Comparison of bench scale testing methods for nitrifier growth rate measurement

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Abstract
The maximum specific nitrifier growth rate was determined for two wastewater treatment plants (WWTPs) using sequencing batch reactors (SBRs) and high F/M exponential growth batch tests. Higher nitrifier growth rates were obtained from the exponential growth batch tests. Operating SRT and aeration mode (fully aerobic versus anoxic/aerobic) significantly impacted the nitrifier’s growth rate in the SBRs with lower SRT and anoxic/aerobic operation resulting in higher specific nitrifier growth rates.

Keywords
Nitrifier growth rate; wastewater characterization

Introduction
As an increasing number of wastewater treatment plants are required to achieve nutrient removal, the ability to accurately predict the nitrification capacity of an existing facility becomes critical in order to optimize biological nitrogen removal. In combination with the wastewater inherent factors such as the diurnal loading and the wastewater characteristics, the nitrifier growth and decay rates will determine a facility’s nitrification performance for a given set of plant operating conditions.

Traditionally, the maximum specific nitrifier growth rate, $\mu_A^{\text{MAX}}$ is believed to be the the primary kinetic parameter (Henze, 1987; Randall, 1992; Barker and Dold, 1997) that needs to be measured in order to determine a WWTP’s nitrification capacity and permit development of nitrification process configuration alternatives. When activated sludge models are used, as is typically the case today, calibration for $\mu_A^{\text{MAX}}$ and solids production is considered adequate for satisfactory model performance. This approach to modeling of the nitrification process has resulted in the development of various methods for the determination of the value of $\mu_A^{\text{MAX}}$ so that the calibrated model can be used to simulate wastewater treatment plant performance. The use of different methods has created a large database of values that vary by as much as an order of magnitude.

In this study four bench scale protocols were used for measurement of the maximum nitrifier growth rate:
1. long-term concurrent operation of 6 L oxic sequencing batch reactors (SBRs) using 12 hour cycles at Solids Retention Times (SRTs) of 8 and 20 days at 20°C,
2. long-term operation of SBRs operated in an anoxic/oxic, and in a fully oxic mode at an SRT of 15 days at 20°C,
3. operation of an SBR in a multiple-feed/multiple-cycle mode intended to simulate the operation of the step feed nitrogen removal process (ongoing) at an SRT of 15 days, and
4. batch nitrifier exponential growth experiments at low mixed liquor concentrations (Antoniou et al., 1990; Stensel, 1992; Sozen et al., 1996) using the SBR biomass.
Experimental setup and methods

SBR setup and operation

The SBRs were operated for a sufficient time to acclimate the biomass to the wastewater and achieve pseudo steady-state conditions (5–10 SRTs). Subsequently, kinetic studies were performed to measure the nitrification rate, and thus calculate $\mu_A^{\text{MAX}}$. Table 1 outlines the operating conditions for the SBRs used in this study to measure $\mu_A^{\text{MAX}}$ in several New York City WWTPs, which treat weak, primarily domestic, wastewater (COD~180 mg/L, TKN~20 mg/L).

Each SBR was fed with composite primary settling tank effluent from the WWTP, which was collected three times a week using an ISCO 6700 refrigerated automatic sampler (ISCO, Lincoln, Nebraska USA). The samples were trucked to CCNY and stored at 3–4°C. Prior to feed, the appropriate volume was removed and equilibrated to 20°C. The mixed liquor volume wasted from each SBR was calculated daily, taking into account the SS in the decant, to maintain the target SRT. DO was monitored and controlled between 4.5 and 5.5 mg/L using a DO Controller (Model 01972, Cole-Parmer, IL, USA) and automated oxygen uptake rate (OUR) data collection system (DasyLab, CT, USA). The pH was controlled between 7.1 and 7.3 with a pH Controller (Model 05652, Cole-Parmer, IL, USA) utilizing a concentrated solution of sodium bicarbonate (5 gr/L). Mechanical stirrers were used to assure continuous suspension of the solids during the react period.

Pseudo steady-state conditions were considered to be established when full nitrification of the ammonia was achieved, and the MLSS concentration and nitrogenous OUR stabilized (approximately 2–3 SRTs, barring any major disruptions). During the stabilization period, influent and effluent samples were analyzed three times a week for COD$_T$, TSS, NH$_3$-N and NO$_X$-N and TKN$_T$. Alkalinity and RBCOD in the influent were also measured once a week. In addition, effluent NO$_X$-N, effluent TSS and reactor MLSS samples were analyzed on a daily basis. All analyses were conducted at the City College of New York Environmental Engineering Laboratory in accordance with Standard Methods (20th Edn).

Once the SBRs reached pseudo steady-state, an intensive sampling and analysis period was initiated in order to perform the nitrogen balance around the reactor and collect sufficient data to determine the nitrifier growth rates. During the intensive sampling period, daily additional analysis of COD$_T$ and COD$_S$, RBCOD, TKN$_T$ and TKN$_S$, NH$_3$-N and NO$_X$-N, on the influent and effluent were carried out to facilitate the nitrogen mass balance. The TKN concentration in the MLVSS was also measured.

Four kinetic experiments ("sampling events") were then carried out on each reactor over a 1 to 2 month period. Each of the kinetic experiments was conducted over a 3-day period covering six consecutive cycles. One batch of approximately 60 litres of PSTE was used from each of the respective WWTPs during this 3-day period to ensure that the feed for

<table>
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<tr>
<th>Table 1</th>
<th>SBR reactor configuration and operating mode</th>
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<tr>
<td>Reactor configuration</td>
<td>Operating mode</td>
</tr>
<tr>
<td>Reactor Volume: 6.0 L</td>
<td>Aeration Cycle: Fully Aerobic – 11 hrs</td>
</tr>
<tr>
<td>Feed Volume: 4.5 L</td>
<td>Anox/Aerob. – 2 hr Anox./9 hr Aerob.</td>
</tr>
<tr>
<td>SBR Cycle Time: 12 hrs</td>
<td>Aerobic Stage DO: 5.0 mg/L</td>
</tr>
<tr>
<td>React Period: 11 hrs</td>
<td>pH: 7.2–7.4</td>
</tr>
<tr>
<td>Settling Period: 0.667 hrs (40 min)</td>
<td>Alkalinity Source: 5 gr/L NaHCO$_3$</td>
</tr>
<tr>
<td>Decant Period: 0.250 hrs (15 min)</td>
<td>Mixing: Continuously Stirred</td>
</tr>
<tr>
<td>Idle Period: 0.083 hrs (5 min)</td>
<td>SRT: 100% Aerobic: 8, 15 and 20 days</td>
</tr>
<tr>
<td>Feed Period: 0.083 hrs (5 min)</td>
<td>Anoxic/Oxic: 15 days</td>
</tr>
<tr>
<td>SRT Control: Eff. TSS + Manual Wasting Once Every Second Cycle</td>
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</table>
all 6 cycles was from the same sample batch. Profiles of NH$_3$-N and NO$_X$-N were then obtained on alternate cycles. The profile was started at the beginning of the cycle and consisted of collecting 20 ml samples from the SBRs, which were filtered and acidified for later analysis of NH$_3$-N and NO$_X$-N using flow injection analysis (Lachat QuickChem 8000, WC, USA). Samples were collected at 20-minute intervals for the first two hours and at 30-minute intervals for the third hour. DO, pH, and temperature were recorded during sample pickup and alkalinity was measured at the start and end of the profile. The oxygen uptake rate was automatically recorded.

Operating data quality was verified on each SBR by running an inert suspended solids balance. On the fully oxic SBRs a nitrogen balance was also conducted. The BioWin dynamic activated sludge modeling package, based on the General Model (Barker and Dold, 1997) was used to model the operation of each SBR. Usage of the computer model permitted accounting of the variations in influent COD and nitrogen concentrations, as well as reactor MLSS that were observed during the course of the study. The model output was compared to the actual operating data and the non-biodegradable particulate fraction of the influent’s COD was varied as the primary solids production calibration parameter until a good correlation between actual and model mixed liquor concentrations was obtained. The effluent model NO$_3$-N was then compared to the actual effluent NO$_X$-N data. The calibration was accepted when no bias in either the reactor MLSS or decant NO$_X$-N concentrations were observed. The only parameter requiring modification to achieve this, was the non-biodegradable particulate fraction of the influent COD.

The concentration of the sludge components as a function of time, from the model output, was retained and used when modeling the corresponding nitrification profiles obtained during the kinetic experiments. For each SBR, $\mu_A^{MAX}$ was calculated for two nitrifier decay rate values, the General Model default decay rate of 0.04 d$^{-1}$ and the experimentally determined value.

### Nitrifier decay rate measurement

The decay rate was measured for the SBR mixed liquor using the protocol outlined by Lesouef et al. (1992). A portion of the SBR mixed liquor was removed from the SBR and placed in a separate reactor (Decay Reactor) within the environmental chamber (20°C). The nitrifying sludge was maintained without an external ammonia source for 2–5 days. During this period mixed liquor subsamples were removed from the Decay Reactor, spiked with ammonia to a concentration of approximately 10 mgN/L and the nitrification rate was measured. The protocol was modified in that multiple measurements of the decaying biomass’s nitrification rate were obtained over the course of 2–5 days (Siegrist et al., 1999) and direct measurement of NOx-N and NH$_3$-N was used instead of oxygen uptake rates. The pH and dissolved oxygen was controlled at 7.3 ± 0.1 and 5.0 ± 0.3 mg/L, respectively, in both the Decay Reactor, as well as the nitrification rate measurement reactor using a setup similar to that used for the SBRs. A combination of CO$_2$ and NaHCO$_3$ was used to maintain a stable pH.

Assuming exponential decay with negligible nitrifier growth in the decay reactor, the nitrification rate, $R_N$, as a function of time, will follow the equation:

$$R_N(T) = \frac{d(NOx-N)}{dH} = \mu_A^{MAX} \frac{X_A(T)}{Y_A} = \mu_A^{MAX} \frac{X_A^{INL}e^{(-b_A T)}}{Y_A} = \mu_A^{MAX} \frac{X_A^{INL}}{Y_A} e^{(-b_A T)}$$

(1)

where $b_A$ is the nitrifier decay rate (d$^{-1}$), $X_A$ is the nitrifier concentration (mg VSS/L), and $Y_A$ is the nitrifier yield (mg VSS/mg N utilized). Linearizing the above equation:

$$\ln R_N(T) = \ln \left( \mu_A^{MAX} \frac{X_A^{INL}}{Y_A} \right) - b_A T$$

(2)
the nitrifier decay rate was thus obtained as the slope of the natural log of the nitrification rate versus time.

A certain amount of ammonia is liberated during the course of the decay experiment due to endogenous biomass activity and the hydrolysis of particulate TKN to soluble TKN and finally ammonia. This is reflected by the slow increase in NOX-N concentrations in the Decay Reactor during the course of the experiment. Where necessary, the decay rate was obtained by modeling the decay experiment using the General Model (Barker and Dold, 1997). This allowed accounting for the change in nitrifying biomass due to the nitrification of the small quantities of ammonia liberated from the hydrolysis of the particulate matter.

Exponential batch nitrifier growth experiments

Exponential growth batch tests were conducted using biomass from the fully aerobic SBRs. The low VSS batch test was conducted using the procedure guidelines outlined in Antoniou et al. (1990) and Sozen et al. (1996). Two litres of filtered (0.45 µm) primary effluent were added to a small volume of mixed liquor of known VSS concentration, such that the mixture had an initial MLVSS of 40–80 mg/L. Filtration of the primary effluent was deemed necessary to avoid seeding from nitrifiers that may be present in it. The pH and DO was controlled in the batch reactor. DO was controlled at 5 ± 0.3 mg/L with automated intermittent air addition. A 1 g/L NaHCO₃ solution was utilized to maintain the pH at 7.3 ± 0.1. Addition of CO₂ was necessitated when the pH increased above the maximum allowable of 7.4. The reactors were placed in an environmental chamber, where temperature is controlled at 20 ± 0.2°C. A 10 gN/L NH₄Cl solution was used to intermittently spike the reactor with ammonia, such that the ammonia concentration was in the range of 10–20 mgN/L. Continuous mixing was provided by a magnetic stirrer. A 1 cm thick piece of foam between the bottom of the beaker and the magnetic stirrer eliminated heat transfer from the stirring mechanism to the batch reactor contents. Each experiment was typically simultaneously conducted in duplicate.

Each reactor was sampled at 1 to 3 hour intervals for NH₃-N and NOx-N, over a 55 to 72 hour period in order to develop a well defined exponential growth curve. The time, reactor temperature and pH was recorded at each sampling interval. This was a modification of the method as applied by Antoniou and Sozen who sampled the batch reactor once or twice per day for 3–5 days. The purpose of this modification is to permit better definition of the exponential growth curve, and allow calculation of the initial nitrifier concentration. The MLSS and MLVSS were measured in the first and last sample. Data analysis was conducted using the non-linear parameter estimation method utilized by Sozen (1996). The experimental data, NOx-N(t), was fitted to the exponential equation:

\[
[NO_X - N](t) = [NO_X - N]_{INI} - \frac{\mu_A^{MAX} X_A^{INI}}{(\mu_A^{MAX} - b_A)Y_A} + \frac{\mu_A^{MAX} X_A^{INI}}{(\mu_A^{MAX} - b_A)Y_A} e^{(\mu_A^{MAX} - b_A)it} \tag{3}
\]

such that a best fit curve was obtained. This permitted calculation of the net growth rate (\(\mu_A^{MAX} - b_A\)). Reproducibility of the method was evaluated by conducting simultaneous duplicate measurements using the same primary effluent and equal volumes of the same seed sludge. The increased sampling, relative to the original protocols, as defined by Antoniou et al. (1990) and Sozen et al. (1996), allowed estimation of the initial nitrifier concentration, \(X_A^{INI}\) in the batch reactor. This was obtained by solving equation (1) for \(X_A^{INI}\):

\[
X_A^{INI} = \frac{[NO_X - N](t) - [NO_X - N]_{INI} e^{(\mu_A^{MAX} - b_A)it}}{\frac{(\mu_A^{MAX} - b_A)Y_A}{\mu_A^{MAX}}} \tag{4}
\]
Using the General Model default value for nitrifier yield, $Y_A$, and the experimentally determined decay rate for the seed source SBR, the initial nitrifier concentration in the batch reactor, and thus the concentration in the seed source was estimated (Figure 1). However, using this technique for estimation of the initial nitrifier concentration proved difficult, as the NOx-N data must be of excellent quality. Scatter in the data, particularly in the first 24 hours of the batch experiment significantly impacted our ability to confidently calculate $X_A^{INI}$.

Results and discussion

The maximum specific nitrifier growth rates obtained from the fully oxic SBRs operated at 8 and 20 days SRT are shown in Table 2. Each data point constitutes the average of three nitrification rate measurements over a 3 day period, as previously described. Comparison of the growth rates calculated using the General Model (Barker and Dold, 1997) default decay values, shows the 8 day SRT SBR consistently exhibiting a higher nitrifier growth rate. For the measured SBR nitrifier decay rate, the higher than model default decay rates further exacerbated the difference. A lower than average growth rate was observed during the fourth sampling event in both reactors, suggesting the possible presence of a nitrifier inhibitor in that day’s influent wastewater.

In Figure 2 the specific nitrifier growth rates obtained from the exponential growth batch experiments that were conducted in parallel to the SBR sampling events are shown, along with the $\mu_A^{MAX}$ values calculated for the SBRs, using the measured nitrifier decay rates. For the exponential batch tests, the reported maximum specific growth rates were

![Figure 1](https://iwaponline.com/wst/article-pdf/46/1-2/289/476960/289.pdf)  
Figure 1 Typical batch nitrifier net growth rate and initial nitrifier concentration measurement

![Table 2](https://iwaponline.com/wst/article-pdf/46/1-2/289/476960/289.pdf)  
Table 2 Comparison of calculated maximum specific nitrifier growth rates from fully aerobic SBRs operated at 20°C and SRT of 8 and 20 days (WWTP I)
calculated by adding the measured decay rate to the net specific growth rate obtained from the batch test. Each exponential growth batch test data point (B1 and B2) in Figure 2 represents the average of two parallel measurements. The growth rates observed in the exponential growth batch tests are significantly higher than the rates determined using the fully aerobic SBRs, even after the values obtained using the SBRs were adjusted upwards due to the higher than model default nitrifier decay rate.

The operating SRT may thus affect the culture’s nitrifier growth rate, with a higher growth rate occurring at the lower SRT. One possibility is that operation at the higher SRT allowed accumulation of less active nitrifiers in the culture that would otherwise have been washed out at the lower SRT. The higher growth rates measured using the exponential growth batch test may be a result of reduction or elimination of (1) diffusional limitations and (2) competition for nutrients between nitrifiers and heterotrophs within the floc, as no visible floc was formed during the course of the exponential growth batch test. Furthermore, the soluble organic substrate in the weak NYC wastewater’s filtered primary effluent was expended within the first few hours of the experiment, as attested by the reduction in oxygen uptake rate (data not shown) 5 to 6 hours into the experiment.

**Fully oxic versus anoxic/oxic SBR**

In the next phase of the study an anoxic/oxic SBR was run in parallel with a fully aerobic SBR. Both reactors were run at a SRT of 15 days. The nitrifier decay rate was measured twice during the 4 months the reactors were operated and was determined to be 0.09 d\(^{-1}\) for

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**Table 3**  Comparison of calculated maximum specific nitrifier growth rates from fully aerobic versus anoxic/oxic SBR operated at 20°C and SRT of 15 days (WWTP II)

<table>
<thead>
<tr>
<th>Samp. event</th>
<th>Aerobic SBR</th>
<th>Anoxic/Oxic SBR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model default decay ((b_a = 0.04) d(^{-1}))</td>
<td>Measured decay ((b_a = 0.09) d(^{-1}))</td>
</tr>
<tr>
<td>WI1</td>
<td>0.48</td>
<td>0.75</td>
</tr>
<tr>
<td>WI2</td>
<td>0.45</td>
<td>0.66</td>
</tr>
<tr>
<td>WI3</td>
<td>0.60</td>
<td>0.90</td>
</tr>
<tr>
<td>WI4</td>
<td>0.42</td>
<td>0.62</td>
</tr>
<tr>
<td>Aver.</td>
<td>0.49</td>
<td>0.73</td>
</tr>
</tbody>
</table>

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**Figure 2**  Comparison of \(\mu_A^{\text{MAX}}\) values measured during SBR Sampling Events E1 and E2 and the exponential growth batch experiments (B1 and B2) using the respective SBR’s sludge for seed
both SBRs. The nitrifier growth rates were significantly higher in this phase of the study (Table 3), as wastewater from a different NYC WWTP was used. Furthermore, the SBR that was operated in the anoxic/oxic mode consistently exhibited a higher nitrifier growth rate than the fully aerobic SBR.

Conclusions
Care must be exercised in selecting the appropriate bench scale test for determining nitrifier growth rates for BNR processes. Utilization of data obtained from batch nitrifier growth test experiments at low MLVSS concentrations may result in a significant over prediction of nitrification capacity in a WWTP, as the test conditions do not reflect activated sludge operating conditions. However, with further refinement the low MLVSS batch tests may be useful for estimating the concentration of nitrifiers in a WWTP’s sludge. Long-term SBR operation at conditions (SRT and anoxic/oxic ratio) approximating the WWTP is preferred. The nitrifier decay rate, \( b_A \), should be measured, and reported along with the specific nitrifier growth rate value, as the combination of the two will impact both data analysis, and the ability to extrapolate the growth rate to predict performance as a function of SRT.

References