Models for nitrification process design: one or two AOB populations?

ABSTRACT

Models for engineering design of nitrifying systems use one ammonia oxidizer biomass (AOB) state variable. A simple extension using two AOB populations allows a more accurate prediction of nitrification systems at switching process environments. These two AOB subpopulations are characterized by two different sets of kinetic parameters. Selection pressure and competition between the two functional AOB populations are determined by process conditions as demonstrated by three case studies: Case study I describes dynamics of two AOB populations showing different temperature sensitivities (modified Arrhenius term on growth and decay) when bioaugmented from the warm sidestream treatment environment to the cold mainstream and vice-versa. Case study II investigates competition between fast growing μ-strategists and k-strategists adjusted to low ammonia levels depending on the internal mixed liquor recycle rate (IMLR). Case study III shows that AOB transferred from the waste activated sludge of an SBR to the parallel continuous flow system with different decay kinetics can overgrow or coexist with the original population.

Key words | ASM, AOB, population dynamics, bioaugmentation, nitrification, process design

INTRODUCTION

Ammonia oxidizer biomass (AOB) in wastewater treatment plants consists of a diverse population from a microbiological and phylogenetic standpoint. For engineering design the accepted practice is to use a lumped parameter model, including one AOB state variable with its kinetic and stoichiometric parameters (maximum specific growth rate \( \mu_{A,\text{max}} \), decay rate \( b_A \), oxygen and substrate half-saturation constants \( K_{O_{2}}, K_{N_{H4}} \), temperature sensitivities \( \theta \) for growth and decay). This model has been successfully used for activated sludge (AS) plant design and upgrades for over 30 years (Marais & Ekama 1976). Some kinetic variations have been reported when taking the parameter values into consideration, particularly the critical \( \mu_{A,\text{max}} \) and \( b_A \) linked to plant capacity (Henze et al. 2000; Rieger et al. 2001; Bollmann et al. 2002; Rönner-Holm et al. 2006), but have otherwise been found to be relatively stable (WERF 2003).

External disturbances such as toxic chemicals, phages and storm weather conditions can cause a severe drop in performance of WWTPs. Since the mass of nitrifying organisms in activated sludge is low and the required sludge age is high, the nitrification rate mainly suffers from a wash-out of these populations. FISH techniques have shown that nitrifying populations vary in different WWTPs and after temperature shifts (Daims et al. 2004; Siripong & Rittmann 2001). In SBR plants, a higher diversity of nitrifying organisms often corresponds to higher process stability (Daims et al. 2004).

In lumped parameter models with one AOB state variable the modelled nitrifier wash-out will occur almost immediately, whereas models with more than one AOB subpopulations better reflect real world conditions. This is especially true for AOB bioaugmentation situations, where
different AOB subpopulations are derived from different plant sections.

In this paper, full scale experimental observations indicate that consistent differences can be found in AOB kinetic parameters under certain circumstances. Three case studies and general modelling approaches including different AOB subpopulations and different bioaugmentation and sludge cycling strategies for engineering design will be described and discussed.

**METHODS**

**Model extension**

Available simulators in wastewater engineering apply models that differ a lot in complexity and in various aspects of process description. The description of aerobic ammonia oxidation shows broad consensus – typically considering growth and decay of one AOB population. Equations (1) and (2) represent generally accepted approaches for AOB growth and decay. Two widely acknowledged models – ASM3 in combination with the EAWAG-BioP model (Henze et al. 2000; Rieger et al. 2001) set up in SIMBA® 6.1 for case study III and the general Activated Sludge-Digestion Model (ASDM, Jones et al. 2007) in the BioWin® simulator for case study I and II – are used to simulate three specific nitrification scenarios. The two model approaches differ in inhibition and limitation terms specified in the following kinetic expressions:

\[ r_{growth} = X_{AOB} \cdot \theta^{T-20} \cdot \mu_{max} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{NH4}}{K_{NH4} + S_{NH4}} \cdot A \]  
\[ A(ASM3) = \frac{S_{Alk}}{K_{Alk} + S_{Alk}} \]  
\[ A(ASDM) = I_{pH} \cdot \left( \frac{K_{LHNO2}}{K_{LHNO2} + S_{HNO2}} \right) \frac{1}{1 + \exp((K_{HC03} - S_{HC03})/S_{HC03})} \]  
\[ r_{decay} = X_{AOB} \cdot \theta^{T-20} \cdot \left( b_{aerobic} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} + b_{anoxic} \cdot B \right) \]  
\[ B(ASM3) = \frac{K_{O2}}{K_{O2} + S_{O2}} \cdot \frac{S_{NOx}}{K_{NOx} + S_{NOx}} \]  
\[ B(ASDM) = \frac{K_{O2}}{K_{O2} + S_{O2}} \]  
(2b)

In ASM3, AOB growth is limited by a Monod term for alkalinity Equation (1a) while in ASDM the process rate is slowed down outside an optimum pH-range Equation (1b). Additionally ASDM considers inhibition by nitrous acid and inorganic carbon limitation which can become relevant especially in high-strength ammonia conditions (Wett & Rauch 2003). Both ASM3 and ASDM models define reduced decay rates under anoxic conditions. Under anaerobic conditions the ASM3 model maintains anoxic decay rates (Equation 2b) different from the ASM3 decay rate which drops to zero without NOx as electron acceptor (Equation 2a). Table 1 lists those parameters which are addressed by the definition of two different AOB populations in the presented case studies.

**Case study I: bioaugmentation from sidestream to mainstream at the WWTP Salzburg**

The WWTP at Salzburg, Austria, operates a 2-stage system – high-rate (A) for carbon and low-rate stage (B) for N-removal – to treat a design-load of 680,000 PE. From an existing nitrification/denitrification sidestream SBR system for treatment of sludge liquors, nitrifying biomass was transferred to the mainstream to improve N removal capacity. In summer bioaugmentation of the high rate A-stage promises partial upstream N removal; while in winter bioaugmentation of the B-stage can substantially reduce minimum sludge retention time requirements. Both strategies have been piloted in cold and warm seasons at increasing pumping rates of recycled WAS to the SBR and of enriched biomass to the mainstream (Figure 1). Operating temperature in the mainstream ranged from 11 to 16 °C and in the sidestream SBR from 26 to 36 °C.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Definition of kinetic parameters for two separate AOB populations depending on site-specific setup</th>
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<tbody>
<tr>
<td>scenario</td>
<td>target parameter</td>
</tr>
<tr>
<td>case study I</td>
<td>( \Theta(a), \Theta(b) )</td>
</tr>
<tr>
<td>case study II</td>
<td>( \mu_{max}, K_{NH} )</td>
</tr>
<tr>
<td>case study III</td>
<td>( b, K_{NH} )</td>
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</table>
Nitrifier activity measurements had been performed \textit{ex-situ} at 10 and 22 °C on sludge samples from all three biological systems. A 2h test procedure was applied on each original sample and a control sample spiked with a nitrification inhibitor – oxygen uptake rate (OUR) was monitored at the beginning and the end of the test and nitrogen compounds (NH$_4$, NO$_2$ and NO$_3$) were measured on an hourly basis (Schön \textit{et al.} in preparation).

Additionally the phylogenetic footprint of the nitrifying organisms in these samples was analyzed by applying denaturing gradient gel electrophoresis (DGGE) and real-time PCR melt curve analysis (Podmirseg \textit{et al.} 2010). The DGGE-bands and the DNA melt-curve analysis (dissociation pattern of DNA strings at increasing temperature) confirm that at zero sludge recycling rate there is a difference in nitrifier populations in the separate systems. At increasing sludge cycling rates the SBR samples were assimilated with the B sample. Nitrifiers in the A-stage (SRT of about 0.5 days) show similarities with the SBR-biomass at increasing bioaugmentation rates (Figure 2a). Regarding bioaugmentation to the B stage (Figure 2b), the non-influenced A samples are similar to the B samples and also the SBR samples adapt immediately to the B fingerprint pattern at higher sludge recycle rates. Obviously the sludge transfer rate between both systems rules the blending of the two AOB populations.

\textbf{Case study II: The effect of the degree of mixing on AOB selection}

To investigate the influence of the degree of mixing on the maximum specific growth rate of AOB ($\mu_{A,\text{max}}$), samples were collected from an 8 MGD (ca. 30,000 m$^3$/day) treatment plant, at different internal mixed liquor recycle rate (IMLR) conditions. The plant schematic is shown in Figure 3. The IMLR was set to 0, 1, 2, 4, 6 and 8Q, where Q is the influent flow rate (Jimenez \textit{et al.} 2009). The maximum growth rate $\mu_{A,\text{max}}$ was determined by \textit{ex-situ} high F/M tests where the exponential depletion of spiked ammonia concentrations is measured (Dold & Takacs 2004). IMLR usually shows impacts on the sludge volume index (SVI) which are not considered here.

\textbf{Case study III: Bioaugmentation from an SBR to a parallel continuous flow system}

The municipal, modernized and extended Weisstal plant was put into operation in 2004. With regard to its size (20,500 PE, including WAS from a neighbouring plant),
The Weisstal WWTP is a relatively complex plant. A continuous flow AS plant and an SBR plant are operated in parallel (Figure 4). The continuous plant consists of one combined tank with upstream denitrification, 1,149 m³, and nitrification stage 1, 1,702 m³, and a second combined tank with nitrification stage 2, 1,346 m³, and secondary clarifier, 2,046 m³. The SBR plant comprises one buffer tank, 900 m³, and two SBR tanks, 1,559 m³. The continuous plant treats 55% and the SBR plant 45% of the influent after a primary clarifier in normal operation mode. The primary sludge and waste activated sludge (WAS) are anaerobically stabilized in a digester. Depending on the operation mode chosen, sludge liquor can be fed into both plants or directly into the denitrification basin, or into both SBR tanks respectively (Figure 4).

Dynamic simulation studies were performed in accordance with the HSG and STOWA guidelines (Roeleveld & van Loosdrecht 2002; Langergraber et al. 2004). Further descriptions are outlined in Rönner-Holm et al. (2006, 2009). The objectives of the project were the development of control strategies for optimisation and bioaugmentation analysis using SBR WAS to reduce sludge retention time (SRT) and SVI in the continuous section of the WWTP.

Since routine data was insufficient, three intensive (spring, summer, autumn) measuring campaigns were carried out under different influent situation in accordance with Rönner-Holm et al. (2006, 2009). AOB kinetics such as yield, half-saturation coefficient and decay rate were measured according to Petersen et al. (2005). One model was set up for the continuous section and one model for the SBR plant. After optimisation of the treatment performance and operating costs, AOB enrichment in both models and bioaugmentation using SBR WAS in the continuous model with two AOBs using similar and differing kinetics were analysed. Optimisation strategies and bioaugmentation of SBR sludge were implemented stepwise on the plant and characteristics such as effluent values, SVI and operational costs were monitored.

RESULTS AND DISCUSSION

Case study I: bioaugmentation from sidestream to mainstream treatment

The temperature sensitivity (Arrhenius coefficients) of sidestream biomass compared to the mainstream biomass was significantly higher based on AOB activity measurements at 10 and 22 °C. At increasing sludge cycling rates the difference in temperature sensitivity became less significant (Schön et al. in prep.). For modelling the sludge cycle regime between the cold mainstream environment to the high temperature sidestream reactor and reverse, the conventional single Arrhenius term approach was not appropriate. Therefore a two-sided temperature function was developed to consider activity peaks at low temperature for AOB(L) subpopulation and at high temperature for AOB(H). The starting point for this concept was the following question: at temperatures beyond the optimum operating range, does a sharp increase

Figure 3 | Plant schematic providing a recycle of nitrate from the aerobic- to the upstream anoxic compartment at a variable rate (IMLR).

Figure 4 | Weisstal WWTP schematic in normal operation mode (left) and bioaugmentation mode (right).
In AOB decay reduce the net AOB process rate, or does a decline in growth-rate dominate the net decrease in AOB activity?

A prior study on long-term nitrifier decay rates by Salem et al. (2006) could not reveal conclusive results: in activated sludge typical decay rates of 0.2 per day at 20°C under aerobic conditions, and 0.1 and 0.06 under anoxic and anaerobic conditions, respectively, were shown. For laboratory-enriched cultures of nitrifiers much lower decay rates without any temperature dependency were reported while for digested sludge (mesophilic temperature and anaerobic conditions) no remaining nitrifying activity at all was detected. Other nitrifier decay tests based on OUR revealed a significant acceleration at higher temperatures (Slazer 1992: 0.4 at 30°C; Nowak et al. 1994: 0.2 at 20°C and 0.43 at 28°C).

A physiological slow-down in growth at high temperature has been observed, e.g., for Nitrosomonas europaea at 37°C (Beyer et al. 2006). On gene level the temperature optimum for the reaction of hydroxylamin conversion by ammonia monooxygenase is even higher – at 45°C (Hetzelt et al. submitted).

In Figure 5 (left) the conventional Arrhenius approach ($\theta(b) = 1.029$) is shown resulting in exponential net-growth rates which obviously overestimate process rates at high temperatures. A simple concept for improved high temperature behaviour is the superposition with high decay ($\theta(b) = 1.16$; Figure 5, right). The other option is a two-sided growth function using logistic terms in order to reduce the growth rate at excessive temperature. Another approach in the literature uses a quadratic temperature dependency expression combined with an exponential turn-down on high temperatures (e.g. Ratkowsky et al. 1983). Due to the historical acceptance of the Arrhenius term in low temperature wastewater engineering this concept was maintained.

Figure 6 (left) shows growth characteristics of high temperature AOB(H) established in sidestream treatment systems with a steeper slope in the low temperature range and a distinct peak in the high temperature range (function given by Equation (3)). Low temperature AOB(L) show less temperature sensitivity in the low temperature range and only moderate rates at high temperatures and therefore are adapted to cold mainstream conditions. The comparison
of both 2-AOB concepts (Figure 6, right) indicates a very similar shape of net-growth profiles.

A significant difference between the two concepts concerns the mass of AOBs accumulated in each treatment system. In both the sidestream and the mainstream treatment systems almost complete ammonia oxidation is achieved. This means that AOB-accumulation at sufficient SRT and at certain substrate availability does not depend on growth rates but exclusively on the yield coefficient and decay-rates. In the case when assumed decay rates are high (‘high b’-concept; θ(b) = 1.16) the accumulation of AOB biomass will be underestimated especially in the high-temperature SBR. High recycle rates of AOB(L) from mainstream WAS can compensate decay. In the case of low decay assumptions (ASDM default of θ(b) = 1.029) massive AOB-accumulation in the SBR will occur. To match the actual AOB(H)-mass (Figure 7) in the SBR an intermediate decay rate of 1.06 had to be selected.

Since the ‘high-b’ approach could not be applied, the ‘logistic-μ’-concept to reduce the growth-rate at high temperature was chosen as more appropriate. The authors recognize that the function chosen is quite complex, containing a correction factor to the original Arrhenius term both at low and high temperatures. Simpler functions were not able to describe the observed temperature sensitivity. If a simpler, more general function is found in the future that describes the observed behaviour, it could be calibrated to the double-logistic function presented in Equation (3) and Table 2. Kinetic parameters have been selected (Table 2) to match AOB-accumulation simulated by the ASDM whole-plant model and specifically contributions to measured nitrogenous oxygen uptake in the sidestream versus mainstream mixed liquor samples (Figure 7). The parameters of the logistic terms in Equation (3) have been calibrated to match the slope of the function to measured temperature sensitivity of AOB (L) and AOB(H). Ex-situ activity measurements have been performed at high DO and high substrate conditions and therefore half-saturation parameters play a negligible role in this calibration procedure. Model calibration has been validated by the simulation of increasing nitrate production in the SBR at higher cycling rates (Figure 8).

$$R_{growth} = X_{AOB} \cdot \frac{\mu_{max} \cdot \theta \cdot T}{1 + \exp(-L_{step} * (T - L_{cross}))}$$

$$\cdot \frac{1}{1 + \exp(H_{step} * (T - H_{cross}))}$$

$$\cdot F(O_2) \cdot F(NH_4) \cdot F(pH) \cdot F(HNO_2) \cdot F(HCO_3)$$

(3)

Figure 7 | Calculated OUR-contribution by AOB(L), AOB(H) and NOB in the A-stage (left), B-stage (middle) and the sidestream-SBR (right) in comparison with total nitrogenous oxygen uptake NOUR determined by ex-situ tests.

Table 2 | Kinetic parameters of AOB-subpopulation AOB(L) and AOB(H) in all 3 process environments for growth, decay and temperature sensitivity according to Equation (3)

<table>
<thead>
<tr>
<th>Core parameters</th>
<th>Logistic parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>θ(b)</td>
</tr>
<tr>
<td>AOB(L)</td>
<td>0.17</td>
</tr>
<tr>
<td>AOB(H)</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Case study II: The effect of the degree of mixing on AOB selection

The measured nitrifier $\mu_{\text{max}}$ decreased by 15% when the internal recycle rate (IMLR) was increased in a plug-flow tank (Figure 9). This phenomenon can be explained as the selection of growth ($r$- or $\mu$-) strategists in environments where substrate (ammonia in this case) is not limiting (such as the head of plug-flow tanks), and of k-strategists where only low levels of substrate are available.

An extended model with two AOB populations was coded in BioWin. The composite (measurable) growth rate of the plant was well simulated using different $\mu_{\text{max}}$ values ($\mu_{\text{max}} = 0.95$ and $\mu_{\text{max}} = 0.75$/day) for the 2 AOB-subpopulations ‘$\mu$-strategists’ and ‘K-strategists’. Figure 10 demonstrates how the extended model selects AOB populations with lower or higher $\mu_{\text{max}}$ values depending on the IMLR.

Obviously at increasing internal recycle rates (IMLR up to 800%) ammonia concentration gets equalised towards a low level in the range between 0.5 and 3.0 mg/L (Figure 11, right). This low ammonia level throughout the flow-path represents favourable conditions for k-strategists which are adapted to low half-saturation values ($K_{\text{NH3}} = 0.3$ mgN/L compared to 0.7 for $\mu$-strategists). Therefore $\mu$-strategists are out-competed and represent only a share of 34% of the total AOB-population in the simulation at 800% IMLR (Figure 10). The gradual population shift at increasing recycle rates causes a corresponding gradual decrease of the averaged maximum growth-rate (Figure 11, left).

Case study III: bioaugmentation from SBR to parallel continuous flow system

AOB kinetics, calibration and validation of the models

The half-saturation coefficient and decay rate of AOB during measuring campaigns differed slightly depending on the operation mode, influent load and temperature. Whether this corresponds with nitrifying population variations as described by Daims et al. (2001) and Siripong & Rittmann (2007) is still under investigation. Nevertheless, the kinetic model parameters which led to the best match with measured data are shown for the following operation modes in Table 3: SBR plant with 45% influent plus 100% sludge liquor and for continuous plant with 55% influent only. Calibration results under this condition for SBR1 are shown in Figure 12, left.
Enrichment of AOB

The enrichment of AOB using increasing amounts of digester liquor in both models using the kinetics in Table 2 is shown in Figure 12, right. It is obvious that the enrichment of AOB in the continuous plant is higher. In this respect it has to be considered that no sludge liquor was fed into the continuous plant during the measuring campaign whereas the SBR plant was loaded with 100% of the sludge liquor. The measured and adjusted decay rate of AOB in the SBR plant model was higher than that in the continuous plant, probably due to this loading difference, and this implies that the load with sludge liquor might have an effect on AOB kinetics (Table 3). Nevertheless, since a similar growth rate ($\mu_{max}$) was assumed for this analysis, more measurements under different sludge liquor loading situations in the plant are necessary to analyse how this might affect the growth rate of AOB under long term operation and for validation of the measured decay rate ($b_{aerobic}$).

Bioaugmentation

The two AOB model for the continuous plant was used for bioaugmentation analysis. Two situations were investigated. First it was assumed that the AOB kinetics depend on the operation mode and loading situation. Thus the AOB in SBR WAS (X$_{AOBS}$) should have similar kinetics (here K1) once transferred compared to the original AOB of the continuous plant (X$_{AOBC}$). In this case the X$_{AOBS}$ can overgrow X$_{AOBC}$ which is nearly washed out (Figure 13, left), the removal efficiency and operational costs remaining almost unaffected. In a second but worse case, the X$_{AOBS}$ should keep the original kinetic K2 as in the SBR model. As shown in Figure 13, right, the X$_{AOBS}$ and X$_{AOBC}$ can coexist, which has a slight effect on the effluent values but operational costs are lower. Unfortunately, with

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### Table 3 | AOB kinetics used in the models

<table>
<thead>
<tr>
<th>Kinetic</th>
<th>No.</th>
<th>$\mu_{max}$</th>
<th>Y$_a$</th>
<th>$b_{aerobic}$</th>
<th>$K_{ass}$</th>
<th>$K_{DO}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous plant</td>
<td>K1</td>
<td>1</td>
<td>0.24</td>
<td>0.04</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>SBR plant</td>
<td>K2</td>
<td>1</td>
<td>0.24</td>
<td>0.20</td>
<td>0.7</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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*Figure 11* | Averaged $\mu_{max}$ according to the composition of AOB subpopulations depending on IMLR (left) and corresponding ammonia concentration profiles in a sequence of tanks along the flow path (right).

*Figure 12* | Comparison of simulated and measured online data SBR1 of SBR plant (left) and enrichment of AOB with increasing amounts of sludge liquor in continuous plant with kinetic K1 and SBR plant kinetic K2 from Table 2 (right).
bioaugmentation of SBR WAS, heterotrophic and inert fractions increase in the continuous plant, yielding lower SRT, therefore the amount of total AOB is lower no matter which kinetics were used.

**Reduction of MLSS and sludge retention time (SRT)**

In a third investigation, the effect of the AOB amount and performance was analysed when MLSS was reduced in the continuous plant. Without bioaugmentation, AOB in the continuous section was washed out at MLSS concentrations lower than 1 g/L (Figure 14). With bioaugmentation, $X_{AOB}$ from SBR WAS stayed available even at MLSS of 0.5 g/L (Figure 14) and $N_{total}$ effluent values were nearly in the range of monitoring values at 12 °C, which indicated sufficient nitrification activity.

### Evaluation of success

The SBR WAS (with stable SVI in a SBR plant of ~80 mL/g) has been fed into the continuous plant influent instead of wasting it into the digester since July 2008. The SVI in the continuous plant has been reduced from 170 to ~110 mL/g after approx. five weeks. This very clearly demonstrates that pure SVI bioaugmentation is possible, though this impact is not described by state-of-the-art models. We assume one reason for this successful bioaugmentation is that both biological systems have been adapted to the same wastewater. If the SBR biology were treating completely different wastewater (for example only sludge liquor with or without external carbon sources), a similar bioaugmentation success would be uncertain. During bioaugmentation operation of the plant no drastic changes in the treatment performance or operation costs have been reported up to now. Therefore decreasing MLSS in stages in the continuous plant will be performed to analyse the effect of reduction of SRT.

### CONCLUSIONS

There are widely accepted experiences that the kinetics of nitrifying organisms vary with different reactor configurations and process conditions. However, there are two potential answers on how parameters change when biomass is transferred from one reactor environment to the other:

1. Kinetic parameters belong to the biomass and are maintained during transfer.
2. Kinetic parameters are bound to the process and transferred biomass switches parameters.
In this paper three different case studies have been presented which follow concept 1 requiring two different AOB populations with different parameter sets. This new model approach can explain observed parameter differences as an AOB population shift induced by selection under specific process conditions. In addition, this paper has introduced bioaugmentation and sludge recycling as a process engineering tool to interfere in the competition of different AOB-subpopulations. In other words, a 2-AOB-model can be used to develop strategies and designs to improve nitrification capacity.

The comparison of model behaviour of both concepts clearly demonstrated the limitation of using only one AOB population. Procedures for calibrating growth and decay have been affected by identifiability issues as discussed for the comparison of the ‘high-b’ and the ‘logistic-μ’ concept of case study 1. Thus it is recommended that kinetic tests be performed to determine both μmax and bA parameters and their temperature sensitivities.

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