

# Chemical Inhibition of Biological Nutrient Removal Processes

C. KNOETZE, T.R. DAVIES\* and S.G. WIECHERS

National Institute for Water Research, P.O. Box 395, Pretoria, 0001

## Abstract

The inhibitory effects of various heavy metal ions and organic inhibitors were studied using laboratory-scale activated sludge and biological rotating disc units.

All the heavy metal ions studied were tolerable in raw sewage at concentrations below  $1 \text{ mg l}^{-1}$ . Oxidation of carbonaceous matter in the activated sludge units was unimpaired, even when nitrification and denitrification were severely inhibited and some of the mixed liquor suspended solids were killed and lost in the effluent. Given sufficient time to adapt, all the unit processes developed resistance to the inhibitors studied.

Guidelines are given regarding permissible concentration levels of inhibiting materials in the feed to biological wastewater purification systems.

## Introduction

Local authorities and government departments are becoming increasingly concerned about the polluting effect of wastewaters and sophisticated processes are being developed and used to purify them more effectively. Two main methods of treatment can be distinguished; namely –

- (1) physical-chemical methods, which are applicable especially to industrial wastewaters, and
- (2) biological methods, which are used by most municipalities for the purification of domestic wastewater.

The presence of partially treated or even untreated industrial effluents in sewage has given rise to concern about the potential inhibitory effects of heavy metal ions, phenols, cyanides, pesticides and other chemicals on biological treatment processes. The purpose of this study was to establish the critical concentration levels above which selected inhibitors would cause significant malfunctioning of representative biological treatment processes. The following aspects were investigated:

- (1) The threshold concentrations of various chemical inhibitors below which no inhibition was observed.
- (2) Adaptation of microbiological life to inhibition induced by prolonged exposure to sublethal concentrations of chemical inhibitors.

Laboratory-scale units were used throughout this study.

Although useful information on establishing guidelines and problem areas could be found in the literature (Davies, 1975), there was little dealing specifically with chemical inhibition of wastewater purification systems.

## Materials and Methods

### Denitrification Growth Studies

Denitrification growth studies in Erlenmeyer flasks were carried out before the laboratory-scale studies to determine the concentration range of the chemical inhibitors to be investigated. Due to the very low cellular yield of autotrophic nitrifying bacteria and because all nitrifying-denitrifying wastewater treatment systems rely mainly on facultative anaerobic bacteria, denitrifying bacteria were selected for the growth studies.

After routine analyses of samples taken from the influent to several municipal sewage works, the following inhibitors were found most likely to occur and were therefore selected for these studies:

Barium chloride ( $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ); Cadmium chloride ( $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ ); Chromium chloride ( $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ); Lead chloride ( $\text{PbCl}_2$ ); Mercury chloride ( $\text{HgCl}_2$ ); Nickel chloride ( $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ); Phenol ( $\text{C}_6\text{H}_5\text{OH}$ ); Potassium cyanide (KCN); Potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ); Silver sulphate ( $\text{Ag}_2\text{SO}_4$ ); Sodium arsenite ( $\text{NaAsO}_2$ ); and Zinc chloride ( $\text{ZnCl}_2$ ).

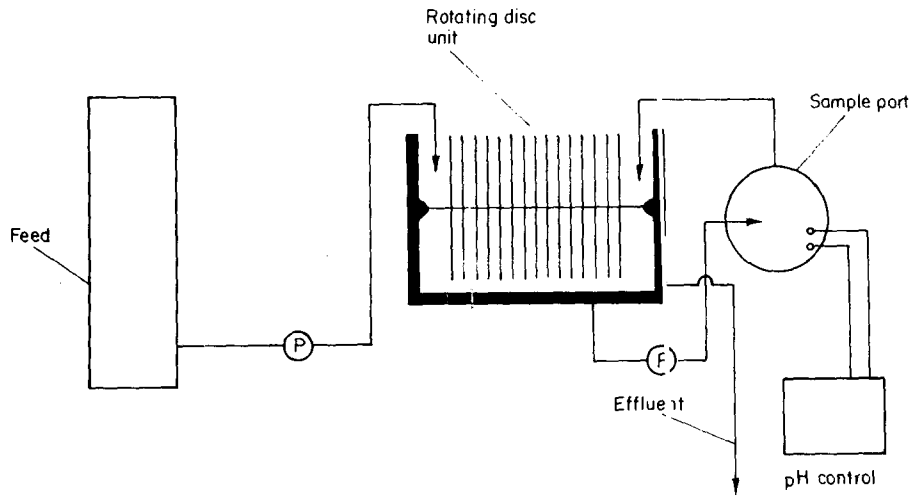
Phenol was the only organic inhibitor investigated.

The bacteria were cultivated in a synthetic growth medium (Davies, 1973) to prevent interference by external factors. Bacteria from the same culture grown in media containing no inhibitor were used as controls.

A mixed population of denitrifying bacteria was obtained from a denitrifying rotating disc unit and inoculated into 200 ml of sterile growth medium. The bacteria on the disc unit had been fed with the settled effluent from trickling filters, with methanol being used as hydrogen donor. The inoculated medium was incubated at  $20^\circ\text{C}$  under a nitrogen atmosphere acquired by gassing the medium with high purity nitrogen. When it was determined (by means of an Eppendorf line spectrophotometer) that the bacterial suspension was in the logarithmic growth phase,  $10^{-4} \text{ l}$  aliquots of this suspension were used to inoculate the experimental flasks containing the growth medium and a chosen concentration of inhibitor. The inoculated media in the flasks were subsequently gassed with high purity nitrogen to remove oxygen. All cultures were then incubated at  $20^\circ\text{C}$  and shaken continuously by means of a shaking machine.

Growth rate was determined by measuring the turbidity

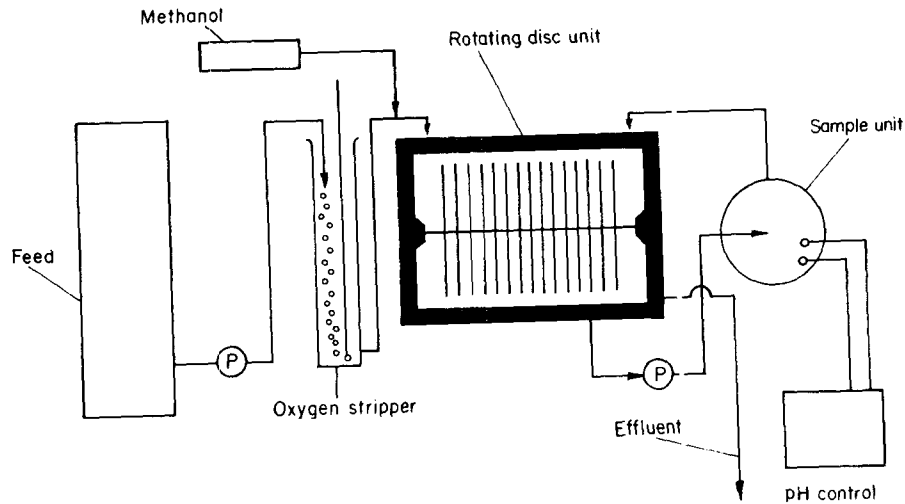
\*Present address: International Consulting and Laboratory Services (Pty) Ltd, Earigo Building, Cnr. Kloof St. and Park Road, Cape Town 8001.



Number of discs = 30  
 Disc diameter = 410 mm  
 Surface area per disc = 0,2641 m<sup>2</sup>  
 Total disc surface area = 7,922 m<sup>2</sup>  
 Total available area for attached growth = 8,1 m<sup>2</sup>

Liquid volume of container with discs 45 % submerged = 15 l  
 Ratio of surface area to volume with discs 45 % submerged  
 = 540 m<sup>2</sup> m<sup>-3</sup>  
 Operating speed = 6 r min<sup>-1</sup>  
 Operating temperature = 20 °C

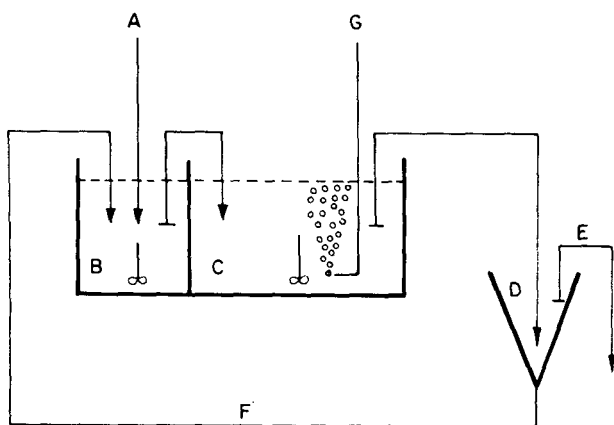
Figure 1  
 Experimental layout and design specifications of an aerobic nitrifying rotating disc unit



Number of discs = 43  
 Disc diameter = 406 mm  
 Surface area per disc = 0,2589 m<sup>2</sup>  
 Total disc surface area = 11,1327 m<sup>2</sup>  
 Total available area for attached growth = 11,25 m<sup>2</sup>

Liquid volume of container with discs 45 % submerged = 14,25 l  
 Ratio of surface area to volume with discs 45 % submerged  
 = 789,5 m<sup>2</sup> m<sup>-3</sup>  
 Operating speed = 6 r min<sup>-1</sup>  
 Operating temperature = 20 °C

Figure 2  
 Experimental layout and design specifications of an anoxic denitrifying rotating disc unit



- A Feed
- B Anoxic zone (1,1 l)
- C Aerobic zone (3,0 l)
- D Settler
- E Effluent
- F Recycle of anoxic zone
- G Aerator (sintered glass)

Figure 3

Experimental layout and design specifications of the laboratory-scale activated sludge units

of an aliquot from a well-shaken sample on the Eppendorf line spectrophotometer at 578 nm every 4 h. After withdrawing each aliquot, the growth flasks were again gassed with high purity nitrogen and incubated on the shaking machine at 20 °C. This procedure was continued until cell multiplication had ceased.

### Chemical Inhibition Studied on Laboratory-Scale Units

#### Biological reactors

On the basis of previous experimentation by Davies and Pretorius (1975), two rotating disc units (Figs. 1 and 2) were constructed to study nitrification and denitrification processes separately, while an activated sludge plant was used to study these processes in succession (Fig. 3).

FIGS. 1, 2 and 3

#### Feed

Humus tank effluent from the Daspoort sewage works, Pretoria, was used as feed for the nitrifying and denitrifying rotating disc units. Ammonium bicarbonate ( $\text{NH}_4\text{HCO}_3$ ) was used to adjust the ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) content of the feed to the nitrifying rotating disc units to 60 mg  $\text{l}^{-1}$ . At a later stage, the feedstock was adjusted further to increase the rate of bacterial establishment on the perspex discs of the nitrifying rotating disc units. Sufficient raw sludge, pasteurized at 73 °C for 15 min, was added to enrich the feed with a variety of nutrients and to ensure a chemical oxygen demand (COD) of 60 mg  $\text{l}^{-1}$ . Potassium nitrate ( $\text{KNO}_3$ ) was added to the feed of the denitrifying rotating disc unit to provide a nitrate nitrogen ( $\text{NO}_3\text{-N}$ ) concentration of 60 mg  $\text{l}^{-1}$ . Methanol was used as electron donor and was added in undiluted form directly into the feedstream to obtain a C:N ratio of 3:1.

Settled sewage with a COD of 400 mg  $\text{l}^{-1}$  was obtained from the Daspoort sewage works to serve as feed for the activated sludge units. The total Kjeldahl nitrogen (TKN) values of the feed varied between 30 mg  $\text{l}^{-1}$  and 75 mg  $\text{l}^{-1}$ , while the ammonia concentrations ranged from 25 mg  $\text{l}^{-1}$  to 50 mg  $\text{l}^{-1}$ .

#### pH control

The nitrifying and denitrifying rotating disc units were automatically pH controlled by introducing appropriate quantities of either a 1 mol  $\text{l}^{-1}$  sodium hydroxide (NaOH) or a 1 mol  $\text{l}^{-1}$  hydrochloric acid (HCl) solution. In the later stages of the study, pH control was effected by adding 200 mg  $\text{l}^{-1}$  sodium bicarbonate ( $\text{NaHCO}_3$ ) to the various feedstocks as a buffer, thus eliminating the need for automatic pH control. The pH was maintained at 7.2 in the nitrifying rotating disc units and at 7.0 in the denitrifying rotating disc unit.

The activated sludge units were not pH controlled because changes in the performance of these units to pH variations are relatively minor. Each activated sludge unit was left to attain its own pH level.

#### Dosage of chemical inhibitor

Various quantities were added from stock solutions to the feedstreams to the experimental units. Inhibitor dosage contributed less than 1 % of the total inflow to the units.

#### Method of operation

To initiate growth on the nitrifying rotating discs, they were supplied with secondary humus tank effluent. When the established growth on the discs removed approximately 45 mg  $\text{l}^{-1} \text{m}^{-2} \text{h}^{-1}$   $\text{NH}_3\text{-N}$ , the hydraulic load was increased until a steady 10 mg  $\text{l}^{-1}$   $\text{NH}_3\text{-N}$  was measured in the effluent. When the  $\text{NH}_3\text{-N}$  concentration remained constant for at least four hydraulic displacements (approximately 12 h), inhibitor addition was commenced. Addition continued until inhibition had ceased to increase for four hydraulic displacements.

Growth on the discs of the denitrifying rotating disc unit was initiated by inoculation of active denitrifying cells taken from the anoxic zone of an experimental activated sludge unit. Initially the feed rate to the rotating disc units was 0,6  $\text{l h}^{-1}$ , but, as growth on the discs increased, the hydraulic load was gradually increased until the  $\text{NO}_3\text{-N}$  removal was in the order of  $1,6 \times 10^5$  mg  $\text{l}^{-1} \text{m}^{-2} \text{h}^{-1}$ , at which point the experiment was begun. Inhibitor addition was continued until the  $\text{NO}_3\text{-N}$  concentration in the effluent no longer increased.

The activated sludge units were operated in a constant temperature room at 20 °C. To prime the units, active nitrifying-denitrifying sludge was obtained from a pilot-scale activated sludge plant at the Daspoort sewage works. The units were then operated for 2 sludge ages (30 d) to allow acclimatization. To ensure good denitrification, the mixed liquor from the aerobic basins was collected in settlers where the active nitrifying-denitrifying sludge was separated and recycled to the anoxic zone at twice the feed rate.

In order to obtain a sufficient rate of nitrification and denitrification without a high concentration of inactive volatile suspended solids, the sludge age used in the laboratory-scale units was limited to 15 d, but 10 and 20 d were used occasionally. With the chemical oxygen demand value set at either 500 or 400 mg  $\text{l}^{-1}$  and a volatile suspended solids concentration of 2 000 mg  $\text{l}^{-1}$ , the hydraulic residence time was calculated by means of

the following relationship (Marais, 1973):

$$X_v = \frac{Y(S_i - S) \cdot R_s \cdot (1 + 0.2bR_s)}{(1 + bR_s) R}$$

where  $X_v$  = concentration of volatile suspended solids  
 $Y$  = growth yield coefficient  
 $S_i$  = influent substrate concentration  
 $S$  = effluent substrate concentration  
 $b$  = endogenous respiration coefficient  
 $R$  = hydraulic residence time  
 $R_s$  = sludge age.

Routine microscopic examination of the nitrifying sludge in the aerobic basin was also carried out to detect changes in the morphological appearance of the microfauna present in the sludge (Knoetze and Davies, 1976).

#### *Dissolved oxygen (DO) control and measurement*

Oxygen for the nitrifying bacteria on the nitrifying rotating disc units was obtained through exposure to the atmosphere. As a result of the relatively low cellular yield of autotrophic bacteria, the maximum thickness of the microbiological film on the perspex discs seldom exceeded 2 mm and the space between the discs was thus largely unoccupied.

The dissolved oxygen concentration in the liquid phase never dropped below 1 mg l<sup>-1</sup>.

Denitrifying bacteria are facultative anaerobic organisms, and denitrification will only take place in the total absence of free oxygen. In order to reduce the amount of free oxygen entering the rotating disc unit, the feed to the denitrifying unit was stripped of dissolved oxygen, using high purity nitrogen gas. The pH value of the feed was never affected by this stripping because the oxidation of free nitrogen to nitrate ions, and hence the formation of nitric acid, never occurred. This method of oxygen stripping always resulted in a well-established bacterial population on the discs, with the desired denitrification rate of 1.6 x 10<sup>5</sup> mg l<sup>-1</sup> m<sup>-2</sup> h<sup>-1</sup> NO<sub>3</sub>-N removed.

The oxygen concentration in the aerobic zone and the condition of the anoxic zone of the activated sludge units were monitored with a Yellow Springs dissolved oxygen meter. Throughout the study, the dissolved oxygen concentration of the aerobic zone was maintained at between 1.5 and 2.5 mg l<sup>-1</sup> by means of aeration via sintered glass diffusers (Fig. 3).

#### *Analytical methods and calculations*

For the nitrifying rotating disc units, influent and effluent samples were taken once during every hydraulic displacement period and analysed immediately for ammonia nitrogen (National Institute for Water Research, 1974a).

The influent and effluent of the denitrifying rotating disc units were monitored for NO<sub>3</sub>-N concentration by means of an Orion 401 specific ion electrode and meter.

For the activated sludge units, growth was monitored regularly by analysing the mixed liquor wastage for the concentration of COD, NH<sub>3</sub>-N, NO<sub>3</sub>-N, nitrite nitrogen (NO<sub>2</sub>-N), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS). With the exception of MLSS and MLVSS, all were analysed by Technicon Autoanalyzer (National Institute for Water Research, 1974b).

To establish the degree of inhibition and acclimatization experienced by the activated sludge units during the period of

prolonged exposure to sublethal concentrations of chemical inhibitors, the following mathematical calculations were used for MLVSS, COD, nitrification and denitrification:

#### (1) Volatile suspended solids

$$\text{Inhibition} = 100 (1 - \text{VSS}_E / \text{VSS}_R) \%$$

Where:  $\text{VSS}_E$  = average volatile suspended solids concentration of the experimental unit during the period of experimentation

$\text{VSS}_R$  = average volatile suspended solids concentration of the reference unit during the period of experimentation.

#### (2) Chemical oxygen demand

$$\text{Inhibition} = 100 [1 - (400 - \Delta\text{COD}_E) / (400 - \Delta\text{COD}_R)] \%$$

Where:  $(400 - \Delta\text{COD}_E)$  = average COD removed by the experimental unit during the period of experimentation

$(400 - \Delta\text{COD}_R)$  = average COD removed by the reference unit during the period of experimentation.

#### (3) Nitrification

$$\text{Inhibition} = 100 [1 - (\text{NO}_3)_E / (\text{NO}_3)_R] \%$$

Where:  $(\text{NO}_3)_E$  = average amount of NH<sub>3</sub> nitrified by experimental unit during the period of experimentation

$(\text{NO}_3)_R$  = average amount of NH<sub>3</sub> nitrified by reference unit during the period of experimentation.

#### (4) Denitrification

$$\text{Inhibition} = (100 - \frac{cx}{y}) \%$$

Where:  $x$  = mg l<sup>-1</sup> NO<sub>3</sub> formed by reference unit during the period of experimentation

$y$  = mg l<sup>-1</sup> NO<sub>3</sub> removed by reference unit during the period of experimentation

$c$  = percentage NO<sub>3</sub> removed by experimental unit during the period of experimentation

## Results and Discussion

### Denitrification Growth Studies

On the basis of the results given in Table 1, it was concluded that the potential chemical inhibitor should be studied in the concentration range of 0.005 to 1 mg l<sup>-1</sup>. Considering the long lag phase (period of no appreciable multiplication of cells), as well as the reduction in cell yield induced by 10 mg l<sup>-1</sup> of chemical inhibitor, Cr<sup>6+</sup>, Hg<sup>2+</sup>, Ag<sup>2+</sup>, Ni<sup>2+</sup> and CN<sup>-</sup> were ex-

pected to be most inhibitory and special attention was therefore to be devoted to these substances. It was obvious that slug doses of the chemical inhibitors tested, to concentrations exceeding 10 mg l<sup>-1</sup>, even over periods shorter than 30 h, would seriously inhibit biological wastewater purification systems and further studies to quantify the effect were therefore considered justified.

### The Threshold Concentrations for Chemical Inhibitors Studied

The threshold concentrations below which no inhibition was observed on the biological laboratory scale units tested are summarized in Table 2.

The results show that the units differed considerably in their ability to withstand the effect of different inhibitors.

The denitrifying rotating disc unit was inhibited only when Cr<sup>6+</sup> concentrations were up to 9 mg l<sup>-1</sup>, compared with a corresponding value of 1 mg l<sup>-1</sup> for the nitrifying rotating disc and activated sludge units. This greater resistance to inhibition of the denitrifying unit was also noted for Ni<sup>2+</sup> salts. On the other hand, the nitrifying rotating disc and activated sludge units (threshold concentrations 5 mg l<sup>-1</sup> or higher) tolerated Zn<sup>2+</sup> and Cd<sup>2+</sup> salts much better than the denitrifying rotating disc unit (threshold concentration 1 mg l<sup>-1</sup>). Both rotating disc units were equally affected by mercury salts (threshold concentration 1 mg l<sup>-1</sup>), and comparatively much more severely than the acti-

TABLE 1  
THE EFFECT OF CHEMICAL INHIBITORS ON THE GROWTH RATE AND YIELD OF DENITRIFYING BACTERIA

Inhibitor	Ion	Max. conc.	Lag phase in-	Reduction in	Suppression of
		producing no significant effect	duced by 10 mg l <sup>-1</sup> inhibitor	cell yield due to to 10 mg l <sup>-1</sup> inhi- bitor	overall growth rate by 10 mg l <sup>-1</sup> inhibitor
		mg l <sup>-1</sup>	h	%	%
BaCl <sub>2</sub>	Ba <sup>2+</sup>	0,1	0	64,3	77,1
CdCl <sub>2</sub>	Cd <sup>2+</sup>	1,0	0	56,2	86,6
CrCl <sub>3</sub>	Cr <sup>3+</sup>	0,01	0	52,0	87,4
PbCl <sub>2</sub>	Pb <sup>2+</sup>	0,05	0	57,3	90,8
HgCl <sub>2</sub>	Hg <sup>2+</sup>	0,005	24	97,5	97,5
NiCl <sub>2</sub>	Ni <sup>2+</sup>	5,0	25	29,8	75,2
KCN	CN <sup>-</sup>	0,1	20	98,3	98,3
K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	Cr <sup>6+</sup>	0,05	120	98,0	97,0
Ag <sub>2</sub> SO <sub>4</sub>	Ag <sup>+</sup>	0,01	0	92,0	97,8
NaAsO <sub>2</sub>	AsO <sub>2</sub> <sup>-</sup>	1,0	0	48,0	79,6
ZnCl <sub>2</sub>	Zn <sup>2+</sup>	0,1	0	69,5	95,3
C <sub>6</sub> H <sub>5</sub> OH		0,1	0	65,3	85,4

TABLE 2  
SUMMARY OF THE THRESHOLD CONCENTRATIONS BELOW WHICH CHEMICAL INHIBITORS CAUSED NO INHIBITION TO BIOLOGICAL LABORATORY SCALE UNITS (mg l<sup>-1</sup>)

Chemical inhibitor	Denitrifying RDU	Nitrifying RDU	Activated sludge unit	Sludge age (d)
Chromium (Cr <sup>6+</sup> )	9	1	1	15
Cadmium (Cd <sup>2+</sup> )	1	5	10	15
Copper (Cu <sup>2+</sup> )	20	Not tested	20	15
Cyanide (CN <sup>-</sup> )	0,1	0,1	0,1	10 and 20
Chlordane (C <sub>10</sub> H <sub>6</sub> Cl <sub>8</sub> )	10	10	10	15
Carbaryl (C <sub>12</sub> H <sub>11</sub> N <sub>2</sub> O <sub>2</sub> )	10	10	10	15
Carbamate (C <sub>9</sub> H <sub>18</sub> FeN <sub>3</sub> S <sub>6</sub> )	Not tested	0,5	0,5	10 and 20
Dithane (C <sub>4</sub> H <sub>6</sub> N <sub>2</sub> Na <sub>2</sub> S <sub>4</sub> )	10	0,075 - 0,10	Not tested	
Dichlorophenol (C <sub>12</sub> H <sub>8</sub> Cl <sub>2</sub> O <sub>3</sub> S)	Not tested	5	0,5	10 and 20
Lead (Pb <sup>2+</sup> )	20	Not tested	20	15
Mercury (Hg <sup>2+</sup> )	1	1	5	15
Mercaptothion (C <sub>10</sub> H <sub>19</sub> O <sub>6</sub> PS <sub>2</sub> )	10	10	10	15
Nickel (Ni <sup>2+</sup> )	10	Not tested	1	15
Phenol (C <sub>6</sub> H <sub>5</sub> OH)	0,5	Not tested	Not tested	
Zinc (Zn <sup>2+</sup> )	1	10	10	10

vated sludge units (threshold value  $5 \text{ mg l}^{-1}$ ). Copper and lead salts had a similar effect on all three types and were tolerated at concentrations of up to about  $20 \text{ mg l}^{-1}$ .

The organic chemicals chlordane, carbaryl and mercaptothion became inhibitory only at concentrations higher than  $10 \text{ mg l}^{-1}$  for all units. The inhibitory effect of carbamate was similar in the nitrifying rotating disc and activated sludge units, but occurred at a much lower concentration ( $0,5 \text{ mg l}^{-1}$ ).

Of the organics studied, only dithane and dichlorophenol had significantly different effects on the units investigated. The denitrifying rotating disc unit had a high tolerance for dithane (threshold concentration  $10 \text{ mg l}^{-1}$ ), which was severely inhibitory to the nitrifying rotating disc unit (threshold concentration  $0,1 \text{ mg l}^{-1}$ ). Dichlorophenol had no effect on the nitrifying rotating disc unit at concentrations less than  $5 \text{ mg l}^{-1}$ , but inhibited the activated sludge units at a concentration of  $0,5 \text{ mg l}^{-1}$ .

These results emphasize the importance of using laboratory-scale units similar in concept and operation to the large-scale system being simulated, when studying the likely effects of a particular inhibitor.

#### Adaptation of Nitrifying Rotating Disc and Activated Sludge Units to Chemical Inhibitors

It is well known that biological life tends to adapt to imposed external stress. This adaptation characteristic is illustrated by the results presented below.

#### Nitrifying rotating disc units

The inhibitory effect of cyanide on nitrification in a rotating disc unit is shown in Figure 4. In these tests the rotating disc unit was not cleaned and sterilized between experiments, in order to allow for development of resistance by the microflora to repeated addition of cyanide.

During test 1 (Fig. 4), in which cyanide was added gradually until a final concentration of  $0,5 \text{ mg l}^{-1}$  had been reached, a maximum inhibition of 61 % was obtained after 28 h, after which inhibition levelled off to 32 %.

In the second test (five days after completion of test 1), still using the same unit, method of operation and concentration of cyanide, the maximum inhibitory effect was 26 %, and inhibition was negligible after 70 h. Test 3, which was performed 15 d after completion of test 2, resulted in a maximum inhibition of 57 %, which was 4 % lower than that of test 1. The most striking feature, however, was that after 60 h inhibition was negligible. Test 4 was done directly after test 3 and, although the inhibitor concentration had been doubled, the maximum inhibition attained was only 18 %, which disappeared completely after 45 h.

It is concluded that, if the bacterial population is left to multiply in the absence of cyanide, the whole population tends to lose the ability gained to resist inhibition. On the other hand, if the bacterial population is in constant contact with cyanide, adaptation is maintained.

It was found that a nitrifying culture established on a ro-

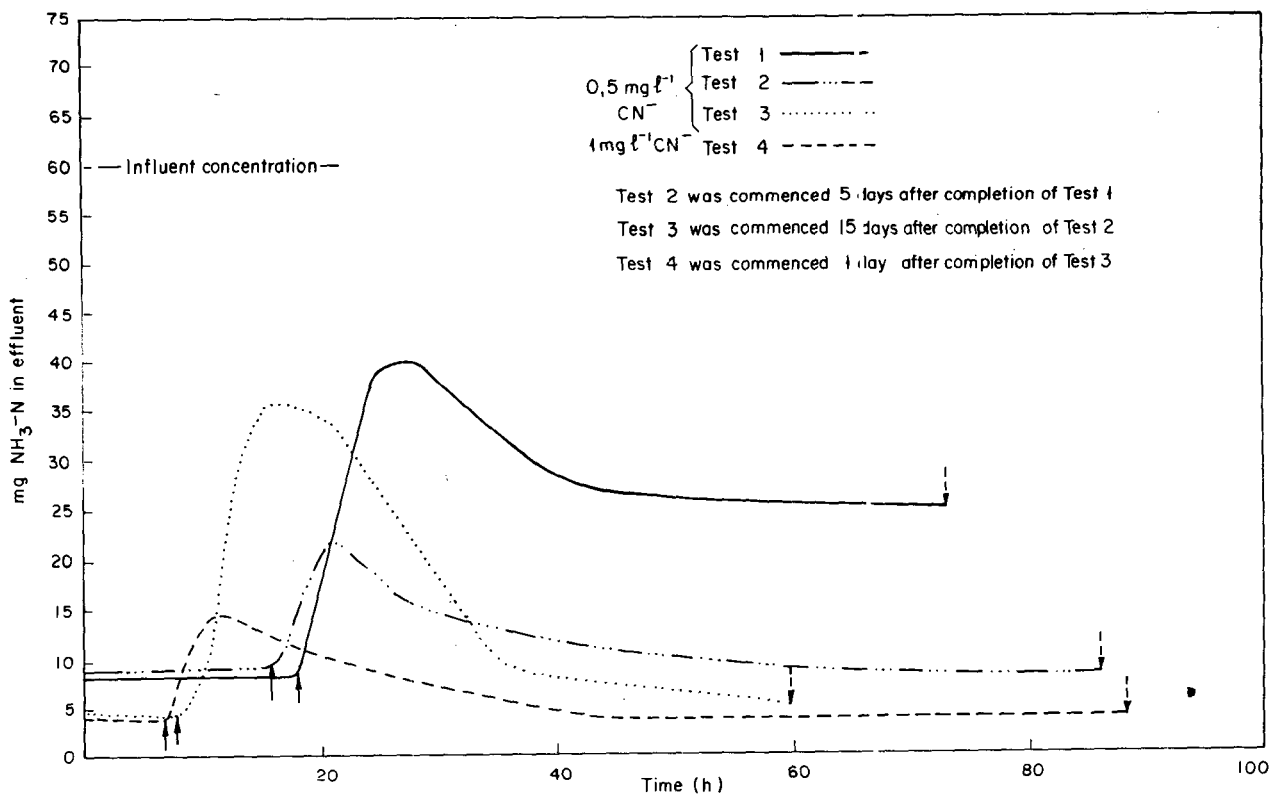


Figure 4  
The inhibitory effect of cyanide on a biological rotating disc unit. Solid arrows indicate commencement of inhibition and broken arrows, end of inhibition

tating disc unit will develop resistance to the unfavourable condition of  $\text{Hg}^{2+}$  addition. Figure 5 (test 1) gives results on the inhibitory effect of  $3 \text{ mg l}^{-1} \text{ Hg}^{2+}$  on a nitrifying culture previously exposed to cyanide. A maximum inhibition of 46 % was observed after 40 h, but this decreased to 20 % after 90 h. Test 2 was carried out with the same concentration of  $\text{Hg}^{2+}$ , but on a nitrifying rotating disc unit previously cleaned and sterilized with sodium hypochlorite. Even after 90 h had been allowed for adaptation, inhibition was still 56 %. In the first test, resistance, compared with that of the fresh culture, was considerably greater.

#### Activated sludge process

The nitrification stage of an activated sludge process was severely inhibited by the alternate introduction of pesticides, when relatively short recovery periods were allowed. The results of an experiment carried out on unit 1 with a sludge age of 10 d are illustrated in Figure 6. Dichlorophenol ( $0,5 \text{ mg l}^{-1}$ ) had an initial inhibitory effect of 7 % on nitrification and 5 % on denitrification. The COD removal was virtually unaffected, while no active biomass was lost in the effluent. After a recovery period of 5 d,  $0,5 \text{ mg l}^{-1}$  dichlorophenol was again introduced, resulting in nitrification inhibition of 12 %. When the inhibitor was changed to  $0,5 \text{ mg l}^{-1}$  carbamate, nitrification was further impaired to 49 % inhibition. The concentration of active biomass (MLSS) was reduced by 7% lost in the effluent, while 2,5 % less carbonaceous matter was removed (Table 3). This sequence of events

was typical of the severe repression suffered by the nitrification stage when alternate pesticides were introduced and recovery periods were relatively short.

The sludge age of an activated sludge unit is of crucial importance in determining the effect of inhibitors. Its role is illustrated by the results of an experiment performed on unit 2 (Fig. 7) with a sludge age of 20 d, where  $0,5 \text{ mg l}^{-1}$  dichlorophenol resulted in 5 % of the biomass being killed and lost in the effluent, without inhibition. When the inhibitor was changed to  $0,5 \text{ mg l}^{-1}$  carbamate, nitrification was suppressed by 7 % (Table 3). The percentage inhibition in this case was low because an adequate adaptation period had been allowed. In the experiment carried out with  $0,5 \text{ mg l}^{-1}$  dichlorophenol and  $0,5 \text{ mg l}^{-1}$  carbamate on an activated sludge unit with a sludge age of 10 d, the short sludge age prevented adaptation of the micro-biological population to the recurring doses of pesticides.

The response of activated sludge units may be markedly influenced by the particular mode of occurrence of inhibitors. This statement may be illustrated by the results of an experiment in which  $0,5 \text{ mg l}^{-1}$  carbamate was added in the feed-stream to unit 3 (Sludge age 20 d; Fig. 8). Initially nitrification was inhibited by 18 %, while 12 % of the active biomass were killed and lost in the effluent. After a recovery period of 5 d, the sludge had adapted itself to the carbamate and a recurring dosage of  $0,5 \text{ mg l}^{-1}$  resulted in only 13 % of the biomass being killed and lost in the effluent. A most important observation, however, was that when the pesticide was changed to  $0,5 \text{ mg l}^{-1}$  dichlorophenol, which proved non-toxic in unit 2 (Fig. 7), inhi-

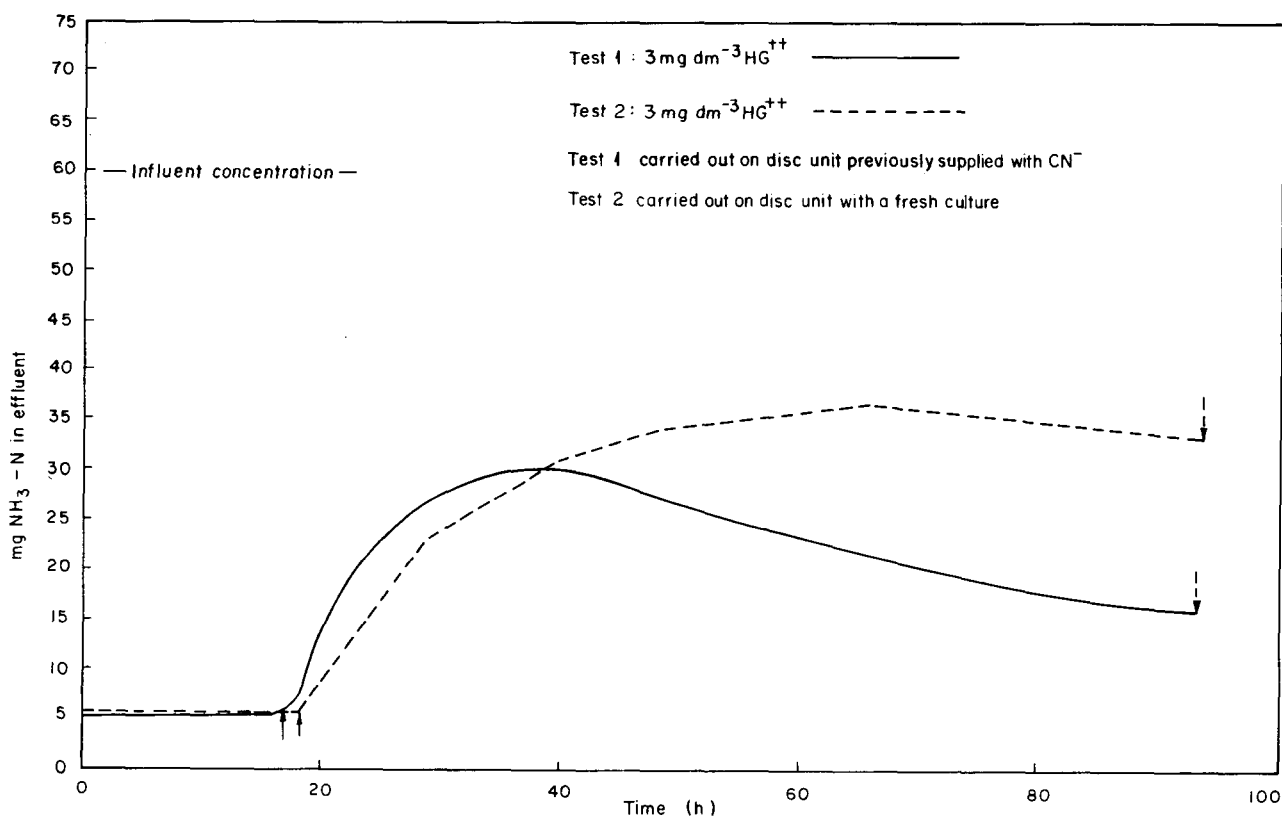


Figure 5  
The inhibitory effect of mercury on a biological rotating disc unit. Solid arrows indicate commencement of inhibition and broken arrows, end of inhibition

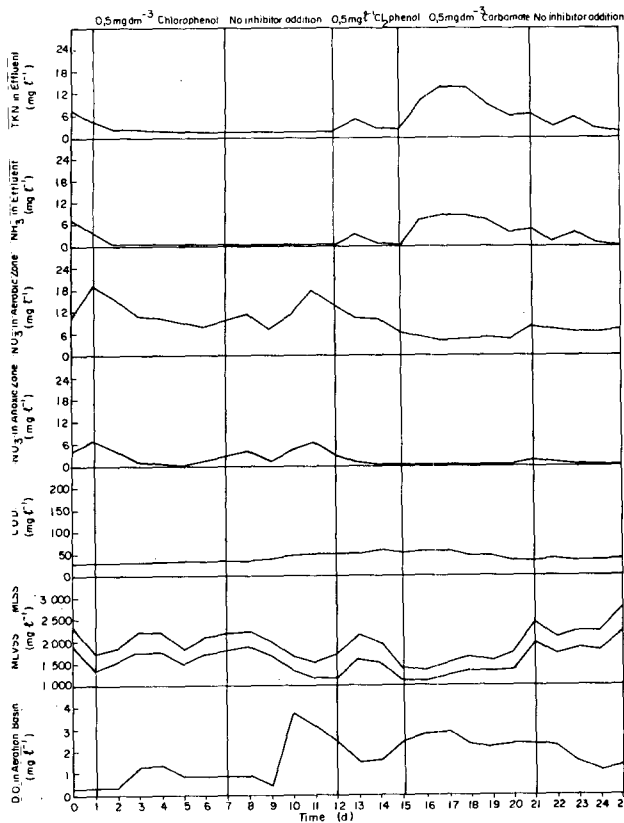


Figure 6

The inhibitory effect of recurring addition of  $0,5 \text{ mg l}^{-1}$  dichlorophenol and periodic addition of  $0,5 \text{ mg l}^{-1}$  carbamate on an activated sludge unit (sludge age 10 d)

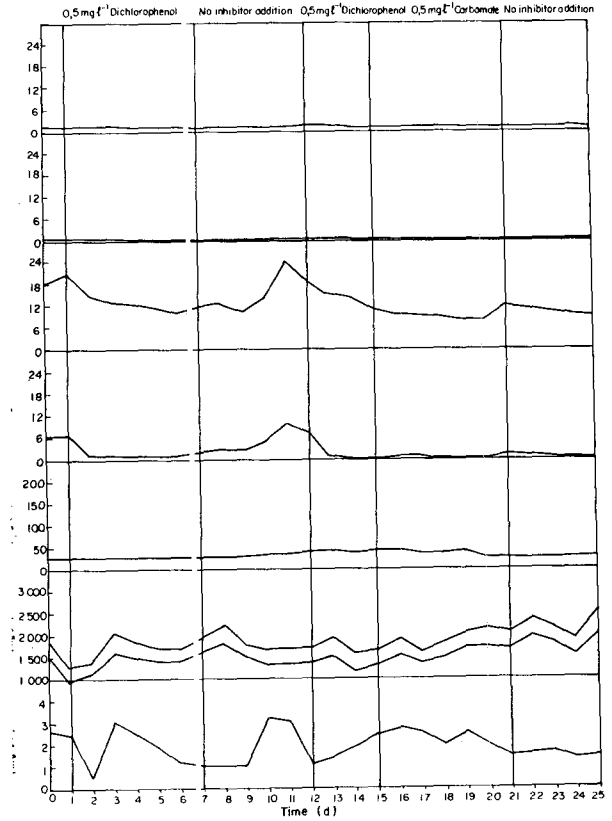


Figure 7

The inhibitory effect of recurring addition of  $0,5 \text{ mg l}^{-1}$  dichlorophenol and periodic addition of  $0,5 \text{ mg l}^{-1}$  carbamate on an activated sludge unit (sludge age 20 d)

TABLE 3  
SUMMARY OF PERCENTAGE INHIBITION EXPERIENCED BY ACTIVATED SLUDGE UNITS

Toxin	Concentration ( $\text{mg l}^{-1}$ )	Sludge age (d)	Percentage inhibition			
			Nitrification	Denitrification	COD Removal	MLVSS
Dichlorophenol (unit 1)	0,5	10	6,80	5,20	1,20	0
Recovery period: 5 d						
Dichlorophenol	0,5	10	12,20	0	1,80	0
Carbamate	0,5	10	49,30	0	2,50	6,6
Dichlorophenol (unit 2)	0,5	20	0	0	0	4,71
Recovery period: 5 d						
Dichlorophenol	0,5	20	0	3,60	0	0
Carbamate	0,5	20	7,30	0	0,60	0
Carbamate (unit 3)	0,5	20	17,90	0	0	11,60
Recovery period: 5 d						
Carbamate	0,5	20	0	0	0	13,20
Dichlorophenol	0,5	20	12,50	0	0,40	23,60



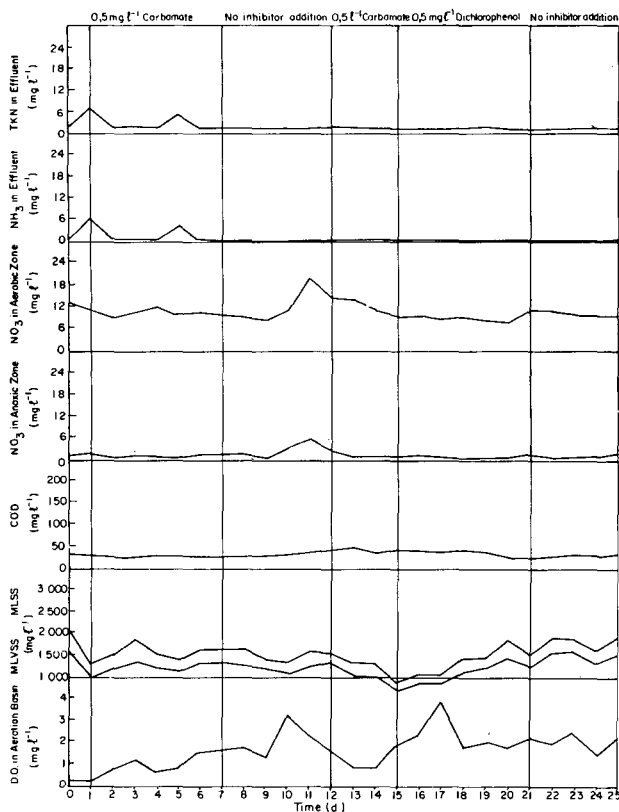


Figure 8

The inhibitory effect of recurring addition of  $0,5 \text{ mg l}^{-1}$  carbamate and periodic addition of  $0,5 \text{ mg l}^{-1}$  dichlorophenol on an activated sludge unit (sludge age 20 d)

bition of nitrification and the loss of active biomass in the effluent were exceptionally high for this relatively inefficient inhibitor. Nitrification was inhibited by 12 % and denitrification by 24 %. When this information is compared with the results for unit 2, it is confirmed that the resistance developed by the nitrifying flora to carbamate in a suspended growth system, did not improve resistance to dichlorophenol; in fact, sensitivity to dichlorophenol was increased.

No general rule can be given as to the possible enhancement or reduction in sensitivity to inhibition by compound inhibitors, compared with that displayed by single inhibitors. The particular conditions expected at the site where potential inhibition is considered to be an important factor, would have to be studied individually.

### Summary of Conclusions

The main findings of this study may be summarized as follows:

- (1) All the heavy metals investigated were tolerable in raw

sewage at concentrations below  $1 \text{ mg l}^{-1}$ . Depending on the unit process and the metal involved, concentrations of up to  $20 \text{ mg l}^{-1}$  had no inhibitory effect.

- (2) Biological wastewater purification systems will develop resistance to the chemical inhibitors studied, even if the threshold concentrations indicated in Table 2 are exceeded, provided that the concentrations of the inhibitors are not lethal and the contact periods long enough to induce resistance.
- (3) Activated sludge systems with short sludge ages are more receptive to inhibition than those with long sludge ages.
- (4) In activated sludge units, the oxidation of carbonaceous matter in the feed remained excellent even when nitrification and denitrification were severely inhibited and as much as 20 % of the mixed liquor suspended solids were killed and lost in the effluent.
- (5) Microscopic examination of sludge from an activated sludge unit showed that suppression of the grazing activity of the microfauna was noticeable immediately after the chemical inhibitors studied were introduced. This method was found to be a useful quantitative method in detecting chemical inhibition of biological organisms.
- (6) The insecticides investigated; i.e. chlordane, carbaryl and mercaptothion, proved to be tolerable in the biological wastewater purification reactors tested, even at concentration levels as high as  $10 \text{ mg l}^{-1}$ .
- (7) The fungicides Ferbam (carbamate) and dithane, as well as the acaricide dichlorophenol, proved highly inhibitory to activated sludge systems and rotating disc units at concentrations as low as  $0,5 \text{ mg l}^{-1}$ .
- (8) The response of an activated sludge unit may be markedly influenced by the particular mode of occurrence of pesticides in the influent to the particular unit; i.e. whether one or many occurred the same time.
- (9) Similar experiments on nitrifying and denitrifying rotating disc units, as well as nitrifying-denitrifying activated sludge units, had completely different results. Evaluation of the likely effects of a particular inhibitor on laboratory scale therefore necessitates a facility similar in concept and operation to the large-scale system being simulated.

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