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Stoichiometric and kinetic characterisation of *Nitrosomonas* sp. in mixed culture by decoupling the growth and energy generation processes

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Abstract

A novel method that relies on the decoupling of the energy production and biosynthesis processes was used to characterise the maintenance, cell lysis and growth processes of *Nitrosomonas* sp. A *Nitrosomonas* culture was enriched in a sequencing batch reactor (SBR) with ammonium as the sole energy source. Fluorescent in situ hybridization (FISH) showed that *Nitrosomonas* bound to the NEU probe constituted 82% of the bacterial population, while no other known ammonium or nitrite oxidizing bacteria were detected. Batch tests were carried out under conditions that both ammonium and CO_2 were in excess, and in the absence of one of these two substrates. The oxygen uptake rate and nitrite production rate were measured during these batch tests. The results obtained from these batch tests, along with the SBR performance data, allowed the determination of the maintenance coefficient and the in situ cell lysis rate, as well as the maximum specific growth rate of the *Nitrosomonas* culture. It is shown that, during normal growth, the *Nitrosomonas* culture spends approximately 65% of the energy generated for maintenance. The maintenance coefficient was determined to be 0.14–0.16 mgN $mgCOD_{biomass}^{-1}$ h^{-1}, and was shown to be independent of the specific growth rate. The in situ lysis rate and the maximum specific growth rate of the *Nitrosomonas* culture were determined to be 0.26 and 1.0 day^{-1} (0.043 h^{-1}), respectively, under aerobic conditions at 30 °C and pH 7.

Keywords: *Nitrosomonas*; Maintenance; Specific growth rate; Lysis rate; High nitrogen load; Yield

1. Introduction

Ammonia oxidation plays an important role in the nitrogen cycle occurring in natural environments, and is also a key step in engineered biological wastewater treatment plants. The reactions are performed by ammonia oxidising bacteria (AOB), which are obligate chemolithoautotrophs gaining both energy and reducing power from oxidising ammonia and using CO_2 as carbon source. Inorganic carbon is fixed via the Calvin cycle for the synthesis of new biomass. The growth kinetics of AOB have been researched extensively pri-

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marily due to their critical importance in modern nitrogen removal wastewater treatment processes. In particular, many studies have been carried out to determine the maximum specific growth rate of AOB (μ_{Amax}) using both pure and mixed cultures. The reported μ_{Amax} values fall in the range of 0.02–0.09 h^{-1}, which gives a generation time of 8–36 h (Sharma and Ahlert, 1977; Yoshioka et al., 1982; Alleman, 1985; Keen and Prosser, 1987). The large variation in the reported values is likely due to the use of different strains and/or different growth conditions in these studies. For similar reasons, the affinity constant of AOB with respect to ammonia/ammonium has also been shown to vary considerably (0.027–0.85 mgNH_3-N L^{-1}) (Koops and Pommerening-Roser, 2001).

Not all the energy generated from ammonia oxidation results in production of new biomass. AOB, like many other organisms, spend considerable energy on functions that are not directly related to growth, commonly referred to as maintenance activities (Keen and Prosser, 1987). The maintenance energy refers to the energy consumed for various cell survival activities other than formation of new biomass (Nystrom and Gustavsson, 1998; Minkevich et al., 2000). This includes re-synthesis of damaged cellular material, maintenance of the necessary concentration gradients across the cell membrane, cell motility and other non-growth related processes. Based on a thermodynamic model, Poughon et al. (2001) predicted that AOB should spend about 75% of the energy gained from ammonium oxidation to fulfil their maintenance requirement. However, experimental information investigating the maintenance processes of AOB, and indeed of all microorganisms, is scarce. To our best knowledge, there have been only two experimental studies reported in literature concerning the maintenance energy demands of AOB. Tappe et al. (1999) determined a maintenance energy consumption rate of 0.013 gN $gVSS_{biomass}^{-1}$ h^{-1} for a *Nitrosomonas* culture grown in ammonia limited conditions in a retentostat (with complete biomass retention). Due to the infinite retention time of cells in the reactor, it was assumed that there was no net cell growth when the reactor reached steady state, and therefore all the energy generated through ammonia oxidation was assumed to be used only for cell maintenance. This value is far lower than the values reported by Keen and Prosser (1987) from their chemostat studies (0.42 and 0.061 gN $gVSS_{biomass}^{-1}$ h^{-1}). However, it should be noted that the two values in Keen and Prosser (1987) were obtained from the same data set with two different data analysis algorithms. The large discrepancy between the two values was likely a consequence of the fact that only a limited number of data points were available.

In situ cell lysis rate of AOB is another important parameter that has not been well characterised. The most commonly used technique for the determination of this parameter has been to measure the decrease in respiration activity over time when bacteria are kept under starvation conditions (Siegrist et al., 1999). Generally it is assumed that there is no growth under starvation conditions and the loss of activity is caused by cell lysis leading to decrease in cell mass or numbers. The cell lysis rate thus determined may not necessarily represent the in situ cell lysis rate due to the application of an unusual environmental condition. The application of this method has revealed that the lysis rate is greatly influenced by the availability and the type of electron acceptors. Nitrifiers have been found to decay at higher rates under aerobic than under anoxic or anaerobic conditions (Nowak et al., 1994; Siegrist et al., 1999; Lee and Oleszkiewicz, 2003).

In the kinetic and stoichiometric characterisation of *Nitrobacter*, a common nitrite oxidizing bacteria, Vadivelu et al. (in press publication-b) developed a model-based approach that allowed the determination of the maintenance coefficient, the in situ lysis rate as well as the growth kinetics of *Nitrobacter*. In this study, the same method is applied to characterising these parameters of *Nitrosomonas* sp., an important AOB commonly found in biological wastewater treatment plants (Wagner et al., 1996). A lab scale sequencing batch reactor was operated to selectively grow an enriched culture of AOB. The maintenance coefficient (m), the in situ lysis rate (b), as well as the maximum specific growth rate (μ_{Amax}) of the culture were determined.

2. Materials and methods

2.1. Operation of a sequencing batch reactor (SBR) to enrich AOB

A sequencing batch reactor was operated to selectively grow an enriched culture of AOB. Mixed liquor