Treatment of effluents from ammonia plants, Part IV Denitrification of an inorganic effluent from a nitrogen-chemicals complex using methanol as carbon source

F G NEYTZELL-DE WILDE, G R NURSE and J GROVES
[AECI POLLUTION RESEARCH GROUP, DEPARTMENT OF CHEMICAL ENGINEERING, UNIVERSITY OF NATAL, KING GEORGE V AVENUE, DURBAN]

Abstract

Denitrification of an inorganic effluent containing approximately 2 800 mg ℓ^{-1} NO_3-N can be reliably carried out over a wide range temperature range (10 -31°C) using methanol as carbon source in a continuous stirred tank reactor.

Sludge age is the controlling factor and this varies from a minimum of 3,6–3,8 days at 28°C to 45-50 days at 10°C.

Methods for achieving high sludge ages (while still retaining low hydraulic retention times) involved (a) separation of the biomass in the effluent by centrifugation and return of solids or (b) retention of biomass in the reactor by use of suspending media, such as diatomaceous earth, in the reactor.

The experimental yield obtained for methanol ranged between 0,18–0,22 g biomass g^{-1} methanol.

pH plays an important role and a value of 7,2–7,4 was selected to avoid formation of free ammonia, which inhibits significantly. H_2S generation must be avoided when operating at very low nitrate-N values in the effluent, because this compound is toxic and cannot be controlled by pH alone.

Introduction

The aqueous effluent from a nitrogen-chemicals complex amounts to 1,64–1,7 Mℓd^{-1}. It contains approximately 1 400 mg ℓ^{-1} ammonium nitrogen and an equivalent amount of nitrate nitrogen.

Careful consideration of various physico-chemical and biological methods led to the conclusion that bacterial nitrification followed by denitrification was the most suitable method to adopt for effective nitrogen removal.

Investigations have already been carried out on the successful nitrification of this effluent (Neytzell-de Wilde, 1975; 1977).

This study covers some of the investigations which have been carried out on denitrification of the nitrified effluent containing approximately 2 800 mg ℓ^{-1} nitrate-nitrogen.

Nitrification is carried out by autotrophic bacteria. Denitrification, however, occurs with chemo-organotrophs and therefore an organic substrate is required as carbon and energy source. An exception here is the chemo-lithotroph *Thiobacillus denitrificans* (Mann *et al*, 1972). Investigations are proceeding at this University on the use of this bacteria with sulphur as the energy source and carbon dioxide as the carbon source.

Methanol was selected as the carbon and energy source for the present denitrification study because

(1) it is completely oxidised to CO_2 and H_2O thereby not directly increasing the COD or BOD of the resulting effluent.

(2) it is completely miscible with water, and

(3) its composition is constant and the stoichiometry of the reactions is relatively straight forward.

General

Methanol has been used as carbon source in investigations on the production of protein. At present the following strains have been investigated:

for dimethylamine the ratio

$(CH_3)_2NH_2^+/(CH_3)_2NH$ is approximately 60,3

and for monomethylamine,

$(CH_3)NH_3^+/(CH_3)NH_2$ is approximately 42,7.

The influence of the CH_3 – groups (electron release effect) will affect the reactivity of the N: group and trimethylamine will therefore respond most readily to oxidation. In the case of ammonia, the ratio of NH_4^+/NH_3 at pH 9, is of the order of 1,8, but reactivity is limited because in the absence of methyl groups, hydrogen bonding will occur more readily with the N: grouping.

It is evident then that the conjugate acids of TMA, DMA and MMA are much less susceptible to oxidation than the amines themselves, and effective oxidation of an effluent containing these products will have to be carried out under alkaline conditions.

However, operation under alkaline conditions produces further complications in that the decay rate of O_3 in water at pH values above 9 becomes quite fast (Hoigne and Bader 1976, Peleg 1976, Stumm 1954). The decay is enhanced by some substances but reduced by the addition of aliphatic alcohols and carbonate ions.

Hydroxyl radicals are formed with the hydroxide ion catalysed decomposition of ozone in water and Hoigne and Bader (1976) give a value of up to 0,55 mol of hydroxyl radicals produced from one mol ozone at pH 10,5.

Beyond a critical value, $OH^{\cdot}$ radicals therefore become important oxidants, and the critical value depends on both the rate with which ozone reacts directly with the substrates and on the solutes, including the reaction products, which enhance or retard decomposition.

On the basis of the work by Hoigne and Bader, it is likely that at pH values below 9,5, the main reactions with the amines (particularly trimethylamine) will be due to O_3 oxidation where neither methanol nor carbonate will consume the ozone.

At higher pH values however, the ozone may well decompose before reacting with the amines and the OH radicals will then react. Since $OH^{\cdot}$ is less selective as an oxidant and since only half of the ozone produces $OH^{\cdot}$ the oxidation yield is likely to be poorer under these conditions. Further, since $OH^{\cdot}$ is scavenged by $CO_3^{=}$ and HCO_3^- by producing a radical of lower reactivity it becomes likely that the oxidation of substrates may decrease even further in the presence of carbonate.

In this respect instead of modifying the pH of solutions containing CO_2 with sodium hydroxide, lime should be considered because of the additional advantage of carbonate removal. However the precipitation of calcium carbonate on the packing of absorption columns may prove to be a disadvantage.

More detailed work along these lines is desirable and should include the effects of hydroxyl ions, carbonate and bicarbonate ions, methanol and ammonia, and temperature so that optimum conditions can be selected for treatment of the effluent concerned.

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System parameter	Steady state equation without sludge retention	Steady state equation with sludge retention (or sludge recycle)	Eqn. No.
Substrate utilisation rate SUR = $\frac{\text{Mass substrate oxide per time}}{\text{Mass MLVSS}}$	$\frac{k^1S}{K_s+S}$	$\frac{k^1S}{K_s+S}$	5
Microbial solids concentration X	$\frac{Y_t\,(S_r-S_e)}{1+b\,R_s}$		6
	(where $R_s = R$)	$\frac{Y_t\,(S_r-S_e)}{1+b\,R_s}\,\frac{R_s}{R}$	7
XV = mass of solids	$Y_t\,\frac{\text{(Mass of S oxidised)}}{(1+b\,R_s)}$		8
		$Y_t\left[\frac{\text{mass of S oxidised}}{(1+b\,R_s)}\right]R_s$	9
Solids retention time $^{-1}$ i.e. R_s^{-1}	$\frac{Y_tk^1S}{K_s+S}-b$	$\frac{Y_tk^1S}{K_s+S}-b$	10
		$\frac{Y_t\,(S_r-S_e)}{XR}-b$	11
Effluent substrate conc. S_e	$\frac{K_s\,[1+bR_s]}{R_s\,(Y_tk^1-b)-1}$		12
	(where $R_s = R$)	$\frac{K_s\,[1+bR_s]}{R_s\,(Y_tk^1-b)-1}$	13
Limiting minimum R_s (when $S >> K_s$	$\frac{1}{Y_tk^1-b}$	$\frac{1}{Y_tk^1-b}$	14
Y	$\frac{X_v}{(S_r-S_e)}$	$\frac{X_v}{(S_r-S_e)}\,\frac{R}{R_s}$	15
Y_t	$\frac{Y\,[1+bR_s]}{[1+0{,}15\,bR_s]}$	$\frac{Y\,[1+bR_s[}{[1+0{,}15\,bR_s]}$	16

Experimental

Standard conditions (Continuous culture tests)

Temperature

Tests were carried out at fixed temperatures 10-12°C, 17°C, 28-30°C, 30-31°C.

pH

All tests were conducted under controlled pH conditions. The alkali produced during reaction was neutralised with 4,6 N hydrochloric acid (and in some cases the liberated CO_2 was used as effectively as possible by recirculation of the gas). The choice of acid was arbitrary, sulphuric acid would be equally satisfactory. Except where otherwise stated, pH was controlled between 7,2-7,4. The range was selected to keep free HNO_2 and free NH_3 to low values (Figure 1).

Synthetic Medium

The medium for synthetic effluent tests was made up from commercial chemicals in tap water.

Component	g ℓ^{-1}	Medium used in tests marked GN	
KH_2PO_4	0,125	KH_2PO_4	1,56
		NA_2HPO_2	1,4
$CaCl_2.2H_2O$	0,150		0,025
$MgSO_4.7H_2O$	0,10		0,20
Trace elements-iron, manganese and molybdenum.	Trace		Trace
Sodium nitrate	17,00 ≡	2 800 mgℓ^{-1} NO_3-N	

Trade effluent medium

The trade effluent was nitrified as described by Neytzell-de Wilde (1975) and the nitrified effluent was used as influent medium for denitrification tests. (The nitrate concentration was adjusted slightly to bring the nitrate values in line with the synthetic medium, but no other additions were made).

Feed

Feed was made up by adding the required volume of pure methanol or crude methanol to the above media. The mixtures were then stored at 5°C to minimise bacterial attack.

Analytical techniques

Selective electrodes were used for routine measurement of ammonia, nitrate and oxygen. Due precautions were taken with regard to interfering ions, and ionic strength. Nitrite was determined by the α-naphthylamine method. Mixed liquor suspended solids were determined by turbidmetric and gravimetric methods. Gas chromatography methods were used for the determination of methanol, carbon dioxide and nitrogen. Total organic carbon was determined on a Beckman TOC analyser.

Reaction vessels

Two types of CST reactor were used and these are shown in Figures 2 and 3. Thorough mixing of the culture was obtained by mechanical and/or gas agitation.

Micro-organisms

Hyphomicrobium sp 27484 was obtained in pure culture from the American Type Culture Collection and maintained on an inorganic salts medium containing methanol (0,2%) and agar (1,0%) (Attwood and Harder, 1972).

Organisms were also grown in liquid media as described by Harder *et al,* (1973). These bacteria were used for identification purposes only and were not used for the kinetic studies.

Bacteria used in continuous and respirometer tests were obtained by enrichment in a chemostat from an inoculation of actively digesting sewage sludge fed on synthetic feed under anaerobic conditions. When the entire mass was pale pink and no longer showed traces of the original sludge, the biomass was used for inoculation of various reactors used in this investigation. Details regarding the preparation of the biomass are given by Nurse (1977).

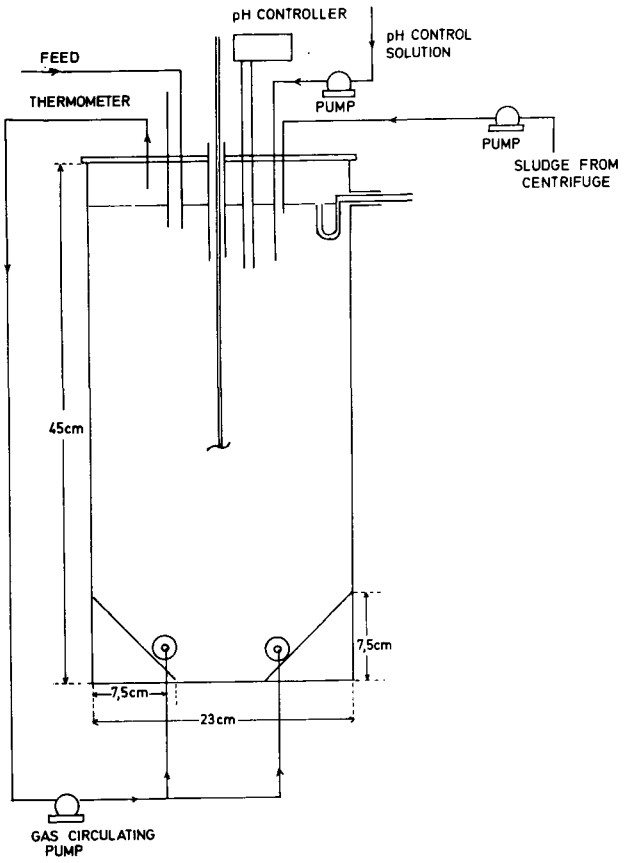


Figure 2
Laboratory scale (10ℓ) CSTR with or without sludge return. Dimensions approx. 23 cm × 12 cm × 45 cm.

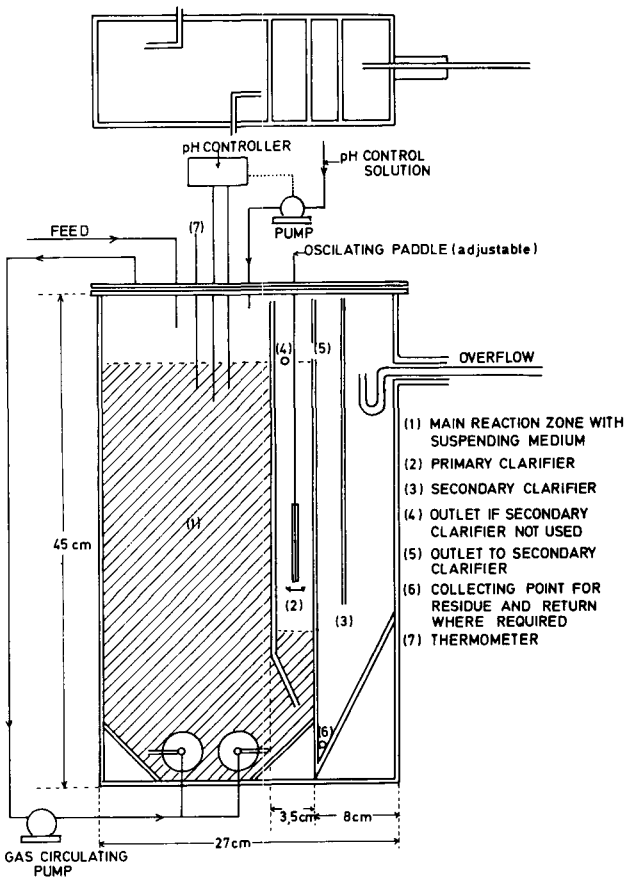


Figure 3.
Laboratory scale CSTR with sludge retention. Overall dimensions 45 cm × 27 cm × 12 cm.

Experiments and Results

Identification of Bacteria

Identification of the bacteria was based mainly on their characteristic morphology. Growth on various substrates aerobically and anaerobically in comparison with a control also indicated that the enrichments obtained consisted essentially of *Hyphomicrobium* species (Nurse, 1977). These results confirm the findings of Sperl and Hoare (1971) and Attwood and Harder (1972) that denitrification with methanol is a selective enrichment for *Hyphomicrobium* species.

Owing to the size and type of treatment envisaged for the destruction of nitrate in the effluent, it would be impractical to maintain a particular species of bacteria or pure culture since this would require sterilization of reactors and effluent. The fortuitous specific enrichment for *Hyphomicrobium* species therefore requires that no special precautions be taken to maintain a virtually pure culture.

The results obtained in the various experiments may thus be ascribed to a particular species and not an unidentified biomass, Figures 4 and 5 illustrate the bacteria found, for example, in experiments 18 and 22.

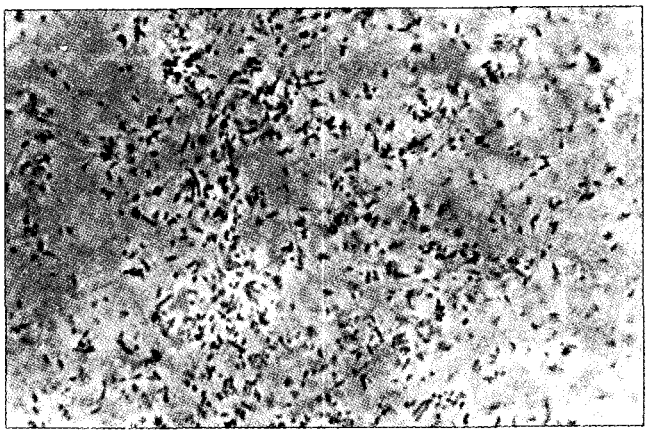
Elemental composition of biomass

A sample of anaerobically cultured *Hyphomicrobium* spp taken from continuous operating chemostats was washed with distilled water then freeze-dried. An elemental analysis of the material yielded results from which the following empirical formula was derived: $C_4H_7O_2H$.

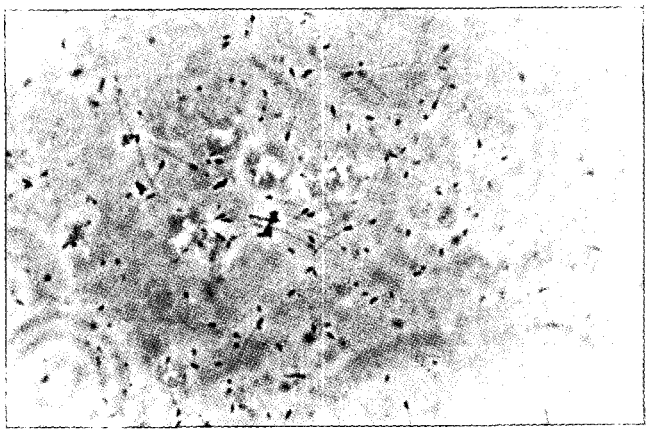
The composition ($C_4H_7O_2N$) has been used as a good approximation in the calculations given previously. The ash-content of the bacteria used in the above elemental analysis was found to be 3,7%.

Determination of saturation constant K_s (NO_3-N)
(Batch tests)

Values for K_s are low and special techniques have to be employed to obtain meaningful results. Nurse (1977) employed a respirometer similar to van Kessel's (1975) and obtained an estimate of 0,295 mg NO_3-N ℓ^{-1} for K_s (NO_3-N), at 28°C and pH 7,4. The results are given in Figure 6. The rate of utilisation in such a batch experiment will not compare necessarily with that in continuous operation because of alcohol inhibition.

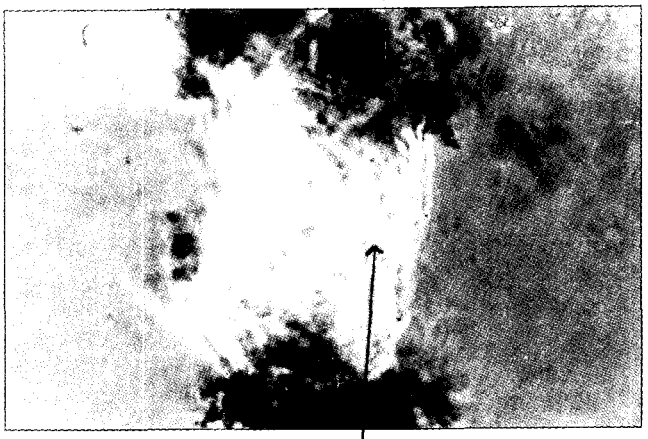


Typical *Hyphomicrobium* Growth
Exp. 18

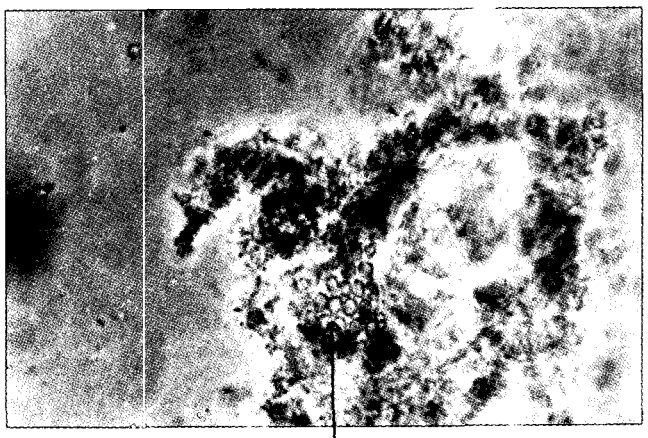


Typical *Hyphomicrobium* Growth
Exp. 18
Figure 4

Bacteria (stained with crystalviolet)



Diatomaceous earth



Diatomaceous earth surrounded by bacteria
Figure 5

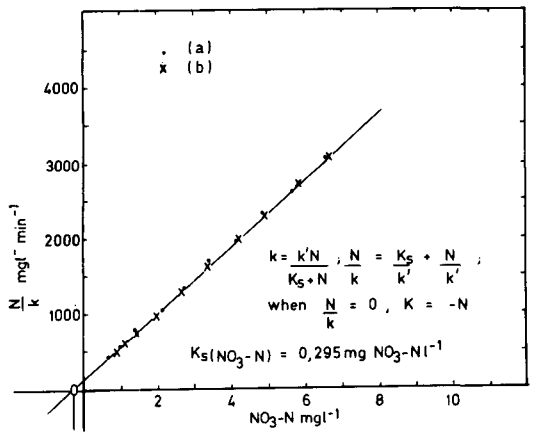


Figure 6.
Graphical determination of $K_s(NO_3\text{-}N)$.

Since the K_s value is inversely related to the affinity of the organism for the substrate, in effluent treatment the low value is most desirable in that high removal rates can be obtained even at low concentrations.

Continuous culture tests carried out in this investigation have shown that nitrate-N does not become the growth limiting substrate until the concentration approaches zero. However once zero concentration has been reached, sulphate is utilised by the *Hyphomicrobium* and H_2S is generated, which at very low concentrations causes inhibition and further nitrate is not denitrified. Recovery of a reactor where H_2S has been generated could however be achieved by removal of the free H_2S by addition of an iron salt to the reactor.

Continuous culture experiments – with and without sludge recycle

The feed to the reactors (Figure 2) consisted of the medium to which methanol was added to yield a ratio of CH_3OH:NO_3-N of approximately 2,5.

Oxygen was excluded from the culture by maintaining a positive pressure of gases (N_2 and CO_2) in the volume above the liquid in the reactor.

Methanol, nitrate, nitrite and biomass were monitored regularly and checks were made on dissolved oxygen. In later experiments the redox potential in the reactor was also measured.

In tests where sludge was returned to the reactor, every 24 hours a fixed volume of the effluent was centrifuged and the

TABLE 1

TESTS AT VARIOUS DILUTION RATES (NO RECYCLE OR SLUDGE RETENTION)

Test No.		°C	pH	Feed including acid MeOH mg ℓ⁻¹ S_i	Feed including acid NO_3-N mg ℓ⁻¹ N_i	Ratio MeOH/ NO_3-N mg/mg	Effluent Volume ml	X_v mg ℓ⁻¹	Effluent Composition MeOH mg ℓ⁻¹ S_e	Effluent Composition NO_3-N mg ℓ⁻¹ N_e	Effluent Composition NO_2-N mg ℓ⁻¹	R_s d	$\frac{1}{R_s}$ d^{-1}	R d	$\frac{S_i-S_e}{X_vR}$	$\frac{N_i-N_e}{X_vR}$	NO_3-N removed %
(1)	A_1	29	7,4	6 889	2 755	2,5	2 848	1 438	ND	75	—	3,51	0,285	3,51	1,365	0,546	97,3
(2)	B_1	29	7,4	6 887	2 755	2,5	2 850	1 358	ND	37	—	3,51	0,285	3,51	1,44	0,57	99,5
(3)	A_2	11,8–12	7,4	6 633	2 749	2,4	330	1 112	ND	41	<1	30,3	0,033	30,3	0,197	0,08	98,5
(4)	A_3	16,8–17,3	7,4	7 605	2 716	2,8	702	1 567	ND	13	0,1–20	14,25	0,070	14,25	0,341	0,121	99,5
(5)	B_2	17,5	7,4	7 699	2 760	2,79	725	1 567	ND	<5	<1	13,79	0,073	13,79	0,356	0,128	>99,8
(6)	B_3	11,8–12	7,4	6 810	2 755	2,47	312	1 235	ND	79	<2	32,1	0,031	32,1	0,172	0,068	97,1
(7)	B_4	17	7,4	6 908	2 767	2,5	837	1 368	ND	(300)	<2	11,95	0,084	11,95	0,423	—	—
(8)	C_1	11	7,4	7 717	2 756	2,8	320	1 382	ND	<5	—	31,25	0,032	31,25	0,179	0,064	>99,8
(9)	C_2	11	7,4	7 690	2 746	2,8	314	1 553	63	<5	<1	31,84	0,031	31,84	0,154	0,055	>99,8
(10)	C_3	28	7,4	6 636	2 750	2,4	2 600	1 330	ND	73	<1	3,85	0,026	3,85	1,3	0,52	97
(11)	E_1	30	7,4	6 216	2 745	2,26	3 400	1 240	ND	250	<1	2,94	0,34	2,94	1,71	0,68	90,9
(11a) (Commercial methanol)	E_3	30	7,4	6 455	2 748	2,35	3 300	1 273	ND	220	<1	3,03	0,33	3,03	1,67	0,66	92,0
(12)	GN_1	28	7,4	6 886	2 755	2,5	1 480	1 200	ND	15	—	6,76	0,148	6,76	0,849	0,34	99,8
(13)	GN_2	28	7,4	6 861	2 745	2,5	1 825	1 190	ND	23	—	5,48	0,183	5,48	1,05	0,42	99,7
(14)	GN_3	28	7,4	6 886	2 752	2,5	2 351	1 220	ND	77,5	—	4,25	0,235	4,25	1,33	0,52	99,0
(15)	GN_4	28	7,4	6 902	2 761	2,5	2 792	1 220	ND	65	—	3,58	0,279	3,58	1,58	0,62	99,1
(16)	GN_5	28	7,4	7 119	2 744	2,69	2 666	1 290	198	<10	—	3,71	0,27	3,71	1,45	0,57	99,9
(17)	GN_6	28	7,4	6 840	2 725	2,69	1 687	1 220	ND	<10	—	5,87	0,17	5,87	0,96	0,38	99,9
(18)	E_2	30	7,4	6 489	2 747	2,36	3 286	1 201	ND	195	<1	3,04	0,33	3,04	1,78	0,75	92,9

*ND = not detectable

TABLE 2

SLUDGE RECYCLE TESTS

Test No.		°C	pH	Feed including acid MeOH mg ℓ^{-1} s_i	Feed including acid NO_3-N mg ℓ^{-1} N_i	Ratio MeOH/ NO_3-N mg/mg	Effluent Volume ml	Sludge return ml	X_v mg ℓ^{-1}	Effluent Composition MeOH mg ℓ^{-1} S_e	Effluent Composition NO_3-N mg ℓ^{-1} N_e	NO_2-N mg ℓ^{-1}	NH_3-N feed mg ℓ^{-1}	NH_3-N effl. mg ℓ^{-1}	R_s d	$\frac{1}{R_s}$ d^{-1}	R d	$\frac{S_i-S_e}{X_vR}$	$\frac{N_i-N_e}{X_vR}$	NO_3-N removed
(19)	D_1	28,3	7,2	6 217	2 471	2,52	9 930	7 000*	4 600	ND	43	<1,0	—	—	3,41	0,293	1,01	1,34	0,53	98,3
(20)	D_2	28,5	7,2	6 211	2 477	2,51	10 053	7 000*	4 660	ND	140	<1,0	159	1,0	3,28	0,305	0,99	1,35	0,51	94,4
(21)	D_3	29,3	7,2	6 307	2 505	2,52	11 390	8 000*	4 440	ND	58	<1,0	742	590	2,95	0,339	0,88	1,61	0,63	97,8

*return sludge centrifuged and residue made up to 1 ℓ

TABLE 3

SLUDGE RETENTION TESTS

Test No.	°C	pH	Feed including acid MeOH mg ℓ^{-1} S_i	Feed including acid NO_3-N mg ℓ^{-1} N_i	Ratio MeOH/ NO_3-N mg/mg	Ef-fluent Volume mℓ	X_v in react. mg ℓ^{-1}	Residue in react. mg ℓ^{-1}	Effluent Composition MeOH mg ℓ^{-1} S_e	Effluent Composition NO_3-N mg ℓ^{-1} N_e	NO_2-N mg ℓ^{-1}	X_v in effl. mg ℓ^{-1}	Resi-due in effl. mg ℓ^{-1}	R_s d	$\frac{1}{R_s}$ d^{-1}	R d	$\frac{S_i-S_e}{X_vR}$	$\frac{N_i-N_e}{X_vR}$	NO_3-N removed %
(22) Diat[1] (Prelim. – not steady state)	23–35	7,4	6 333	2 753	2,3	9 590	16 864	11 776	ND	trace	<1,0	1 376	1 052	* 6,44	* 0,155	* 0,54	0,7	approx 0,3	>98
(23) Diat[2] (Commercial methanol)	31–31,3	7,4	6 017	2 703	2,23	9 583	13 612	10 766	ND	** 13–205	<1,0	978	238	* 7,29	* 0,137	* 0,54	0,81	approx 0,36 99,5**	92,4–

*Effective reactor volume 5,2 ℓ; deliberate sludge withdrawal = 0,025 ℓ

**Varied with actual feed (methanol to nitrate ratio) during test extending over days.

Note:

Diat[1] and Diat[2] results are given as examples only. At the high flow rates and the small capacity of the reactor considerable errors can arise in the estimation of MLSS, MVLSS and residue. The results do however, demonstrate the promise of the CST reactors with sludge retention by means of the addition of a suitable enmeshing or suspending medium to the reactor. The settling characteristics of a typical reactor contents and also of the effluent are demonstrated in Figure 9. From this it is also evident that a sludge return system could be operated without the use of a centrifuge, but using instead a suitable settler if cognisance is taken of the natural loss of bacteria which are not 'enmeshed'. This will limit the operation at very very low temperatures to that sludge age which is achieved.

ND = not detectable.

solids made up to a suitable volume (1 ℓ) for return to the reactor via a peristaltic pump over 24 hours.

Steady state conditions were assumed when biomass and nitrate concentrations remained constant with methanol taken to a "not detected" value. These conditions were achieved after three sludge retention times.

The experimental results for the above series of tests are given in Tables 1 and 2.

Continuous culture experiments – with sludge retention

Since denitrifiers are relatively slow growing, light in weight and easily washed out of a suspended growth system, experiments were carried out in reaction vessel (Figure 3) to which a quantity of diatomaceous earth was added. In this way the bulk of the biomass was retained in compartment (1). The bacteria appear to be 'enmeshed' by the diatomaceous earth (Figure 5). The height of the concentrated biomass/diatomaceous earth mixture varies with flow rate and concentration of mixture in compartment (2).

Any loss of diatomaceous earth can be collected in compartment (3) where the earth/biomass mixture settles readily. The material can of course be returned to the reaction vessel if required. The overflow from compartment (3) is practically free from diatomaceous earth but does contain a small amount of the culture.

In the experiments described here, tests were conducted with compartment (3) by-passed. The results of these tests are given in Table 3.

In practice it is likely that a quantity of suspended inorganic matter will form from the calcium content of the effluent and the material will assist in forming heavy flocs as was the case in the tests with the trade effluent described in the previous section.

The addition of a suspending medium from the start of bringing a reactor on line, will avoid the need to use a centrifuge for separating biomass from reactor effluent for purposes of returning biomass to the reactor. The separation in the presence of a suspending medium can be effected by conventional thickening or settling.

The diatomaceous earth used in these experiments gave the following size analysis on wet screening (see Table 4).

TABLE 4

SIZE ANALYSIS ON DIATOMACEOUS EARTH (WET SCREENING)

Sieve opening	% mass on sieve	cumulative % mass finer
μm		
300	0,1	99,9
212	1,0	98,9
150	1,3	97,6
106	2,0	95,6
75	5,1	90,5
53	10,3	80,2
38	17,0	63,2

Gas evolution

During the course of operation (experiment D, Table 2) the gas evolved was measured and analysed.

From equation (4) on a mass basis:

$$1\ CH_3OH + 0{,}426\ NO_3\text{-}N \rightarrow 0{,}188\ \text{cells} + 0{,}3999 N_2 + 1{,}0474\ CO_2$$

For a feed containing 7 039 mg ℓ^{-1} methanol and 2 800 mg ℓ^{-1} NO_3-N at a flow of 9,260 ℓd^{-1}, theoretically 26 000 mg N_2 should be evolved per day.

From gas sampling and analysis 25 000 mg N_2 was recovered.

The evolved gas gave the following composition: 73,2% N_2, 24,1% CO_2, and 2,6% H_2O.

The above results were characteristic for a feed containing no ammonia-N and a reactor operated at pH 7,2-7.4

When approximately 180-800 mg ℓ^{-1} NH_4-N was added to the feed, the amount of gas evolved and the composition altered to yield more nitrogen (75-76%). When the pH was changed from 7,2 to 7,5 – 7,6 the toxic effect of free NH_3 occurred and gas evolution decreased with, of course, increase in unchanged nitrate and alcohol.

Ammonia usage

In experiment D (Table 2) where ammonia-N was added to the feed, this was clearly assimilated into biomass as was shown by depletion from the feed and estimation from nitrogen in the biomass wasted per day – confirming the course of the reaction as shown in equations (1) and (2).

For purposes of nutrient supply the requirements for ammonia-N and for phosphate-P can readily be calculated (Speece and McCarty, 1962).

$$\text{Nitrogen requirement} = \frac{N_v}{R_s(SUR)}$$

$$\text{But } \frac{1}{R_s} = Y(1+0{,}15bR_s)SUR - b$$

$$\text{and } \frac{1}{R_s(SUR)} = Y(1+0{,}15bR_s) - \frac{b}{SUR}$$

since $b \geqslant O$

$$N \text{ requirement} = N_v\, Y(1+0{,}15bR_s)$$

Similarly

$$P \text{ requirement} = P_v\, Y(1+0{,}15bR_s)$$

During the course experiment D3, with approximately 700 mg ℓ^{-1} NH_3-N in the feed, the pH was varied. At pH 7,2 to 7,4 operation remained satisfactory. When the pH was increased to 7,5-7,6 the reactor gradually failed due to the pressure of free NH_3-N. After restoring the pH to 7,2, recovery of the reactor was gradually effected.

Heat evolution

In experiments with high biomass (and low hydraulic retention times) sufficient heat was evolved to increase the temperature of the reactor above the surrounding temperature. This aspect must not be overlooked when designing a high rate plant to ensure that temperatures do not exceed say 35°C.

Temperature dependency of denitrification

The temperature dependency of biological denitrification is depicted in Figure 7. The relation may be given as follows

$$R_{s_{T^o}} = R_{s_{28^o}}\, 10^{-0{,}057\,(T-28)}$$

Dawson and Murphy (1972) using sodium citrate as carbon source and a dominant culture of *Pseudomonads denitrificans* found $\mu_{m_T} = \mu_{m_{20}}\, 10^{0{,}05\,(T-20)}$. Christensen and Harremoës (1972) in their literature study give values derived from data of various investigators.

Sludge age and utilisation

The results of tests given in Tables 1, 2 and 3 are also reflected in Figures 8a and 8b. The hydraulic retention times varied from 0,5 d to 32 d and the sludge retention times from 2,9 d to 32 d. Temperature was varied from 10° – 31,5°C.

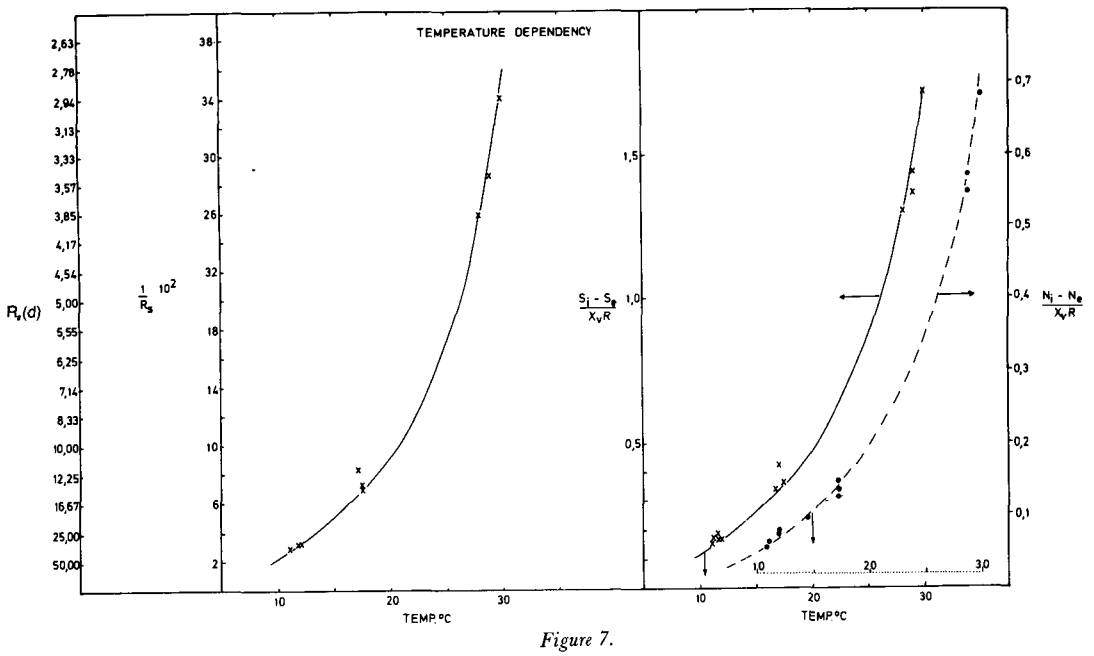


Figure 7.

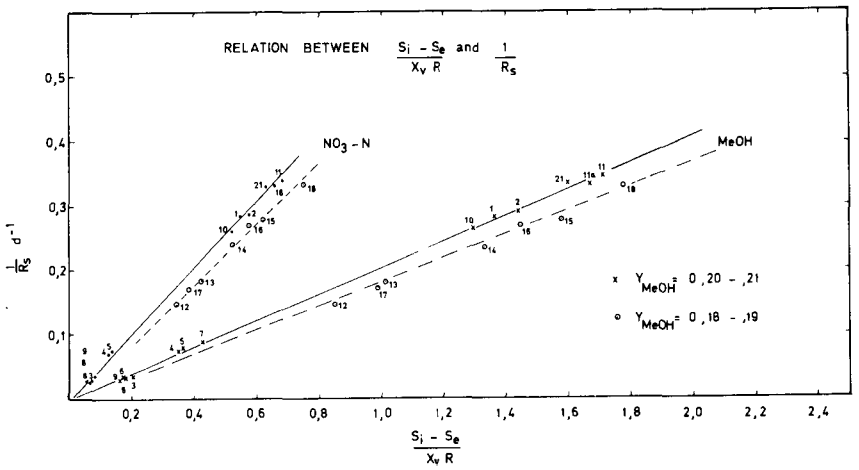


Figure 8a.
Graphical determination of Y.

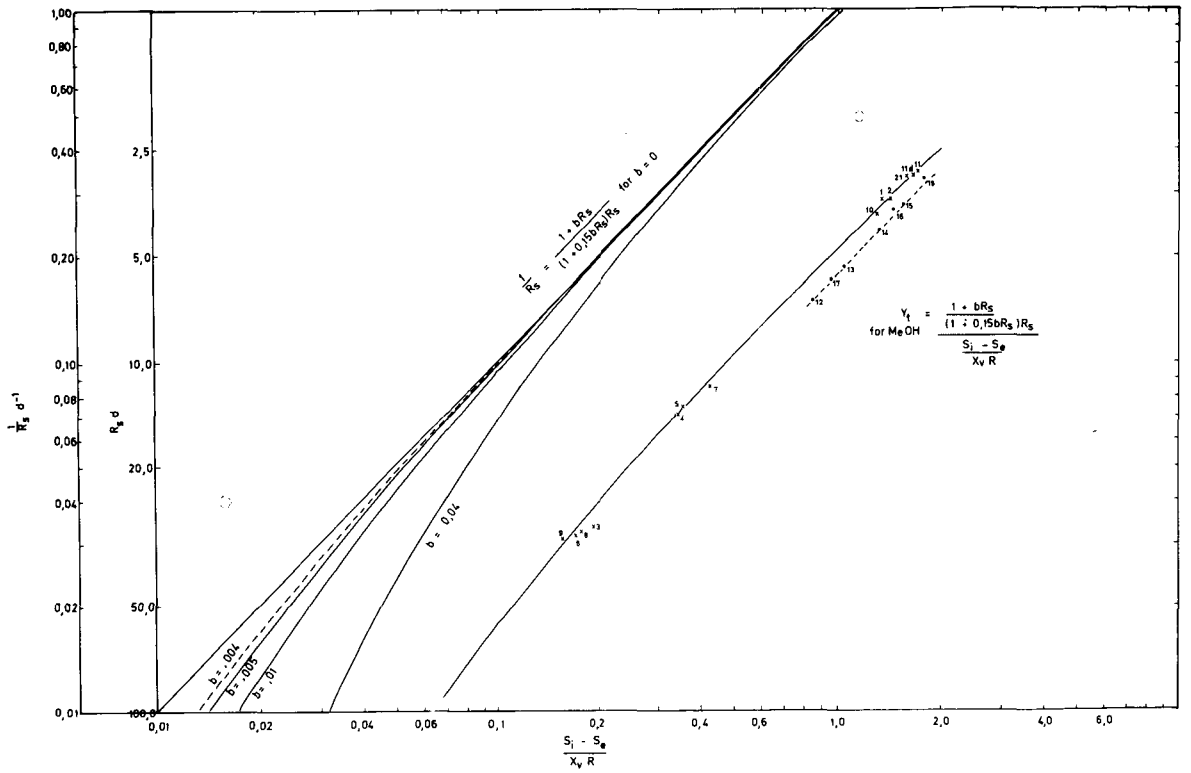


Figure 8b.
Graphical determination of Yt and b.

From these tests at pH 7,2 – 7,4 the following information was obtained:

$Y_{t\ (methanol)}$ = 0,18 – 0,22

b ≤ 0,005

R_{smin} 28° = 3,6 – 3,8 d from which $\mu_{max\ 28^\circ} = 0{,}278\text{-}0{,}263$

$R_{s\ min 10^\circ}$ = 45 – 50 d

Temperature dependency:

$R_{s_T} = R_{s\ min\ 28^\circ}\ 10^{-0{,}057(T-28^\circ)}$

methanol:nitrate-N ratio: approx. 2,5
(uncorrected for oxygen in feed)

The experimentally observed yield of biomass per gram methanol utilized was compared with a theoretical yield of $0{,}195 \frac{\text{mg cells}}{\text{mg } CH_3OH}$ (including ash) calculated from the biochemical knowledge of methanol oxidation on assimilation and the expected adenosine triphosphate (ATP) yields under anaerobic conditions. (Nurse 1977). The results obtained indicate good agreement and show the value of using biochemical knowledge to assess and explain the value obtained experimentally for an important kinetic parameter, since it is the inherent properties of the organism which limit the parameter.

Oxygen levels and redox potential

Dissolved oxygen was measured in the reactors from time to time, and levels less than 0,1 ppm were observed. Dissolved oxygen concentration is a satisfactory control variable for aeration in the presence of excess oxygen as in aerobic processes. The redox potential of a culture is probably a more useful guide in anaerobic processes (Wimpenny, 1969; Jacob, 1970).

The oxidation/reduction potential of continuous culture tests 18 and 22 was measured using platinum and saturated calomel electrodes. The platinum electrode was polished before use for quick response.

	pH	Temp	mV (sat.cal/Pt)
Test 18	7,4	30	between – 114 to – 131
Test 22	7,4	31-31,5	between – 110 to – 129

The reactor potential of a solution is a function of pH as well as of the presence of oxidising and reducing species. The above results are quoted at pH 7,4. The feed solution (without alcohol) gave a value of +205 mV at pH 6,54 and 30°C whereas the feed including alcohol gave +280 mV at pH 6,9.

The measured redox of the culture indicates the change as a result of bacterial activity in anoxic conditions. The feed used in the tests did however contain some oxygen and this oxygen will account for unneccessary consumption of methanol according to the following relation

(assuming Y_t = 0,188)

$$0{,}4375\ CH_3OH + 1\ O + 0{,}0261\ HNO_3 \rightarrow 0{,}0261\ C_4H_7O_2N + 0{,}333\ CO_2 + 0{,}797\ H_2O$$

Hence 1 mg $O_2 \equiv$ 0,875 mg CH_3OH

No nitrite was present in the feed used in these investigations and accumulation of nitrite during culture did not occur to any extent in well operated reactors. Should feed however, contain nitrite, then methanol will be consumed according to the relation

$$0{,}6162\ CH_3OH + 1\ HNO_2 \rightarrow 0{,}0367\ C_4H_7O_2N + 0{,}469\ CO_2 + 0{,}963N + 1{,}604\ H_2O$$

Hence 1 mg NO_2-N ≡ 1,41 mg CH_3OH

These consumption values may be compared with nitrate only in the feed where 1 mg NO_3-N ≡ 2,35 mg CH_3OH (from equation 4)

General

MAINTENANCE OF 'SINGLE' CULTURE

In all the chemostat type tests the bacteria showed the typical *Hyphomicrobium* form – the length of the hyphae, however, varied with environmental conditions (temperature, pH, growth rate, presence of foreign suspended matter).

In the return sludge operations, some contamination of the biomass occurred and the presence of protozoa was observed. However the bulk of the biomass again showed the typical *Hyphomicrobium* form except that hyphae were shorter and the dumb-bell form was more prevalent.

It is of interest therefore to note that the experiments which started with inocula from an enrichment of *Hyphomicrobium* from sewage sludge, maintained the 'single' culture throughout a period of 3 years during which the experiments were conducted.

USE OF TRADE EFFLUENT IN EXPERIMENTS

After experiment D (19, 20, 21) had been operating for 300 days on synthetic feed, the feed was switched to nitrified trade effluent in which the NO_3-N content only had been adjusted to the same value as the synthetic feed. Operation proceeded normally. The observed difference was in the biomass: the flocculation was improved and after about 60 days operation, the flocs were quite granular. This is not unexpected since the trade effluent is high in calcium.

After 90 days operation on trade effluent the reactor was run again on synthetic feed and except for the continued presence of a granular biomass the reactor continued normally. It is likely therefore that the trade effluent will enable the natural formation of suspended inorganic matter which will behave in a similar manner to the diatomaceous earth used in experiments 22 and 23 (See also Figure 9).

During the course of the various runs in the D series experiments (19, 20, 21) when nitrate in effluent was run close to zero, H_2S was liberated which immediately upset conditions by inhibition. The removal of H_2S with iron sulphate restored conditions rapidly and by decreasing slightly the methanol to nitrate ratio, to increase the nitrate in the effluent, conditions remained satisfactory.

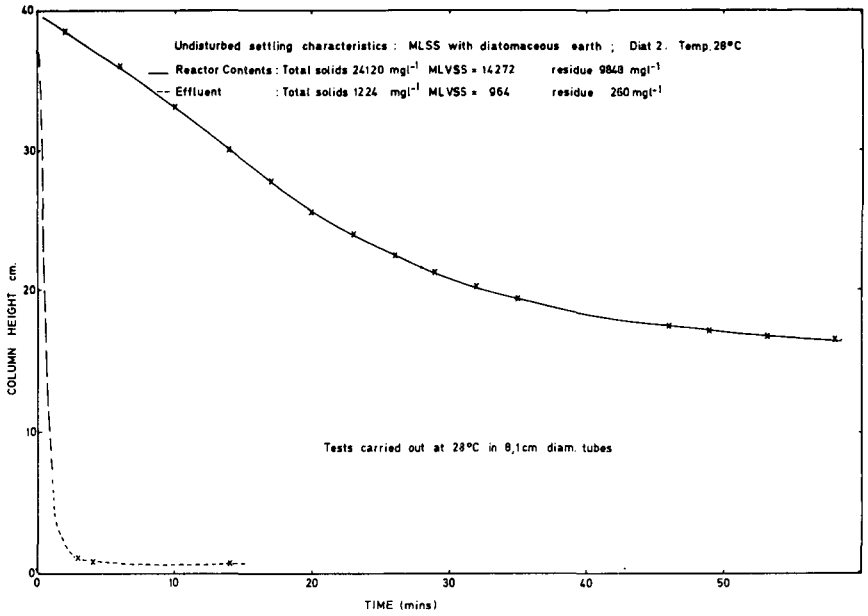


Figure 9.
Undisturbed settling test

USE OF COMMERCIAL METHANOL

In most of the tests described in this report pure methanol (S.G. 0,791 @ 24°C) was used as carbon source. In some tests however, commercial methanol conforming to the following analysis was used (Tests 11a and 23).

There was no noticeable difference in results.

Methanol	67,8 – 70,5% (S.G. 0,870 @ 24°C)
Nitrogen compounds as N	2 mg ℓ^{-1} m/V
Pentane	<3 mg ℓ^{-1} v/v
Hexane	3 mg ℓ^{-1}
Higher paraffins	<3 mg ℓ^{-1}
Ethanol	80 mg ℓ^{-1}
Propanol	35 mg ℓ^{-1}
Butanol	10 mg ℓ^{-1}
Higher alcohols	<3 mg ℓ^{-1}
Acetaldehyde	200 mg ℓ^{-1}
Acetone	40 mg ℓ^{-1}
Methylethylketone	5 mg ℓ^{-1}
Higher aldehydes and ketones	<3 mg ℓ^{-1}

Conclusions

An effluent containing approximately 2 800 mg ℓ^{-1} NO_3-N made up in a suitable medium from commercial chemicals was successfully denitrified to low residual values of NO_3 -N and NO_2-N. The nitrified trade effluent was also successfully denitrified. The denitrification was carried out in CST reactors where pH and sludge age can be readily controlled. At pH 7,2 – 7,4 the following data for design purposes was obtained:

$Y_{t(\text{methanol})}$	= 0,18 – 0,22
b	≤ 0,005
$R_{smin\ 28°}$	= 3,6–3,8 and $\mu_{max}{}^{28°} = {}^{0,278-0,263}$
$R_{smin\ 10°}$	= 45 – 50 d
Temperature dependency	: $R_{sT} = R_{s\ min}{}^{28°}\ 10^{-0,057(T-28°)}$
Methanol : nitrate-N ratio	= approx. 2,5

Free ammonia inhibits the reaction and it is considered that best results can be obtained by denitrifying a fully nitrified effluent to which a portion of original effluent containing both nitrate-N and ammonia-N is added to give that concentration of ammonia-N which is required for assimilation only. Operation under these conditions gives the best methanol: total N ratio. However the microbial yield will be greater (see section "nature of reactions"). Free H_2S is toxic to bacteria but can be controlled by operating with a small residual nitrate in the effluent and no methanol.

The equations, based on the Monod relation (equation 5) apply to the denitrification reaction.

It is proposed to run a CST reactor pilot plant on the trade effluent to ensure that with normal variation in the actual effluent, satisfactory denitrification can be achieved and maintained, both under summer and winter conditions.

It will be necessary to ensure that toxic materials such as detergents and heavy metals do not enter the feed stream to the reactor.

Nomenclature

b	= endogenous mass loss rate coefficient (d^{-1})
E_{sat}	= millivolt referred to platinum/saturated calomel electrodes (mV)
k	= utilisation rate constant (d^{-1})
k'	= maximum utilisation rate constant (d^{-1})

K_s = substrate saturation constant (mg ℓ^{-1})

MeOH = methanol

MLVSS = mixed liquor volatile suspended solids (mg ℓ^{-1})

N = concentration of NO_3–N substrate surrounding the organisms (mg ℓ^{-1})
(S is used for methanol or the general case)

N_i = initial NO_3–N concentration (mg ℓ^{-1})

N_e = NO_3–N in effluent (mg ℓ^{-1})

N_v = organic N fraction of MLVSS

P_v = organic P fraction of MLVSS

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

R = hydraulic retention time = $\frac{V}{Q}$ (d)

R_s = holding or retention time of biomass

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

S = growth limiting substrate concentration (mg ℓ^{-1})

S_i = initial substrate concentration (mg ℓ^{-1})

S_e = substrate concentration in effluent (mg ℓ^{-1})

SUR = specific substrate utilisation rate

$$= \frac{\text{mass of substrate utilised per day}}{\text{mass MLVSS}}$$

X = sludge concentration or concentration of bacteria generally (mg ℓ^{-1})

X_v = MLVSS (mg ℓ^{-1})

V = volume of reactor (ℓ)

Y = apparent growth yield

Y_t = total growth yield or total mass of organisms/mass of substrate fully converted

μ_m = maximum specific growth rate constant (mg $\ell^{-1}d^{-1}$)

CSTR = continuous stirred tank reactor

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