(7) Modelling NOB activity under different dissolved oxygen, ammonia and nitrite concentrations

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Abstract
Batch tests were conducted on sludge samples drawn from a nitrifying/denitrifying SBR fed on a synthetic wastewater to search for causes of the observed nitrification unbalancing, focusing on the effects of dissolved oxygen and ammoniacal and nitrous nitrogen concentrations on nitrite oxidising bacteria. Ammonium and nitrite oxidation rates were assessed by pH/DO-stat titration, i.e. by measuring the rate of addition of an alkaline solution and of an oxygen-reach solution required to maintain constant, at a selected set-point level, both pH and dissolved oxygen (DO) concentration of the sludge sample. According to experimental results, nitrite oxidising bacteria (NOB) were penalised under DO limitation (DO half saturation constant for ammonia and nitrite oxidation were 0.1 and 0.4 mg l^{-1}, respectively). Several inhibition functions were found to describe the effect of increasing nitrite and ammonia concentrations on NOB allowing to assess the EC50 values for both chemical species.
Keywords
dissolved oxygen, free ammonia, free nitrous acid, inhibition functions, nitrite oxidising bacteria

INTRODUCTION

Nitrite is known to be a common intermediate in the biological nitrogen oxidation/reduction cycle. While nitrite accumulation within conventional nutrient removing wastewater treatment plants has to be avoided, favouring nitrite production *versus* oxidation plays a relevant role in novel processes for nitrogen removal, like the short-cut nitritation/denitritation and the fully autotrophic N removal process (partial nitritation/anammox). The nitrite level is mostly defined by the activity of nitrite oxidising bacteria (NOB). Several environmental parameters have been appointed for playing a role in affecting the NOB activity. The pH of the mixed liquor is also known to affect bioprocesses by influencing ionisation of weak acid/bases and thus their possible inhibiting effect on bacterial activity. Free ammonia (FA) is known to be more toxic to NOB than the ionised ammonium form, while inhibition of NOB by nitrite is a typical example of substrate inhibition as firstly described by Anthonisen et al. (1976). These authors identified the un-ionized nitrous acid (FNA) as the inhibiting form, which prevails at low pH values and reported a threshold inhibiting level as low as 0.2 mg_N l^{-1}, although higher concentrations were later suggested (e.g. Carrera et al., 2004). However, even ionised nitrites were found to have an inhibiting effect on nitrite oxidisers (Buday et al., 1999; Jeniček et al., 2004, Mosquera-Corral et al., 2005). As for dissolved oxygen (DO) concentration, several authors claim that AOB have a higher affinity for oxygen (Hanaki et al., 1990 among others).

Aim of this work was to study the effects of some relevant conditions (dissolved oxygen, ammonia and nitrite concentrations) on activity of NOB in sludge samples cultivated in a SBR fed on a synthetic wastewater. Batch tests were performed in order to get sufficient experimental data to test mathematical models describing the effect of those factors on NOB activity.

MATERIAL AND METHODS

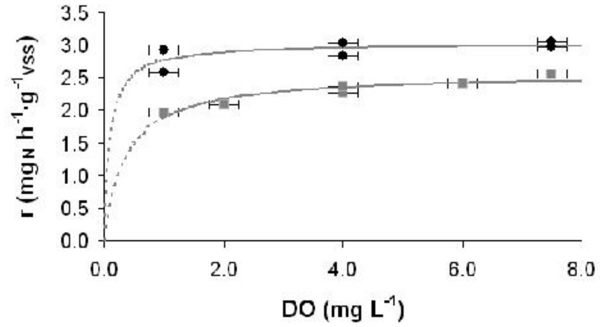
A 20-litre volume SBR was operated according to 4 cycles per day, fed on a synthetic influent (OECD, 2001) having a COD and N content of 880 mgCOD l^{-1}, and 100 mgN l^{-1}. The organic and nitrogen loading rates were 0.53 kgCOD m^{-3} d^{-1}, and 0.06 kgN m^{-3} d^{-1}, respectively. The aerobic sludge age was 20 to 24 d. Temperature and pH were respectively 20-22°C and 7.2-8.3 pH units.

Nitritation rates were determined by applying the pH/DO-stat titration technique (Ficara et al. 2000, Artiga et al., 2005), which allowed easy calculation of the reaction rates, which are directly related to titration rates, under careful control of DO and pH. Specifically, NaOH addition rate is used to assess the activity of ammonium oxidising bacteria (AOB), while nitrite oxidation rate is assessed from O_2 addition rate. Tests were performed at constant temperature (22±0.5°C). The titration instrument (MARTINA) has been provided by SPES s.c.p.a. (Fabriano, AN, Italy). Set-point titration tests were performed to determine AOB and NOB activity under various environmental conditions. An initial set of environmental parameters was fixed and a test was performed by adding the appropriate substrate for the bacteria group whose activity had to be determined (ammonium or nitrite, 5 mgN l^{-1}). When the added nitrogen was fully oxidized, all parameters were kept constant but the one whose influence was under study, which was increased or decreased. The appropriate substrate was spiked again to assess the nitrification activity under the new condition. From a minimum of two up to five different conditions were tested on one sludge sample. The maximum nitrification activity was measured after each substrate spike and, then plotted against the level of the varied parameter.

RESULTS

First, the effect of DO concentration between 1 and 7.5 mg/L on nitrification activity was considered. pH was set at the optimal value for the bacteria group under study, i.e.: 8.3 for AOB and 7.5 for NOB. Results are plotted in Figure 1 where horizontal error bars indicate that DO concentration oscillated around the set-point value during the activity test. The best-fitting Monod curves are plotted, and estimated parameters are: maximum AOB activity = 3.0 $mg_N\ g^{-1}_{VSS}\ h^{-1}$; half saturation constant for AOB: $K_{O,AOB}$ = 0.1 $mg_{O2}\ L^{-1}$; maximum NOB activity = 2.6 $mg_N\ g^{-1}_{VSS}\ h^{-1}$; half saturation constant for NOB: $K_{O,NOB}$ = 0.4 $mg_{O2}\ L^{-1}$. These results confirm the higher affinity of AOB for oxygen.

In the second experimentation, the effect of the free ammonia concentration on NOB activity was assessed by measuring the decrease in NOB activity under increasing free-ammonia (FA) concentration and constant nitrite concentration (5 mgN L^{-1}). Allylthiourea was added to inhibit ammonia oxidisers. The FA level was modified either by increasing the total ammoniacal nitrogen (TAN) up to 1000 mgN/L, or by increasing the pH value (from 7.5 to 8.3). The FA molar concentration was assessed from that of TAN By taking into account the dissociation equilibrium of ammonium. By plotting the percentage inhibition of NOB activity against both TAN and FA concentrations similar inhibition responses were detected in the presence similar FA concentrations (S_{FA}), confirming that FA more than TAN is responsible for the observed inhibition. For data interpretation, inhibition (I) versus data were fitted with several inhibition functions (Kroiss et al., 1992). General dose-response functions were considered: linear: $I = a \cdot S_{FA} + b$; exponential: $I = 1 - e^{-a \cdot S_{FA}}$; S-curve: $I = 1 - 1 \Big/ \left[1 + \left(\frac{S_{FA}}{a} \right)^b \right]$ where: *a* and *b* are the fitting parameters. Moreover, the non-competitive and competitive inhibition model were also tested: $I = \frac{1}{1 + K_{I,FA}/S_{FA}}$; $I = S_{FA} \Big/ \left[K' + K_{I,FA} \cdot \left(1 + \frac{S_{FA}}{K_{I,FA}} \right) \right]$ where: $K_{I,FA}$ and K' are fitting parameters. Fitting results are plotted in Figure 2. According to the adjusted coefficient of determination (R^2_{adj}), all tested models appear to satisfactorily fit experimental data (R^2_{adj} included between 0.8714 and 0.9716), the exponential curve appears to be the best one. On this curve, the estimated EC_{50} (50% effect concentration) is 45.3 $mg_N\ L^{-1}$.



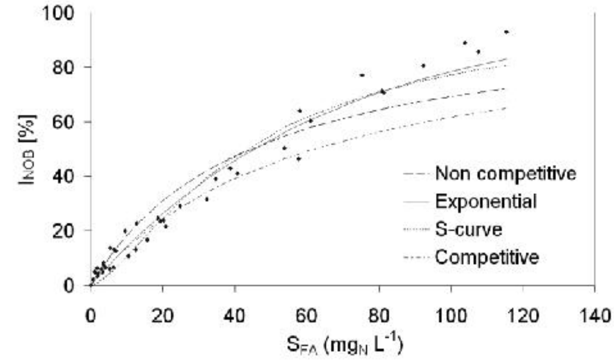


Figure 1: Effect of DO on the oxidation rate of AOB (●) and NOB (■).

Figure 2: Fitting experimental data by several inhibition curves.

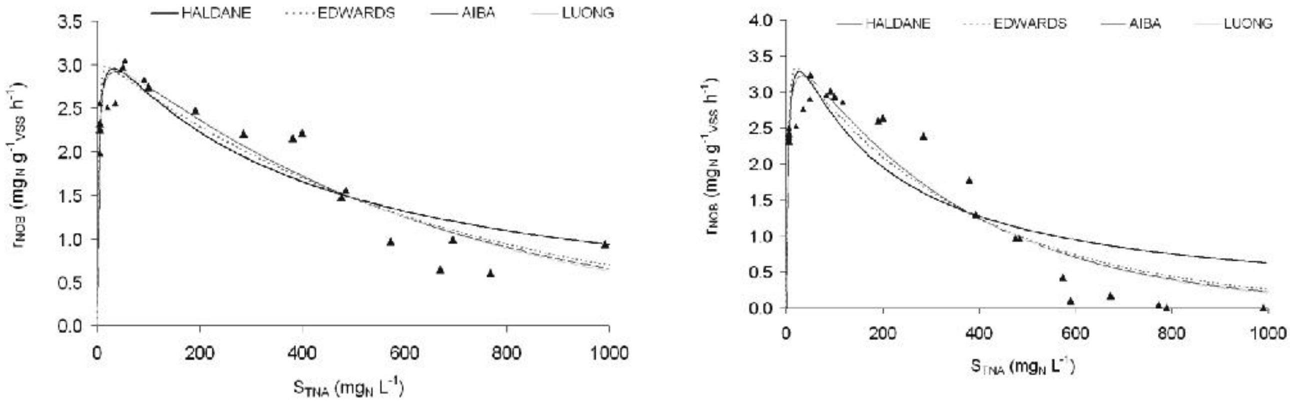


Figure 3: Fitting experimental data by several inhibition curves (pH = 8.3 on the left and at pH = 7.5 on the right).

In the third experimentation, the effect of total nitrous acid (TNA) concentration and of the un-ionized nitrite acid (FNA) concentration was evaluated by measuring the decrease in NOB activity under increasing total nitrite concentration, achieved by spiking nitrites at 30-50 min intervals under DO-stat conditions and by working at two pH values (7.5 and 8.3). The actual TNA during the test was computed by taking into account the total amount of nitrite added and the amount of nitrite oxidised to nitrate, as estimated from the oxygen dosed by titration. TNA was increased from 0 to 1000 mg_N L^{-1}. The FNA concentration was calculated from that of TNA, by considering its dissociation equilibrium. The NOB activity was plotted against both TNA and FNA concentrations. Contrary to what observed with FA concentration, the TNA and not the FNA concentration appeared to correlate with the observed inhibition. Possibly, at the tested pH, FNA concentrations were too low (<0.01 mg_N L^{-1}) to be relevant for inhibition. Thus, NOB activities versus TNA concentrations data were fitted with four inhibition functions known to describe substrate inhibition, namely Haldane, Edwards, Aiba and Luong (for more details on these functions please refer to: Carrera et al., 2004). The R^2_{adj} values varied between 0.7892 and 0.8987, suggesting that all models could explain data trends satisfactorily. The Aiba model provided the best fit (Figure 3). This model can be written as:

$r_{NOB} = \hat{r}_{NOB} \cdot \frac{S_{TNA}}{K_S + S_{TNA}} \cdot e^{\left(-\frac{S_{TNA}}{K_{I,TNA}}\right)}$, where r_{NOB} is the nitrite oxidation rate, S_{TNA} is the concentration of total nitrous acid and $K_{I,TNA}$ is the inhibition coefficient. According to this model, the EC_{50} TNA concentration is 430 mg_N l^{-1} at pH 8.3 and 240 mg_N l^{-1} at pH 7.5.

CONCLUSIONS

The experimentation lead to the following conclusions:

(1) The applied pH/DO-stat titration technique proved to be a simple and convenient technique to assess the activity of nitrite oxidising bacteria (NOB) under various operational conditions.

(2) The tests revealed that the half saturation constant of NOB for dissolved oxygen in Monod kinetics was $K_{DO,NOB} = 0.4\ mg_N\ l^{-1}$, and confirmed it is higher than that of ammonia oxidising bacteria ($K_{DO,AOB} = 0.1\ mg_N\ l^{-1}$).

(3) Data of NOB activity under various free ammonia (FA) concentrations could be best-fitted by an exponential-like dose-response curve ($R^2_{adj} = 0.9716$) with an EC_{50} of 45 $mg_N\ l^{-1}$.

(4) The effect of nitrite concentration (substrate inhibition) on NOB was assessed; total nitrous acid (TNA) more than free nitrous acid (FNA) was found to correlate well with the observed inhibition; Aiba model provided the best fit of the experimental data, with an EC_{50} TNA concentration of 430 $mg_N\ l^{-1}$ at pH 8.3 and 240 $mg_N\ l^{-1}$ at pH 7.5.

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