Treatment of Effluents from Ammonia Plants – Part II Biological Oxidation of Carbon-One Compounds in an Effluent from an Ammonia Plant

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

Abstract

The conditions for the biological oxidation of mono-, di- and trimethylamine and methanol present in a condensate from an ammonia synthesis gas stream have been investigated in continuous units.

The amines were completely destroyed to ammonia and CO_2 and the methanol to CO_2. At high flow rates and low sludge retention times very little ammonia was converted to nitrites and nitrates and no nitrosoamine was detected.

The relationship $\frac{1}{R_s} = Y_c \frac{(S_i - S_e)^*}{XR}$ can be applied. The biomass produced contains a high protein content and a satisfactory balance of amino acids and may prove to be satisfactory as a single cell protein source.

Introduction

In the process stream making ammonia synthesis gas from refinery gas or naphtha, an aqueous condensate arises which contains methylamines, methanol, ammonia and carbon dioxide.

An examination of the condensate has shown that the contaminants can be present up to the following levels:

methanol	2 000 mg ℓ^{-1}
ammonia	600 mg ℓ^{-1}
methylamines (mono-, di- and tri-)	200 mg ℓ^{-1}
carbon dioxide	saturated

The quantity of condensate in the plant investigated is of the order of 30 m^3h^{-1} and the concentration of amines varies with operating conditions. The pH of the solution is in the range of 8 – 8,7 and the temperature of the condensate leaving the plant is about 80°C.

The fishy odour of the effluent (under alkaline conditions) is a nuisance to the community. Since the odour is associated with the free amines, keeping the effluent pH sufficiently low to avoid the free species would be adequate for control of odour. For various reasons it was however considered desirable to investigate methods for removal or destruction of the amines.

Processes which were investigated included reverse osmosis, ion-exchange and chemical oxidation. In the case of reverse osmosis, it was possible to remove 98% of the amine into a concentrate approximately 1/3 of the original volume. Ion exchange similarly, results in concentrated solutions containing the amines and ammonia.

Oxidation by ozone showed considerable promise and this method will be described elsewhere.

During the course of investigations on the biological oxidation of ammonia (Neytzell-de Wilde, 1975) the presence of micro-organisms capable of oxidising methylamines was observed. It was decided therefore to investigate the destruction of amines by biological means. At the same time Steenkamp undertook to investigate the enzyme trimethylamine dehydrogenase and considerable progress is being made (Steenkamp *et al*, 1976). This will assist in the fuller understanding of the reactions.

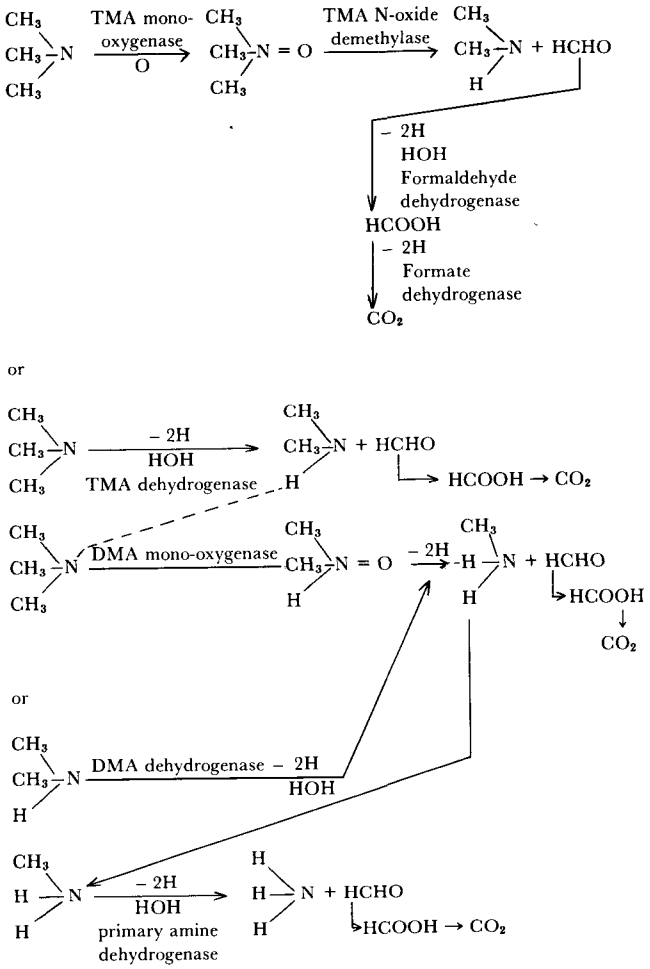
Review and Development

Ribbons *et al* (1970) and Quayle (1972) have reviewed the metabolism of one-carbon compounds and have reported in some detail on the physiology and biochemistry of the organisms concerned.

Colby and Zatman (1972) refer to heterotrophic micro-organisms capable of growth on carbon compounds containing one or more carbon atoms but no carbon-carbon bonds as methylotrophs. The cellular material of these organisms must

be synthesised from C-1 units obtained from their growth medium. Organisms capable of growth only on trimethylamine, dimethylamine, methylamine and methanol are termed obligate methylotrophs. Organisms which can grow on a variety of C-1 compounds as well as other carbon compounds are termed facultative methylotrophs.

The oxidation of trimethylamine and enzymes involved are summarised below (see also Colby and Zatman 1973).



The enzymes participating in the scheme shown above have been studied to various extents and the pathways for the formation of cells from C-1 compounds have been proposed by a number of workers in this field for bacteria including *Pseudomonas* species and *Hyphomicrobium*. (Large and Quayle 1963; Bellion and Hersch 1972; Harder, Attwood and Quayle 1973; Large and Carter 1973; Cox and Zatman 1973; Salem *et al* 1973 and Hersch 1975).

Kouno and Ozaki (1975) have shown that a large number of methanol-utilizing bacteria and methanol – and methylated amine – utilizers are widely distributed in aquatic and soil environments. There should therefore be little difficulty in developing suitable cultures for effluent treatment, but although much has been reported on the biological utilisation of methylamines, the literature is not rich in the application of these studies to the degradation of amines.

Chudoba *et al* (1969), however, have reported that methylamines, dimethylamines and trimethylamines decomposed satisfactorily with acclimated actived sludge.

Formation of nitrosoamines

Sander (1968) demonstrated that nitrate reducing bacteria are able under suitable conditions to synthesize nitrosoamines. The nitrosation occurred at such pH values which do not permit a spontaneous reaction between secondary amines and alkali nitrites.

Ayanaba *et al* (1973) have also reported on the possible formation of nitrosoamines. Voets *et al* (1975) however, reported that the formation of nitrosoamines was not evident in their work.

The potential exists for the microbial formation of nitrosoamines and because of their carcinogenic properties it will be desirable to assess whether such compounds are generated in any treatment system involving or generating the precursors.

Methanol as C-1 substrate

In recent years, there has been considerable interest in single cell protein (SCP) production and the literature contains a great deal of information on studies on the mechanism of utilisation of C-1 compounds and also on the use of methanol as a substrate for industrial fermentation.

It has been shown that methanol-utilizing micro-organisms oxidise methanol into CO_2 for energy generation (Cooney and Levine 1972).

$$CH_3\,OH \rightarrow HCHO \rightarrow HCOOH \rightarrow CO_2$$

It appears that the enzymes of methanol-utilizing bacteria are methanol dehydrogenases which require ammonia or methylamine as activator (Quayle 1972). Formaldehyde and formate dehydrogenases complete the conversion to CO_2.

The metabolic rates for the assimilation of C-1 substrates involve either direct incorporation of formaldehyde into phosphorylated sugar (ribulose phosphate pathway) or a condensation of formaldehyde with glycine to form serine (serine pathway). In single cell protein production, high cell yields are desirable. In effluent treatment, however, low yields are preferred because of the need to dispose of biomass, unless for example, the biomass can be used as feed or food source.

Thermophilic cultures

The optimum temperature range for the growth of most methanol-utilizing micro-organisms is normally about 30°C (Topiwala *et al* 1972). Snedecor and Cooney (1974) describe the selection of micro-organisms capable of utilizing methanol as sole carbon source at high temperatures and at growth rates comparable with those of mesophilic cultures at lower temperatures. The thermophilic cultures were more sensitive to alcohol, displaying complete inhibition at concentrations above 0,1% methanol. However in the content of effluent treatment, this should not pose a problem since steady state methanol levels should be extremely low in continuous operation mode. Problems will arise with respect to the transfer of oxygen, however, even in the treatment of effluents with relatively low methanol and amine concentrations.

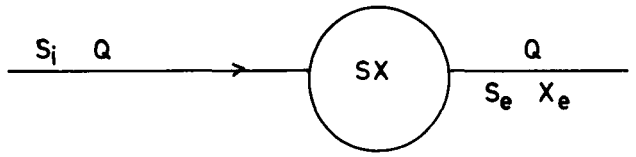


Figure 1a
Reactor without sludge return

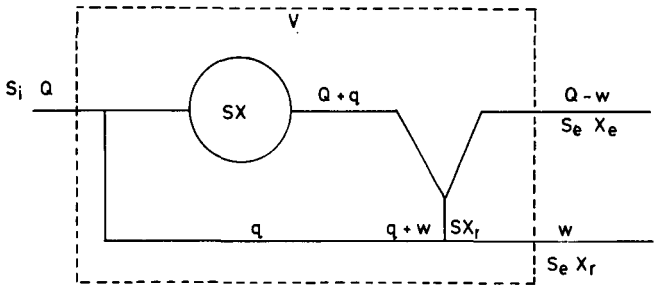


Figure 1b
Reactor with sludge retention or sludge recycle

Kinetics

The steady state biological kinetic equations for a completely mixed reactor (Figures 1a and 1b) are given below. These are similar to the equations used by the author (1975 and 1977).

System parameter	Steady state equation without sludge retention	Steady state equation with sludge retention (or sludge recycle)	Eqn. No.
Substrate utilisation rate SUR = $\frac{\text{Mass S oxidised per time}}{\text{mass MLVSS}}$	$\frac{k'S}{K_s+S}$	$\frac{k'S}{K_s+S}$	(1)
Microbial solids concentration X	$\frac{Y(S_r-S_e)}{1+b\,R_s}$		(2)
		$\frac{Y(S_r-S_e)}{1+b\,R_s}\,\frac{R_s}{R}$	(3)
XV = *mass of solids*	$\frac{Y \text{ mass of S oxidised}}{(1+b\,R_s)}$		(3a)
		$Y\left[\frac{\text{mass of S oxidised}}{(1+b\,R_s)}\right]R_s$	(3b)
Solids retention time $^{-1}$ i.e. R_s^{-1}	$\frac{Yk'S}{K_s+S}-b$	$\frac{Yk'S}{K_s+S}-b$	(4)
		$=\frac{Y(S_r-S_e)}{XR}-b$	(4a)
Effluent substrate conc. S_e	$\frac{K_s(1+bR_s)}{R_s(Yk'-b)-1}$		(5)
	(where $R_s = R$)	$\frac{K_s(1+b\,R_s)}{R_s(Yk'-b)-1}$	(6)
Limiting minimum R_s (when $S >> K_s$)	$\frac{1}{Yk'-b}$	$\frac{1}{Yk'-b}$	(7)

The equations based on the Monod relation for utilization of substrate by bacteria cultures, show that the effluent microbial solids and substrate concentration are dependent on the coefficients k', K_s, Y and b and the solids retention time of the process.

Equation (2) shows that there is a unique value for the microbial solids concentration at a given solids retention time and in equation (3), X is dependent only on R. Equations (5) and (6) show that S_e is dependent only on R_s.

By carrying out experiments at different solids retention times by varying the liquid flow rate and then using a linear regression analysis with equations (1) and (4) the value for k', K_s, Y and b can be derived. From the process operation aspect, the value of K_s determines how close the biological degradation process can be operated for maximum removal of substrate (S). The smaller the value, the more easily can the unit be operated at maximum rates of removal.

Oxygen consumption

Assuming the only products of metabolism are the cells themselves, carbon dioxide and water and that the source of nitrogen is ammonia, then for methanol it can be shown that

$$\frac{\text{g oxygen}}{\text{g cells}} = 16\,\frac{2C+\frac{H}{2}-O}{Y_{MeOH}\,M_{MeOH}}+\frac{O'}{1600}-\frac{C'}{600}+\frac{N'}{933}-\frac{H'}{200}$$

or

$$\frac{32C+8H-16O}{Y_{MeOH}\,M}+0{,}01\,O'-0{,}267\,C'+0{,}01714\,N'-0{,}08\,H'$$

where C, H and O represent the number of atoms of carbon, hydrogen and oxygen respectively in each molecule of methanol source; C', H', O' and N' represent the percentage of carbon, hydrogen, oxygen and nitrogen respectively in the cells; Y_{MeOH} represents the yield of cells based on the methanol source, gram cells per gram methanol consumed and M_{MeOH} represents the molecular weight of methanol.

Similarly for amines assuming that the only products of metabolism are the cells themselves, carbon dioxide, water and ammonia and that the nitrogen source is the N in the amine and released as NH_3, then for trimethylamine

$$\frac{\text{g oxygen}}{\text{g cells}}=\frac{32\,C+8\,(H-3)}{M_{TMA}\,Y_{TMA}}+0{,}01\,O'-0{,}0267\,C'-0{,}08\,(H'-3)$$

(The above equations are derived from the stoichiometric oxygen requirements for the complete combustion of methanol or amines and cells, and

$$Y_{MeOH}=\frac{\text{g cells}}{\text{g methanol}}\text{ or }Y_{amine}=\frac{\text{g cells}}{\text{g amine}}).$$

Assuming cell composition of $C_4H_8O_2N$ (van Dijken and Harder, 1975), the following can be calculated

$$\frac{\text{g oxygen}}{\text{g cells}} = \underset{\text{Methanol}}{\frac{1,5}{Y_{MeOH}}} - 1,34 \underset{\text{TMA}}{\frac{2,44}{Y_{TMA}}} - 1,34 \underset{\text{DMA}}{\frac{2,13}{Y_{DMA}}} - 1,34 \underset{\text{MMA}}{\frac{1,55}{Y_{MMA}}} - 1,34$$

$$= \frac{4}{Y_c} - 1,34$$

where $Y_c = \dfrac{\text{g cells}}{\text{g substrate as equiv. carbon}}$

or mg Oxygen $\ell^{-1} = 4(S_i - S_e)^*$ mg $\ell^{-1} - 1,34$ (cells) mg ℓ^{-1} (8)

where $(S_i - S_e)^*$ = mg ℓ^{-1} substrate as equiv. carbon.

Considering the flow diagram (Figure 1b) a mass balance at steady state on biomass gives:

$$Q(S_i - S_e)\ Y\text{-}b\ XV - wX_r + (Q\text{-}w)\ X_e = 0$$

from which $(S_i - S_e)\ Y = \left[b + \frac{1}{R_s} \right] XR$ (9)

Oxygen requirements, mg oxygen per litre reactor capacity per day (including endogenous respiration)

$$= 4\ \frac{(S_i - S_e)^*}{R} - 1,34\ Y \frac{(S_i - S_e)}{R} + 1,34\ Y \frac{(S_i - S_e)}{R} \quad (10)$$

and by substituting (9) in (10)

$$= 4\ \frac{(S_i - S_e)^*}{R} - \frac{1,34\ X(1 + bR_s)\ (1 - b)}{R_s} \quad (11)$$

This equation can be modified further in order to allow for that portion of the endogenous mass which is not biodegradable.

In single cell protein production, the specific oxygen requirement is such that the energy costs for supply of oxygen is considerable. Systems have been described involving air-lift type fermenters to achieve not only air for biological oxidation but also driving force for liquid circulation, Kuraiski *et al* (1975), MacLennan *et al* (1973), Gow (1973), Hines *et al* (1975).

The latter investigators have adapted such a scheme to effluent treatment, where the duties normally involve much lower micro-organism concentration and where the required oxygen transfer intensity is very much lower than for SCP production.

Acid/alkali production

Assuming a microbial composition of $C_4H_8O_2N$, for methanol the energy reaction may be represented by

$$CH_3OH + 3\ 0 \rightarrow CO_2 + 2H_2O$$

and the cell synthesis reaction by

$$4CH_3OH + NH_3 + 3,5\ O \rightarrow C_4H_8O_2N + 5,5\ H_2O$$

In a theoretical study, van Dijken and Harder (1975), using the serine pathway and assuming that in the dissimilation of methanol 1, 2 and 3 moles ATP are produced in the oxidation of methanol to formaldehyde, formate and carbon dioxide respectively, obtained the following equations: for energy

$$[6,9\ CH_3OH + 20,7\ O \rightarrow 6,9\ CO_2 + 13,8\ H_2O]$$

for cell synthesis

$$12\ CH_3OH + 3NH_3 + 10,5\ O \rightarrow 3C_4H_8O_2N \rightarrow 16,5\ H_2O$$

Assuming the serine pathway is used in the assimilation of amines (Hersch 1975) the following stoichiometry will apply, where n, is a factor modifying the basic energy equation

For TMA, energy:

$$n'(2,3\ (CH_3)_3N + 20,7\ O \rightarrow 6,9\ CO_2 + 2,3\ NH_3 + 6,9\ H_2O)$$

cell synthesis:

$$4(CH_3)_3N + 10,5\ O \rightarrow 3\ C_4H_8O_2N + 4,5\ H_2O + NH_3$$

For DMA, energy:

$$n''(3,45\ (CH_3)_2\ H\ N + 20,7\ O \rightarrow 6,9\ CO_2 + 3,45NH_3 + 6,9H_2O)$$

synthesis:

$$6(CH_3)_2HN + 10,5\ O \rightarrow 3C_4H_8O_2N + 4,5H_2O + 3NH_3$$

For MMA, energy:

$$n'''(6,9CH_3H_2N + 20,7\ O \rightarrow 6,9CO_2 + 6,9NH_3 + 6,9\ H_2O)$$

synthesis:

$$12CH_3H_2N + 10,5\ O \rightarrow 3C_4H_8O_2N + 4,5H_2O + 9NH_3$$

From the stoichiometry it is seen that the methanol and amine constituents all produce the same amount of CO_2 per unit equivalent of carbon. The alcohol constituent of the effluent will consume NH_3 but the amines will release excess ammonia, the amount increasing from TMA → DMA → MMA.

Experimental

The experimental work was primarily designed to show the effective destruction of methanol and methylamines in the presence of ammonia and CO_2 by micro-organisms.

Experimental conditions

Temperature all tests were carried out under controlled temperature conditions as shown in Table 1.

pH From a consideration of the dissociation of amines and ammonia, a pH of 7 ± 0,2 was selected for normal continuous operation. Fig. 2.

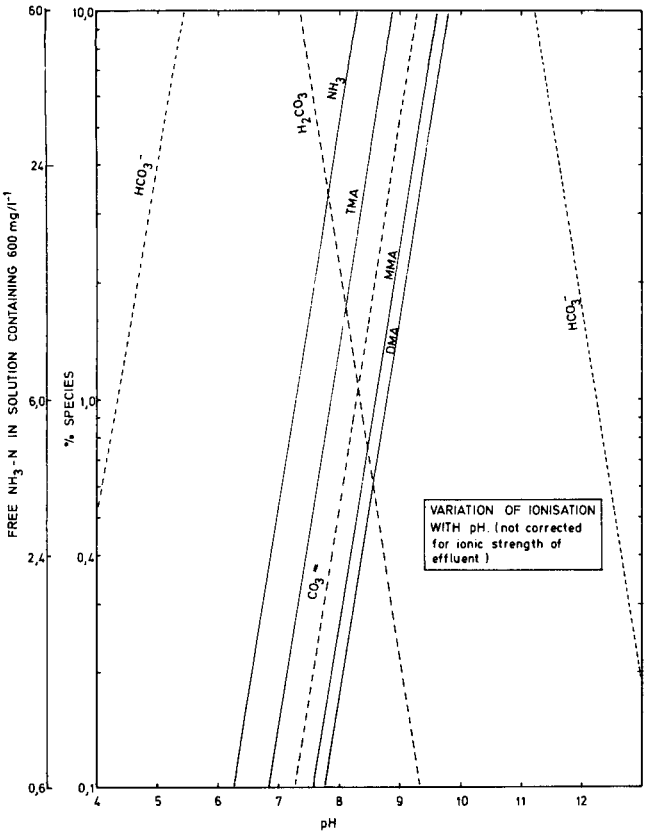


Figure 2

Oxygen The supply of air through diffusers was sufficient to give a minimum of 2 mgℓ^{-1} in the reactor at any time.

Substrate/medium

A.	Methanol ... as required by test, usually	2 000 mgℓ^{-1}
	TMA ..... as required by test, usually	100 mgℓ^{-1}
	DMA ..... as required by test, usually	50 mgℓ^{-1}
	MMA..... as required by test, usually	50 mgℓ^{-1}
B.	Potassium dihydrogen phosphate	62 mg ℓ^{-1}
	Magnesium sulphate (commercial)	50 mg ℓ^{-1}
	Calcium chloride (commercial)	75 mg ℓ^{-1}
	Sodium chloride (commercial)	100 mg ℓ^{-1}
	Ferrous sulphate (commercial)	trace
	Dilution, tap water	

Concentrates of A were added in the required quantity to B. The solution was then neutralised with H_2SO_4 to pH 7 and then saturated with CO_2. Sufficient substrate was made for 24 hour operation and this material stored at 5-10°C to reduce bacterial attack.

Actual plant effluent

Plant effluent was transferred to the laboratories in sealed drums and allowed to cool to ambient temperature. The material was transferred to a large refrigerated tank (5-10°C).

TABLE 1
RELATION BETWEEN SLUDGE AGE AND UTILIZATION OF SUBSTRATE

Exp. No.	Days	Temperature °C	$(S_r-S_e)^*$ mg ℓ^{-1}	X mg ℓ^{-1}	Effluent Volume ml d^{-1}	R_s d	R d	$Y_c = \frac{X}{(S_r-S_e)^*}\frac{R}{R_s}$ mg cells mg C^{-1}	$\frac{(S_r-S_e)^*}{XR}$ mg C mg Cells^{-1} d^{-1}	$\frac{1}{R_s}$ d^{-1}	FEED (approximate values) Carbon equiv. mg ℓ^{-1} TMA	DMA	MMA	Me OH	Total C	NH_4-N mg ℓ^{-1}	Total C based on effluent volume mg ℓ^{-1}
1	4–20	25–26	102,5	35	2200	4,55	4,55	0,34	0,64	0,22	102,5				102,5	280	102
	34–50	25–26	102,5	35	4450	2,25	2,25	0,34	1,30	0,44							
	56–	25–26	102,5	35	5850	1,71	1,71	0,34	1,71	0,58							
2	50–80	25–26	874	580	2200	4,55	4,55	0,66	0,33	0,22	93			770		274	874
3	20–26	39,5–40	373	400	7825	3,54	1,28	0,39	0,73	0,28	5,5	2,9	8,0	384	400,4	660	
	29–34	39,5–40	365	390–400	8000	3,33	1,25	0,39	0,73	0,30							
	36–44	39,5–40	357	700–820	16500	2,86	0,61	0,42–0,49	0,84–0,71	0,35							
	46–88	42	364	700	16240	3,09	0,62	0,39	0,84	0,32							
4	22–35	39,5–40	406	300	7372	2,97	1,36	0,34	1,0	0,34							
5	20–28	38,5–40	396	260	7970	2,01	1,25	0,41	1,22	0,5							
	28–34					2,12	1,29	0,40	1,18	0,47							
	40–44	39–40	413	380	7570	2,79	1,32	0,42–0,44	0,85–0,82	0,36							
6	5–15	40–42	842	1040–1060	8800	1,59	1,14	0,89–0,90	0,71–0,70	0,63	62	28	20	713	823	600	842
	35–42	25,5–26,5	832	680	4000	2,94	2,5	0,69	0,49	0,34							
	47–57	25,5–26,5	838	780	4600	2,5	2,17	0,69	0,50	0,4							
7	50–60	25,5–26,0	833	900	4950	2,4	2,02	0,90	0,46	0,42	approx.						
				800				0,81	0,52	0,42							
	105–110	34,5–35	900	950	9300	1,2	1,08	0,95	0,88	0,83							
	5–8	40–42	867	1100–1200	8900	1,56	1,12	0,95	0,67	0,64	approx.						
8	5–8	41–42	831	920	8400	1,56	1,19	0,84	0,76	0,64							
	72–75	41–42	840	720	5700	1,96	1,75	0,80	0,65	0,51							
	40–50	41–42	837	630	990	11,11	11,11	0,75	0,12	0,09							
	65–70	41–42	847	780	4600	2,5	2,17	0,80	0,51	0,40							

Note: $(S_r-S_e)^*$, R and R_s all based on effluent volume from reactor
Tests 3, 4, 5 carried out on trade effluent.

In one series of tests the material was fed to the reactors at the original pH value. In all other tests the bulk was neutralised to pH 6,8-6,9 with sulphuric acid.

Lots of 30ℓ were withdrawn from the bulk feed and modified by the addition of medium and trace elements before being fed to the reactors. Trace elements were added to give 0,05 mgℓ$^{-1}$ of each of Fe, B, Co, Cu, Mn, Mo, and Zn. This feed was also kept at 5-10°C to decrease bacterial activity.

Two bulk lots were tested, the compositions of which were:

Component	mgℓ$^{-1}$	equivalent C mgℓ$^{-1}$	mgℓ$^{-1}$	equivalent C mgℓ$^{-1}$
TMA	6,8	4,2	9,1	5,5
DMA	4,2	2,2	5,5	2,9
MMA	19,0	7,3	20,6	8,0
MeOH	989	371	1024	384
NH_4-N	660	—	660	—
		384,7		400,5

Analytical techniques

Selective electrodes were used for routine measurement of ammonia (in absence of amines only), nitrate and dissolved oxygen. Nitrite was determined by the α-naphthylamine – sulphanilic acid method. Mixed liquor suspended solids (MLSS) were determined by both turbidimetric and gravimetric procedures. Total carbon was determined occasionally using a Beckman TOC analyser. Gas Chromatographic methods were used for the determination of methanol, methylamines and dimethyl-nitrosoamine.

Nitrogen balance

A nitrogen balance across the reactors of the three forms of nitrogen (after complete destruction of amines), namely NO_2^-, NO_3^- and NH_4^+ was carried out to act as a check on any unexpected losses. The nitrogen incorporated into protoplasm was taken into account.

Reaction vessels

The reaction vessels used in the tests are shown in Fig. 3.

The stirrer was so designed and placed that a slight vortex was created and the pattern of flow resulting was such that accumulation of biomass and foam at the surface was reduced to a large extent.

The overflow from the reactor was collected in a receiver and kept aerobic by an air sparge. Every 24 hours a fixed volume of the contents of the receiver was centrifuged and the solids so recovered returned to the reactor in one dose or continuously by means of a peristaltic pump over 24 hours.

A volume equivalent to that withdrawn from the reactor for analytical purposes was withdrawn from the receiver and returned to the reactor after each sampling event.

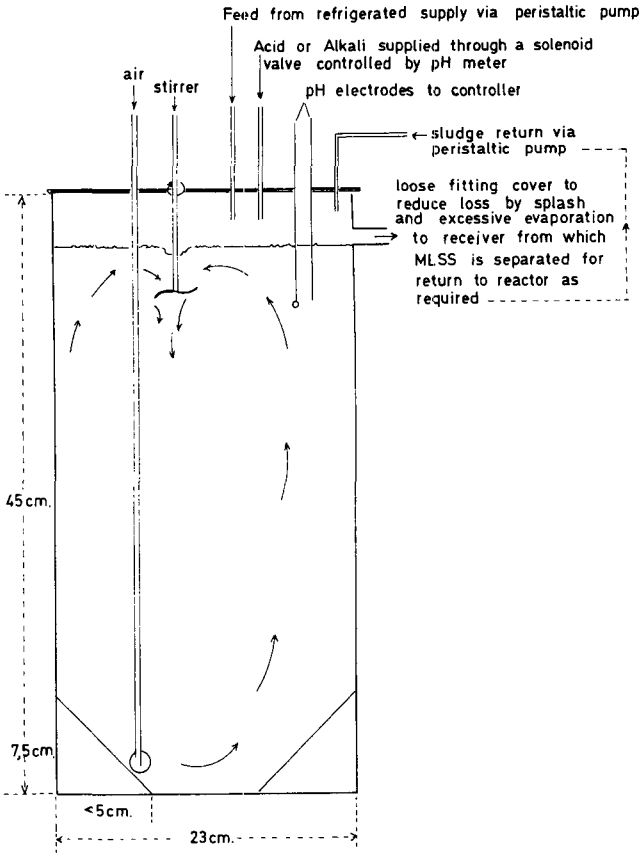


Figure 3
10 ℓ tank for continuous tests.

Results and discussion

Preliminary chemostat-type continuous tests

Two samples of sludge taken from a reactor, in which nitrate was being denitrified anaerobically with methanol as carbon source, were transferred to two reactors charged with medium but without methanol and amine.

Reactor 1 was then fed at a low rate with medium containing 2 000mgℓ$^{-1}$MeOH, 200mgℓ$^{-1}$TMA and over 200mgℓ$^{-1}$$NH_4$-N.

Reactor 2 was fed with medium containing 200mgℓ$^{-1}$TMA and over 200mgℓ$^{-1}$$NH_4$-N. pH was controlled with sodium bicarbonate at 7 ± 0,2 and temperature at 25-26°C.

It was found that the yield from TMA was only 0,34 mg cells per mg C equivalent and that a relatively low sludge age (less than 1,7 days) was required for complete destruction of the amine (Exp. 1, Table 1.).

The yield in the presence of methanol and amine was 0,66 mg cells per mg carbon equivalent and the indications from this test were that R_s of over 2 days would be necessary for destruction of the mixture at 25-26°C. (Exp. 2. Table 1.)

A nitrogen balance over the reactor indicated that the analytical techniques were satisfactory and that the amine was completely degraded to ammonia.

A microscopic examination of the sludge indicated the presence of *Hyphomicrobium* spp in both the mixed cultures (Figure 4).

Continuous tests with synthetic effluent and trade effluent

Three reactors were operated under different conditions using the medium and substrate shown earlier.

Flow, temperature, pH and recycle rates were varied to obtain information which could be applied to the treatment of the actual trade effluent. (In addition, the actual content of the medium was halved during the latter stages of the experiments without any effect on results). The inoculum for these tests was taken from Reactor 2 after this reactor had been fed for some days with feed containing TMA, DMA, MMA, methanol and ammonia.

The results of these tests, 6, 7 and 8 are given in Table 1.

On completion of the above tests, the synthetic feed was changed over to the actual effluent (modified as indicated earlier). Tests were run at high temperatures 38-40°C and at high flow rates since such conditions only would be of interest in the treatment of the effluent concerned. Complete degradation of amines and methanol was achieved at reasonable hydraulic retention times (0,6-1,3 d) and at sludge ages between 2-3,5 days (Exp. 3,4 and 5).

The results are shown in Table 1 and Figure 5. The marked difference in utilization rates in experiments with synthetic effluent and industrial effluent was not investigated further since for the purposes of the project satisfactory degradation of the amines had been achieved.

The scatter in results may be due to difficulties in returning sludge in accurate amounts in tests on the scale used in the experiments. The biomass physical characteristics varied considerably with environmental conditions – pH and temperature. The most satisfactory sludge was produced at the higher temperatures 39-40°C. At 25-26°C the sludge was slimy and difficult to separate.

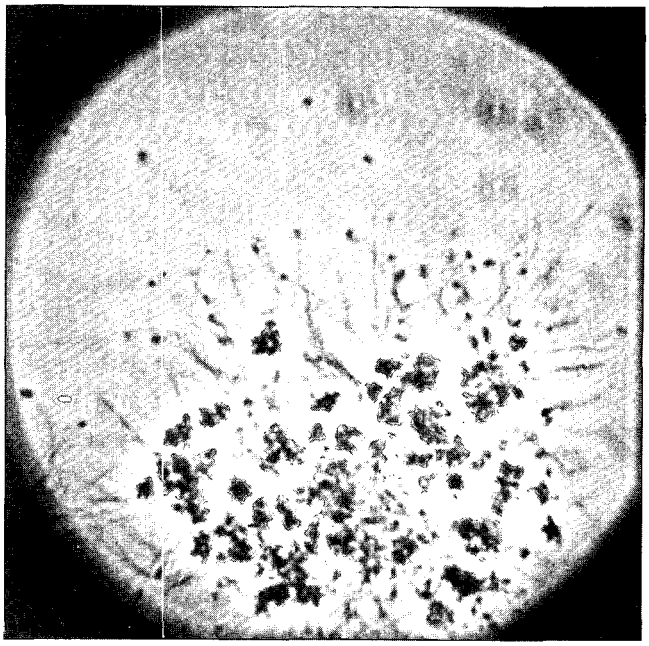


Figure 4
Typical microbial growth

It was found that operation at near pH 7 gave stable operation. Not only were rates decreased at pH values below 7 but also at pH values beyond pH 7, when free ammonia is released.

With regard to temperature, the sludge age required for complete degradation decreases from 25°C to 35°C and then increases at temperatures from 35°C-40°C. Above about 42°C the decrease in biological activity is marked and damage to the biomass probably occurs.

Composition of cells

A sample of the cells from Experiment 6 was centrifuged, washed and freeze dried. At the same time cells from an experiment in which nitrate was being anaerobically denitrified

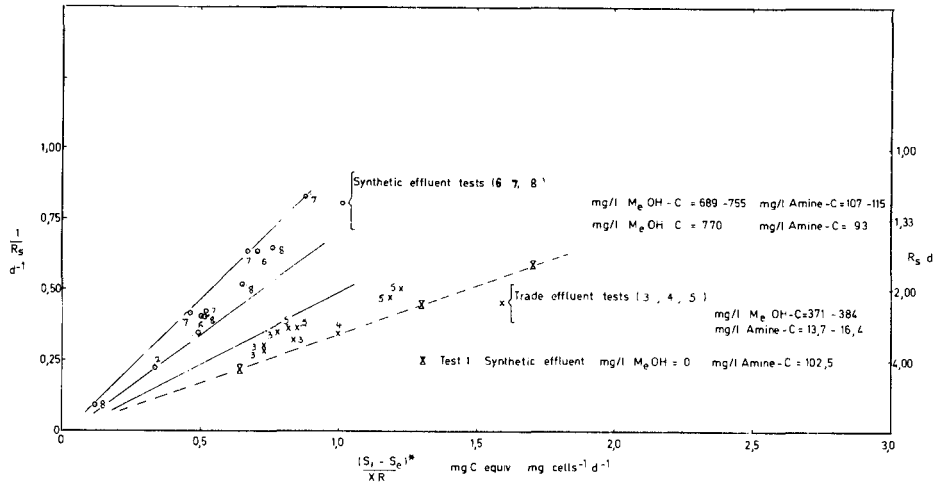


Figure 5
Relation between sludge age and utilisation of substrate

with methanol as the C-source were similarly treated. Elemental analysis on these samples gave the following results on an ash free basis.

	Aerobic cells	Anaerobic cells
C	4,1	4,03
H	7,49	7,19
N	0,74	0,74
O	2,05	2,03

Protein analysis

In considering the nutritional value of bacteria as a feed or feed supplement, it is the protein quality which primarily determines its value. The value of the anaerobically grown cells (*Hyphomicrobium* spp) obtained in denitrification studies is discussed by Nurse 1976. The amino-acid composition of the aerobically grown cells was found to be practically identical (Neytzell-de Wilde, 1975)

General

LOSS OF CONSTITUENTS FROM FEED AT HIGH TEMPERATURES AND/OR HIGH pH VALUES

Since it would be desirable to operate at the highest temperature at which the biomass will function satisfactorily tests were conducted to assess the loss of constituents from an aerated reactor at about 43°C.

A batch feed (10ℓ) containing approximately 60 mgℓ^{-1} TMA, 30 mgℓ^{-1}DMA, 30mgℓ^{-1}MMA, 1000 ppm methanol and 600 mgℓ^{-1} NH_4-N was placed in a 10ℓ reactor maintained at about 43°C. Chromium (sulphate) was added to inhibit any bacterial activity. The reactor was aerated at the rate of 0,8ℓ min^{-1}. It was found that the loss of all amines was negligible at pH 7-7,1; between pH 7,4-7,5 loss of trimethylamine occurred, at pH 8-8,3 loss of all amines occurred (Figure 6).

In continuous reactors at high temperatures 35-40°C and with hydraulic retention times of one day or less, loss of amines at pH 7-7,2 would not be noticed. Some small loss of ammonia may occur as this material in the feed is not changed in the reactor and is in fact increased by the conversion of amines to ammonia. Loss of methanol is likely to be very small since retention time is low and concentration at any instance is low because of bacterial activity and dilution.

RESIDUAL ORGANIC CARBON

The effluent from the reactors showed residual organic carbon of between 30-50 mgℓ^{-1} after centrifigation at 5000-6000 g. Since neither methanol nor amines could be detected, this organic material probably arises from products of the biomass.

NITRATES AND NITRITES

The formation of nitrites and nitrates arises from biological oxidation of ammonia by contaminating nitrifiers. Values for nitrite-N were however generally low (<1 mgℓ^{-1} and nitrate-N varied between 3 and <1 mgℓ^{-1}; the lower the sludge age, the lower the nitrite and nitrate content of the effluent.

NITROSOAMINE

Dimethyl-nitrosoamine was prepared by the method described by Blatt (1944). Standard solutions were prepared and analysed by gas chromatographic methods. In all the experi-

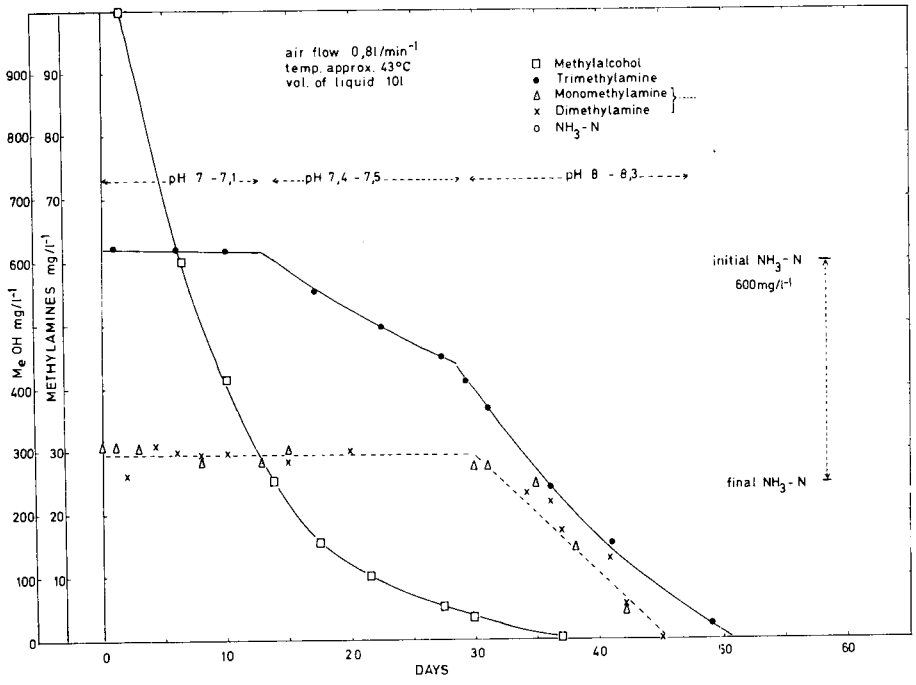


Figure 6
Stripping of components

ments carried out in both synthetic and also the trade effluent, no dimethylamine nitrosoamine was detected in any samples taken from any of the reactors.

Application

To illustrate the size of treatment plant required consider an effluent of 30 m^3h^{-1} of the following composition

	$mg\ell^{-1}$	equivalent C $mg\ell^{-1}$
NH_4-N	600	—
TMA	10	6,1
DMA	6	3,2
MMA	20	7,7
MeOH	1050	393,8
		410,8

The effluent is acidified to pH 7-7,2 (with hydrochloric acid) and the necessary minerals added and allowed to cool to 40-42°C.

Expected MLSS (neglecting endogenous respiration)

From Section 2.5, since $X = Y_c(S_i\text{-}S_e)^* \frac{R_s}{R}$; $(S_i\text{-}S_e)^*$ is known, Y is fixed and R_s is selected, a plot of X versus R for a series of R_s values will enable selection of R for a given R_s value and give the expected X value. Assume Y_c = 0,4, hydraulic retention time = 1 day, a sludge retention time of 3 days is known to be adequate and under these conditions at 39-40°C the mixed liquor concentration will be 493 $mg\ell^{-1}$.

Oxygen requirements

From Section 2.6 omitting maintenance requirements, mg O_2 per litre reactor capacity

$$= \frac{4(S_i\text{–}S_e)^*}{R} - \frac{1{,}33\ X}{R_s} = 1425\ mg\ \ell^{-1}\ d^{-1}$$

The total oxygen for 30 m^3d^{-1} = 1026 kg d^{-1}, which is equivalent to a load of approximately 20 Kw for a surface aerator under standard conditions (20°C and 101,3 kPa).

Reactor size This is dependent on the hydraulic retention time selected, for R = ld a tank 14m x 14m x 4m deep would probably be adequate.

Control procedures Automatic pH control in the reactor will be necessary to achieve consistent rates and reliable degradation of components.

For the selected sludge age, irrespective of the flow through the reactor, the MLSS in the effluent volume equivalent to $V_w = V/R_s$ may be discarded and the remainder returned to the reactor.

With a suitable sedimentation tank in circuit, the MLSS should concentrate in about 20% of the original volume. By centrifuging this volume, the MLSS can be further concentrated and the correct proportion returned to the reactor. The remainder can be discarded but consideration should be given to processing the residue as a SCP feed supplement.

Nomenclature

b	microbial decay coefficient (d^{-1})
k'	maximum specific substrate removal rate per unit weight of organisms $= \frac{1}{X}\frac{ds}{dt}$ (d^{-1})
K_s	substrate concentration when $\mu = \frac{1}{2}\mu_{max}$ or substrate saturation constant of an organism (mg ℓ^{-1})
n	a factor, modifying the basic energy equation
q	return flow ($\ell\ d^{-1}$)
Q	base flow through reactors ($\ell\ d^{-1}$)
R	hydraulic retention time = V/Q (d)
R_s	holding or retention time of biomass = sludge age $= \frac{\text{sludge in reactors}}{\text{sludge wasted per day}}$ (d)
S	substrate surrounding the micro-organisms (mg ℓ^{-1})
S_e	substrate in effluent (mg ℓ^{-1})
S_i	substrate in influent (mg ℓ^{-1})
$(S_i\text{–}S_e)^*$	carbon equivalent of substrate consumed (mg ℓ^{-1})
V	reactor volume as per Fig. 1b (ℓ)
w	sludge waste flow ($\ell\ d^{-1}$)
X	sludge concentration (mg ℓ^{-1})
X_e	sludge concentration in supernatant flow (mg ℓ^{-1})
X_r	sludge concentration in return sludge (mg ℓ^{-1})
Y	growth yield $= \frac{\text{mass of bacteria formed}}{\text{mass of substrate consumed}}$
Y_c	growth yield $= \frac{\text{mass of bacteria formed}}{\text{mass of substrate consumed expressed as C}}$
μ	specific growth rate (d^{-1} or h^{-1})
μ_{max}	maximum specific growth rate (d^{-1} or h^{-1})
MLSS	suspended solids in liquor (mg ℓ^{-1})
SCP	single cell protein
TMA	trimethylamine
DMA	dimethylamine
MMA	monomethylamine
MeOH	methanol
ATP	adenosine 5'triphosphate

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