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Nitrifying activity monitoring and kinetic parameters determination in a biofilm airlift reactor by respirometry

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Abstract

The applicability of batch respirometry, as a simple technique for monitoring off-line nitrifying activity and kinetic parameters, was evaluated using two sets of ammonia and nitrite concentrations. The O_2 uptake rate (OUR) profiles obtained from the assays were adjusted to a substrate inhibition model. The maximum specific ammonia-oxidizing biomass activity (r_{Smax}) was 0.079 g N-NH_4^+ g VSS^{-1} d^{-1} with a half saturation coefficient (K_S) of 11 mg N-NH_4^+ l^{-1} and an inhibition coefficient (K_i) of 3300 mg N-NH_4^+ l^{-1}. Besides, the maximum specific value of nitrite-oxidizing activity was 0.082 g N-NO_2^- g VSS^{-1} d^{-1} with a K_S of 4.1 mg N-NO_2^- l^{-1} and K_i of 1400 mg N-NO_2^- l^{-1}.

Introduction

Most of the methods to determine kinetic parameters and biomass activity were developed for suspended biomasses and not for attached growth systems. As the physiology and composition in biofilm systems differs from suspended growth systems, other methods have measured kinetic parameters after disruption of the biofilm structure, being treated as a pseudo-suspended growth (Cao & Alaerts 1995). Within this type of methodology it is uncertain if the disruption of the structure of the biofilm affects the estimation of kinetic parameters. Only a few methods have been developed to determine kinetic parameters and biomass activity without affecting the biofilm. For aerobic, attached growth systems, many of the problems and limitations of the applied methods can be resolved by indirect determination of the substrate uptake profile by the associated OUR (Riefler *et al.* 1998).

This methodology can be applied in a nitrifying airlift biofilm reactor because it has a good mixing regime neglecting external mass transfer restrictions (Tijhuis *et al.* 1995). Another aspect is the internal mass transfer limitation which would be the same in an airlift reactor and in an agitated respirometer. These internal restrictions are only attributable to the biofilm structure and composition. Based on these facts, the nitrifying activity measured and the kinetic parameters established by the respirometric methodology, could be applied to the nitrifying airlift biofilm reactor. In this study batch respirometric tests are proposed as a simple technique for off-line monitoring of nitrifying airlift biofilm reactor activity and kinetics parameters estimation.

Materials and methods

Airlift reactor

An acrylic concentric-tube airlift reactor of 3.25 l working volume was set up. A three-phase separator was located at the top of the reactor in order to retain biofilm particles. Biofilm was developed on small-suspended particles of 0.3 mm mean diameter.

The airflow coming through a porous glass diffuser at the bottom of the reactor provided internal mixing of wastewater and biofilm particles, as well as an efficient aeration. Temperature and pH were maintained at 23 ± 1 °C and 7.5 ± 0.1.

Respirometer

The respirometer consisted in a glass vessel of 300 ml internal volume with three ports at the top for insertion of a dissolved O_2 (DO) probe (WTW, Cellox 325), a pH probe and the injection of the test compounds. A magnetic stirring bar and a stirring plate provided internal mixing of the liquor and biofilm particles. Aeration was provided through a porous glass diffuser at the bottom of the respirometer.

Methodology

For every respirometric test, 12 ml settled biofilm nitrifying particles were obtained from a continuous airlift reactor and added to the respirometer. The reactor was kept without substrate for 5 h to establish endogenous respiration; the endogenous respiration was evaluated at the beginning of each experiment as a constant OUR. Temperature was kept at 23 ± 1 °C in the respirometer and pH at 7.5 ± 0.1 using 2 M NaOH. The respirometer is aerated until the DO concentration of the mixed liquor reached a constant value. Then, the aeration is stopped and substrate is added to the liquor. DO depletion was monitored for 3 min and the OUR was determinate twice for every substrate concentration, considering as statistical error two times the standard deviation.

Analytical procedures

Ammonia was measured using an ion selective electrode (Orion 95-12 Ammonia, USA). Nitrite was measured by a colorimetric method (Greensberg *et al.* 1992).

Results and discussion

Two different experiments were carried out for nitrite-oxidizing and ammonia-oxidizing populations. For the ammonia-oxidizing populations, the respirometric test evaluates the OUR at several ammonia concentrations between 1 and 2500 mg $N\text{-}NH_4^+$ l^{-1}. The resulting OURs are illustrated in Figure 1. Each OUR value is related to a specific ammonia-oxidizing rate by the biomass concentration in the respirometer (2.8 g VSS l^{-1}) and the stoichiometry of the nitrification reactions. In order to transform the OUR to ammonia-oxidizing rates it was considered that the second step of the nitrification process (oxidation from nitrite to nitrate) was completely inhibited at over 1 mg $N\text{-}NH_3$ l^{-1} (Anthonisen *et al.* 1976). At the moment of the experiments, the airlift reactor was operating with an Ammonia Loading Rate (ALR) of 0.11 g $N\text{-}NH_4^+$ g VSS^{-1} d^{-1}, corresponding to an influent ammonia concentration of 180 mg $N\text{-}NH_4^+$ l^{-1}, Hydraulic Retention Time (HRT) of 5 h^{-1} and biomass concentration of 7.3 g VSS l^{-1}. The effluent ammonia concentration of the airlift was 70 mg $N\text{-}NH_4^+$ l^{-1}. The activity obtained at 70 mg $N\text{-}NH_4^+$ l^{-1} with the respirometer was identical to the ammonia-oxidizing rate of the airlift reactor. According to this result, batch respirometry technique can be a simple and accurate methodology for nitrifying airlift biofilm activity monitoring.

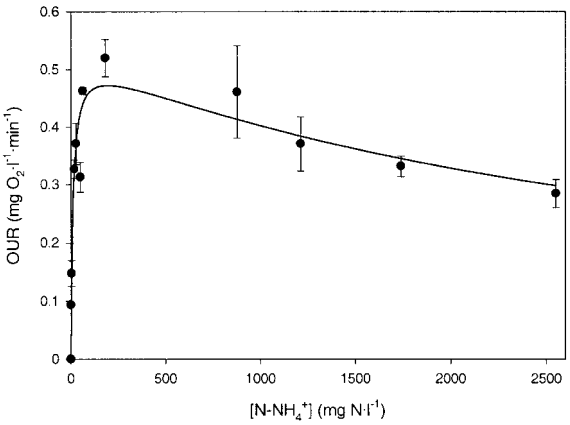

Fig. 1. O_2 uptake rate (OUR) profile of the ammonia-oxidizing organisms, obtained to ammonia concentrations from 1 to 2500 mg $N\text{-}NH_4^+$ l^{-1}. Experimental data (•) and model prediction (—). At pH = 7.5 ± 0.1 and T = 23 ± 1 °C.

Nitrifying organisms are inhibited by their own substrates, such as ammonia and nitrite. Inhibition phenomenon depends on the temperature and pH, because the inhibitors are acid forms of those substrates: free ammonia and nitrous acid (Lee *et al.* 2000). Substrate inhibition model has found wide applicability in modelling substrate inhibition (Mulchandani & Luong 1989):

$$r_S = \frac{r_{S\,\max}\,S}{K_S + S + \dfrac{S^2}{K_i}} \quad [gN - NH_4^+ gVSS^{-1}d^{-1}],$$

Table 1. Kinetic parameters for ammonia-oxidizing and nitrite-oxidizing biofilm populations at pH = 7.5 ± 0.1 and T = 23 ± 1 °C.

	Ammonia-oxidizing organisms		Nitrite-oxidizing organisms	
$r_{S\,max}$	0.079 ± 0.007	g $N\text{-}NH_4^+$ g VSS^{-1} d^{-1}	0.082 ± 0.004	g $N\text{-}NO_2^-$ g VSS^{-1} d^{-1}
K_S	11 ± 5	mg $N\text{-}NH_4^+$ l^{-1}	4.1 ± 0.9	mg $N\text{-}NO_2^-$ l^{-1}
K_i	3300 ± 1400	mg $N\text{-}NH_4^+$ l^{-1}	1400 ± 150	mg $N\text{-}NO_2^-$ l^{-1}

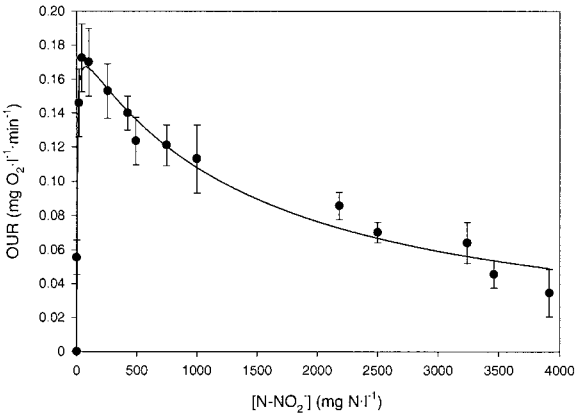

Fig. 2. OUR profile of the nitrite-oxidizing organisms, obtained to nitrite concentrations from 1 to 4000 mg $N\text{-}NO_2^-$ l^{-1}. Experimental data (•) and model prediction (—). At pH = 7.5 ± 0.1 and T = 23 ± 1 °C.

where r_S is the specific rate of substrate depletion, $r_{S\,max}$ is the maximum specific rate of substrate depletion, S is the substrate concentration, K_S is the half saturation coefficient and K_i is the inhibition coefficient.

With the activities calculated at different ammonia concentrations, kinetics of substrate consumption for ammonia-oxidizing organisms in the biofilm can be obtained. The kinetic model was previously applied for nitrification suspended growth by other authors (Gee *et al.* 1990, Rozich & Castens 1986). The results obtained for the validation in our case are presented by the continuous curve illustrated in Figure 1, which supplies a correlation coefficient (r^2) of 0.91. Kinetic parameters obtained from the model are represented in Table 1.

In order to estimate the kinetic parameters of the nitrite-oxidizing organisms present in the biofilm, a second experiment using nitrite as substrate was carried out. The OUR was monitored at nitrite between 1 and 4000 mg $N\text{-}NO_2^-$ l^{-1} (Figure 2). The nitrite-oxidizing rate was calculated from the OURs obtained from the respirometric test. Nitrite-oxidizing biomass activity showed an important enhancement with the increase of the nitrite concentration, as it occurred for the ammonia-oxidizing biomass.

The maximum value of the nitrite-oxidizing activity was 0.082 g $N\text{-}NO_2^-$ g VSS^{-1} d^{-1}. At higher nitrite concentrations the biofilm activity decreased as a result of substrate inhibition. The continuous curve illustrated in Figure 2 represents the kinetic model. The response was modelled considering substrate inhibition kinetics ($r^2 = 0.98$). Kinetic parameters obtained from the model fitting are represented in Table 1.

Kinetic parameters obtained from a substrate inhibition model are very useful for airlift biofilm characterisation. The half saturation coefficients (K_S) found (Table 1), are larger than coefficient values reported for nitrifying suspended biomass systems ($K_S = 0.3 - 1$ mg N l^{-1}) (Kong *et al.* 1996, Drtil *et al.* 1993); as a consequence of internal mass transfer restrictions in the biofilm structure. The inhibition coefficient (K_i) quantifies the substrate inhibition effect on the biofilm.

The biofilm system evaluated reached a K_i of 64 mg NH_3 l^{-1} for ammonia-oxidizing populations, and 0.35 mg HNO_2 l^{-1} for nitrite-oxidizing organisms. These values are in the range of the inhibitory concentrations described in the literature. Ammonia-oxidizing organisms are inhibited when free ammonia is between 10 and 150 mg NH_3 l^{-1}, and nitrite-oxidizing organisms are inhibited at 0.2 to 2.8 mg HNO_2 l^{-1} (Anthonisen *et al.* 1976). However, the K_i obtained for the ammonia-oxidizing organisms is closer to the higher values of the inhibitory concentrations; and the K_i obtained for the nitrite-oxidizing organisms is closer to the lower values. These results could indicate that in our system the ammonia-oxidizing organisms are less affected than the nitrite-oxidizing organisms by substrate inhibition. The ammonia-oxidizing organisms are more adapted to high substrate concentrations than the nitrite-oxidizing organisms, as a consequence of the usual environmental conditions of the airlift reactor where there is a certain ammonia concentration and not nitrite.

Conclusions

Batch respirometry technique is an adequate methodology for kinetic parameters determination and activity monitoring in an airlift biofilm reactor.

Apparently, biofilm ammonia-oxidizing organisms are less affected than the nitrite-oxidizing organism by inhibition of its substrate, attributable to the ammonia synthetic wastewater fed to the reactor during its operation.

The resulting kinetic parameters are important tools for airlift nitrifying biofilm characterisation and modelling its behaviour.

References

Anthonisen AC, Loehr RC, Prakasan TBS, Srineth EG (1976) Inhibition of nitrification by ammonia and nitrous acid. *J. Water Poll. Con. Fed* **48**: 835–852.

Cao YS, Alaerts GJ (1995) Influence of reactor type and shear stress on aerobic biofilm morphology, population and kinetics. *Water Res.* **29**: 107–118.

Drtil M, Nemeth P, Bodik I (1993) Kinetic constants of nitrification. *Water Res.* **27**: 35–39.

Gee CS, Suidan MT, Pfeffer JT (1990) Modeling of nitrification under substrate- inhibiting conditions. *J. Environ. Eng. ASCE* **116**: 18–31.

Greensberg AE, Clesceri LS, Eaton AD, Franson MAH (1992) *Standard Methods for the Examination of Waters and Wastewater*, 18th edn. Washington DC: American Public Health Association.

Kong Z, Vanrolleghem P, Willems P, Verstraete W (1996) Simultaneous determination of inhibition kinetics of carbon oxidation and nitrification with a respirometer. *Water Res.* **30**: 825–836.

Lee SM, Jung JY, Chung YH (2000) Measurement of ammonia inhibition of microbial activity in biological wastewater treatment process using dehydrogenase assay. *Biotechnol. Lett.* **22**: 991–994.

Mulchandani A, Luong JHT (1989) Microbial inhibition kinetics revisited kinetics revisited. *Enzyme Microb. Technol.* **11**: 66–73.

Riefler RG, Ahlfeld DP, Smets BF (1998) Respirometric assay for biofilm kinetics estimation: parameter identifiability and retrievability. *Biotechnol. Bioeng.* **57**: 35–45.

Rozich AF, Castens DJ (1986) Inhibition kinetics of nitrification in continuous-flow reactors. *J. Water Poll. Con. Fed.* **58**: 220–226.

Tijhuis L, Huisman JL, Hekkelman HD, van Loodsdrecht MCM, Heijnen JJ (1995) Formation of nitrifying biofilms on small suspended particles in airlift reactors. *Biotechnol. Bioeng.* **47**: 585–595.