation of CH_3OH. The quantity of heat generated by the oxidation of CH_3OH can be calculated in the following manner. The observed denitrification rate was 36.3 mg NO_3/s. Methanol analysis of the influent and effluent showed that 0.52 g of CH_3OH was required to denitrify a gram of nitrate. Thus, the oxidation rate of CH_3OH was 18.9 mg CH_3OH/s. If the heat of combustion for CH_3OH is taken as 0.71 MJ/mol then the rate of energy released on oxidizing is 422 J/s. Thus, the rate of heat generated is nearly twice that reflected by the increase in influent temperature. A portion of the remaining energy is

Table 5. Element removal during denitrification in a tapered column packed with anthracite coal

Element	Concentration Influent	Effluent	Removal
	g/m³		%
Calcium	645	7.0	99
	792	4.6	99
	615	5.7	99
Phosphorus	3.0	1.3	57
	5.1	0.52	90
	7.1	1.2	83
Zinc	0.15	< 0.005	> 96
Nickel	0.30	< 0.1	> 66
Copper	0.20	0.035	82
Cadmium	0.06	< 0.005	> 91

used for microbial growth while the remainder is lost as heat to the surrounding environment.

A large unit, one capable of denitrifying 5 metric tons of nitrate daily and operating at this efficiency will generate considerable energy 58.1 GJ, which is equivalent to 54.6×10^6 BTU. Quite likely the heat loss in a larger unit will be much less which means the heat generated may limit (up to a certain temperature may increase) the rate of denitrification. It appears that some type of cooling of larger units will be necessary if they are to operate at maximum efficiency.

Polypropylene Raschig Rings

Microbial populations were established faster on polypropylene rings than on the anthracite coal packing, viz., < 2 weeks on rings compared to 3-4 weeks on the coal. Maximum denitrification rates (Table 6) based on initial bed size were similar (1.0 - 1.2 g $NO_3/m^3/s$). However, on prolonged operation (> 40 days) denitrification rates began to decrease to 0.3 - 0.7 g $NO_3/m^3/s$ after 50-60 days (Table 6). The reduction in denitrification rates was attributed to hydraulic short circuiting in the column due to excessive microbial growth. For instance, after 30-35 days, areas of dark colored microorganisms were observed in the lower portion of the column. Healthy denitrifiers are pink colored, and they rapidly turn black if they do not receive a sufficient supply of nitrate. Initially, the dark colored organism were suspected to be sulfate reducing microorganisms even though no evidence of H_2S could be detected. Sulfate concentration in the influent was reduced by a factor of 10 but the dark colored areas continued to grow. On the 45th day the column was flushed for 0.33

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Table 6. Denitrification in a column packed with 1.6 cm dia. polypropylene raschig rings

Time Days	NO_3 Influent kg/m^3	NO_3 Effluent kg/m^3	Flow Rate cm^3/s	Denitrification Rate mg NO_3/m^3/s
10	2.43	0.71	0.73	54
11	2.21	1.20	0.26	11
12	2.21	1.33	0.73	28
13	2.08	0.95	0.57	27
14	3.10	1.20	0.80	65
15	2.21	0.93	0.82	45
16	2.74	0.75	0.83	71
17	2.30	0.55	1.01	76
18	2.26	0.06	0.75	70
19	2.21	< 0.05	1.02	> 97
20	2.61	< 0.05	1.86	> 209
22	2.26	< 0.05	2.73	> 265
23	3.19	< 0.05	5.37	> 737
24	3.10	0.82	5.30	520
25	3.45	0.66	5.23	629
27	2.66	< 0.05	5.20	> 585
31	2.83	0.56	5.15	503
36	4.87	< 0.05	4.78	> 998
37	5.09	0.16	5.92	1260
38	5.31	0.93	5.33	1000
41	4.98	0.80	5.17	930
42	4.65	0.83	5.20	856
43	5.53	0.74	5.17	1070
45	4.03	1.90	5.22	477
46	4.43	2.26	5.17	483
47	4.21	2.79	5.17	315
49	5.53	3.32	4.12	392
52	5.53	3.50	5.37	471
56	5.20	2.21	5.07	652
57	3.59	2.43	5.00	247

1/ Denitrification rate based on initial bed volume, 23.2 dm^3.

hr at a flow rate of 115 cm^3/s. Visual observation of the effluent or microbial mass in the column indicated that little biomass had been removed and denitrification rates the following 10 days were not appreciably changed. At the end of the experiment, mixed liquor suspended solids, MLSS, were 28.7, 37.6 and 83.0 kg/m^3 at the top, center and bottom of the column, respectively. These MLSS values are significantly higher than the maximum MLSS values of 5.55 kg/m^3 observed in stirred reactors fed NH_4NO_3 (Francis and Malone 1975a). With the 1.6 cm diameter polypropylene ring, microbial growth fills the center of the ring and reduces the surface area exposed to nitrate solution. Possibly, larger diameter rings would be more effective for long term use.

DENITRIFICATION KINETICS

A number of investigators (Requa and Schroeder 1973; Moore and Schroeder 1971; Stensel, Loehr, and Lawrence 1973) have evaluated the kinetics of denitrification at low nitrate substrate concentrations (< 250 g NO_3/m^3) and have concluded that as long as there is a sufficient supply of a carbon substrate the rate of denitrification does not decrease until the nitrate concentration approaches < 0.05 g NO_3/m^3. To our knowledge, no one has evaluated denitrification kinetics at nitrate substrate concentrations > 1000 g NO_3/m^3. The major purpose in evaluating denitrification kinetic data is that it can be used to determine nitrate concentrations for optimum dentrification rates.

Proper evaluation of denitrification kinetics in columnar studies is difficult because the concentration of substrate nitrate in an upward

flow column will decrease with increasing column height. In addition, denitrification rates in this study were based on the volume of initial packing media rather than current hydraulic residence times and microbial concentrations. Thus, any treatment of denitrification kinetics here should be viewed as a tool to evaluate the influence of nitrate substrate concentrations on rates of denitrification and not as establishing maximum specific substrate removal rates per unit weight of microorganism or a specific value for the Michaelis-Menten constant such as described by Monod (1949). A more formal treatment of denitrification kinetics is presented in the continuous flow stirred reactor work (Francis and Malone 1975a).

If an active microbial-nitrate compound (MS) is formed in the reaction between a microbial enzyme (M) and a specific nitrate concentration (S) at equilibrium, the reaction may be represented as

$$K_S = (M)\ (S)/(MS) \qquad (1)$$

where K_S = the dissociation constant. Thus, only at low nitrate concentrations is the denitrification rate proportional to nitrate concentrations. If the observed denitrification rates (R) are expressed in terms of the Mechaelis and Menten equation, then

$$R = R_{max}\ (S)/[K_S + (S)] \qquad (2)$$

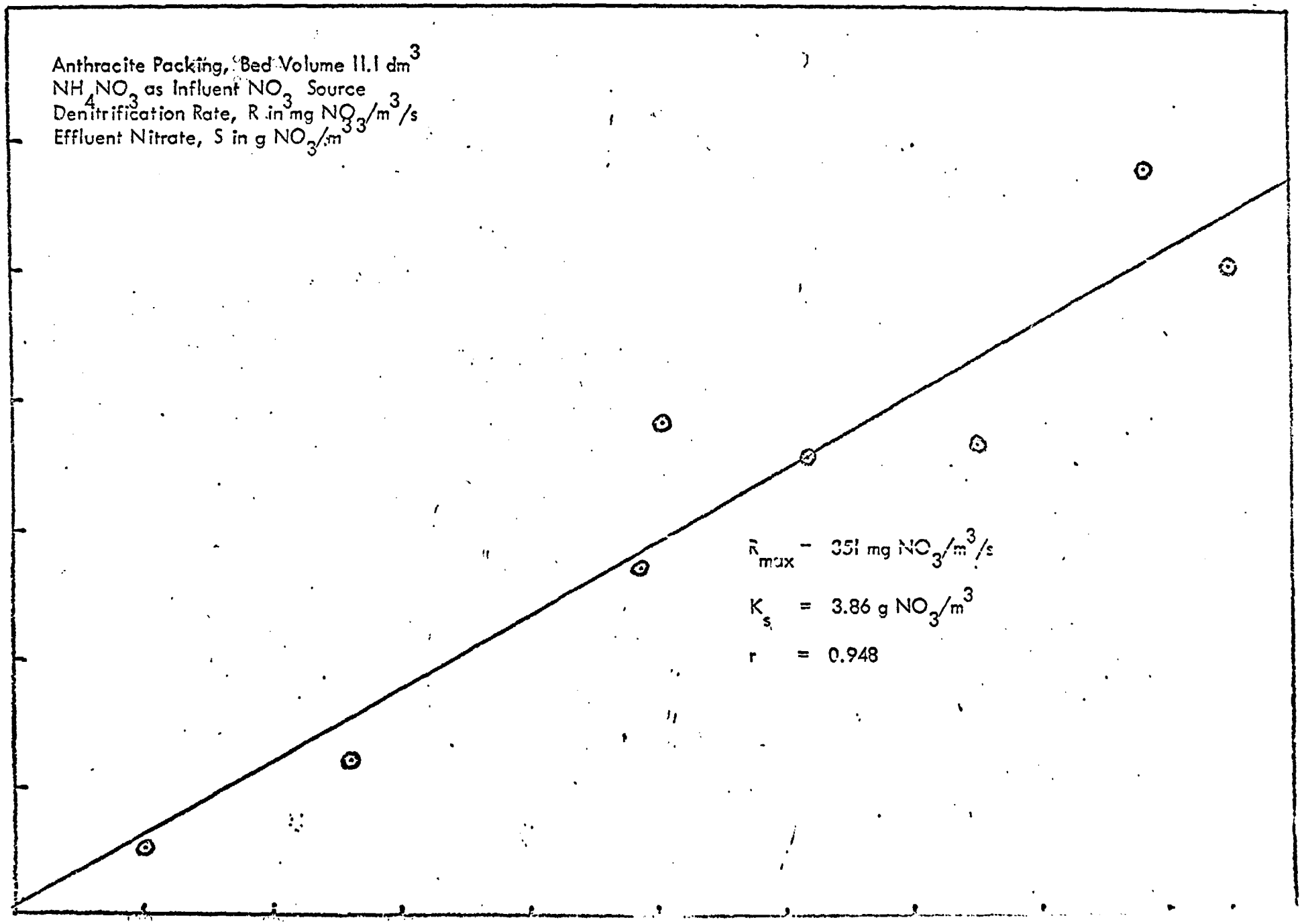
where R_{max} is the maximum denitrification rate obtained only when all the microbial enzyme (M) is combined in the form (MS) and K_S is equal

to the nitrate concentration at 1/2 R_{max}. R_{max} and K_s can be solved graphically by plotting the Lineweaver and Burk equation, that is

$$(S)/R = (S)/R_{max} + K_s/R_{max}. \quad (3)$$

The Lineweaver-Burk equation is formed by taking the reciprocal of both sides of Eq. 2 and multiplying the equation through by (S). Thus, R_{max} and K_s can be graphically solved by plotting (S)/R on the linear ordinate verses (S) on the abscissa; the intercept is K_s/R_{max} and the constant slope is $1/R_{max}$. This form is more appropriate for R_{max} and K_s determinations at high substrate concentrations (Lineweaver and Burk 1934) and has been used to determine values of K_s and R_{max} at nitrate substrate concentrations (< 5 g NO_3/m^3) by Requa and Schroeder 1973. Nitrate concentrations on the order of 5 g NO_3/m^3 are very low relative to nitrate concentrations used in our work; however, 5 g NO_3/m^3 is high relative to concentrations which limit rates of denitrification, i.e., < 0.05 g NO_3/m^3.

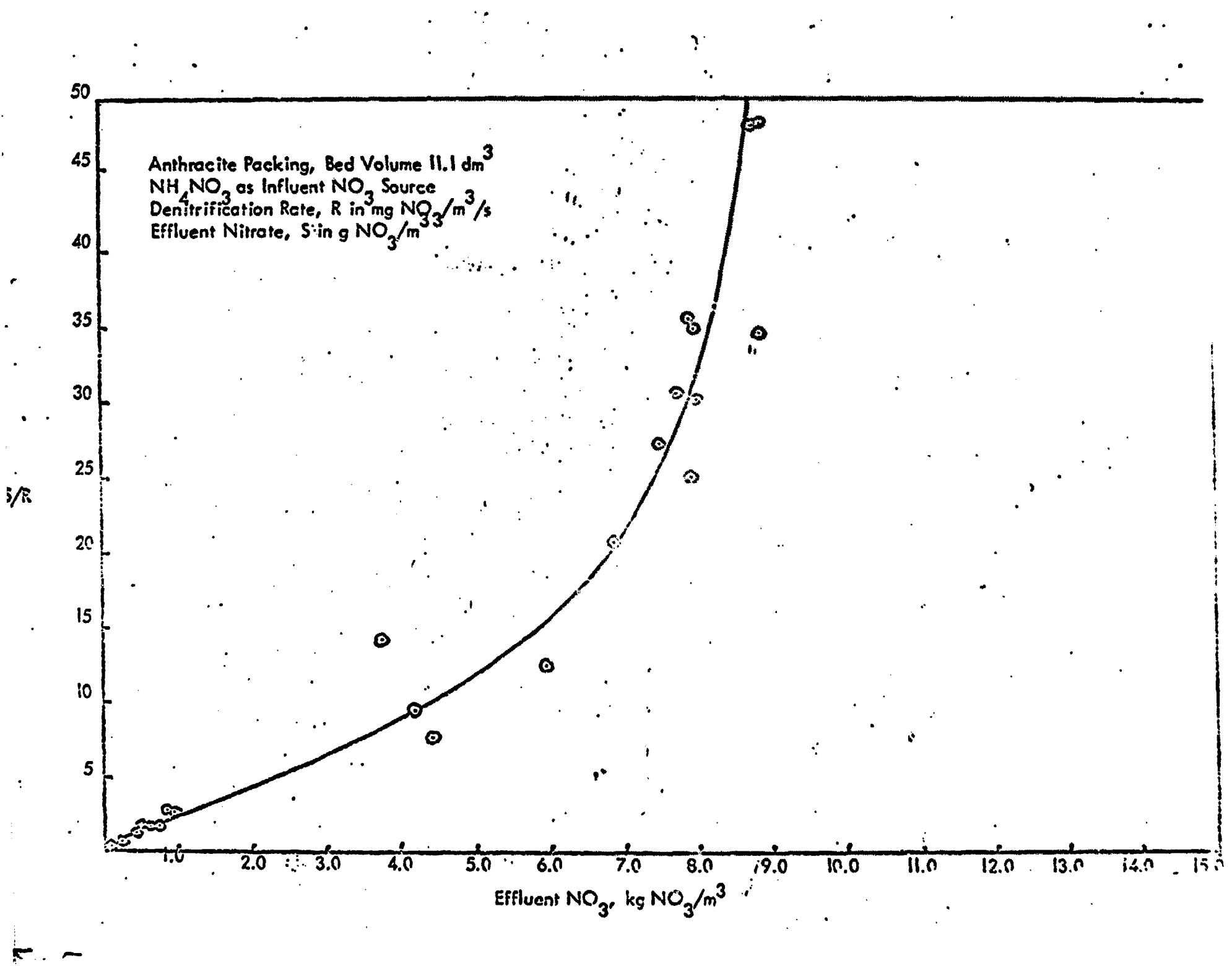
To test if R_{max} and K_s could be evaluated, effluent nitrate concentrations were treated as substrate concentrations. If denitrification mechanisms follow the Michaelis and Menten equation, a straight line should result on plotting (S)/R verses (S). With NH_4NO_3 as effluent concentrations < 1000 g NO_3/m^3 (Fig. 4), the linear regression analyses ($r = 0.948$) resulted in values for R_{max} and K_s of 351 mg NO_3/m^3 and 3.86 g NO_3/m^3, respectively. The value for K_s is slightly 10 times larger than 0.35 /g NO_3/m^3 determined by Reqa and Schroeder (1973) at substrate nitrate concentrations < 5 g NO_3/m^3. Considering the error involved in such a determination where substrate concentrations range


Anthracite Packing, Bed Volume 11.1 dm3
NH4NO3 as Influent NO3 Source
Denitrification Rate, R in mg NO3/m3/s
Effluent Nitrate, S in g NO3/m3
3
2
R
1
Rmax = 351 mg NO3/m3/s
Ks = 3.86 g NO3/m3
r = 0.948

from 100 to 1000 times higher than those used by Rega and Schroeder, 3.86 g NO_3/m^3 is probably not a bad estimate. At nitrate substrate concentrations > 1000 g NO_3/m^3 the curve becomes concave upward (Fig. 5), implying reaction mechanisms different than the simple reaction, $M + S \rightleftarrows MS$. Further detailed analyses of the kinetic data are necessary to determine what mechanisms may, but not necessarily, hold.

The upward curvature of the curve in Fig. 5 represents the formation of an inactive enzyme-substrate (Lineweaver and Burk 1934) formed because of excessive substrate, viz., the reaction is substrate inhibited at high nitrate concentrations. Using the methods presented by Lineweaver and Burk for substrate inhibition, the relative concentrations of free, active, and inactive enzyme forms are calculated at various substrate nitrate concentrations (Fig. 6). As effluent nitrate concentrations exceed 5 kg NO_3/m^3 the inactive enzyme form rapidly increases. For example, at 10 kg NO_3/m^3 < 40% of the enzyme is in the active form. The accuracy of such calculations is difficult to validate. The important conclusions from these kinetic calculations are that nitrate at excessively high substrate concentrations apparently inhibit the rate of denitrification and maximum denitrification per reactor volume can be achieved by increasing flow rates and keeping influent nitrate concentrations < 6.5 kg NO_3 kg/m^3 (assuming constant microbial concentration is maintained).

Other factors such as CH_3OH and ammonium concentrations may also be responsible for observed inhibition and should not be


Anthracite Packing, Bed Volume 11.1 dm3
NH4NO3 as Influent NO3 Source
Denitrification Rate, R in mg NO3/m3/s
Effluent Nitrate, S in g NO3/m3
S/R
50
45
40
35
30
25
20
15
10
5
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
Effluent NO3, kg NO3/m3

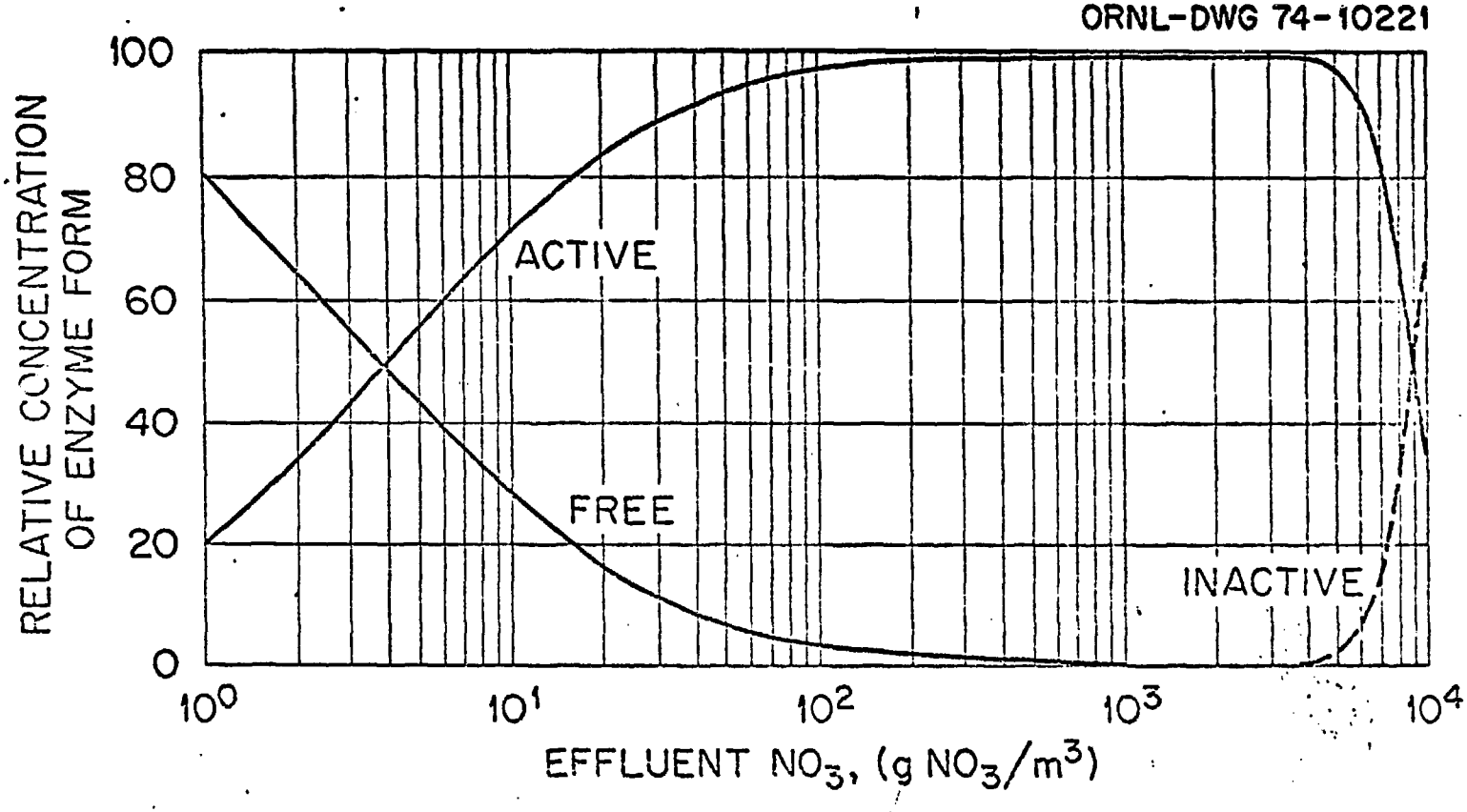

ORNL-DWG 74-10221
RELATIVE CONCENTRATION OF ENZYME FORM
100
80
60
40
20
0
ACTIVE
FREE
INACTIVE
10^0
10^1
10^2
10^3
10^4
EFFLUENT NO_3, (g NO_3/m^3)

Fig. 6

entirely discounted. However, stirred reactor data at CH_3OH substrate concentrations as high as 10 kg/m^3 did not inhibit denitrification rates (Francis and Malone 1975a). The influence of ammonium has not been fully evaluated.

Other factors such as physical alterations that take place in the anthracite bed should be considered as variables that might cause responses similar to that illustrated in Fig. 5. One alteration is the influence of increasing denitrification rates on the mean hydraulic residence time in the anthracite bed. For example, as denitrification increases, the generation of N_2 increases, which may decrease the residence time of nitrate substrate; flow-rate is unchanged. Thus, for the same amount of nitrate denitrified, as the residence time decreases R increases and (S)/R decreases which is opposite to the response observed in Fig. 5.

Another factor that should be considered is heat. Influent temperature was maintained at 301° K. Effluent temperature was not recorded in the kinetic studies; however, earlier work in the same column with a thermocouple located in the middle of the bed, temperatures were recorded from 304-310° K when influent temperatures ranged between 293-295° K. Effluent temperatures as high as 307° K were recorded in a tapered column packed with 32.5 dm^3 of anthracite. Heat inhibition would result in a response similar to that illustrated in Fig. 5.

Kinetics of denitrification were evaluated in a similar manner in continuous flow stirred reactors using influents containing NH_4NO_3 (Francis and Malone 1975a). In this case denitrification

rates were expressed in terms of specific removal rates (U) determined by the difference between influent and effluent nitrate concentrations at current hydraulic residence times and mixed liquor volatile suspended solids (MLVSS). Lineweaver-Burk plots at nitrate substrate concentrations < 6 kg NO_3/m^3 resulted in a very high linear regression coefficient (r = 0.997) and a maximum specific removal rate (U_{max}) of 1.97×10^{-5} s^{-1}, a value very close to 1.59×10^{-5} s^{-1} reported by Moore and Schroeder (1971) in continuous flow stirred reactors. At nitrate substrate concentrations 6 to 10 kg NO_3/m^3 denitrification rates appeared to be substrate inhibited. The relative concentrations of free, active and inactive enzyme forms were calculated according to Lineweaver and Burk as in the case of the columnar denitrification data. In both cases, columnar and continuous flow stirred reactor studies, approximately 50% of the microbial-enzyme population was calculated to be in the inactive form at substrate nitrate concentrations between 9 and 10 kg NO_3/m^3. Recent work using influents containing $Ca(NO_3)_2$ in continuous flow stirred reactors also show that at concentrations > 7 kg NO_3/m^3 the rate of denitrification rapidly decreases.

METHANOL UTILIZATION

Numerous denitrification studies at low nitrate concentrations (< 250 g NO_3/m^3) have shown that 0.6 g of methanol is required to denitrify one gram of nitrate (McCarty et al. 1969; Smith et al. 1972). This work at high nitrate concentrations (> 1000 g NO_3/m^3) indicates that less CH_3OH is required (Table 7). The stoichiometric CH_3OH requirement to denitrify one gram of nitrate is 0.43 g

Table 7. Denitrification methanol requirements

	CH_3OH Utilization[1]	Sample Number
Columnar Denitrification		
Anthracite packing	0.48 ± 0.35[2]	18
Anthracite packing in tapered column	0.45 ± 0.14	16
Polypropylene Raschig Rings	0.29 ± 0.14	14

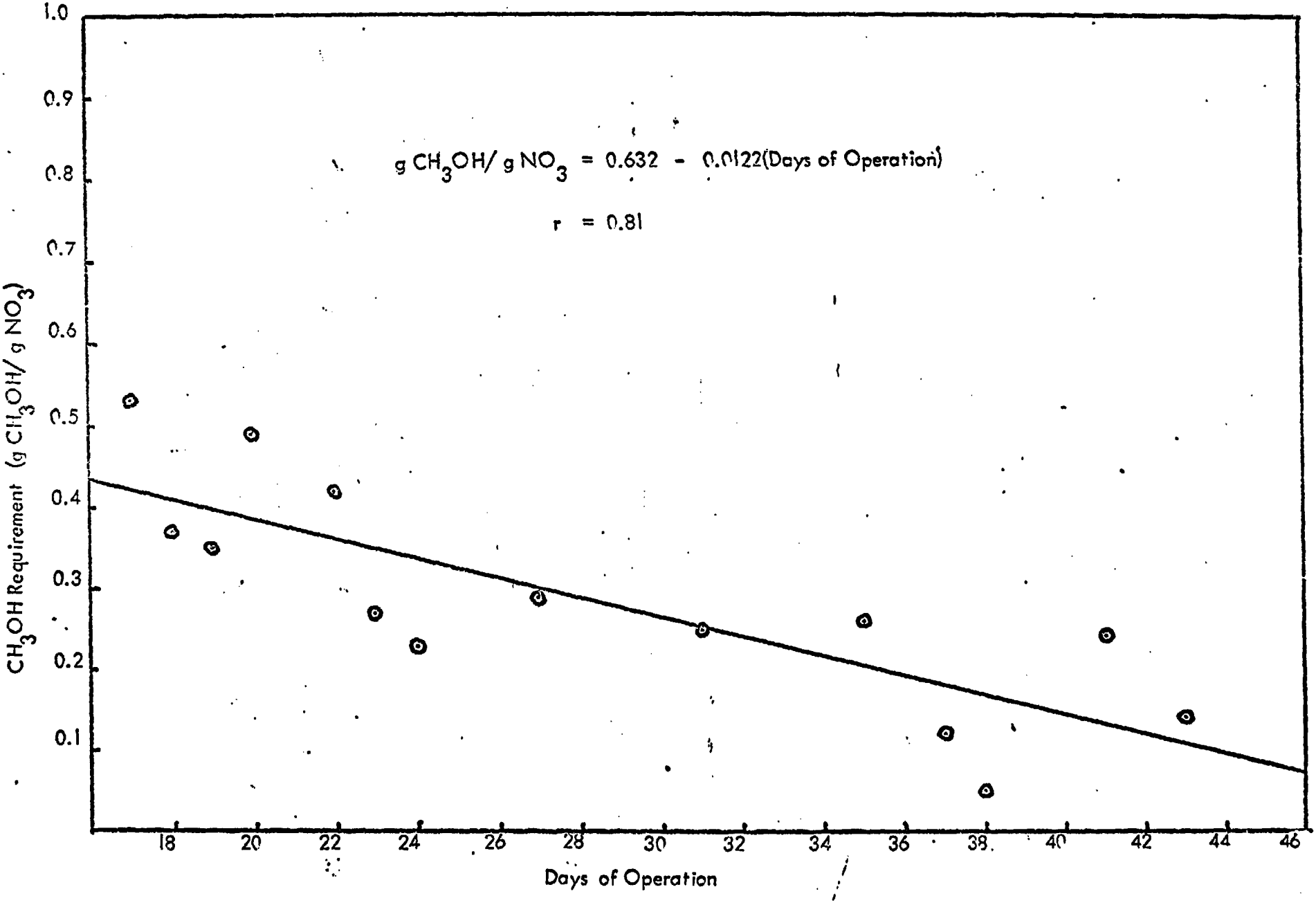
1/ Grams of CH_3OH required to denitrify one gram of nitrate

2/ Standard deviation

CH_3OH; however, it does not provide sufficient carbon for microbial growth or deoxygenation of any dissolved oxygen present in the system.

The CH_3OH requirement for denitrification with polypropylene Raschig ring packing appears to be approximately 50% lower than with anthracite packing (Table 7). In the case where 1.6 cm diameter polypropylene rings were used for packing, excessive microbial growth caused hydraulic short circuiting in the column which resulted in appreciably lower denitrification rates after operation of the unit in excess of 40 days. The CH_3OH utilization constant observed with the polypropylene rings was below the stoichiometric CH_3OH requirement for denitrification, indicating that the microbial biomass (maximum of 83 kg MLSS/m^3 at the bottom of the column) was being utilized as an endogeneous carbon source. The CH_3OH requirement decreased in proportion to the length of operation (Fig. 7).

It appears that denitrification at high nitrate concentrations requires approximately 25% less CH_3OH than denitrification at low nitrate substrate concentrations. For example, the work of McCarty et al. 1969 and Smith et al. 1972 where nitrate substrate concentrations were in the order of 5-10 g NO_3/m^3 required 0.6 g of CH_3OH/g of NO_3 while, in our work, where nitrate substrate concentrations were on the order of 500 to 5000 g NO_3/m^3 the CH_3OH requirement for each g of nitrate was on the order of 0.4 to 0.5 g. This may be a result of two possible relationships. One, in the denitrification at high nitrate concentrations higher microbial buildup occurred in the reactors than at low nitrate concentrations and a portion of the carbon


g CH_3OH/ g NO_3 = 0.632 - 0.0122(Days of Operation)
r = 0.81
CH_3OH Requirement (g CH_3OH/ g NO_3)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
18
20
22
24
26
28
30
32
34
36
38
40
42
44
46
Days of Operation

requirement was due to an endogeneous carbon source rather than added CH_3OH. Two, at high nitrate substrate concentrations (500 to 5000 g NO_3/m^3) a greater proportion of the microbial-enzyme form is in the active form than at nitrate substrate concentrations < 10 g NO_3/m^3 (Fig. 6). Thus, at low nitrate concentrations CH_3OH must be utilized to support both a free and active form (approximately 50% each at 5 g NO_3/m^3, Fig. 6) while at nitrate substrate concentrations between 500 and 5000 g/m^3 the enzyme form is > 95% in the active form.

SUMMARY AND CONCLUSIONS

Anaerobic columns packed with anthracite coal particles appear to be an effective method for the treatment of nitrate wastes associated with uranium recovery operations which use the ammonium diuranate process. This process is used in many of the presently operated UO_2 fuel fabrication plants and will be used in future fuel reprocessing facilities. Nitrate wastes generated in this process are predominantly nitric acid and NH_4NO_3. The quantity of nitrates in the waste streams from these facilities are expected to be as high as 5 metric tons of nitrate a day.

Using the denitrification rate of 1 g $NO_3/m^3/s$ (Table 3), the rate for one day would be 86.4 kg $NO_3/m^3/day$. Thus, to denitrify 5 metric tons of nitrate a day (5×10^6 g NO_3) would require a bed volume of 57.9 m^3. For a bed 8 m in diameter the required height would be slightly over one meter (1.2 m).

Denitrification kinetic data indicate that the most effective influent concentrations range between 500 and 5×10^3 g NO_3/m^3. If

5 kg NO_3/m^3 is selected as the influent concentration the required volume for 5 metric tons of nitrate a day would be 10^3 m^3 or a flow rate of 11.6 dm^3/s. The cross-sectional flow into a 1.2 x 8 m diameter column would be 0.23 $dm^3/m^2/s$, about 10 fold less than that in the tapered column used in these studies. Mechanical stirrers could be used to maintain bed integrity or the bed could be fluidized in a tapered column by using anthracite of smaller particle size. A recycle system could be used if pH levels and ammonium concentrations could be maintained at nontoxic conditions.

Extrapolations of laboratory bench experiments to production size facilities are often haphazardous and speculative. However, data presented in this paper certainly justify pilot plant tests at facilities where the disposal of large quantities of nitrate wastes are a problem.

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Figure Legends

Fig. 1. Tapered column dimensions.

Fig. 2. Influence of nitrate and carbon source, bed expansion and time on denitrification in a tapered column.

Fig. 3. Schematic of the tapered columnar denitrification system.

Fig. 4. Lineweaver-Burk plots of effluent nitrate concentrations < 1000 g/m^3.

Fig. 5 Lineweaver-Burk plots of effluent nitrate concentration to 10 kg NO_3/m^3.

Fig. 6. Relative concentration of enzyme form as influenced by effluent nitrate concentration.

Fig. 7. Influence of time on CH_3OH requirements in a column packed with 1.6 cm diameter polypropylene raschig rings.