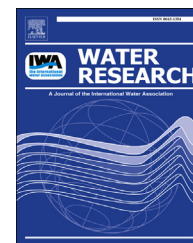


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# Outcompeting nitrite-oxidizing bacteria in single-stage nitrogen removal in sewage treatment plants: A model-based study

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## ARTICLE INFO

### Article history:

Received 4 June 2014

Received in revised form

16 August 2014

Accepted 20 August 2014

Available online 28 August 2014

### Keywords:

Microbial community interactions

Anammox

Nitrification

Biofilm

Granular sludge

Mathematical modeling

## ABSTRACT

This model-based study investigated the mechanisms and operational window for efficient repression of nitrite oxidizing bacteria (NOB) in an autotrophic nitrogen removal process. The operation of a continuous single-stage granular sludge process was simulated for nitrogen removal from pretreated sewage at 10 °C. The effects of the residual ammonium concentration were explicitly analyzed with the model. Competition for oxygen between ammonia-oxidizing bacteria (AOB) and NOB was found to be essential for NOB repression even when the suppression of nitrite oxidation is assisted by nitrite reduction by anammox (AMX). The nitrite half-saturation coefficient of NOB and AMX proved non-sensitive for the model output. The maximum specific growth rate of AMX bacteria proved a sensitive process parameter, because higher rates would provide a competitive advantage for AMX.

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## 1. Introduction

In the search for energy neutral (or even energy positive) wastewater treatment plant (WWTP) configurations, the use of anammox for nitrogen removal from pretreated sewage is seen as an imperative (Jetten et al., 1997; Siegrist et al., 2008; Kartal et al., 2010). The pretreatment of sewage would remove most of the organic matter, and the remaining liquid

would contain mainly ammonium, that could be treated by anammox-based technologies. In particular, the single-stage nitrification-anammox biofilm (SNAB) process is considered the more convenient way of implementation, since a single reactor devoted to nitrogen removal would decrease both the investment and the operational costs (Kartal et al., 2010; De Clippeleir et al., 2013; Wett et al., 2013). Challenges associated to such a sewage treatment process are related to (i) low wastewater temperatures, (ii) low nitrogen concentration of

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<http://dx.doi.org/10.1016/j.watres.2014.08.028>

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the wastewater, and (iii) the high effluent quality required (Kartal et al., 2010; Winkler et al., 2011; Wett et al., 2013; Hu et al., 2013; De Clippeleir et al., 2013).

Many laboratory studies have investigated the use of a sequencing batch reactor (SBR) for implementation of the SNAB process. SBR operation allows for easy granulation and enables different operational strategies, such as aerated and non-aerated periods, or intermittent aeration or feeding. It furthermore facilitates selective granular sludge removal and easy automation and control (Winkler et al., 2011; Wett, 2007; Joss et al., 2009; Hu et al., 2013). Nevertheless, in full scale installations a continuous mode of operation would be preferred due to simpler and more economic operation and more effective use of the aeration equipment (among others).

The major challenges in selecting the desired microbial community are related to the competition between anammox (AMX) and nitrite-oxidizing bacteria (NOB) for nitrite, and between ammonia-oxidizing bacteria (AOB) and NOB for oxygen (Winkler et al., 2011). Nitrate accumulation due to nitrite oxidation by NOB has become a clear symptom of undesired reactor performance, the repression of NOB in single-stage nitrification-anammox biofilm reactors has been identified as one of the main challenges for successful implementation of the SNAB process for sewage treatment (Volcke et al., 2010; Winkler et al., 2011; Volcke et al., 2011; De Clippeleir et al., 2013; Wett et al., 2013).

Successful implementation of the SNAB process thus relies on the definition of the operational conditions that enable effective enrichment of AOB and AMX dominated biofilms and repression of the NOB population. In the present study a computational biofilm model is used to investigate the development of a microbial community consisting of ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB) and anammox (AMX) in bioreactors operated in continuous mode. The main aim is to understand how these microbial community interactions evolve at low temperatures and low nitrogen concentrations and to identify the most sensitive parameters leading to NOB repression. A second objective is to determine the domain of operating conditions in which a granular sludge based reactor operated in continuous mode achieves stable nitrogen removal, while long term NOB repression is assured.

## 2. Model description

### 2.1. Biofilm model, kinetics and parameters

A dynamic model was developed to simulate the granular sludge reactor performance, based on the one-dimensional multispecies biofilm model of Wanner and Reichert (1996), implemented in the software package AQUASIM v.2.1d (Reichert, 1998). The reactor volume (including the bulk liquid phase and the granular sludge) was considered constant at an arbitrary volume of 2000 m<sup>3</sup>, with a fully-mixed liquid phase. The inflow composition was set to represent pretreated sewage, with a temperature of 10 °C, without soluble nor particulate COD and with an ammonium concentration of 70 g N/m<sup>3</sup>.

Four particulate components were considered in the biofilm matrix: AOB, NOB, AMX and inert biomass (Table S1). Initial weight fractions of active biomass in the biofilm were assumed to amount 40% AOB, 40% NOB, and 20% AMX. The initial fractions of the biomass do not impact the results obtained with the model at steady state, which are the results presented in the graphs. The porosity of the biofilm (80%) and the total biomass concentration in the granule (90 kg VSS/m<sup>3</sup>) were kept constant during all the simulations. The total biofilm area was defined as a function of granule size and number of granules, calculated from the total biomass concentration in the reactor (3 kg VSS/m<sup>3</sup>) and the assumed granule diameter and density. Initial granule size was set to 180 μm in all simulations. A variable detachment rate was used to reach a constant granule size set to 1.5 mm. A single granule size was selected, without including a granule size distribution, to ease the interpretation of results. Although the standard case was investigated for a granule size of 1.5 mm, the effect of granule size was also assessed with the model, by using sizes of 1 and 2 mm in additional simulations. Detached biomass from the biofilm was active in suspension and following the same kinetics as the biomass in the biofilm, but it was assumed to be washed out with the effluent. Attachment of biomass onto the biofilm surface has been neglected.

Four soluble components were considered: oxygen (O<sub>2</sub>), ammonium (NH<sub>4</sub><sup>+</sup>), nitrite (NO<sub>2</sub><sup>-</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>). The microbial kinetics and the stoichiometry used are detailed in Tables S1–S3 in the supplementary information. Growth of AMX was inhibited by O<sub>2</sub>. Decay of all active biomass types to inert material was included. Lower diffusivity was assumed in the biofilm (Table S4) and external mass transfer resistance has been neglected.

### 2.2. Closed process control loops

To determine the effect of ammonium and oxygen bulk liquid concentrations on the microbial interactions, two different control loops were implemented as proposed by Jemaat et al. (2013). Including the control loops in the mathematical model is very convenient, since it allows for investigating the impact of both ammonium and dissolved oxygen (DO) concentrations independently. For the mathematical description of the DO control loop, aeration was introduced as a dynamic process only active in the bulk liquid phase:  $dS_{O_2}/dt = k_L a ((S_{O_2})_{SP} - S_{O_2})$  with  $S_{O_2}$  the dissolved oxygen concentration in the bulk liquid and oxygen solubility  $(S_{O_2})_{SP}$  as the set point. A high value for the volumetric gas–liquid oxygen transfer coefficient ( $k_L a = 10000 \text{ d}^{-1}$ ) was selected.

For the ammonium concentration control loop the wastewater flow-rate ( $Q_{in}$ ) was used as manipulated variable:

$$Q_{in} = Q_{in,0} + Q_{in,0} a \left( \frac{(S_{NH_4^+})_{SP} - S_{NH_4^+}}{(S_{NH_4^+})_{SP}} \right) \quad (1)$$

where  $Q_{in,0}$  is the bias of the control action, i.e. the default value of flow-rate. The controller always acts either increasing

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