Treatment of Effluents from Ammonia Plants — Part V Denitrification of an Inorganic Effluent from a Nitrogen Chemicals Complex using Sulphur as the Energy Source

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Abstract

The literature on chemo-autotrophic sulphur-oxidising bacteria is briefly reviewed. The use of such bacteria (thiobacteria) in the denitrification of an inorganic effluent containing high levels of nitrate (2 800 mg l^{-1} NO_3^- —N) has been shown to be feasible. Further studies are necessary to develop techniques to increase the biomass in the reactor and so to decrease the hydraulic retention time necessary for the completion of the denitrification.

Introduction

The aqueous effluent from a nitrogen-chemicals complex amounts to 1,64 — 1,7 Ml d^{-1}. It contains approximately 1 400 mg l^{-1} ammonium nitrogen and an equivalent amount of nitrate nitrogen. Nitrification of this effluent has already been described by Neytzell-de Wilde (1977). Denitrification of the nitrified effluent using methanol as carbon source has also been investigated (Neytzell-de Wilde *et al.*, 1977).

Although denitrification with methanol does not directly increase the COD or BOD of the resulting effluent, the cost of methanol in the denitrification process is high. Cheaper sources of carbon have been investigated, and molasses was selected as an alternative carbon source by investigators using fluidised and packed bed reactors in order to decrease reactor size (Baskir *et al.*, 1976 and Bosman *et al.*, 1978).

Carbon sources other than methanol are however likely to produce a high cell biomass and, in the case of molasses, will directly increase the COD of the resulting effluent. Any increase in BOD_5 of the resulting effluent will depend on the control of the ratio of effective carbon to NO_3^- —N ratio in the denitrifying reaction.

Chemo-autotrophic sulphur oxidising bacteria, such as *Thiobacillus denitrificans* (Mann *et al.*, 1972) are capable of using nitrate as an oxidant. It was decided therefore to investigate the use of sulphur as an energy source using thiobacillus as the organism to carry out the denitrification.

The cost per ton (at Durban) of the three energy sources mentioned above are: methanol R310, molasses R18 and sulphur R47.

Literature Review

Baalsrud and Baalsrud (1954) gave an excellent review and set out to show that the organism *Thiobacillus denitrificans* can oxidise a number of inorganic sulphur compounds either aerobically, or anaerobically with nitrate as the oxidant. It was also shown that this bacterium cannot develop in media devoid of ammonium salts and that iron is required for growth. Nitrite was found to be highly toxic to the nitrate-reducing enzyme system: concentrations as low as $3{,}5 \times 10^{-4}$ M inhibited denitrification in the presence of sulphur. In the presence of thiosulphate however nitrite was rapidly decomposed to nitric oxide (NO).

More recent reviews, with particular reference to the knowledge of the energy metabolism of thiobacilli were undertaken by Trudinger (1967, 1969). While the work has not led to unequivocal conclusions, more precise information on enzymatic transformations of S^0 and sulphur compounds by thiobacilli (including *T. denitrificans*) is beginning to accumulate.

Studies on the practical application of sulphur bacteria for the destruction of nitrate by denitrification have been reported only recently.

Mann *et al.*, (1972) have studied increased denitrification in soils by additions of sulphur as energy source. Soil columns amended with sulphur and limestone were used in tests. A solution of 425 mg l^{-1} NO_3-N was perfused through the column continuously. Highly anaerobic conditions were developed and nitrate was successfully reduced; nitrite concentrations were found to be negligible.

Aleem (1974), carried out investigations to elucidate the physiology and metabolism of sulphur bacteria since this approach appeared to be the key to the development of pollution control measures. Unfortunately he did not use S^o in his tests. In his work with *T. denitrificans*, however, complete oxidation of sulphide, thiosulphate, tetrathionate, and sulphite to sulphate occurred with stoichiometric reduction of nitrate, nitrite, nitric oxide, and nitrous oxide to nitrogen gas. These nitrogen compounds are the possible intermediates in the denitrification of nitrate. This process was mediated by the cytochrome electron transport chain and was sensitive to the electron transfer inhibitors.

Sikora and Keeney (1976) conducted tests on column reactors to evaluate the use of sulphur and *T. denitrificans* as a means of denitrifying septic tank effluent. The reactors were columns packed with a 1:1 mixture of sulphur and dolomite chips, and were pretreated by recycling an enrichment culture of *T. denitrificans* through the columns. Almost complete denitrification of effluent (40 mg l^{-1}) was obtained and denitrification kinetics closely resemble first order in the range of NO_3^- concentrations employed.

Batchelor (1976), studied the feasibility of employing autotrophic bacterial denitrification as an alternate to heterotrophic bacterial denitrification in waste water treatment, and a program of theoretical and experimental investigations is given in his thesis. Enrichment cultures similar in appearance to *T. denitrificans* were developed. Experimental work involving both batch and continuous growth modes was planned to determine process stoichiometry and kinetics. In addition to experimental studies, theoretical calculations of microbial growth yield based on thermodynamic concepts are given. As a corollary to these calculations, a hypothesis is offered to explain the roles of electron donors and acceptors in an autotrophic anaerobic, biochemically mediated environment.

In their work on autotrophic micro-organisms McFadden and Denend (1972) grew *T. denitrificans* anaerobically with nitrate as an acceptor in both sterile and non-sterile media. They found that *T. denitrificans* can be grown in mass cultures almost free from heterotrophic contamination without sterilisation of the media.

In the inorganic effluent under consideration, therefore, it is likely that the main organism will be *T. denitrificans*.

The chemistry of denitrification and sulphur oxidation

The reduction of $NO_3^- - N$ to N_2 and the oxidation of S^o to SO_4^{2-} may be derived from the various half reactions, some of which are given below and in Figure 1 (numbers of the reactions correspond to those in Figure 1). The values given alongside the reactions are taken or calculated mainly from data given by Stumm and Morgan (1970), Mahler and Cordes (1971), Charlott and Manchon (1971) and Meynell and Meynell (1970).

	E_o	$p_e^o = \log K = E_o/0{,}059$	$p_e^o + \frac{nH}{2}\log Kw = p_e^o(w)$
(1) $\frac{1}{3}\ NO_2^- + \frac{4}{3}H^+ + \epsilon =$ $\frac{1}{6}\ N_2(g) + \frac{2}{3}\ H_2O$	1,52	25,68	16,35
(2) $\frac{1}{4}\ O_2\ (g) + H^+ + \epsilon = \frac{1}{2}\ H_2O(l)$	1,229	20,75	13,75
(3) $\frac{1}{5}\ NO_3^- + \frac{6}{5}\ H^+ + \epsilon =$ $\frac{1}{10}\ N_2\ (g) + \frac{3}{5}\ H_2O$	1,24	21,05	12,65
(4) $\frac{1}{2}\ NO_3^- + H^+ + \epsilon =$ $\frac{1}{2}\ NO_2^- + \frac{1}{2}\ H_2O$	0,83	14,15	7,15
(5) $\frac{1}{6}\ SO_4^{2-} + \frac{4}{3}\ H^+ + \epsilon =$ $\frac{1}{6}\ S(s) + \frac{2}{3}\ H_2O$	0,357	6,03	−3,30
(6) $\frac{1}{8}\ SO_4^{2-} + \frac{5}{4}\ H^+ + \epsilon =$ $\frac{1}{8}\ H_2S\ (g) + \frac{1}{2}\ H_2O$	0,31	5,25	−3,50
(7) $\frac{1}{2}\ S(s) + H^+ + \epsilon =$ $\frac{1}{2}\ H_2S(g)$	0,171	2,89	−4,11
(8) $H^+ + \epsilon = \frac{1}{2}\ H_2(g)$	0,0	0,0	−7,00

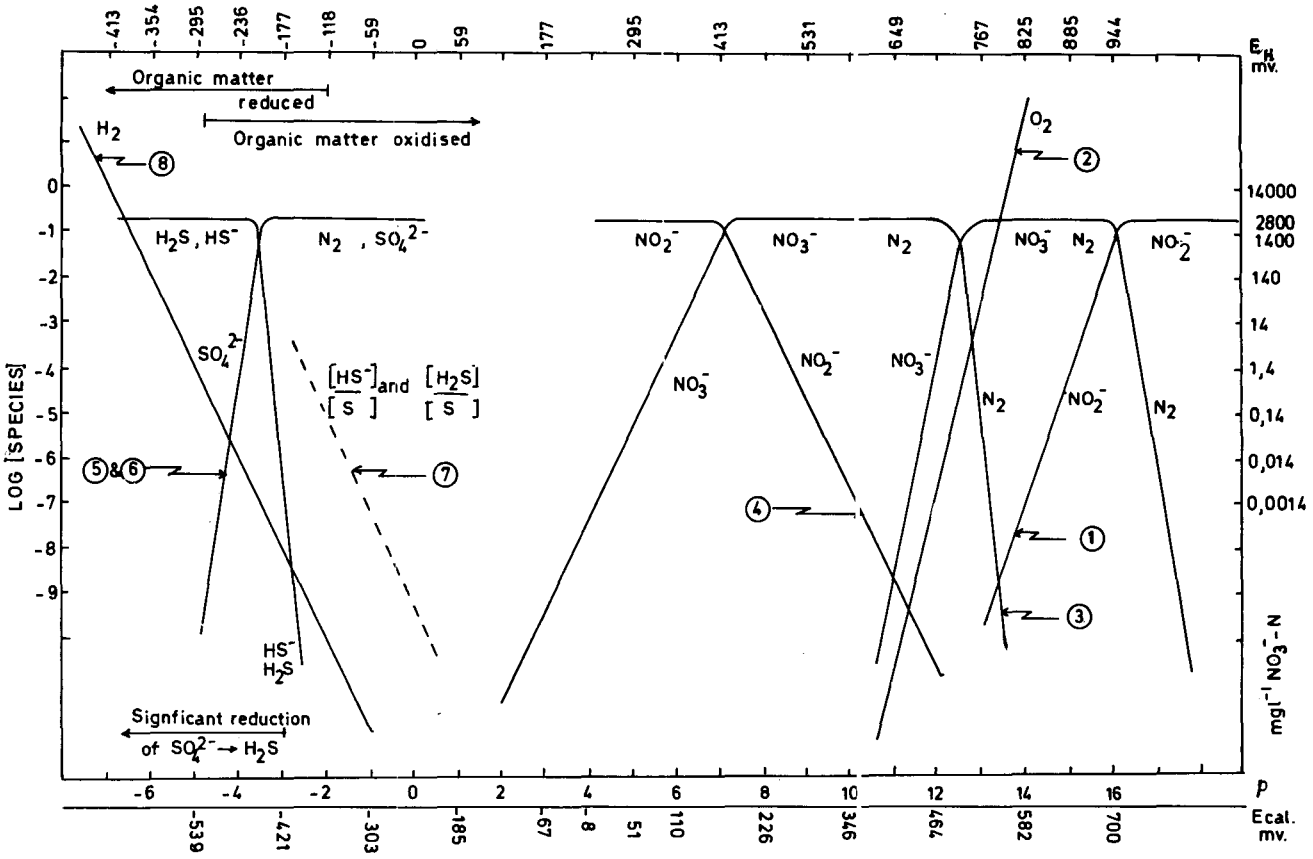

Figure 1
Relation between log (species) and redox potential
NO_3^-—N approx. 2 800 mg^{-1}; SO_4^{2-} approx. 16 000 mg l^{-1}; pH 7; and temp. 25°C

A system will tend to oxidise equimolecular concentrations of any other system of lower $p_e^o(w)$ value. Values represent measures of oxidising intensity and where $\Delta p_e^o(w)$ is positive, the reaction is thermodynamically possible in neutral aqueous solutions at standard conditions.

Micro-organisms mediate the electron transfer in oxidising-reducing reactions and the bacteriological significance of oxidation-reduction potential lies in the dependence of growth on a succession of enzymatic reactions, each of characteristic E'_o and which will not occur unless the medium has a suitable E_H.

Obligate anaerobes for example, will fail to grow in media at E_H greater than say 100 mv. However both aerobes and anaerobes can to some extent bring the E_H of their medium to a value suitable for their growth, but that ability to do so depends on their concentration.

In experimental work, it is often desirable to set the E_H value of the medium to a level favourable to growth. Thioglycollate is often added to a culture medium of anaerobic bacteria to remove dissolved oxygen and to set the medium at the low redox potential required for growth by the micro-organisms.

Daniels *et al.* (1965), have used sparging, with nitrogen and chemical addition with cysteine, ascorbic acid and thioglycollate. (E'_o at 30°C and pH 7 for thioglycollate, for example, is −340 mv).

By combining equations (3) and (5), the oxidation of S° to SO_4^{2-} and reduction of NO_3^-—N to N_2 is given by:-

$$6\,NO_3^- + 5\,S_{(s)} + 2\,H_2O \rightarrow 3\,N_{2(g)} + 4\,H^+ + 5\,SO_4^{2-} \quad \ldots(9)$$

Since Δp_e^o is positive, the reaction is thermodynamically possible. This reaction supplies the energy required for the conversion of carbon dioxide to cell matter (equations 10 and 11).

In the anaerobic conditions, sulphur plays the role of electron donor

$$\tfrac{1}{4}\,CO_2 + {}^1/_{20}\,NH_4^+ + {}^1/_{20}\,OH^- + H^+ + \epsilon \rightarrow {}^1/_{20}\,C_5H_7O_2N + {}^9/_{20}\,H_2O \quad \ldots(10)$$

$${}^1/_6\,S + {}^4/_6\,H_2O \rightarrow {}^1/_6\,SO_4^{2-} + {}^8/_6\,H^+ + \epsilon \quad \ldots(5)$$

Hence by combining equations (10) and (5) the conversion of CO_2 into cell matter is achieved:-

$$\tfrac{1}{4}\,CO_2 + {}^1/_{20}\,NH_4^+ + {}^1/_6\,S + {}^4/_{15}\,H_2O \rightarrow {}^1/_{20}\,C_5H_7O_2N + {}^1/_6\,SO_4^{2-} + {}^{23}/_{60}\,H^+ \quad \ldots(11)$$

In anaerobic environments such as the above, once all the nitrate has been reduced to nitrogen, the potential (EMF) can become poised at a lower value. At the lower value sulphur, in-

completely reduced sulphur compounds such as sulphite, and sulphates can be reduced by micro-organisms e.g. *Vibrio desulphuricans*, provided some organic matter is present. The reaction for the reduction of SO_4^{2-} is given by the following equation

$$2\,C_{\text{(organic substrate)}} + SO_4^{2-} + 2\,H_2O \rightarrow HS^- + HCO_3^- + H_2CO_3$$

Biebl and Pfennig (1977) have also reported on strains of sulphate bacteria that are capable of using elemental sulphur as an electron acceptor for growth.

$$8H_{\text{(organic substrate)}} + H_2SO_4 \rightarrow H_2S + 4\,H_2O$$

$$8\,H_{\text{(organic substrate)}} + 4\,S^o \rightarrow 4\,H_2S$$

Microbial reduction of incompletely reduced materials such as sulphite, thiosulphate, tetrathionate and elemental sulfur is common and according to Starkey (1956) many bacteria and fungi are concerned in these transformations.

In the present study, measurement of the redox potential in growing cultures of sulphur bacteria was used to give an indication of the correct EMF for growth and of the onset of sulphide production after exhaustion of nitrate in the environment.

Overall reaction : stoichiometry

Batchelor and Lawrence (1977) adopted McCarty's (1964) method with slight modification to predict the theoretical autotrophic denitrification stoichiometry. In the overall reaction stoichiometry, sulphur is the apparent electron donor for synthesis (see section on chemistry of denitrification and sulphur oxidation).

Using a value of 0,6 for the efficiency in producing or utilising ATP, and 7,5 k cal/electron equivalent as the ATP energy required to convert one electron equivalent from pyruvate to cells, and assuming a cell composition of $C_5H_7O_2N$, the authors calculate a ratio of 0,336 for the electron equivalent ratio allocated to the synthesis and energy reactions, thus,

$$\text{Yield} = \frac{\text{mol cells}}{\text{mol } NO_3^-} \times 0{,}336$$

For the reactions,

$$^1/_5\,NO_3^- + {}^1/_6\,S + {}^1/_{15}\,H_2O \rightarrow {}^1/_{10}\,N_2 + {}^1/_6\,SO_4^{2-} + {}^2/_{15}\,H^+$$

and $$¼\,CO_2 + {}^1/_6\,S + {}^1/_{20}\,NH_4^+ + {}^4/_{15}\,H_2O \rightarrow {}^1/_{20}\,C_5H_7O_2N + {}^1/_6\,SO_4^{2-} + {}^{23}/_{60}\,H^+$$

$$\text{Molar yield} = \frac{^1/_{20}}{^1/_5} \times 0{,}336 = 0{,}084$$

and the overall stoichiometry becomes:

$$1\,NO_3^- + 0{,}42\,CO_2 + 1{,}1133\,S + 0{,}084\,NH_4^+ + 0{,}7813\,H_2O = 0{,}084\,C_5H_7O_2N + 0{,}5\,N_2 + 1{,}1133\,SO_4^{2-} + 1{,}31\,H^+$$

Hoor (1976) has also considered the energetic aspects of the metabolism of reduced sulphur compounds in *T. denitrificans*, but experimental work was done with thiosulphate and sulphide as energy source. The work is also of interest because of the successful attempt to grow *T. denitrificans* anaerobically in a chemostat with sulphide as the growth limiting factor. Cell yields on sulphide were 0,29 g cells/g $S^=$.

Experimental

Standard conditions

Temperature: Continuous tests were carried out at a fixed temperature (28 – 28,5°C)

pH: All tests were conducted under controlled pH conditions (between 7 – 7,2). The acid produced during reaction was neutralised with approximately 1 molar sodium bicarbonate.

Feed: The medium for synthetic effluent tests was made up from technical grade chemicals in tap water as described below:

NH_4Cl	0,5 g l^{-1}
$MgCl_2.H_2O$	0,5 g l^{-1}
KH_2PO_4	2,0 g l^{-1}
$FeSO_4.7H_2O$	10,0 mg l^{-1}
Fine ground S^o	30 – 80 g l^{-1} (in suspension)

Feed was made up by adding the required amount of sodium nitrate to the medium.

Analytical techniques

Nitrate was measured with an Orion selective nitrate ion electrode. Due precautions were taken with regard to interfering ions, and ionic strength. Nitrite was determined by the α-naphthylamine/sulphanilic acid method. Sulphate estimation was based on the reaction of solid barium chloranilate with sulphate ion at pH 4 in 50% ethyl alcohol solution to liberate the highly coloured acid-chloranilate ion. (Bertolacini and Barney, 1957).

Biomass was calculated from the determination of nitrogen in the solids obtained from centrifuging a sample. It was assumed that the organic solids obtained by centrifugation and washing represents the biomass containing 12,4% nitrogen. (The cell composition was assumed to be $C_5H_7O_2N$). Borgerding (1972) has shown how magnesium ammonium phosphate precipitates in an anaerobic digestion system. This could affect the reliability of the biomass values based on the Kjeldahl determination of nitrogen on the centrifuged solids since the feed contains magnesium, and phosphate salts.

It can be shown by calculation (Stumm and Morgan, 1970) that some precipitation of magnesium ammonium phosphate can occur at pH 7 with the feed solution used in these tests. Feed solutions however, were made up in bulk and allowed to stand for some days before use. Precipitation could therefore occur in the bulk vessel and subsequent precipitation in the biological reactors was probably minimal. Nevertheless future work should be planned with a feed mixture in which the possibility of precipitation of magnesium ammonium phosphate is absent.

Enrichment for thiobacilli

An inoculum of mud from Durban Bay was added to a stirred

reactor charged with medium containing finely ground industrial sulphur and sodium nitrate. The reactor was purged with nitrogen and then operated under anaerobic conditions.

The course of reaction was followed by measurement of NO_3^-—N consumed. When nearly all the nitrate had been consumed the stirring was stopped, the contents allowed to settle and the supernatant liquor withdrawn. This liquor was centrifuged and the biomass from the liquor returned to the reactor which was then recharged with fresh medium. This "fill and draw" procedure was repeated several times in order to obtain a high concentration of actively denitrifying thiobacilli for use in various tests.

Batch tests

Simple cylindrical stirred tank reactors (anaerobic) were not satisfactory for kinetic studies because of wall growth. This could be overcome by the addition of an inert material such as diatomaceous earth to scour the walls clean. Alternatively wall surfaces could be kept clean by pumping the liquor from the reactors (without any inert material) and returning it to the reactor in a series of tangential jets designed to sweep the walls of the reactor. Both methods were used. However the batch tests carried out were intended to show the course of the reaction to obtain the relation between sulphate formed and nitrate converted to nitrogen. Wall growth with this objective in mind is not a serious problem.

Reactors were charged with medium and NO_3^-—N added as required together with finely ground sulphur, 30—50 g l^{-1}. Bacteria from the "fill and draw" stocks or from previous batch or continuous runs were used for inoculation.

Results

The results of three tests are given in Figures 2 to 4. Bacteria for inoculation varied in environmental history. In tests shown in Figures 2 and 3 the inocula had been stored for some time. In the test shown in Figure 4, the biomass from a previous run (not shown, but a series of runs was carried out with reactors of different design, and test results are given for those reactors with minimum wall growth) became the inoculum for the test depicted immediately on completion of that run. It will be noticed that the build up of nitrite is negligible in this test (Figure 4).

The ratio of SO_4^{2-}:NO_3^-—N (g g^{-1}) after all nitrite had been converted was between 6,4 — 6,7 for the three tests. It was noted that the sulphur became wetted shortly after the addition of the biomass inoculum and that dispersion of sulphur in the reactor was very satisfactory thereafter.

Continuous tests

A series of continuous runs was conducted to show the stability of the biomass and to determine whether a high concentration of active biomass could be built up in the reactor to enable the reactor to be run at low hydraulic retention times.

Although wall growth will occur, the bacteria do not form

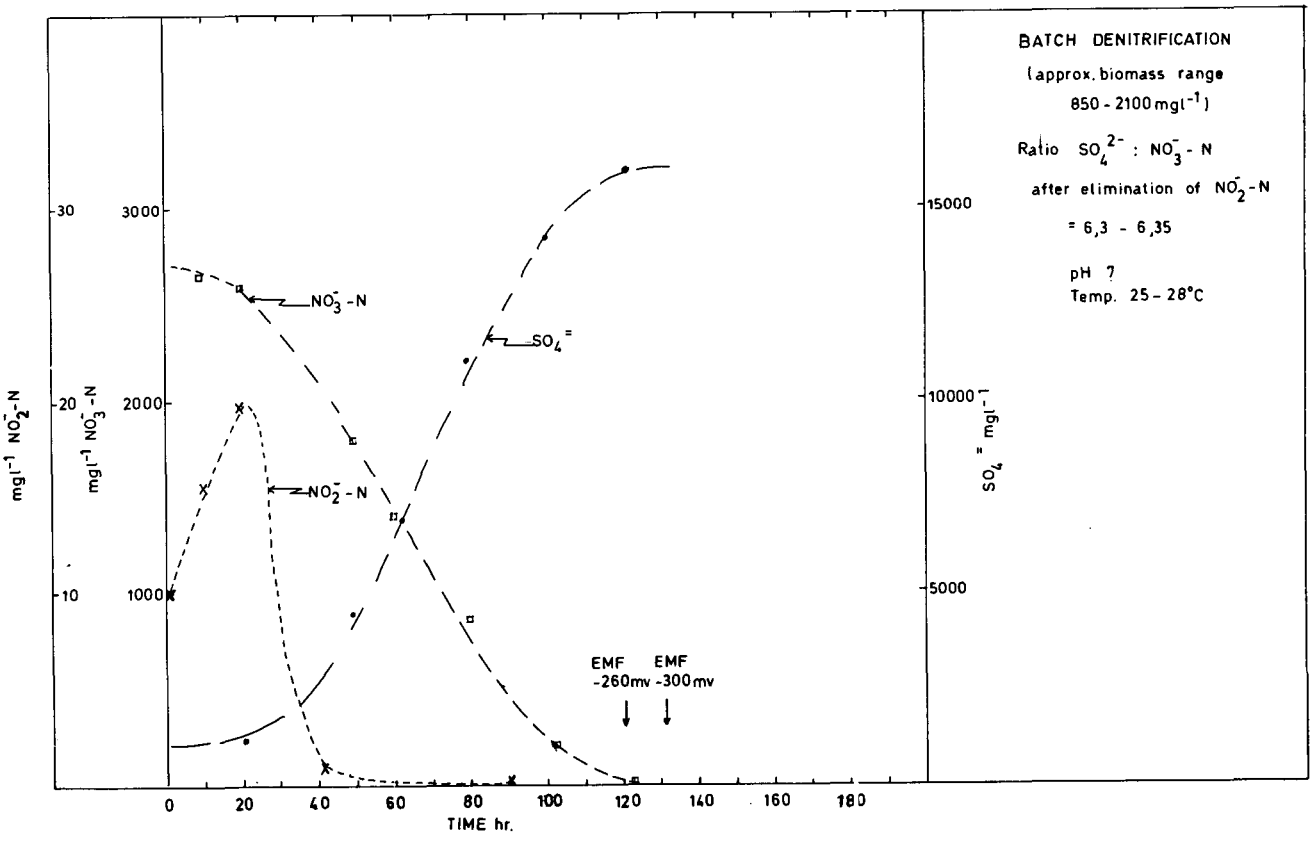

Figure 2
Batch Denitrification (approx. biomass range 850—2100 mg l^{-1})

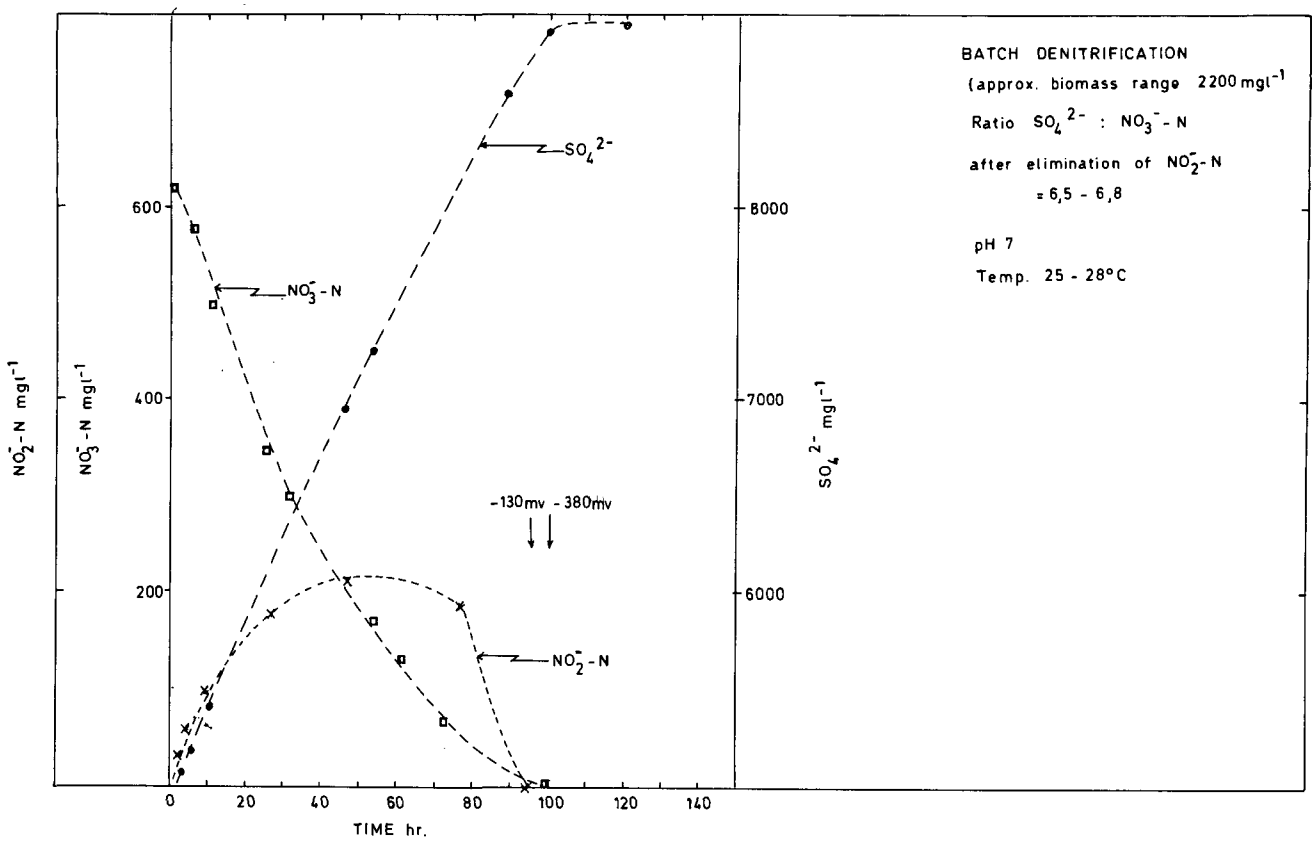

Figure 3
Batch Denitrification (approx. biomass range 2200 mg l $^{-1}$)

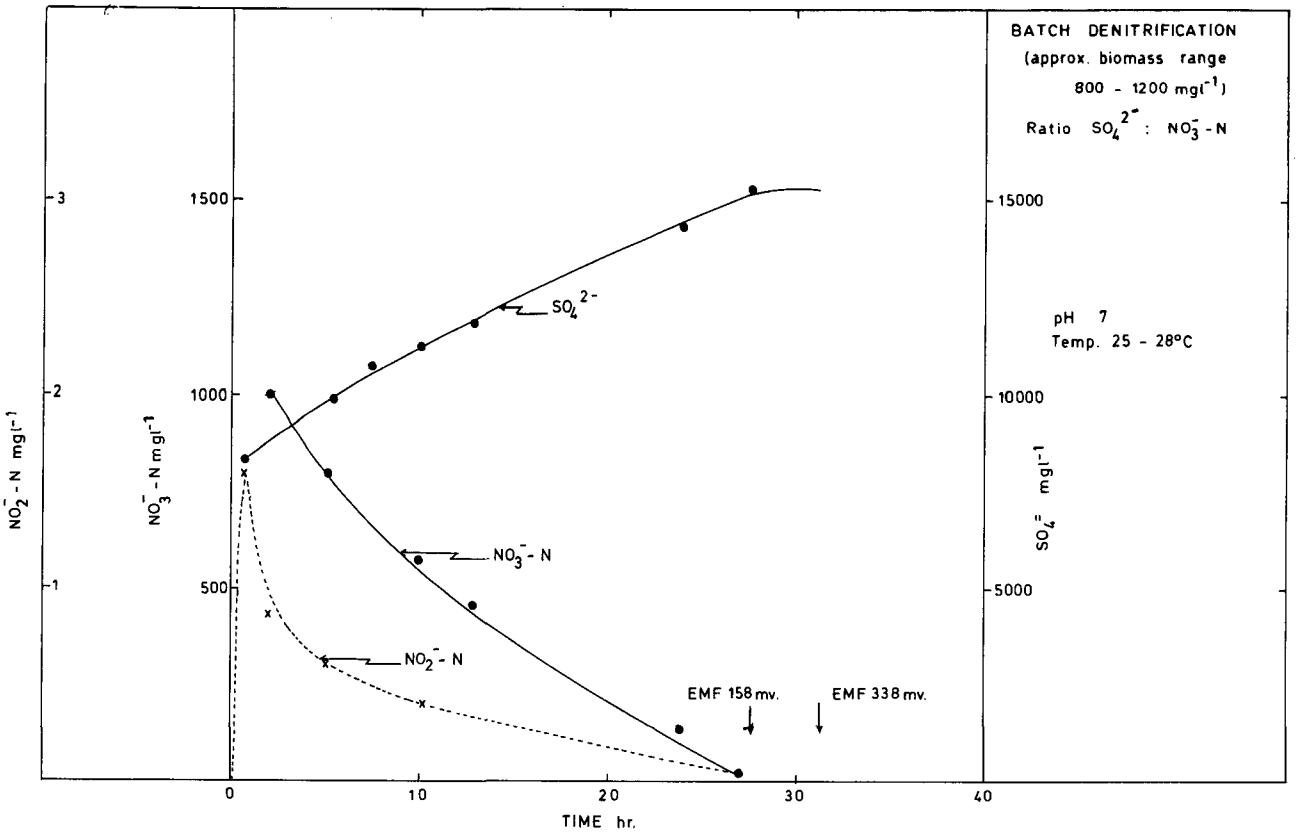

Figure 4
Batch Denitrification (approx. biomass range 800—1200 mg l $^{-1}$)

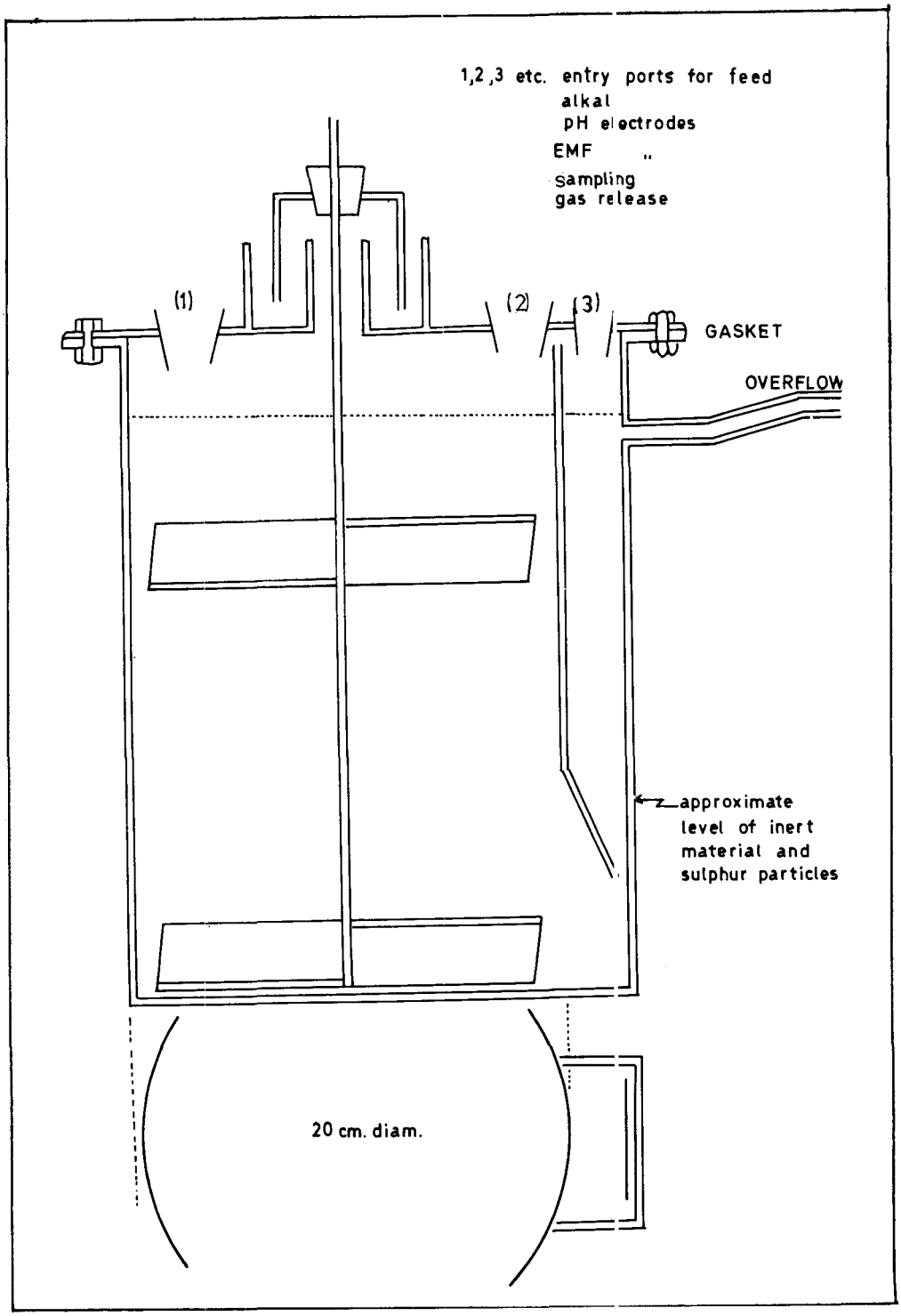

Figure 5
Continuous stirred tank reactor

clusters or flocs in the free medium. Further there is little tendency for the bacteria, which are motile, to settle.

To retain bacteria in a continuous stirred tank reactor (CSTR) it was decided to use an inert solid such as diatomaceous earth to enmesh and hold back bacteria in the reactor. It was found also that the diatomaceous earth kept the walls of the stirred section of the reactor clean.

Tests were thus carried out in a CST reactor (Figure 5) in which 25–40 g l^{-1} diatomaceous earth was suspended in the medium together with the finely ground sulphur (50–80 g l^{-1}). Sulphur was added periodically to the reactor during the course of the runs to maintain an excess in the reactor at all times. The feed to the reactor was the same as the medium described earlier, with NO_3^-—N adjusted to give 2 800 mg l^{-1} NO_3^-—N.

The results of such tests are given in Table 1 and Figure 6.

It will be seen that it was possible to hold back biomass sufficiently to give biomass retention times (R_s) of 25 – 50 days. However, hydraulic retention times of more than 3 – 6 days are still too high for treatment of large volumes of the effluent since reactor costs would be excessive. Further studies will have to be undertaken to increase the biomass retention time, by simple techniques which do not involve centrifugation.

The sulphur which was added to the reactor from time to time, was readily wetted by the reactor liquor and was easily dispersed in the reactor (see also batch tests).

Provided the flow rate at any particular steady state was not suddenly increased nitrite concentration remained low (less than 0,1 mg l^{-1} NO_2^- – N). However when flow rate was increased rapidly beyond a particular steady state condition, nitrite appeared in the reactor followed by nitrate.

When feed was stopped in a well operating reactor (i.e. nitrite levels $<0{,}1$ mg l^{-1}, NO_3^- – N <20 mg l^{-1}) the EMF dropped from an operating figure of say -168 mv to below -300 mv (see Figure 7) over a very short time after which hydrogen sulphide was generated.

Tests were not conducted to determine the micro-organisms responsible for, nor the mechanism for, the generation of H_2S. It is evident however that in any application for the denitrification of an effluent, care would have to be exercised to ensure maintenance of some nitrate in the effluent or a high enough redox potential to avoid H_2S formation.

Ratio of SO_4^{2-} to NO_3^-—N

From the continuous runs the SO_4^{2-}:NO_3^- – N ratio mg mg^{-1} was found to be 6,5 – 6,9. Batch tests gave 6,4 – 6,7. (These values were obtained from the quantity of SO_4^{2-} formed by the destruction of NO_3^- – N at any time during a run).

If the following reactions are accepted as probable, then from the estimated true yield (Y_t) of bacteria calculated from the results of the continuous tests (0,4 mg cells per mg NO_3^- – N, Figure 6), a ratio of SO_4^{2-}:NO_3^- – N of 6,86 is obtained.

Energy reaction

$$NO_3^- - N + 0{,}8333\ S + 0{,}333\ H_2O = 0{,}5\ N_2 + 0{,}6667\ H^+ + 0{,}8333\ SO_4^{2-}$$

Cell formation reaction

$$0{,}25\ CO_2 + 0{,}05\ NH_4^+ + 0{,}1667\ S + 0{,}2667\ H_2O = 0{,}05\ \text{cells} + 0{,}1667\ SO_4^{2-} + 0{,}3833\ H^+$$

or

$$NO_3^- - N + 0{,}25\ CO_2 + 0{,}05\ NH_4^+ + 1{,}000\ S + 0{,}5997\ H_2O = 0{,}05\ \text{cells} + 0{,}5\ N_2 + 1{,}05\ H^+ + 1\ SO_4^{2-}$$

For a yield of 0,4 mg cells/mg NO_3^- – N

$$\text{ratio } SO_4^{2-}:NO_3^- - N \text{ mg mg}^{-1} = \frac{1 \times 96}{1 \times 14} = \underline{6{,}86}$$

The values for yield and ratio of SO_4^{2-}:NO_3^- – N differ from those reported by Batchelor (1977) (Yield = 0,65 mg biomass per mg NO_3^- – N at 30°C and SO_4^{2-}:NO_3^- – N = 7,05 – 9,63).

In both investigations enrichment cultures of denitrifying bacteria were obtained from natural environments. There were however a number of differences in the investigations:

TABLE 1
CONTINUOUS DENITRIFICATION

Day of tests after steady conditions	pH	EMF*	Temp	Total feed (includes alkali)	NO_3^-—N in total feed	SO_4^{2-} in reactor	NO_3^-—N in reactor	NO_2^-—N in reactor	MLSS in reactor (computed mass)	MLSS in effl.	Inert solids in reactor	Sulphur in reactor	R	R_s	$\frac{N_i - N_e}{XR}$	XR $\frac{}{(N_i - N_e)R_s}$ or $\frac{\text{MLSS Effl.}}{N_i - N_e}$ or Y	$\frac{SO_4^{2-}}{NO_3^- - N}$
		mv	°C	m/ d^{-1}	mg/ $^{-1}$	mg/ $^{-1}$	mg/ $^{-1}$	mg/ $^{-1}$	mg/ $^{-1}$	mg/ $^{-1}$	g/ $^{-1}$	g/ $^{-1}$	d	d	d^{-1}		g g^{-1}
Start 23/6/77**																	
6/8/77	7	−168; −133	28,0	1597	2151	14000	10	ND	3596	419	24	—	4,63	39,8	0,129	0,196	6,5
18/9/77	7	−70; −67	28,5	2050	2134	14400	10	ND	3607	503	38	53	3,61	25,9	0,163	0,237	6,7
26/9/77	7	−141; −163	28,3	1982	2163	14050	11	ND	3929	438	—	—	3,73	33,5	0,147	0,203	6,5
4/11/77	7	−173; −206	28,3	1513	2171	14300	23	ND	3298	412	36	54	4,89	39,2	0,134	0,191	6,7
23/11/77	7	−168	28,3	1438	2265	15400	24	ND	3668	364	39	80	5,15	51,9	0,119	0,162	6,9

ND = not detected (less than 0,1 mg l^{-1})

* Platinum/saturated calomel

** Samples of mud from Durban Bay collected June 1976. Thiobacillus grown and used in various denitrification tests; tests described in this report all done with inocula derived from biomass collected from these denitrification tests, and stored until required at 5°C in medium containing sulphur

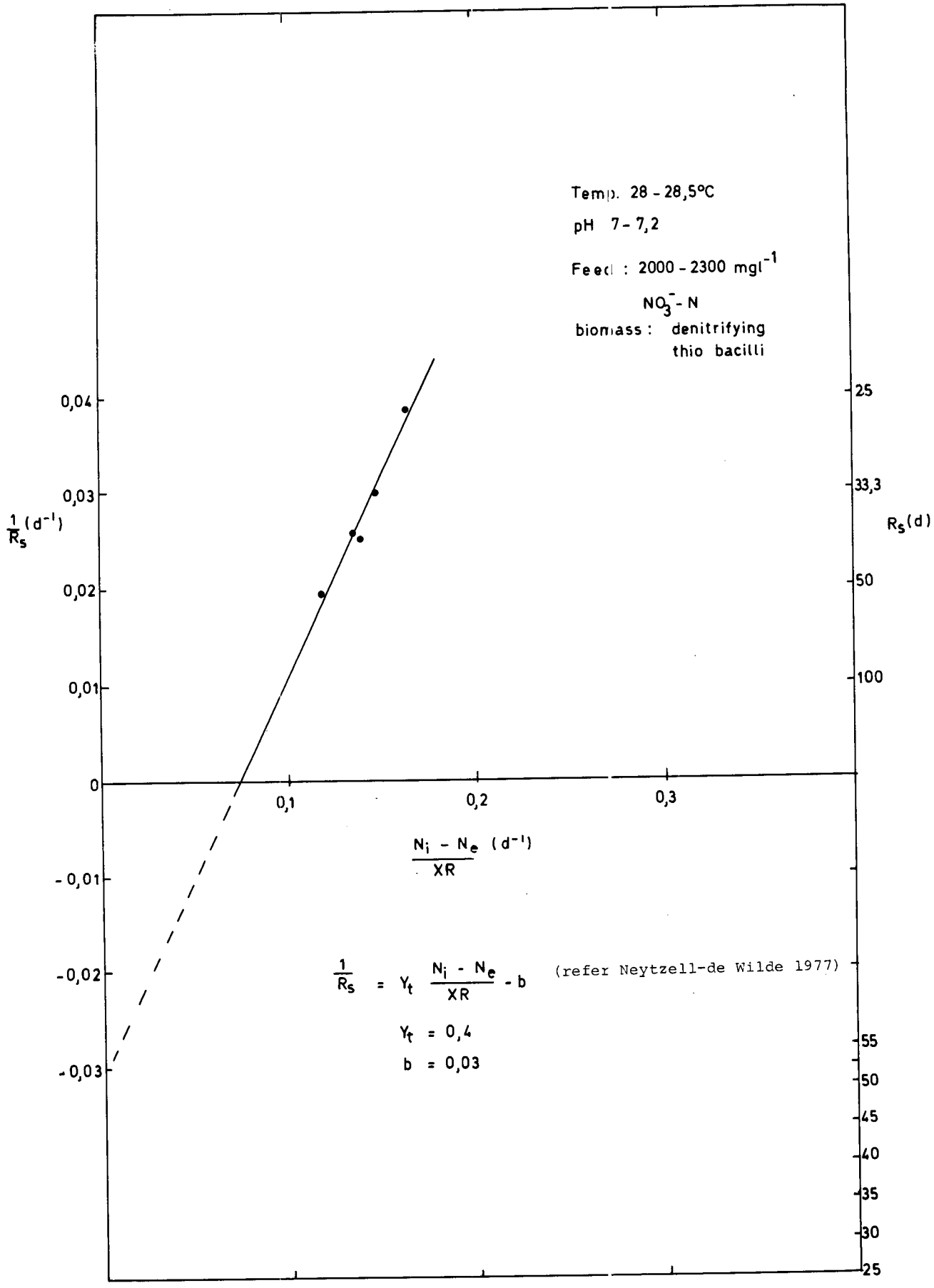

Figure 6
Relation between $\frac{1}{Rs}$ and sludge utilisation rate

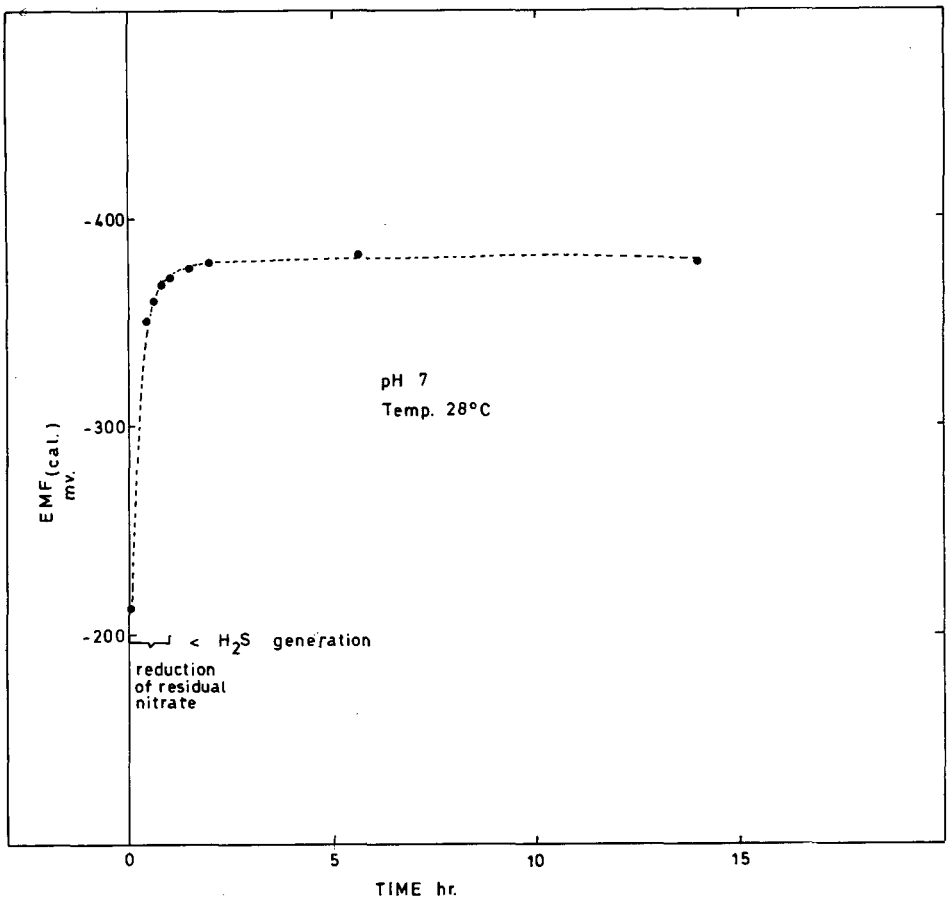

Figure 7
Change of EMF with depletion of NO_3^-—N

1. The present report deals with concentrations of NO_3^-—N between 2 000 and 2 300 mg l^{-1} compared to wastewater concentrations usually of about 30 mg l^{-1}

2. The reactor was designed to eliminate wall growth. In tests using a double cone reactor similar to the one described by Batchelor, a heavy wall growth occurred on the inner surface of the inner cone when tests were conducted with feed at 2 000 mg l^{-1} NO_3^-—N.

3. Sludge age and hydraulic retention times were higher than those used by Batchelor.

4. The sulphur to sludge ratio was lower in the tests described here although the absolute amount of sulphur per litre of reactor content was much higher.

In spite of such differences, it is clear that sulphur can be used effectively in autotrophic denitrification. Active denitrifying biomass can be maintained over long periods. However further efforts must be made to increase the biomass in the reactor in order to decrease hydraulic retention times. The relatively low material cost for this process compared to those requiring carbon sources makes the process more attractive in dealing with high levels of NO_3 —N.

Material usages

The relative material usages per ton NO_3 —N are given below for three different denitrification schemes.

1. Carbon as energy source, using methanol with *Hyphomicrobium* bacteria:
2,35 ton methanol at R310 per ton for conversion of 1 ton NO_3 —N to N_1. (Neytzell-de Wilde, *et al.*, 1977).

2. Carbon energy source, using molasses at R18 per ton with heterotrophic bacteria: 7,6 ton molasses required for conversion of 1 ton NO_3 —N to N_2. (Bosman, *et al.*, 1978).

3. S^o as energy source at R47 per ton with denitrifying thiobacteria:2,29 ton sulphur required for conversion of 1 ton NO_3^-—N to N_2.

Hence for conversion of 1 ton nitrate using methanol, the energy

source cost is R728; using molasses, the energy source cost is R136; and using sulphur energy source the cost is R107.

From the current prices for methanol, molasses and sulphur it is clear that the process using S has an energy source raw material cost advantage.

Nomenclature

b = endogenous mass loss rate coefficient (d^{-1})

E_H = redox potential of a redox couple of an arbitrary but reported composition (mv)

$E_{(satcal)}$ = redox potential measured with Pt/saturated calomel electrode (also E_{cal}) (mv)

E_o = normal potential, all activities equal to one (mv)

E'_o = formal potential for concentrations of oxidising and reducing agents converted to one; medium specified (mv)

K = equilibrium constant, redox reaction

Kw = ionisation constant — water

MLSS = mixed liquor suspended solids (biomass) ($mg\ l^{-1}$)

N = concentration of NO_3 —N substrate surrounding the organisms ($mg\ l^{-1}$)

N_i = initial NO_3 —N concentration ($mg\ l^{-1}$)

N_e = NO_3 —N in effluent ($mg\ l^{-1}$)

$p_e^o = \frac{E_o}{0{,}059}$

$P_e = p_e^o = \log\frac{[oxidant]}{[reductant]}$

$p_e^o(w)$ is analogous to p_e^o, except that H^+ and OH activities in the equilibrium equations are assigned their activities in neutral water. Values for $p_e^o(w)$ for 25 °C thus apply to unit activities of oxidant and reductant at pH7.

$p_e^o(w) = p_e^o + \frac{nH}{2}\log Kw$ (25°C pH 7)

Q = base flow through reactor ($l\ d^{-1}$)

R = hydraulic retention time = $V/$ (d)

R_s = holding or retention time of biomass

= sludge age = $\frac{\text{sludge in reactor}}{\text{sludge lost in effluent per day}}$ (d)

X = concentration of bacteria ($mg\ l^{-1}$)

V = volume of reactor (l)

Y = apparent growth yield = $\frac{XR}{(N_i - N_e)R_s}$

Y_t = total growth yield where allowance is made for b

ATP = adenosine 5' triphosphate

CSTR = continuous stirred tank reactor

COD = chemical oxygen demand

BOD = biological oxygen demand

BOD_5 = biological oxygen demand (5 days)

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