# Induction of nitrite build-up in water by some common disinfectants

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## Abstract

Nitrite build-up in gold-mine service water could be caused by the biotic oxidation of ammonia in the presence of disinfectants such as chlorine, chlorine dioxide or bromine. It has been demonstrated in bench-scale tests on gold-mine service water that complete nitrification can be inhibited. It was shown in these tests that dosages of chlorine between 3 and 13 mg/ℓ, chlorine dioxide between 2 and 8 mg/ℓ, bromine above 8 mg/ℓ and cyanide above 2 mg/ℓ caused selective inhibition of the second stage of the microbiological nitrification process. The nitrifying bacteria appear to be able to adapt to bromine. Nickel (II) did not significantly affect the nitrification process.

## Introduction

It has been found necessary to disinfect the service water at many South African gold mines because this water is being drunk by many of the mine workers in spite of all the discouragement to do so (Pearson et al., 1990). Faecal contamination causes a risk of the water being contaminated with pathogenic organisms (such as typhoid and cholera). This problem can be amplified because of the need to recycle the service water several times before its discharge. During disinfection studies on the service water of the Kloof Gold Mining Company (Pearson et al., 1989; Van Leeuwen and Van der Westhuizen, 1992), it was found that nitrite exerted a significant demand on the oxidising disinfectants such as chlorine and ozone in the service water. Nitrite in the service water, after filtration, was found to average around 0,98 mg N/ℓ (standard deviation 0,21 mg N/ℓ) with a peak value of 11,9 mg N/ℓ. The recommended criterium for nitrite in potable water is 1 mg/ℓ (Kempster and Smith, 1985). However, the magnitude of the problem in terms of the high disinfectant use warranted a closer investigation into the origin of the nitrite.

This paper is aimed at demonstrating the induction of nitrite build-up in the service water by a microbiological kinetics study in a controlled environment.

## Biotic nitrite production

The production of nitrite as the first step in the microbiological nitrification of ammonia and its subsequent oxidation to nitrate has been extensively described in earlier literature (Knowles et al., 1965; Kholdebarin and Oertli, 1977; Painter, 1977; Strom and Finstein, 1977, Benefield and Randall, 1980).

The biotic oxidation of ammonia is accomplished by, among others, the prokaryotes *Nitrosomonas europaea, N. monocella* and *Nitrosococcus*. The species *Nitrobacter winogradsky, N. agilis* and *Nitrocystis, inter alia*, are thought to be involved in the subsequent oxidation of nitrite ($NO_2^-$) to nitrate ($NO_3^-$). Both these groups of organisms are gram-negative chemolithotrophs (Watson et al., 1989). The identification of these organisms is tedious and was not considered essential for the purpose of this investigation.

The build-up of nitrite is not very common and has been ascribed to the breakdown in the microbiological oxidation of nitrite, which is the second step in the biotic mineralisation of ammonia. More recent investigations (Suthersan and Ganczarczyk, 1986; Turk and Mavinic, 1989; Gee et al., 1990a and 1990b) centre around the purposed inhibition of microbiological nitrite oxidation in order to provide a "shortcut" in denitrification during nutrient removal processes. In all the above research it was clearly demonstrated that the nitrite oxidation step is by far the more sensitive step in the nitrification process and is subject to disruption by a number of inhibitive compounds.

## Possible inhibitors

The first step can be inhibited according to the Haldane substrate inhibition model (Haldane, 1965; Painter, 1977) by >2 500 mg/ℓ ammonia as well as by high concentrations (> 2 500 mg/ℓ) nitrite, chelating agents such as thiourea (0,7 mg/ℓ) and 2-chloro-6-trichloromethyl pyridine.

The oxidation of nitrite is inhibited by the simultaneous presence of ammonia (>9 000 mg/ℓ) and nitrite (>170 mg/ℓ) described by a modified Haldane model (Gee et al., 1990 b) as well as by cyanate, chlorate ($ClO_3^-$) and chlorite ($ClO_2^-$) among others. The inhibition level reported for chlorate varies between 0,001 mM (0,08 mg/ℓ; Lees, 1963) and 10 mM (835 mg/ℓ; Belser and Mayes, 1980). Hynes and Knowles (1983) found that chlorate was reduced to chlorite by *Nitrobacter* under both aerobic and anaerobic conditions and that chlorite was the more potent nitrite oxidation inhibitor. It is noteworthy that chlorite is a reduced form of chlorine dioxide, which is often used as a disinfectant for drinking water and has been used as such on the service water at the Kloof Gold Mine.

Optimum growth temperatures of 35°C and 35 to 42°C have been reported for *Nitrosomonas* and *Nitrobacter* species respectively at an optimum pH of 7,8 (Painter, 1977). It has been noticed though, that there is a marked temperature dependence of the observed growth rate ($\mu$) between 10 and 17°C. This differing temperature dependence of $\mu$ of the nitrite-producing and $\mu$ of the nitrite-utilising species can contribute to a nett build-up of nitrite (Randall and Buth, 1984).

Assuming these observations to hold true for mine service water, bench-scale investigations into the mechanics of nitrite

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build-up would centre around the inhibition of nitrifying bacteria. It has been shown that nickel(II), copper(II), arsenic(III), chromium(VI) and fluoride can act as inhibitors (Braam and Klapwijk, 1981; Randall and Buth, 1984; Beg and Hassan, 1987; and Sato et al., 1988). Of these chemical species, only nickel(II) appears in the mine service water to any significant extent - a peak of 2 mg/ℓ as compared to 3 mg/ℓ as observed by Randall and Buth (1984) in an activated sludge.

Since chlorine is being used as disinfectant at dosages less than 50% of breakpoint value, the effect of chlorine as well as other disinfectants currently used at the Kloof Gold Mining Company's No 1 shaft, was investigated. These include chlorine dioxide, ozone and bromine.

## Microbiological kinetics

While not in an endogenous growth phase, the rate at which the substrate, S, is being metabolised at time t, dS/dt, is directly proportional to the rate, dX/dt, at which the population, X, grows [1]. The value of the specific growth rate μ reaches a maximum value μ'.

$$\frac{dS}{dt} = \frac{-1}{Y}\frac{dX}{dt} = \frac{-\mu}{Y}X \qquad (1)$$

where Y is the observed growth yield which is a measure of the amount of cell material formed per unit amount of substrate removed. Equation (1) holds true for continuous mixed reactors. The instantaneous growth rate, μ, can be related to the maximum growth rate, μ', the substrate concentration and the saturation constant K by the Monod Eq. (2).

$$\mu = \mu' \frac{S}{K+S} \qquad (2)$$

Combining Eqs. (1) and (2) yields:

$$\frac{dS}{dt} = -\mu' \frac{S}{K+S}\frac{X}{Y} \qquad (3)$$

Correcting Eq. (3) for the death and decay of bacteria by the term k', which is also proportional to the bacterial population at that instant in time, produces a differential equation, describing the substrate concentration profile:

$$\frac{dS}{dt} = \frac{-\mu'}{Y}\left(\frac{S}{K+S} - k'\right)X \qquad (4)$$

The kinetic constants in this equation are often determined either by directly solving Eq. (4) (Lawrence and McCarty, 1970) or by using various linearising plots as well as simplifications of the kind K<<S or K>>S (Braha and Hafner, 1985; Braha and Hafner, 1987; Lewandowski, 1987). The combinations of 2 conditions in this case make the use of such techniques inadvisable:

- The substrate concentrations (see Table 1) in mine service water are comparable with the saturation constants reported in the literature, which means the simplifications of the kind K>>S or K<<S are invalid.
- The action of 2 bacterial species is coupled by the production/utilisation of nitrite, so the 2 steps in the nitrification process cannot be viewed independently.

Imposing the conditions above on a batch reactor with initial concentrations $A_o$, $N_o$ and $P_o$ and concentrations A, N and P at any future time t for ammonia, nitrite and nitrate respectively, generates a set of simultaneous differential equations by the application of Eqs. (4) and (2).

$$\frac{dA}{dt} = -\mu_m'\left(\frac{A}{K_a+A} - k_m'\right)\frac{M}{Y_m} \qquad (5)$$

$$\frac{dN}{dt} = \mu_m'\left(\frac{A}{K_a+A} - k_m'\right)\frac{M}{Y_m} - \mu_b'\left(\frac{N}{K_n+N} - k_b'\right)\frac{B}{Y_b} \qquad (6)$$

$$\frac{dP}{dt} = -\mu_b'\left(\frac{N}{K_n+N} - k_b'\right)\frac{B}{Y_b} \qquad (7)$$

$$\frac{dM}{dt} = -\frac{M}{Y_m}\frac{dA}{dt} \qquad (8)$$

$$\frac{dB}{dt} = -\frac{B}{Y_b}\frac{dN}{dt} \qquad (9)$$

where the bacterial cell concentrations at time t of *Nitrobacter* or *Nitrobacter*-like nitrite oxidising bacteria and of *Nitrosomonas* or *Nitrosomonas*-like nitrite producing bacteria are B and M respectively, with boundary values $B_o$ and $M_o$ at time $t=t_o$ respectively.

A correction for the mass of substrate used for cell maintenance must be applied. It can be assumed that this amounts to a constant fraction of the metabolised substrate and typically this fraction may be assumed to be 0,01 (Knowles et al., 1965). This means that the solutions A(t) and N(t) of Eqs. (5) and (6) respectively must each be multiplied by 0,99.

## Experimental procedures

### Analytical methods

Nitrite analyses were performed according to the method described in *Standard Methods* (1985) using a Bausch and Lomb Manual Spectrophotometer to measure the absorbance at 536 nm.

Nitrate analyses were performed according to the cadmium

**TABLE 1**
**TYPICAL COMPOSITION OF SERVICE WATER AT THE KLOOF GOLD MINE (PEARSON et al., 1990) OVER THE PERIOD SEPT. 1989 TO FEB. 1990**

| Determinand | Concentration (mg/ℓ) |
|---|---|
| Na | 155 |
| Ca | 107 |
| Mg | 26 |
| $SO_4$ | 370 |
| P | 0,13 |
| Alkalinity | 74 as $CaCO_3$ |

reduction method described in *Standard Methods* (No 418C).

Ammonia was analysed by the phenate method (No 417C) according to *Standard Methods* with absorbance measured at 630 nm.

### Nitrite production on full-scale

Nitrite levels were monitored on various sites within the water system at Kloof Gold Mine.

### Bench-scale biotic nitrite production

A batch reactor was constructed from a 5 ℓ bucket with a lid (Fig. 1). An inverted 250 mℓ polyethylene bottle, with a suitable hole cut into its bottom and filled about halfway with coarse, sterilised, ungraded river sand, retained by stainless steel mesh with 0,8 mm openings, was suspended in the bucket. Provision was made to circulate the reactor contents over the river sand by means of a peristaltic pump at a rate of c. 1 mℓ/min. The reactor content was maintained at a constant temperature (as demanded by the particular experiment) by means of a 100 W immersion-type fish tank heater or by means of a cryostatic unit circulating a cooling liquid through the reactor in 9 mm stainless steel tubing (in the case of sub-ambient temperature experiments).

Air was bubbled into the water above the sand by means of a commercial 15 mm $\phi$ sintered ceramic diffuser at a rate of a about 5 mℓ/min. The reactor was agitated by means of a 40 mm (5 mm $\phi$) Teflon coated follower driven by a magnetic stirrer at about 120 r/min.

The biological culture was established anew for each experiment by circulating 5 ℓ of mine service water through the reactor for 48 h, within 24 h of it being sampled at the point it enters the sand filters in the surface purification works at the Kloof Gold Mining Company's No 1 shaft. The service water was then drained and replaced in the reactor by a nutrient solution as described by Randall and Buth (1984) containing 10 mg/ℓ $NH_3$-N. The pH of this solution was 7,8 ± 0,2. A series of 6 runs was done at a time and all 6 reactors were run on "pure" nutrient until it was established that all reactor substrate curves were within 10% of each other. After refreshing the nutrient solution, one reactor was set aside as a control reactor, while the others were dosed at various concentration levels with suspected bacterial inhibitors.

Chlorine solution was prepared by adding HCl (conc.) dropwise to a 1:10 dilution of commercial NaOCl (3,5%) bleach, until the pH was between 6,5 and 7 and was then standardised using the DPD method in *Standard Methods* (1985).

Chlorine dioxide was prepared by adding a 5% excess of the stoichiometric amount of $NaClO_2$ to a chlorine solution as prepared above.

The other chemicals were obtained as the AR grade and dissolved in suitable volumes of distilled water.

The set of differential equations, (5) to (9), was solved numerically by using the Runge-Kutta-Fehlberg method (Burden and Faires, 1987) with a truncation error of the order of the third power of the step size. The kinetic parameters were chosen as that set of parameters that gave the best fit to the experimental data as measured by a least squares fit. The algorithm used was the derivative-free version of the Levenberg-Marquardt method described by Brown and Dennis (1972), which finds a local minimum of the objective function, defined as the sum of squared differences between the modelled and observed values at each corresponding datum point.

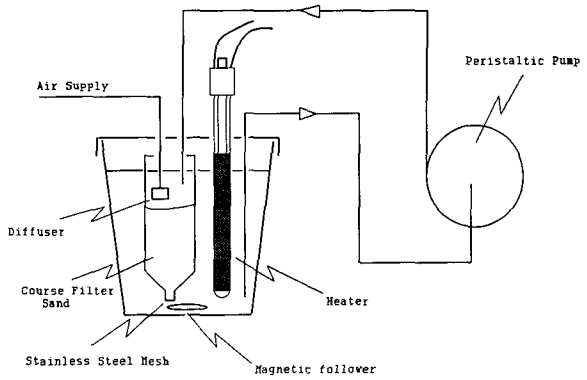


*Figure 1*
*Diagram of the reactor used to determine the kinetic constants of the nitrifying bacteria in the gold mine service water. Detail in text*

## Results and discussion

An increase in nitrite concentration was observed over the sand filters at Kloof Mine. An average ratio of nitrite concentration after the filter to nitrite concentration before the filter of 3,4 ($\sigma$=0,8 with 13 degrees of freedom) was found. The results obtained during this study agree with the observations by Pearson et al. (1989), who found an increase in the nitrite over the sand filters at the same mine by a factor of 3,8. It has been suggested by these authors that inhibition of microbiological nitrification is the reason for the nitrite build-up observed in the filters. A study of the effect of suspended material in the water (Kholdebarin and Oertli, 1977), indicates that nitrification efficiency is enchanced by an increase in surface area of the suspended material, suggesting that nitrification preferably takes place at the solid/liquid interface and consequently that the nitrifying bacteria prefer attached growth conditions. This may explain the prolific increase in nitrite over the sand filters as these offer a large area for bacterial growth.

The data expressed in Fig. 2 indicate that the kinetics of nitrite formation is very different in mine service water compared to that in the pure nutrient solution. This is also reflected in the calculated values of $\mu'$ shown in Table 2. This indicates that a possible cause of nitrite build-up is microbiological inhibition in the mine service water.

Since both groups of organisms are aerobes, the dissolved oxygen (DO) level of the service water in the mine is critical for adequate growth. It has been established (Jooste, 1992) that only at one point in the mine was a DO level of <1 mg/ℓ found but without a concomitant increase in nitrite concentration.

The influent service water temperature at a particular stope was found to be about 5°C, and the drainage water varied between 27 and 45°C.

This temperature variation raised the possibility that a temperature effect (Randall and Buth, 1984; Antoniou et al.,

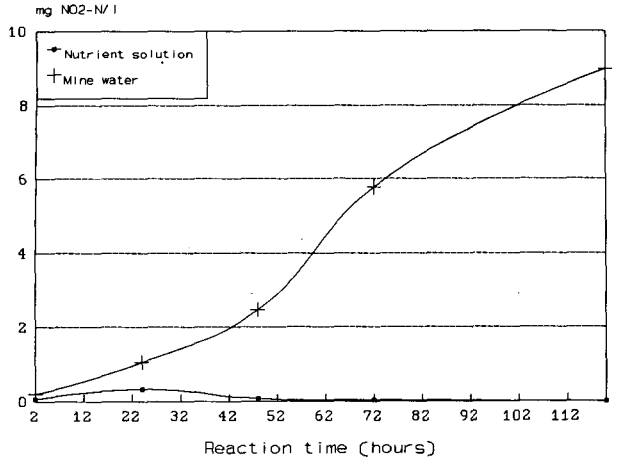


*Figure 2*
*The nitrite concentration in mine service water compared to that in a nutrient solution*

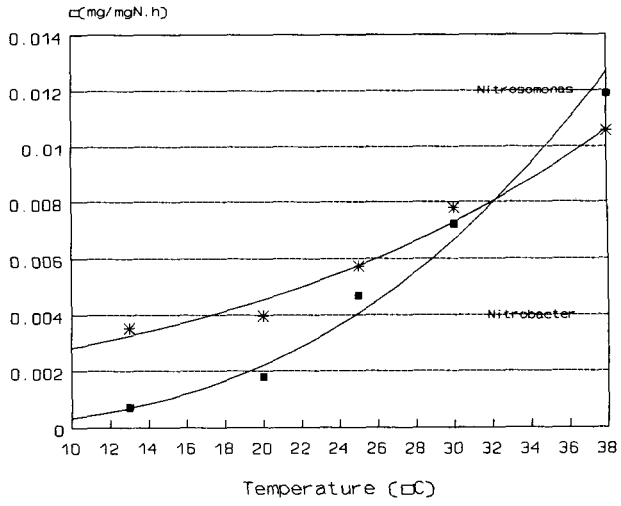


*Figure 3*
*The maximum specific growth rate as a function of temperature for the nitrifying bacteria in the mine service water*

**TABLE 2**
**SPECIFIC MAXIMUM GROWTH RATE (μ') VALUES FOR VARIOUS INHIBITING SUBSTANCES**

| Species | Dosage mg/ℓ | μ'($h^{-1}$) *Nitrosomonas* | μ'($h^{-1}$) *Nitrobacter* |
|---|---|---|---|
| $Cl_2$ (35°C) | 0 | 0,030 | 0,010 |
| | 3 | 0,029 | 0,006 |
| | 4,4 | 0,030 | 0,004 |
| | 5 | 0,029 | 0,002 |
| | 8,8 | 0,028 | 0,001 |
| | 13,2 | 0,0003 | 0,0001 |
| $Cl_2$ (20°C) | 0,5 | 0,030 | 0,010 |
| | 2 | 0,027 | 0,009 |
| | 5 | 0,024 | 0,007 |
| | 8 | 0,024 | 0,004 |
| | 10 | 0,024 | 0,002 |
| | 15 | 0,0003 | 0,0001 |
| $ClO_2$ | 1 | 0,029 | 0,010 |
| | 3 | 0,029 | 0,007 |
| | 9 | 0,008 | 0,001 |
| | 12 | 0,0001 | 0,0001 |
| $Br_2$ (35°C) | 1 | 0,030 | 0,010 |
| | 3 | 0,030 | 0,008 |
| | 10 | 0,025 | 0,003 |
| $CN^-$ (35°C) | 0,05 | 0,030 | 0,007 |
| | 1,5 | 0,026 | 0,002 |
| | 5,1 | 0,013 | 0,002 |
| $Ni^{2+}$ | 1 | 0,027 | 0,009 |
| | 5 | 0,028 | 0,010 |
| | 10 | 0,025 | 0,005 |

1990) could lead to a nitrite build-up. The tendency of the μ' values as a function of temperature reflected in Fig. 3 predicts nitrite build-up above 17°C (based on μ' values alone). The modelled growth yields (Y) at 30°C of *Nitrosomonas* and *Nitrobacter* are 0,08 and 0,01 mg cells/mg N respectively and these values did not vary significantly with temperature. A combination of these factors in a substrate utilisation equation (Eq (1)) predicts a nitrite build-up effect below 17°C, similar to that observed by Randall and Buth (1984). The temperature dependence of the saturation constants ($K_a$ and $K_n$) has not been determined explicitly.

The chemical species that appeared to act as inhibitors together with the calculated μ' values, appear in Table 2. The μ' values determined at 20°C have been corrected to 35°C to correct for the temperature effect.

From the calculated values of μ' it is clear that there is a range of chlorine and chlorine dioxide dosing where nitrite build-up is favoured. Above a chlorine dosage of about 13 mg/ℓ and a chlorine dioxide dosage of about 6 mg/ℓ there appears to be complete inhibition of nitrification. Which chemical species are involved or what the mechanism of inhibition is, has not yet been established. In the case of chlorine it is likely to be chloramines and in the case of bromine it is likely to be bromamines although this is yet to be substantiated.

In order to estimate the effect of various chemical species in the mine service water circuit, it would be advantageous to model parts of the circuit as plug flow reactors (PFRs). This approach would hold true as far as the piping, drainage channels and sand filters are concerned. The integral form of Eq. (4) for a PFR is Eq. (10) (Braha and Hafner, 1987), where Θ is the mean bacterial age (sludge age).

$$\Theta = -\int \frac{Y}{\mu'} \frac{(K+S)}{SX} dS \qquad (10)$$

$$X = (YS_o - X_o) - YS \qquad (11)$$

By substituting the relation Eq. (11) between substrate elimination and bacterial growth (Braun and Berthouex, 1970) in Eq. (10) and integrating between the limits $S_o$ and S, Eq. (12) is obtained:

$$\mu' = \frac{YK}{YS_o + X_o} \ln \frac{S}{S_o} + \left(1 + \frac{KY}{YS_o + X_o}\right) \ln \frac{Y}{X_o} (S_o - S) + 1 \qquad (12)$$

In the context of fixed growth, Θ could be interpreted as the residence time within the reactor. This equation is applicable for a single substrate, S. To extend the application to microbiological nitrification, it is assumed that the mass balance applicable to this situation is:

(Nett nitrite produced) = (Nitrite formed ) – (Nitrite oxidised)

Assuming further that (Nitrite formed) = (Ammonia removed), i.e. the exogenous growth phase for the bacteria and recognising that Eq. (12) is applicable to the removal of a substrate, in this case ammonia or nitrite, then the expression used to calculate the nitrite formation potential, ß, is given in:

$$ß = f(Y_m,A_o,M_o,K_a) - f(Y_b,N_o,B_o,K_n) \quad (13)$$

where $f(x_i)$ refers to the values obtained by solving for the substrate concentration on substituting suitable parameters into Eq. (12).

In order to make the application "semi-dynamic" the function ß is calculated in hourly intervals, i.e. increased by 1. Application of the measured kinetic values to Eqs. (12) and (13) yields the values depicted in Figs. 4 and 5. The values for the parameters have been assumed to be: Θ = 24 h, $Y_b$ = 0,02 mg dry cells/mg N·d$^{-1}$, $Y_m$ = 0,06 mg dry cells/mg N·d$^{-1}$, $M_o$ = $B_o$ = 0,1 mg dry cells/unit area, $K_a$ = 100 mg N/ℓ and $K_n$ = 1 mg N/ℓ.

This suggests that for chlorine dosages (not residuals) between 3 and 13 mg/ℓ and chlorine dioxide dosages between 2 and 8 mg/ℓ there is a possibility for nitrite build-up. The inhibitive effect of bromine appears to be milder and it was found that the inhibitive effect disappeared after about 36 h. In the case of both chlorine and bromine dosing, halo-amines would have been formed. Air stripping and further chemical reaction gradually caused halo-amine concentrations to drop and so reduce the inhibitive effect. However, on a further application of 5 mg/ℓ bromine in the reactor which had the 10 mg/ℓ dosage and with replenished nutrient, no recurring inhibition was observed. This would mean that the nitrite-oxidising bacteria in the mine service water were probably able to adapt to the mild bromination applied here.

At the Kloof Gold Mining Company's surface water treatment works, it was observed that the sand filters are an important area where nitrite is produced (Pearson et al., 1989). Nitrification at this point is understandable in view of the large surface area the sand provides for attached growth. Significant too, is the fact that chlorine dioxide is dosed before the water is filtered. McLaren (1992) confirmed that $ClO_2$ dosing has not solved the nitrite problem and that chlorine dosing at Kloof Mine has led to significant build-up of chloramines during recycling. Chloramines are known to be stable compounds and are likely to build up under recycle conditions (White, 1972).

Although other species such as cyanide and nickel (II) also produced an inhibitive effect, it is probably not of great significance as a microbiological inhibitor in mine service water since inhibitive concentrations of >10 mg/ℓ were generally not encountered. It is possible though that such an effect might be observed in the effluent channels of the metallurgical works. The phenomenon of nitrite build-up may in fact be possible in any water treatment situation where chlorine or chlorine dioxide is used as disinfectant or oxidant at low dosages.

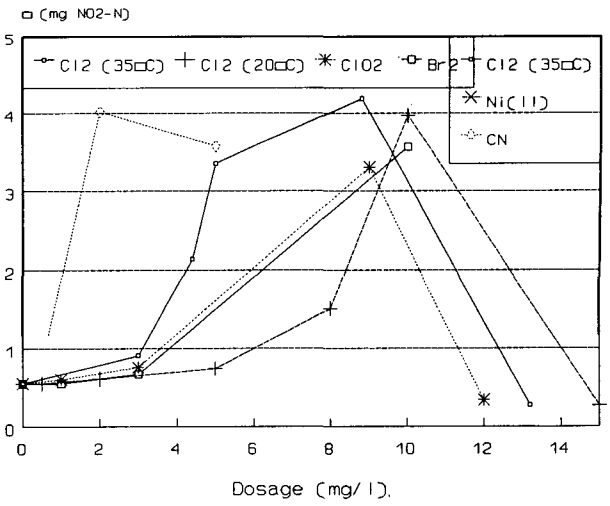


*Figure 4*
*The nitrite formation potential for some halogen based disinfectants*

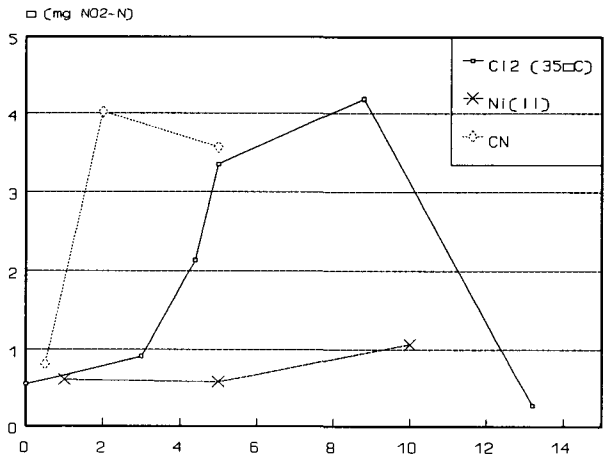


*Figure 5*
*Nitrite formation potential for some non-halogens found in the gold mining context in comparison to chlorine*

## Conclusions and recommendations

Appreciating the limitations of applying limited bench-scale tests to a complex and dynamic situation such as a mine service water circuit, the selective inhibition of microbiological nitrite oxidation by the application of chlorine and/or chlorine dioxide may well merit further investigation as a model for nitrite build-up in mine service water. Water temperatures below 13°C may add to this effect.

In situations where nitrite build-up due to selective inhibition of nitrification is encountered, the use of bromine should be investigated.

Where the use of bromine is not feasible, the use of an oxidant and non-lasting disinfectant, e.g. ozone, followed by the dosage of <4 mg/ℓ chlorine as a residiogenic disinfectant could be used.

The mechanisms of selective inhibition as well as any synergistic/antagonistic effects still need to be investigated before any attention can be given to applying the results obtained here to a full-scale application.

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