



## Effect of ammonia oxidizing bacteria (AOB) kinetics on bioaugmentation

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### HIGHLIGHTS

- ▶ Bioaugmentation with ammonium oxidizing bacteria was tested at bench scale.
- ▶ Nitritation was improved in the seeded reactors.
- ▶ The effectiveness of seeding varied with SRT and substrate concentrations.
- ▶ Conventional activated sludge models overestimated the effect of bioaugmentation.
- ▶ The affinity for ammonia of seeded AOB affected bioaugmentation efficiency.

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### ABSTRACT

Bioaugmentation with ammonium oxidizing bacteria (AOB) was tested for 620 d. A seeding reactor (R1), two seeded reactors (R2 at 21 °C; R3 at 15 °C) and an unseeded-control reactor (R4 at 21 °C) were operated in parallel ( $2.4 < \text{SRT} < 4$  d). The effect of seeding on nitritation efficiency was found to be dependent on solids retention time (SRT), influent ammonia concentration to the seeded reactors and the temperature difference between the seeding and seeded reactors.

Mathematical modeling and batch tests were used to characterize the AOB selected in R1 and the effect of the seeding on AOB kinetics in R2 and R3. The AOB kinetics of R2 and R3 reflected the kinetics of R1 but differed from those in R4. This behavior affected the efficiency of bioaugmentation to varying degrees in the reactors and required a specific approach to represent the experimental results through mathematical modeling.

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### 1. Introduction

Achieving nitrification by means of bioaugmentation involves the enrichment of the recycled activated sludge (RAS) stream with nitrifiers from an outside source. When compared to the conventional approach used in activated sludge biological nutrient removal (BNR) systems to achieve stable and reliable nitrification, bioaugmentation with nitrifier seed from a supplemental source allows the main stream BNR process to operate at a reduced solids retention time (SRT) and reduce the size of the aerobic reactor (Krhutkova et al., 2006). As such, bioaugmentation provides an opportunity for designers to take advantage of the lower SRT and reduce the size of the aerobic reactors required to achieve nitrification in the main process stream. Bioaugmentation can also be used to provide a higher degree of robustness (Parker and Wanner,

2007) to the overall nitrification process, as well as a method for rapid recovery should some transient event negatively impact the nitrification process (Yusof et al., 2010).

Bioaugmentation with nitrifiers has been proven effective in reducing the SRT necessary to meet nitrification in cold temperatures (Head and Oleszkiewicz, 2004; Berends et al., 2005; Krhutkova et al., 2006; Wanner et al., 2009). Seeding with nitrifiers was shown to be effective through bench, pilot and full-scale tests in reducing the start-up time and improving the stability of the nitrification process (Bartrolí et al., 2011; Guo et al., 2010a). Abbreviated start-up times, and correspondingly fast recovery after a partial washout, can reduce the risk of losing nitrification when operating at a low SRT (Satoh et al., 2003). An additional benefit of reducing the SRT is the improvement in the quality of sludge sent to further biological processing such as anaerobic digestion.

An accurate quantification of the beneficial effects of bioaugmentation is still elusive and some phenomena assessments need to be included in the overall evaluation. In some cases bioaugmentation was successfully modeled using conventional IWA activated sludge models (Salem et al., 2002, 2003). However, some phenomena are

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still difficult to be properly characterized and simulated in the current state-of-the-art models. For example, predation of seeded nitrifiers was observed to be the cause of unpredictable failure of bioaugmentation efficiency (Bouchez et al., 2000; Stephenson and Stephenson, 1992; Fu et al., 2009). Predation has never been separately modeled in bioaugmentation studies.

Through molecular techniques it has been demonstrated, that nitrifiers selected in a side stream reactor fed with reject water from sludge dewatering (i.e., centrate) are genetically different from those in the mainstream process of a same plant treating domestic wastewater (Podmirseg et al., 2010). On this basis a model with two separate AOB populations was proposed to simulate and predict the outcome of AOB competition as a function of operating conditions in different bioaugmentation scenarios (Wett et al., 2011).

Large temperature difference between the seeding and seeded reactors has the potential to introduce a thermal shock stress on the nitrifying biomass and diminish the efficiency of bioaugmentation. In certain cases where seeded nitrifiers were added to the main stream process, nitrification rates were satisfactorily described by conventional Arrhenius coefficients (Head and Oleszkiewicz, 2004). However, Plaza et al. (2001) showed that the overall growth kinetics of the main stream nitrifying biomass was very low ( $0.2 \text{ d}^{-1}$  at  $15^\circ\text{C}$ ), compared to the reference values, when nitrifier seed was added to a cooler main stream process environment. The thermal shock stress introduced by transferring nitrifier seed from a warm environment to a cool environment is an important factor that may limit the effectiveness of bioaugmentation. While direct temperature shock appears to be reversible, the initial reaction of nitrifiers is much more pronounced than after a few days of acclimation (Hwang and Oleszkiewicz, 2007) due to a lag period caused by the temperature difference (Lee et al., 2011).

Nitrification and denitrification is a more cost effective process for nitrogen removal (Hellings et al., 1998) than nitrification and denitrification process. When compared with conventional nitrification and denitrification, it consumes less oxygen and requires less electron donors (e.g., methanol, hydrogen, etc.). The reduced need for oxygen translates into less energy demand (i.e., cost savings and reduced greenhouse emissions), and the reduced need for an electron donor has the additional benefit of decreased waste activated sludge (WAS) production.

Bioaugmentation with AOB grown in a side stream reactor can provide the seed necessary to enable partial nitrification in the main stream process and allow for a more sustainable denitrification process as well as to achieve total nitrogen removal requirements. The required AOB can be cultured and grown effectively in a side-stream reactor fed with warm high-ammonia streams, such as centrate from dewatering anaerobically digested sludge.

The goal of this research is to assess the factors affecting the efficiency of bioaugmentation with AOB for different operating conditions between the seeding and seeded reactors, and the predictability of the bioaugmentation effect using activated sludge modeling. The unique aspect of this research is that it couples both mathematical modeling and experiments, to evaluate the AOB kinetic parameters in the seeding and seeded bioreactors and their corresponding roles in affecting the kinetics of the AOB in the bioaugmentation process.

In addition, innovative methods were developed and used to confidently quantify nitrification performance and efficiency in response to AOB bioaugmentation. Since bioaugmentation with AOB alone has been only recently studied (Bartrolí et al., 2011; Zhang et al., 2012), the consistency of modeling and experimental results is very limited and requires validation. This novel research was explicitly designed to assess AOB bioaugmentation response as discussed and described relative to a control reactor in order to place the findings and results into proper context.

## 2. Methods

### 2.1. Experiment design

Four bench-scale sequencing batch reactors (SBR) each with a volume of 3 L were operated and monitored for a total of 620 d. All reactors had a hydraulic retention time (HRT) of 24 h and cycle duration of 4 h. Seeded reactors R2 and R3 were operated at different temperatures,  $21^\circ\text{C}$  and  $15^\circ\text{C}$ , respectively. R2 and R3 were seeded each cycle with a fraction of the excess AOB grown in a partially nitrifying–denitrifying (nitrification–denitrification) reactor R1. A separate control reactor R4 was used as baseline reference and maintained at the same temperature as R2. The operating conditions and ranges in the reactors are summarized in Table 1, and a schematic of the experimental design is shown in Fig. 1.

The experiment was divided into six periods (after the start-up) with the aim of comparing and assessing the effect of seeding as a function of the following operating conditions:

- SRT (d) in the seeded reactors;
- temperature ( $T$ ) difference between seeding and seeded reactors;
- different nitrogen loads (as Total Kjeldahl Nitrogen – TKN) in the seeded reactors;
- amount of seeding ( $\text{mg SS d}^{-1}$ ).

The operating conditions and ranges in the reactors during each experimental period are summarized in Table 2.

The seeding reactor R1 was operated in alternating aerobic and anoxic conditions during its reaction phase and had a sequence of fill, react, waste, settle, and decant. The aerated phase included the filling of the reactor for 20 min, with a total duration of 2 h; the anoxic phase lasted 1.5 h and included the dosing of 400 mg COD as methanol for 50 min was performed at the beginning of this phase; the settling and the decant phases lasted 20 and 10 min, respectively.

In R1, biomass from a previous bench-scale study on nitrification in alternating conditions was used as inoculum, and blended with biomass collected from a bench-scale reactor operating in partial nitrification mode. Blending was done in order to start with mixed AOB and NOB populations. Dissolved oxygen (DO) was in the range  $0.5\text{--}3 \text{ mg O}_2 \text{ L}^{-1}$  during the aerobic phase. Sufficient alkalinity for nitrification was provided and the pH was controlled between 7.3 and 7.6 by dosing of a  $10 \text{ g L}^{-1}$  solution of  $\text{NaHCO}_3$ .

The operating strategy of R1 was purposely developed to maximize the growth of AOB biomass while obtaining a stable washout of the NOB. During startup, the SRT in R1 was increased gradually from 3 to 11 d and methanol was added gradually to avoid possible nitrification inhibition.

**Table 1**  
Summary of the operating conditions in the reactors.

Parameter	Units	R1	R2	R3	R4
Function		Seeding	Seeded	Seeded	Unseeded
SRT	d	$11^b$	4–3.2– 2.5	4–3.2– 2.5	4–3.2– 2.5
DO <sup>a</sup>	$\text{mg O}_2 \text{ L}^{-1}$	0.5–3	$4 \pm 0.2$	$4 \pm 0.1$	$4 \pm 0.1$
$T$	$^\circ\text{C}$	$35 \pm 0.3\text{--}$ $28 \pm 0.3$	$21 \pm 0.3$	$15 \pm 0.3$	$21 \pm 0.3$
TKN	$\text{mg N L}^{-1}$	300	29–45	29–45	29–45
influent					
COD	$\text{mg COD L}^{-1}$	$320^c$	320	320	320
influent					

<sup>a</sup> Specific to aerobic phase only.

<sup>b</sup> Except for start-up when SRT ranged from 3 to 14 d.

<sup>c</sup> Plus  $960 \text{ mg COD L}^{-1}$  of methanol during the anoxic phase.

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