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STUDY OF FACTORS CONTROLLING NITRITE BUILD-UP IN BIOLOGICAL PROCESSES FOR WATER NITRIFICATION

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ABSTRACT

Nitrification processes are well known for certain problems in connection with transient build-up of nitrite ions. Moreover, some works have shown interest in controlling the build-up of this ion, particularly when treatment procedures for nitrogenous pollution are of the nitritation-denitritation type. With this in mind, we have carried out a programme of research to check the main factors responsible for the accumulation of this ion, i.e. $[NH_4]_O$, T°, pH, and dissolved O_2. The main results highlight the key role played by the free form N-NH_3 and by the temperature. This research thus provides answers to a number of practical questions and allows us to envisage setting up new procedures for the treatment of nitrogenous pollution by fixed cultures.

KEYWORDS

Biological treatment; nitritation; free ammonia; nitrite; nitrite build-up; nitritation-denitritation.

INTRODUCTION AND STATEMENT OF PROBLEM

In the biological nitrification of water, nitrite build-up is, in many cases, detrimental to the operation of the water treatment plant, and the concentration of this element in the effluent must be kept low (< 0.5 mg N-NO_2.l^{-1}) because of its great toxicity to some species of fish (Henze, 1991). A certain amount of research has therefore been performed on this subject, with the main aim of controlling the factors inhibiting oxidation of NO_2^- to NO_3^- by Nitrobacter and minimizing transient build-up of this ion. The best known works are those of Anthonisen et al. (1976), Suthersan et al. (1986), Jayamohan et al. (1988), and Hanaki et al. (1990), which principally consist of studies of the influence of a number of factors, such as the ammonium nitrogen concentration, the pH, the temperature, and the dissolved oxygen, on the transient build-up of the nitrite ion. Recent research by Suthersan et al. (1986) and Turk et al. (1989) has shown the interest of mastering the nitritation process with a view to developing new procedures for the elimination of nitrogenous pollution. The work concerns the direct coupling of nitritation and denitritation by performing a nitrate shunt, which has many advantages, notably a reduction of the carbon requirements for denitrification and lower energy consumption for oxygenation.

In the perspective of controlling the permanent build-up of the nitrite ion during biological water nitrification-denitrification processes, we have carried out research into how the production of this ion can be optimized. The experimental method consisted of first mastering the process for elaborating an inoculum from activated sludge and then controlling, on the one hand, the Nitrobacter inhibition mechanisms and, on the other, the Nitrosomonas activation mechanisms.

MATERIALS AND METHODS

State of the art : We give a brief reminder here (figure 1) of the work of Anthonisen et al. (1976) which evidenced the inhibition phenomena connected with the process of nitritation and nitratation by free ammonia (NH_3) and free nitrous acid (HNO_2).

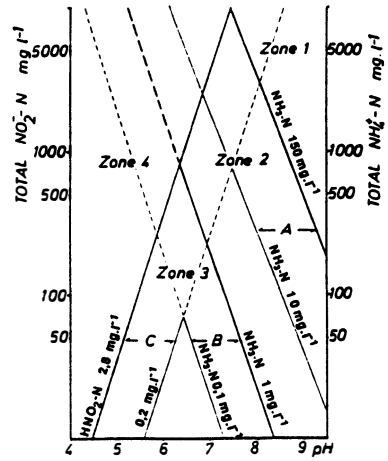


Fig.1 : Nitrification-tolerance graph according to Anthonisen et al. (1976)

This figure shows four zones of interaction :
- zone 1 : inhibition of Nitrobacter and Nitrosomonas by NH_3
- zone 2 : inhibition of Nitrobacter by NH_3
- zone 3 : complete nitrification
- zone 4 : inhibition of Nitrobacter by HNO_2

These zones are delimited by intervals noted A, B, and C, which depend on the various operating conditions, particularly the temperature and the acclimatization of the micro-organisms to the inhibiting substrates which, according to Suthersan et al. (1986) and Turk et al. (1989), play major roles. It is also possible to evaluate the inhibiting forms of reduced and oxidized nitrogen according to the ionic balances in aqueous solution, which Ford (1980) expresses by the following the relationships :

$$[\text{free } NH_3] = \frac{[N\text{-}NH_4^+][10^{pH}]}{(K_b/K_w) + 10^{pH}} \qquad (1)$$

with : [free NH_3] : concentration of the free form of ammonium nitrogen in solution, in mgN.l^{-1}.

[$N\text{-}NH_4^+$] : ammonium nitrogen concentration in the effluent to be treated, in mgN.l^{-1}.

K_b : ionization constant for NH_4^+

$K_b = 10^{-9.24}$ (20°C) ($NH_4^+ + OH^- \rightleftharpoons NH_3 + H_2O$)

K_w : ionization constant for water

$K_w = 0.69 \times 10^{-14}$ (20° C)

$K_b/K_w = \exp(6\,334 / 273 + T)$

T : temperature of solution in °C.

$$[\text{free } HNO_2] = \frac{[N\text{-}NO_2]}{K_a \cdot 10^{ph}} \qquad (2)$$

with : [free HNO_2] = concentration of the free form of nitric nitrogen in solution, in $mgN.l^{-1}$

[$N\text{-}NO_2$] = nitrite concentration in the effluent to be treated, in $mgN.l^{-1}$.

K_a = ionization constant for NO_2^- ($N O_2^- + H_3O^+ \rightleftharpoons HNO_2 + H_2O$)

$K_a = \exp(-2300/273 + T)$ $K_a = 10^{-3.4}$ (20°C)

Experimentation plan

The research being preferentially oriented towards municipal waste water treatment rather than waste water from the food industry, it can easily be shown from the relationships above that inhibition by free HNO_2 will not occur at the ammonium nitrogen concentrations to be treated, but that the effect of free NH_3, on the other hand, will be determining. Under these conditions, an experiment plan was drawn up to study the influence of the following parameters, independently of one another, on the build-up of nitrite ions : initial ammonium nitrogen concentration [range 15-1000 $mgN\text{-}NH_4.l^{-1}$], pH [6-9.5], temperature [10-35°C], and dissolved oxygen [0.5-8 $mg.l^{-1}$]. The analysis techniques used to follow all the parameters were the standardized A.F.N.O.R. or STANDARD METHOD ones.

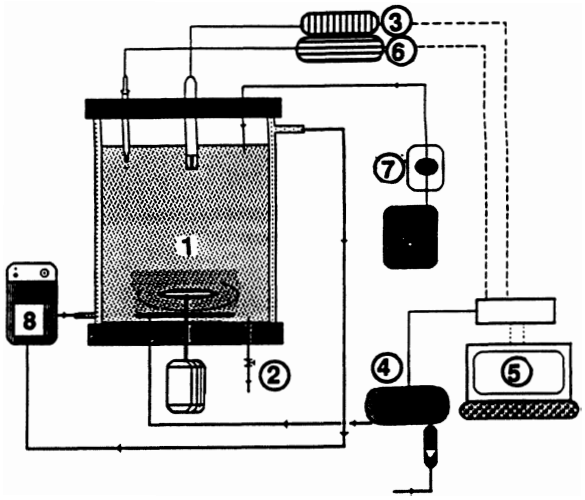
Methods

Our first step was to set up a method for selecting then adapting a mixed population of nitrifiers. This was done in several stages :

• implementation of a continuous activated sludge pilot unit, with recycling, using a very low organic loading (0.08 kg TOC.kg-1MSS.d-1) and a sludge age of 20 days, in order to implant the nitrifiers. This was obtained after about one month using a synthetic substrate, ammonium-sulfate-based, characterized by a TOC/$N\text{-}NH_4$ ratio of 0.125.

• implementation of a semi-continuous S.B.R. pilot unit using the previously obtained biomass, enriched with an essentially mineral synthetic substrate (Watson et al., 1980). A number of oxidation cycles were performed so as to enrich the medium in Nitrosomonas by gradually increasing the ammonium nitrogen concentration (from 100 to 400 $mgN\text{-}NH_4.l\text{-}1$) while keeping all the other parameters constant and favourable to nitritation as indicated in the literature [table 1]. After an analytical check that Nitrobacter was permanently being inhibited and that Nitrosomonas was accumulating, the medium was centrifuged. A microbiological analysis showed that 75 % of the autotrophic bacteria were nitrite producing. The mixed nitrifying population thus obtained was divided into several fractions, preserved in glycerol and deep frozen.

In our second step, these samples were used as the inocula for various experiments in batch reactors (fig.2), supplied with the synthetic substrate described by Watson et al. (1980). Each parameter was studied independently of the others, which were fixed so as to encourage the growth of nitrifiers (table 1).



1. perfectly mixed batch reactor
2 . sampling point
3 . oxygen probe and oximeter
4 . oxygen regulator
5 . Apple II computer controlling oxygen and pH regulation
6 . pH meter
7 . Bioblock pH regulator
8 . Lauda RM6 cryothermostat

Fig. 2. Diagram of batch reactor

TABLE 1 Optimum Parameters For Nitritation

Parameter	Optimum value	Reference
pH	8	Sharma, 1977
T°	25°C	Quinlan, 1986
[HCO3]	8.64 mgHCO_3-.mgN-NH_4^{-1}	Martin, 1979
dissolved O_2	2.5 mg $O_2.l^{-1}$	Knowles, 1965

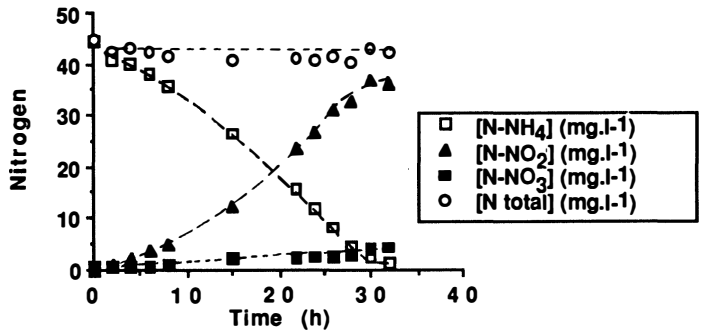
The results were used to evaluate the percentage of Nitrobacter inhibition by expressing the final nitrite build-up relative to the final total oxidized nitrogen through the relationship :

$$\% \text{ inhibition} = \frac{[N\text{-}NO_2^-]}{[N\text{-}NO_2^-] + [N\text{-}NO_3^-]} \times 100. \qquad (3)$$

where $[N\text{-}NO_2^-]$ and $[N\text{-}NO_3^-]$ are the final concentrations of nitrites and nitrates respectively in the solution.It is thus possible to express the capacity of the biological system to accumulate the nitrite ion by determining the maximum mean rate (r_{NH4}) at which the ammonium ion is oxidized. This is done by linear regression in the ([NH_4], t) plane.

EXPERIMENTAL RESULTS AND DISCUSSION

Fig. 3 shows typical variations in the concentrations of the different forms of nitrogen, N-NH_4, N-NO_2,N-NO_3, and total N, with time for an initial N-NH_4 concentration of 40 mg l^{-1}. At this concentration, with a pH of 8.1 and a temperature of 25°C, the free NH_3 concentration is 2.95 mgN.l^{-1}. Under these conditions, we can observe strong inhibition of Nitrobacter, which is expressed by an accumulation of nitrites. Furthermore, if a linear relationship is used as an approximation to the kinetics, we can determine the maximum mean rate (r_{NH4}) at which the NH_4^+ ion is consumed ; this is about 1.2 mgN.l^{-1} h^{-1}.



[Operating conditions : $[N\text{-}NH_4]_O$ = 40 mg.l^{-1} , pH = 8.1, T = 25°C, Free[NH_3]= 2.95 mgN l^{-1} Dissolved O_2 = 2.5 mgN l^{-1}, HCO_3^-/N-NH_4 = 8.64, Initial biomass concentration Xo = 90 ± 10 mgMSS.l^{-1}]

Fig. 3 Variations in the different forms of nitrogen during a nitritation experiment

• Influence of initial ammonium nitrogen $[N\text{-}NH_4]_o$ concentration on nitritation process (fig.4 and 5)

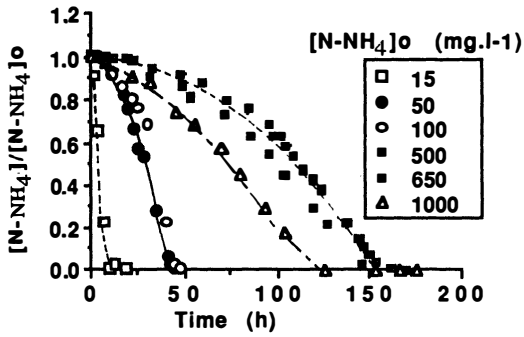


Fig 4 NH_4 consumption kinetics as a function of $[N\text{-}NH_4]_o$

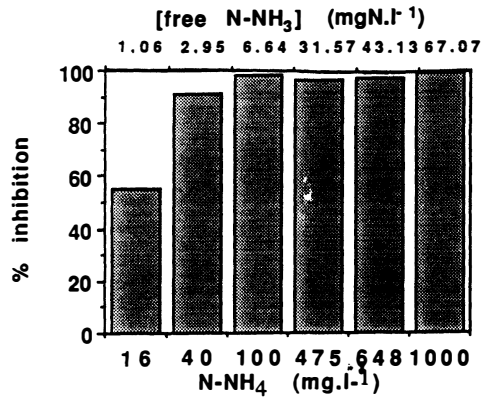


Fig 5 Variations in % inhibition of Nitrobacter with $[N\text{-}NH_4]_o$ and corresponding free $[NH_3]$

[Operating conditions : T = 25°C ; pH = 8.1 ± 0.1 ; dissolved O_2 = 2.5 $mg.l^{-1}$; $[HCO_3^-]_o$ / $[N\text{-}NH_4]_o$ = 8.64 ; initial biomass concentration Xo = 90 ± 10 mgMSS.l^{-1}]

These results confirm those in the literature but with a less noticeable inhibiting effect of free NH_3 than that given in Anthonisen's graph. This can be explained by the different acclimatization conditions. Nevertheless, the inhibition percentages are close to 100 % as soon as the free $N\text{-}NH_3$ concentration approaches 3 $mg.l^{-1}$

• Influence of pH on nitritation process (fig. 6 and 7)

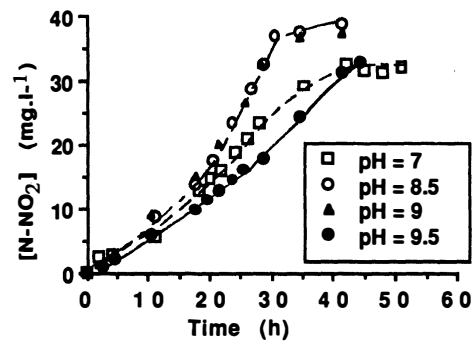


Fig. 6 NO_2 build-up kinetics for various pH values

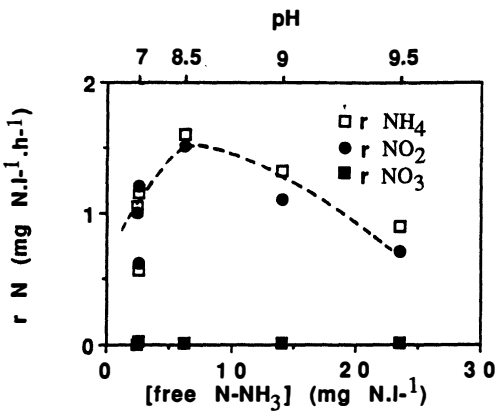


Fig. 7 variation of nitritation rate with pH and corresponding free $[NH_3]$

[Operating conditions : T = 25°C ; dissolved O_2 = 2.5 mg l^{-1} ; $[HCO_3^-]_o$ / $[N\text{-}NH_4]_o$ = 8.64 ; Xo = 80 mgMSS.l^{-1}] ; $[N\text{-}NH_4]_o$ = 40 mg l^{-1}]

These experiments show that, under our operating conditions, nitrite concentration is practically independent of the pH and that it is fixed by the inhibitory effect of the initial free NH_3 concentration, which is situated in

the range 2.5 - 25 mgN-NH_3 l^{-1}. These concentrations lead to 100 % inhibition of Nitrobacter . However, a pH-dependent variation is observed in the rate at which the nitrite ion is produced (r_N) (fig. 7). Our experimental results show that the optimum pH value is around 8.5, which is similar to those reported by Wild (1971) and Jones et al. (1982).

• Influence of temperature on the nitritation process (fig. 8 and 9)

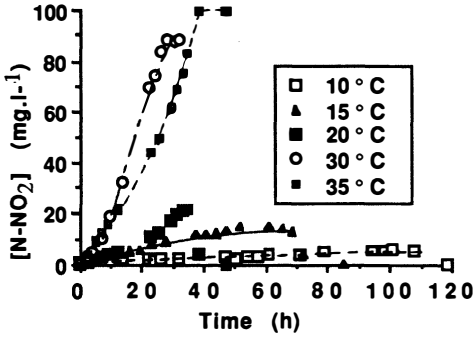


fig. 8 : kinetics of NO_2^- build-up for various T°

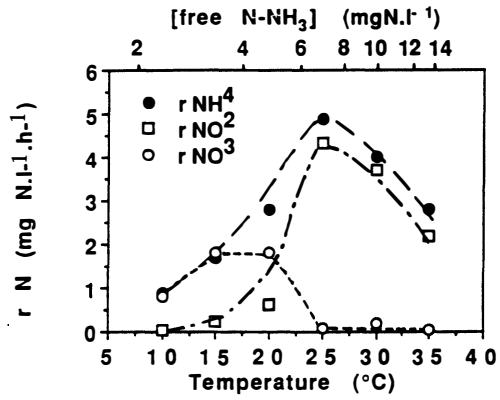


fig. 9 : variations in nitritation and nitratation rates with temperature

[Operating conditions : dissolved O_2 = 2.5 mg l^{-1} ; [HCO_3^-]$_o$ / [N-NH_4]$_o$ = 8.64 ; pH = 8.1 ± 0.1 ; Xo = 80 to 100 mgMSS.l^{-1} ; [N-NH_4]$_o$ = 100 mgNl^{-1}]]

These results show that, in spite of a concentration of free NH_3, normally inhibiting for Nitrobacter, of between 2 and 5 mgN.l^{-1}, obtained by having a pH of 8.1 and an initial NH_4 concentration of 100 mgN l^{-1}, the micro-organism is active over a range of temperatures between about 10 and 20°C. Under these conditions, nitrite build-up remains low, which can be explained by the fact that the effect of Nitrobacter activation by temperature prevails over its inhibition by free NH_3. On the other hand, beyond a temperature of 20-25°C, a slowing of the nitrating activity is observed together with an activation of the nitriting activity, which passes through a maximum at 25°C. These results are in agreement with Quinlan's (1986) data which show high Nitrobacter activity for temperatures below 15°C but are in contradiction with Randall's (1984) work, in which it should also be noted that the temperature effect was not counterbalanced by the inhibiting effects connected with the free form of ammonium nitrogen. With respect to Nitrosomonas, fig. 9 shows that, under these conditions, the inhibiting effect of free NH_3 is preponderant for temperatures higher than 25°C. This result confirms that of Anthonisen, but fairly disparate observations are to be found in the literature, notably those of Ford (1980) which give an optimum for temperatures between 30 and 36°C.

• Influence of free NH_3 on the nitritation process

The above results as a whole show that there is an overall correlation between nitrite build-up and the free form of ammonium nitrogen, as was already indicated by relationship (1). The effects connected with pH, temperature, and the initial ammonium ion concentration, together with additional results obtained from studies on the influence of Xo and dissolved O_2, can be grouped together in the (% inhibition of Nitrobacter, free [N-NH_3]) plane (fig. 10). This figure shows the great sensitivity of Nitrobacter to free NH_3 for concentrations as low as 1 mgN.l^{-1}, with a percentage of inhibition reaching 90 % for about 2 mgN.l^{-1}.

These observations confirm the results obtained under different batch reactor operating conditions, notably by Anthonisen (1976) and Suthersan et al. (1986). However, slightly different results are obtained in reactors with continuous and semi-continuous feeds, in which the acclimatization of the micro-organisms enables higher concentrations of free NH_3, of around 5 mgN-NH_3 l^{-1}, to be tolerated (Turk, 1989). Suthersan (1985) also introduces the concept of "recovery time" to explain the fact that Nitrobacter can

regain its nitrating activity after a return to non-inhibiting conditions. Moreover, it is preferable to express the effect of free NH_3 by normalizing it with respect to the biomass concentration.

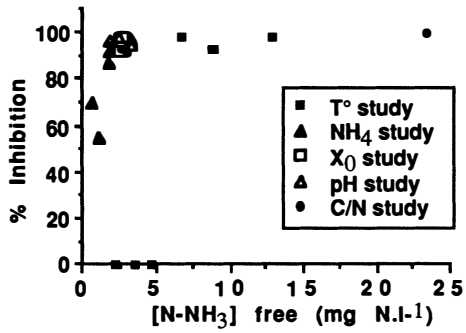


Fig. 10 % inhibition versus free N-NH_3 for the various conditions studied

• Influence of dissolved O_2 concentration on the nitritation process

To complete this study, a series of experiments were carried out at various dissolved oxygen concentrations in the range 0.5 to 8 mg $O_2.l^{-1}$. The operating conditions and curves reported in figure 11 show that Nitrobacter is completely inhibited but that the rates of nitritation or ammonium nitrogen consumption vary depending on the dissolved oxygen concentration.

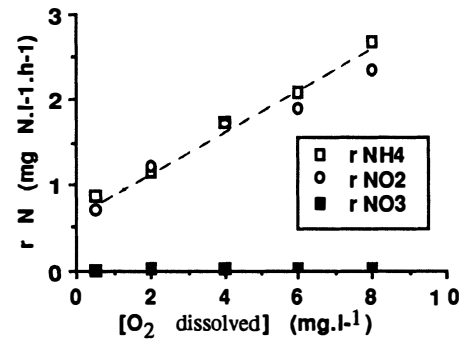


Fig. 11 variation of nitritation rate with dissolved oxygen concentration

[Operating conditions : pH = 8.1 ± 0.1 ; T = 25°C ; $[N\text{-}NH_4]_0$ = 40 mgN l^{-1} ; free $[N\text{-}NH_3]_0$ = 2.6 mgN.l^{-1} ; Xo = 80 mgMSS.l^{-1} ; $[HCO_3^-]_0$ / $[N\text{-}NH_4]_0$ = 8.64]

Thus, we see that optimizing the production of the nitrite ion with the intention of making compact water treatment apparatus of the nitritation-denitritation type is a possibility for the ammonium nitrogen concentrations commonly encountered in urban wastewater, provided that certain conditions are satisfied. In particular, it is necessary to maintain the inhibiting effect of free NH_3 either by introducing an "inhibition chamber" (Suthersan 1986), or by keeping the acclimatization effect to a minimum, as could be done for example in fixed culture reactors in which the hydraulic regime selects biological species in a stable manner.

CONCLUSION

It should be possible to control the build-up of nitrite ions in biological water treatment by controlling certain key parameters, the main one being the concentration of the free form of ammonium nitrogen. We therefore undertook a programme of research having two objectives :
- to minimize the build-up of this ion in conventional nitrification processes,
- to optimize build-up of this oxidized form of nitrogen with a view to making compact nitritation-denitritation systems. The preliminary work by Suthersan et al. (1986) and Turk et al. (1989) showed the main advantages of this approach :
  - reduction of plant volume,
  - reduction of running costs connected with aeration,
  - reduction of carbon requirements for the denitritation, stage, etc.

The main results of our research demonstrate the inhibiting effect of the free form of ammonium nitrogen on Nitrobacter, which may be the result of a combination of several factors, viz.the initial ammonium nitrogen concentration, the pH and the temperature. Although the inhibition effect can generally be observed at concentrations as low as 1 mg $N\text{-}NH_3$ l^{-1}., it should be noted that the effect is attenuated when the temperature is within the optimum range for Nitrobacter growth, i.e. 10-20°C. Thus, to control nitrite ion build-up in conventional procedures, care must be taken to keep the resulting $N\text{-}NH_3$ concentration below this threshold for given characteristics of the effluent, i.e. $[NH_4]_O$, pH and temperature. Furthermore, we have shown, in batch tests, that changes in the O_2 tension of the liquid do not lead to changes in the nitrite ion build-up process when the inhibition effect is present. When this is not the case, it is possible, in order to optimize build-up of this ion, to use permanent Nitrobacter inhibition by favouring the deactivation effect through the ammonium nitrogen concentration and minimizing acclimatization effects, notably by using fixed culture procedures.

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