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Effect of free ammonia on the respiration and growth processes of an enriched *Nitrobacter* culture

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ABSTRACT

The inhibitory effect of free ammonia (FA;NH_3) on the metabolism of *Nitrobacter* is investigated using a method that allows decoupling energy generation from growth processes. A lab-scale sequencing batch reactor (SBR) was operated for the enrichment of *Nitrobacter*. Fluorescent in situ hybridization (FISH) analysis showed that 73% of the bacterial population in the reactor was *Nitrobacter*, while no *Nitrospira* was detected. Batch tests were carried out to measure the oxygen uptake rate (OUR) by the culture at various FA levels, in the presence (OUR_{withCO_2}) or absence ($OUR_{withoutCO_2}$) of inorganic carbon (CO_2, HCO_3^- and CO_3^{2-}). The FA inhibition on the respiration initiated at below 1 mgNH_3–N L^{-1} in both cases. $OUR_{withoutCO_2}$ gradually decreased by 12% when the FA concentration increased from 0 to approximately 4 mgNH_3–N L^{-1} and remained at the same level till an FA level of 9 mgNH_3–N L^{-1} (the highest FA concentration applied in this study). This indicates that FA has a limited inhibitory effect on the respiratory capability of *Nitrobacter*. Starting from a level that is 15% higher than $OUR_{withoutCO_2}$ when no FA was present, OUR_{withCO_2} decreased more rapidly than $OUR_{withoutCO_2}$ reaching the same level as $OUR_{withoutCO_2}$ when FA was between 6–9 mgNH_3–N L^{-1}. This implies that in this range of FA the presence of inorganic carbon did not cause any increase in the respiration activity of *Nitrobacter*. The results suggest that, while still oxidizing nitrite at approximately 75% of the non-inhibited rate, *Nitrobacter* likely ceased to grow at an FA level of above 6 mgNH_3–N L^{-1}. While the real mechanisms remain to be identified, this study indicates that the FA inhibition on *Nitrobacter* is likely much more serious than suggested by previous studies where OUR_{withCO_2} (or the equivalent nitrite oxidation rate) was used as the sole measure of the inhibitory effects.

1. Introduction

Nitrification is a two-step process where the reduced compound of nitrogen, ammonia, is oxidized to nitrite, which is further oxidized to nitrate. These two steps are carried out by two groups of autotrophic microorganisms, namely ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB). Despite the phylogenetic distance between them, AOB and NOB are typically in close physical association in sludge flocs (Daims et al., 2000), with a syntrophic interaction. Under normal growth conditions, nitrate is typically the main product of nitrification, and as such ammonia oxidation is often considered the rate limiting step (Randall and Buth, 1984b, Gil and Choi, 2001). However, incomplete nitrification with various levels of nitrite accumulation has also been widely reported in literature (Hanaki et al., 1990, Yang and Alleman, 1992, Villaverde et al., 2000).

Traditionally, nitrite build up is considered undesirable in biological wastewater treatment (BWT) systems. In recent years, however, several processes have been developed which

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Nomenclature

AOB	ammonia oxidizing bacteria
FA	free ammonia (NH_3)
FNA	free nitrous acid (HNO_2)
m	maintenance energy coefficient (mgN mg $COD_{biomass}^{-1}$ h^{-1}) $m = m^0 + m'(1-\mu/\mu_{max})$, where m^0, m', μ and μ_{max} are defined below
m^0	a component of m that is independent of μ (mgN mg $COD_{biomass}^{-1}$ h^{-1})
m'	a component of m that decreases with μ (mgN mg $COD_{biomass}^{-1}$ h^{-1})
NOB	nitrite oxidizing bacteria
OTR	oxygen transfer rate (mg h^{-1}); OTR_{withCO_2} and $OTR_{withoutCO_2}$ refer to OUR in the presence and absence of inorganic carbon, respectively
OUR	oxygen uptake rate (mg h^{-1}); OUR_{withCO_2} and $OUR_{withoutCO_2}$ refer to OUR in the presence and absence of inorganic carbon, respectively
q	specific uptake rate of a substrate (mgSubstrate h^{-1})
Y_G	true bacterial growth yield on a substrate ($mgCOD_{biomass}$ mgSubstrate^{-1})
Y_O	true bacterial growth yield with respect to oxygen ($mgCOD_{biomass}$ mgO^{-1})
ΔOUR	$OUR_{withCO_2} - OUR_{withoutCO_2}$ (mg h^{-1})
μ	specific growth rate of *Nitrobacter* (h^{-1})
μ_{max}	maximum specific growth rate of *Nitrobacter* (h^{-1})
$\mu_{withoutCO_2}$	specific growth rate of *Nitrobacter* in the absence of externally supplied CO_2 (h^{-1})

remove nitrogen through nitritation (ammonia oxidation to nitrite) and denitritation (nitrite reduction to di-nitrogen gas). Removing nitrogen via this so-called nitrite pathway, in comparison to full nitrification and denitrification, reduces the oxygen and carbon demand by 25% and 40%, respectively (Fux et al., 2003). Examples of such processes include the well-known Single reactor High Activity Ammonia Removal Over Nitrite (SHARON) process (Hellinga et al., 1998) and also a novel sequencing batch reactor (SBR) system reported in Lai et al. (2004). All these processes depend on the elimination or inhibition of NOB so that nitrite accumulates as the end product of nitrification.

Accumulation of nitrite results from higher activities of AOB than NOB (Smith et al., 1997). Over the past few decades much work has been conducted to understand the mechanisms of nitrite accumulation, in order to either stimulate or avoid nitrite build-up during nitrification. Factors such as pH, temperature, and the concentrations of dissolved oxygen (DO), CO_2 and heavy metals were all found to influence the nitrite build-up (Randall and Buth, 1984a, Hanaki et al., 1990, Surmacz-Gorska et al., 1997). However, the main causes are believed to be the inhibitory effects of free ammonia (FA) (Mauret et al., 1996, Villaverde et al., 2000) and free nitrous acid (FNA) (Anthonisen et al., 1976, Philips et al., 2002).

The inhibitory effect of FA on NOB has been widely reported (Balmelle et al., 1992, Philips et al., 2002). While it has been speculated that AOB activities may also be inhibited by FA, NOB has been described to be much more sensitive to FA than AOB (Painter, 1970). Anthonisen et al. (1976) reported that FA initiated inhibition on *Nitrobacter* at about 0.1–1.0 mgNH_3 L^{-1}, while the threshold value for *Nitrosomonas* was about 10–150 mgNH_3 L^{-1}. In a study on nitrogen removal from high strength wastewater via the nitrite pathway, Abeling and Seyfried (1992) found that nitratation (nitrite oxidation to nitrate) was inhibited by FA at a concentration of 1–5 mgNH_3 L^{-1}, while a similar effect was not observed on nitritation.

The literature data summarized above clearly show that FA has a significant inhibitory effect on the metabolism of NOB. However, most studies reported to date relied on the measurement of the oxygen uptake rates (OUR, or equivalently the nitrite oxidation rates) at different FA levels. The levels of inhibition were assessed by comparing these rates with that measured in the absence or low, thus non-inhibitory, level presence of FA. Such studies only revealed the impact of FA on the respiration of NOB. Little information was gained with regard to the impact of FA on the growth of NOB.

In this paper, we report additional experimental information on the inhibitory effects of FA on the metabolic activities of NOB. An enriched *Nitrobacter* culture was used. Batch tests were designed and carried out to measure the oxygen consumption rates of the culture at various FA levels, in the presence and absence of inorganic carbon. The inhibitory effects of FA on the anabolic and catabolic processes of NOB were assessed through comparing the oxygen consumption rates in the two cases and model-based data analysis.

2. Materials and methods

2.1. Operation of SBR to enrich NOB

A SBR was operated to selectively grow an enriched culture of NOB. Mixed liquor from a fully nitrifying wastewater treatment plant in Brisbane, Australia, was used as inoculum for the SBR. This reactor had a working volume of 8 L and was fed with nitrite (synthetic wastewater with 1000 mgNO_2^-–N L^{-1}) as the sole energy source and bicarbonate as the sole carbon source (detailed composition given below). The SBR was operated with a cycle time of 6 h and a hydraulic retention time (HRT) of 1 day. Each cycle consisted of a 270-min aerobic feeding and a 20-min aerobic reaction period, followed by a 60-min settling and a 10-min decanting period. The reactor was operated in a temperature-controlled room (22 ± 1 °C). The DO concentration was maintained within the range of 2.75–3.25 mg L^{-1} using an ON/OFF controller. While not controlled, the pH in the reactor varied in a narrow range between 7.2 and 7.4, due to the strong buffer in the wastewater.