DENITRIFICATION WITH CARBON ADDITION – KINETIC CONSIDERATIONS

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ABSTRACT

The Blue Plains AWTP uses methanol as an external carbon source in a post-denitrification process to achieve low effluent total nitrogen concentrations. This becomes more difficult in winter at lower mixed liquor temperatures and higher flows, as a consequence of the kinetic behavior of the methanol-utilizing heterotrophs. The paper reports on an experimental batch test study conducted on Blue Plains post-denitrification sludge to investigate (a) the maximum specific growth rate of methanol-utilizing heterotrophs (μ_{METH}); (b) the temperature dependency of the growth rate; and (c) the efficacy of alternate substrates (ethanol, acetate, sugar). A limited number of tests were conducted on sludge from two other treatment plants with methanol addition.

KEYWORDS

Denitrification, methanol, ethanol, acetate, sugar, SDNR, kinetics, maximum specific growth rate.

INTRODUCTION

In nitrogen (N) removal activated sludge systems incorporating unaerated zones (or phases) heterotrophic organisms utilize nitrate in place of oxygen as the terminal electron acceptor in the utilization of organic substrate. The nitrate is converted to nitrogen gas (denitrification), reducing the effluent N in the discharge to the receiving water body. In cases where low effluent nitrogen limits must be achieved common practice is to supplement the amount of organic material in the influent by adding organic substrate to the denitrification process. Methanol is the most widely used substrate. Research indicates that methanol added for denitrification is utilized by particular heterotrophic organisms (*Hyphomicrobium* spp.) (Sperl and Hoare, 1971; Carrera *et al*, 2003). Therefore, knowing the kinetic parameters (and stoichiometry) of these organisms is important for the design and optimization of N removal systems with methanol addition. However, measurement of the kinetic parameters (maximum specific growth rate, decay rate, half-saturation coefficient, etc.) has received very little attention.

The Blue Plains Advanced Wastewater Treatment Plant operated by the DC Water and Sewage Authority (DCWASA) is a large-scale facility with methanol addition for denitrification. This essentially is a two-sludge system as shown in Fig. 1. Primary effluent is first treated in a high

rate non-nitrifying (short SRT) system designed for BOD removal (known as the Secondary Stage). The secondary effluent from this system (containing ammonia, very little biodegradable organic material, and approximately 20 mgTSS/L of solids from the high-rate system) is then treated in a nitrification-denitrification suspended growth system with its own final clarifiers (known as the Nitrification Stage). Figure 2 shows a schematic of the nitrification-denitrification system. The system consists of 5 zones in series, with the possibility of stepfeed to the first two zones. In usual operation the first two and the last zones are aerated for growth of nitrifiers; the third zone is unaerated, principally to allow stripping of dissolved oxygen; and methanol is added to the unaerated fourth zone for denitrification. Ferric salts are added to the system for chemical phosphorus (P) precipitation to meet stringent P discharge limits. Mixed liquor solids are a mix of chemical sludge, nitrifiers, methanol-utilizing heterotrophs, endogenous residue, and a limited amount of 'ordinary' heterotrophs. Presumably the contribution to denitrification by the 'ordinary' heterotrophs is limited because no soluble, readily biodegradable substrate is provided for their anoxic growth.

Under typical summer conditions the Blue Plains AWTP achieves low effluent total nitrogen values (approximately 4 to 6 mgN/L). However, it becomes more difficult to attain these 'limit of technology' (LOT) levels in winter when mixed liquor temperatures decrease to 13°C and anoxic retention time is reduced due to higher flows. Therefore a detailed understanding of the kinetics of denitrification is important for optimization of the plant.

Figure 1 – Schematic of the Blue Plains AWTP

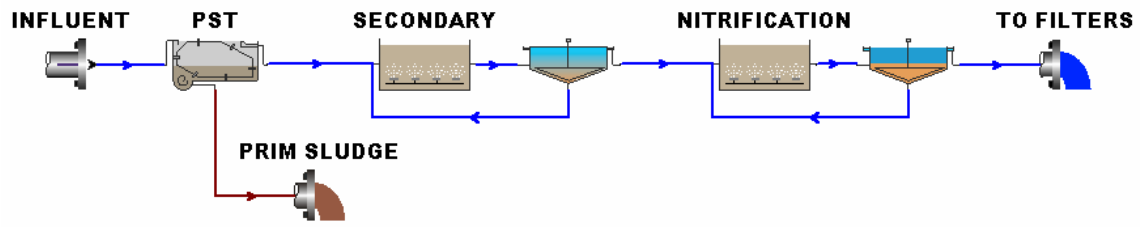
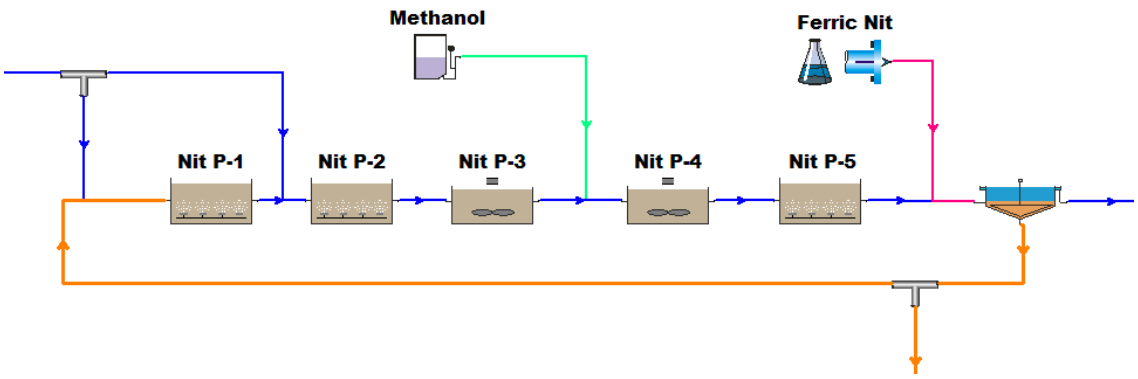


Figure 2 – Schematic of the Blue Plains AWTP 'Nitrification' Stage



Kinetics of the methanol-utilizing heterotrophs (methylotrophs) obviously is crucial for the performance and optimization of the N removal system. A range of questions can be posed regarding the N removal behavior in such a system; for example:

1. With denitrification by methanol, if the methanol flow is increased, will there be increased denitrification? This essentially depends on the maximum specific growth rate of the methanol-utilizing denitrifiers (μ_{METH}). A given μ_{METH} value sets the capacity of the methanol-utilizers to use methanol within the anoxic retention time provided. If the μ_{METH} value is such that methanol is only completely used when the flow exits the anoxic zone, then adding more methanol will not lead to more denitrification. The extra methanol will merely flow out of the anoxic zone unused. This situation will be exacerbated either (a) at low temperatures because the μ_{METH} decreases with decreasing temperature, or (b) at high-flow conditions when the retention time is reduced.

2. What is impact of temperature on μ_{METH}?

3. Is the growth rate of the methanol-utilizers low enough so that there should be a concern about maintaining a sufficiently long anoxic SRT to avoid washout? This is analogous to the concept of a minimum aerobic SRT for nitrification.

4. Are the methanol-utilizers able to use substrates other than methanol in the aerated zones?

5. What is the decay rate of the methanol-utilizers (b_{METH}), and does this differ for aerated and anoxic conditions?

6. If a second soluble substrate (e.g. acetate) is added together with methanol, will the contribution to denitrification of the 'ordinary' heterotrophs reduce the effluent nitrate-N substantially? Alternatively, will there be a slow response while the population of acetate-utilizing heterotrophs is established?

This paper presents results from an experimental batch test study initiated by DCWASA in late 2004 to investigate these factors. This involved conducting two types of batch test over a range of temperatures (10 to 25°C) as the plant mixed liquor temperature has changed:

- **Specific denitrification rate (SDNR) batch tests:** Undiluted mixed liquor from the Nitrification Stage was spiked with nitrate, and a carbon source was added (methanol, ethanol, acetate, sugar). The linear decrease in nitrate concentration was monitored over approximately 6 hours, and the SDNR was calculated from the slope divided by the reactor VSS (mgN/gVSS/hr). SDNR tests were conducted to provide insights on whether mixed liquor from the Nitrifcation Stage (i.e. denitrifying biomass generated from growth on methanol) will utilize alternative substrates.

- **MuMax batch tests**: A batch test method was conducted for estimating the maximum specific growth rate of methanol-utilizing heterotrophs (μ_{METH}). This was a slight

modification of the method proposed by Dold *et al.* (2005). The test protocol was also applied to measuring maximum specific growth rates for biomass utilizing other carbon sources (ethanol, acetate, sugar).

The majority of the data presented here are from tests conducted on sludge from the Nitrification Stage of the Blue Plains AWTP; that is, on sludge from a system where essentially the only carbon input is methanol. A limited number of tests were conducted on sludge drawn from two other plants in the area: (a) the Alexandria Sanitation Authority (ASA) WWTP, a single-sludge plant with methanol addition to a secondary anoxic zone, and (b) the Western Branch WWTP, a three-sludge configuration where methanol is added to a third stage following BOD removal and nitrification stages.

METHODOLOGY

Maximum Specific Growth Rate (μ_{METH}) Test

The bioassays (MuMax tests) were conducted in purpose-built cylindrical (20 cm tall; 13 cm diameter) plexiglass closed vessels placed on a magnetic stirrer plate. The whole apparatus stood in a temperature-controlled chamber.

At the start of the test a small volume (approximately 60 mL) of mixed liquor from the anoxic zone of the full-scale plant was added to 1.6 L of Nitrification Stage clarified effluent. This resulted in an initial batch reactor solids concentration of approximately 50 mgVSS/L. Potassium nitrate (KNO_3) was added to provide an initial concentration of approximately 100 mgN/L. Ammonium chloride (NH_4Cl) and potassium dihydrogen phosphate (KH_2PO_4) were added to provide initial nutrient concentrations of 20 mgN/L and 20 mgP/L. Nitrogen gas was bubbled through the liquid to strip dissolved oxygen. At time zero substrate (methanol, ethanol, acetate or corn syrup) was added to an initial concentration of approximately 600 mgCOD/L. A tight-fitting cover was placed over the reactor, and ORP and pH probes were inserted into the liquid through sealed openings.

The batch tests typically ran for periods of 48 to 72 hours. During the tests ORP and pH were monitored regularly. To offset the pH increase due to denitrification, a few drops of 5M sulfuric acid were added manually at intervals to maintain pH in the range 7.0 to 7.3. The method for pH control was changed during the project; a manually-adjusted flow of CO_2 gas was bubbled through the reactor contents continuously. Samples were withdrawn at intervals of 2 to 3 hours, filtered immediately, and analyzed for nitrate and nitrite concentrations. Very little nitrite accumulation was observed in the tests.

At the start of the project tests were conducted in an open reactor. Seemingly adequate measures to prevent oxygen input were taken; for example, avoiding vortex formation and entrainment of air bubbles into the mixed vessel, and covering essentially 95 per cent of the liquid surface with styrofoam. However, it became apparent that oxygen ingress into the test container was a potential problem. This interfered with the anoxic growth behavior, leading to a reduced apparent growth rate. Hence the modification to include sparging with nitrogen gas and

maintaining a nitrogen blanket on the liquid surface, and monitoring of ORP.

Simulated Batch Test Response

In essence simulation was used to design the test procedure and evaluate the factors controlling the response in the test. This provided useful insights into the method, and was used to decide on volumes of mixed liquor to use, initial concentrations of nitrate and substrate, and so on. The activated sludge model applied in the simulations included a separate population of methanol-utilizing heterotrophs, with the attendant kinetic and stoichiometric parameters.

Simulation allowed rapid assessment of features impacting the batch test behavior, including:

- Initial VSS concentration;
- Initial nitrate concentration;
- Initial methanol concentration;
- Acid addition requirements to control pH to say 7;
- SRT of the system from which sludge is withdrawn;
- Amount of methanol added to the system from which sludge is withdrawn (as this controls the methanol-utilizer fraction of the VSS added to the test);
- Maximum specific methanol-utilizer growth rate (μ_{METH});
- Yield coefficient for the methanol-utilizing heterotrophs (Y_M);
- Methanol-utilizer decay rate (b_{METH});
- Methanol-utilizer half-saturation coefficient ($K_{S,METH}$).

Figures 3 and 4 show an example of the respective nitrate (mgN/L) and methanol (as mgCOD/L) concentration responses in a simulated batch test. The phase of exponential growth when methanol is available in excess is very evident from the shape of the nitrate response. The methanol response is directly related to the nitrate response through the yield coefficient, Y_M. The methanol concentration remains constant once nitrate is depleted.

Figure 3 – Simulated Nitrate Response in Batch Test

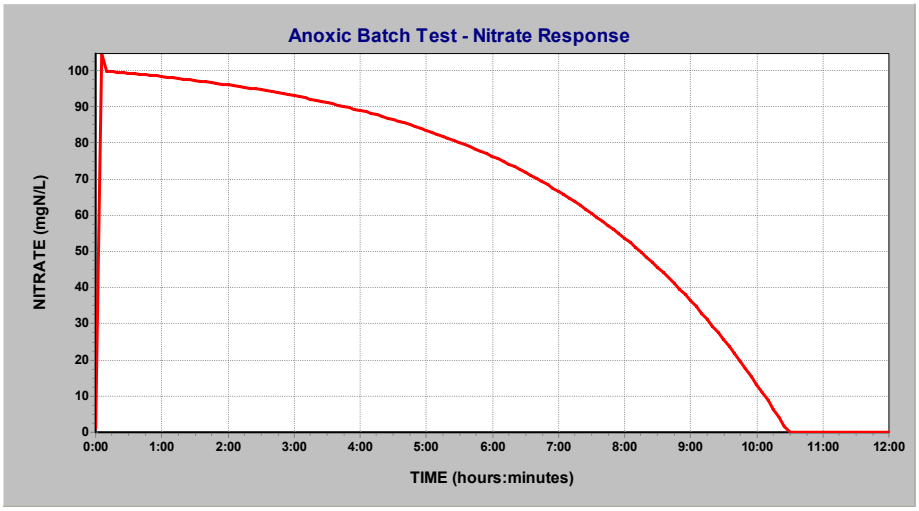
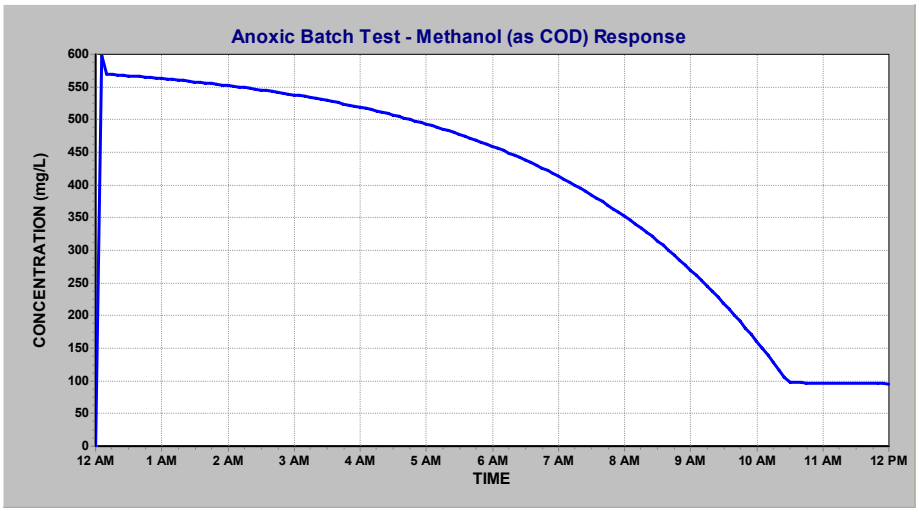


Figure 4 – Simulated Methanol Response in Batch Test



Estimating the Maximum Specific Growth Rate (μ_{METH})

In the bioassay, a relatively low concentration of mixed liquor from an activated sludge plant with methanol addition for denitrification is spiked with nitrate and methanol, and the decrease in nitrate concentration with time is monitored. The basis for estimating the maximum specific growth rate of the methanol-utilizing heterotrophs (μ_{METH}) from the nitrate response in the test is based on a few simple principles:

- The methanol concentration (S_{METH}) in the batch test is high enough (relative to the half-saturation coefficient, $K_{S,METH}$) to ensure that the methanol-utilizer growth rate is at the maximum. That is:

$$\mu = \mu_{METH} \frac{S_{METH}}{K_{S,METH} + S_{METH}} \approx \mu_{METH} \qquad (1)$$

- The initial methanol-utilizer concentration is small, and the rate of growth of methanol-utilizers during the test is exponential.
- The amount of methanol added at the start of the test is in excess of the amount required to denitrify all the nitrate.
- pH and alkalinity are controlled such that growth is not limited by these factors.
- Ammonia and phosphate is added to ensure that growth is not nutrient limited.
- Experimental error in the measured nitrate values should be small relative to the change in nitrate concentration during the test.

At the start of the batch test, with a non-limiting methanol concentration, the nitrate (S_{NO}) utilization rate is:

$$\frac{dS_{NO}}{dt} = -\frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH}}{Y_M} \cdot X_{METH} \qquad (2)$$

The change in biomass concentration, X_{METH}, is the result of growth and decay of methanol-utilizers:

$$\frac{dX_{METH}}{dt} = \mu_{METH} \cdot X_{METH} - b_{METH} \cdot X_{METH} = (\mu_{METH} - b_{METH}) \cdot X_{METH} \qquad (3)$$

Re-arranging Eq. 3 and integrating from time zero to time t:

$$\frac{dX_{METH}}{X_{METH}} = (\mu_{METH} - b_{METH}) \cdot dt$$

$$\int_0^t \frac{dX_{METH}}{X_{METH}} = \int_0^t (\mu_{METH} - b_{METH}) \cdot dt \qquad (4)$$

$$X_{METH,t} = X_{METH,0}\ e^{(\mu_{METH} - b_{METH}) \cdot t}$$

where

$X_{METH,t}$ = methanol-utilizer concentration at time t (mg/L)
$X_{METH,0}$ = methanol-utilizer concentration at time zero (mg/L)
t = time (days)

Substituting Eq. 4 in Eq. 2 and integrating from time zero to time t again:

$$\frac{dS_{NO}}{dt} = -\frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH}}{Y_M} \cdot X_{METH,0}\ e^{(\mu_{METH} - b_{METH})t}$$

$$dS_{NO} = -\frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH} \cdot X_{METH,0}}{Y_M} e^{(\mu_{METH} - b_{METH})t} dt$$

$$\int_0^t dS_{NO} = -\int_0^t \frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH} \cdot X_{METH,0}}{Y_M} e^{(\mu_{METH} - b_{METH})t} dt$$

$$S_{NO,t} = S_{NO,0} - \frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH} \cdot X_{METH,0}}{Y_M \cdot (\mu_{METH} - b_{METH})} e^{(\mu_{METH} - b_{METH})t} + \frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH} \cdot X_{METH,0}}{Y_M \cdot (\mu_{METH} - b_{METH})}$$

$$S_{NO,t} = S_{NO,0} - \frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH} \cdot X_{METH,0}}{Y_M \cdot (\mu_{METH} - b_{METH})} \left\{ e^{(\mu_{METH} - b_{METH})t} - 1 \right\} \qquad (5)$$

where

$S_{NO,t}$ = nitrate concentration at time t (mg/L)
$S_{NO,0}$ = nitrate concentration at time zero (mg/L)

The test leads to an estimate of ($\mu_{METH} - b_{METH}$), not μ_{METH} explicitly. For estimating ($\mu_{METH} - b_{METH}$), non-linear regression is used to fit Eq. 5 to observed nitrate data. The effective SRT in the test is very short, so decay does not have a significant impact on nitrifier concentration *per se*. Nevertheless, any error in b_{METH} will bias the estimate of μ_{METH} by the amount of the error in b_{METH}.

The maximum specific growth rate of methanol-utilizing heterotrophs (μ_{METH}) can be estimated from the batch test data by applying the non-linear equation solver functions available in spreadsheet programs such as Microsoft Excel™. The spreadsheet approach (applied to nitrate concentration values at 20 minute intervals in the simulated batch test) is readily implemented as follows:

- The measured nitrate-time data are entered in two adjacent columns as shown in Table 1.
- The predicted nitrate concentration is calculated by formula directly from Eq. 5. That is:

$$S_{NO,t} = S_{NO,0} - \frac{1-Y_M}{2.86} \cdot \frac{\mu_{METH} \cdot X_{METH,0}}{Y_M \cdot (\mu_{METH} - b_{METH})} \left\{ e^{(\mu_{METH} - b_{METH})t} - 1 \right\} \quad (5)$$

- Initial estimates are specified for μ_{METH} , $X_{N,0}$ and $S_{NO,0}$.
- The spreadsheet solver is applied to minimize the sum of the squares of the residuals by adjusting the three parameters: μ_{METH} , $X_{N,0}$ and $S_{NO,0}$.
- The procedure requires that values are specified for Y_M and b_{METH}. The magnitude of Y_M only impacts the estimate of $X_{METH,0}$; it does not influence the solution for μ_{METH} or $S_{NO,0}$.

Figure 5 shows a fit of the estimated response to the 'observed' (i.e. simulated) nitrated data. The estimated μ_{METH} value from the least squares minimization was 6.29 /day versus the value of 6.40 /day used in the simulation to generate the data. This small discrepancy is due to (a) the simplification in Eq. 1 which excludes the impact of the half-saturation value, and (b) the impact of switching functions and kinetic-pH factors in the simulation model.

Table 1 - Application of the Spreadsheet Least Squares Minimization Method to the 20°C Data Shown in Figure 3 (concentration units: mgN/L)

t (days)	NO_3 measured	NO_3 predicted	Residual	Residual²
0.014	98.94	98.97	-0.026	0.001
0.028	98.36	98.37	-0.012	0.000
0.042	97.72	97.72	0.000	0.000
0.055	97.02	97.06	-0.041	0.002
0.069	96.25	96.29	-0.032	0.001

0.083	95.42	95.45	-0.024	0.001
0.097	94.51	94.53	-0.015	0.000
…	…	…	…	…
…	…	…	…	…
…	…	…	…	…
0.375	44.09	44.02	0.063	0.004
0.389	38.58	38.46	0.118	0.014
0.403	32.59	32.41	0.183	0.034
0.417	26.06	25.80	0.264	0.070
0.430	18.97	19.13	-0.161	0.026
0.444	11.25	11.33	-0.073	0.005
			Sum Squares:	0.436

Specified parameter:

b_{METH} = 0. 04 d^{-1}

Y_M = 0.40

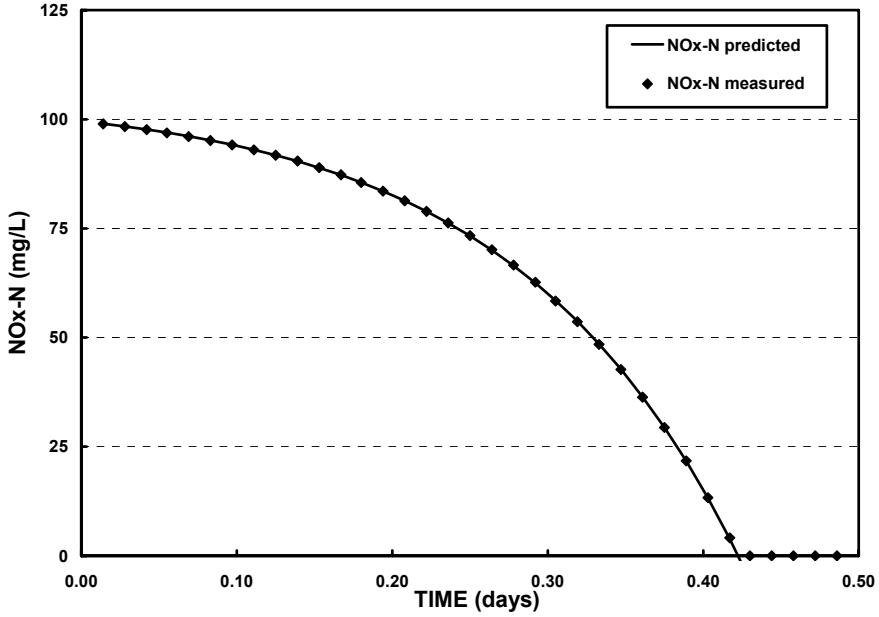
Parameter estimates:

μ_{METH} = 6.29 d^{-1}

$X_{METH,0}$ = 11.38 mgCOD/L

$S_{NO,0}$ = 99.52 mg/L

Figure 5 – Plot of Predicted and Measured Nitrate-Time Data from the Spreadsheet Least Squares Minimization Analysis Method



Estimation of C/N Ratio and Yield (Y_M)

The test can also be used to estimate the C/N ratio for methanol; that is, the amount of methanol required for the removal of nitrate (mgMeOH/mgNO_3-N). This ratio can in turn be used to calculate the yield coefficient for the methanol-utilizing heterotrophs (Y_M). This requires measurement of the COD in filtered samples withdrawn from the batch reactor during the test.

Figure 6 shows a plot of the paired values with COD on the y-axis and nitrate on the x-axis. The slope of the least squares regression line fitted to the experimental data provides an estimate of the C/N ratio for denitrification with methanol addition; that is, the amount of methanol required for the removal of nitrate (mgMeOH/mgNO_3-N). In this (simulated) example the value is 4.73 mgMeOH COD/mgNO_3-N (i.e. 3.15 mgMeOH/mgNO_3-N).

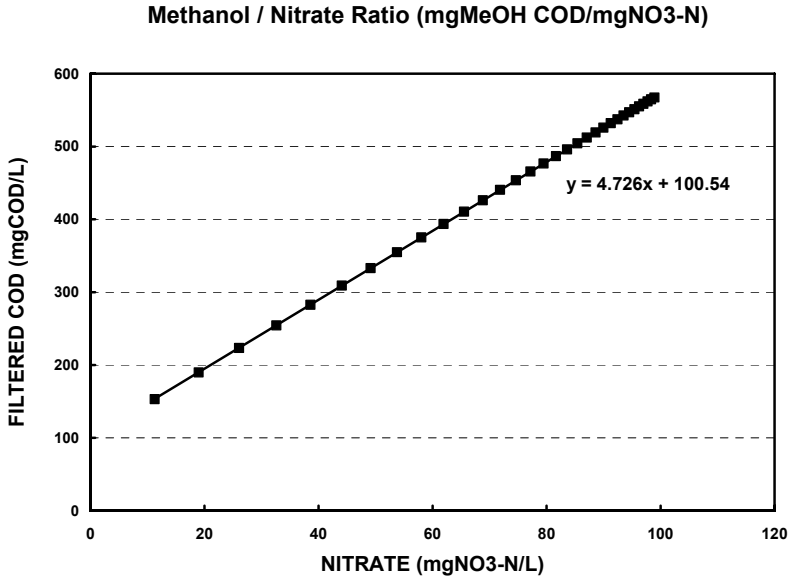
The yield coefficient for the methanol-utilizing heterotrophs (Y_M) can be estimated from the C/N ratio, noting the relationship for the mass of COD utilized per unit mass of nitrate-N denitrified:

$$\mathrm{COD/N} = \frac{2.86}{1 - Y_M}\ \mathrm{mgCOD/mgNO_3 - N} \qquad (6)$$

Re-arranging:

$$\begin{aligned} Y_M &= 1 - \frac{2.86}{\mathrm{COD/N}}\ \mathrm{mgCOD/mgCOD} \\ &= 1 - \frac{2.86}{4.73} \\ &= 0.40\ \mathrm{mgCOD/mgCOD} \end{aligned} \qquad (7)$$

Figure 6 – Plot of Paired Filtered COD and Nitrate-Time Data Measured in the Batch Test



Specific Denitrification Rate (SDNR) Tests

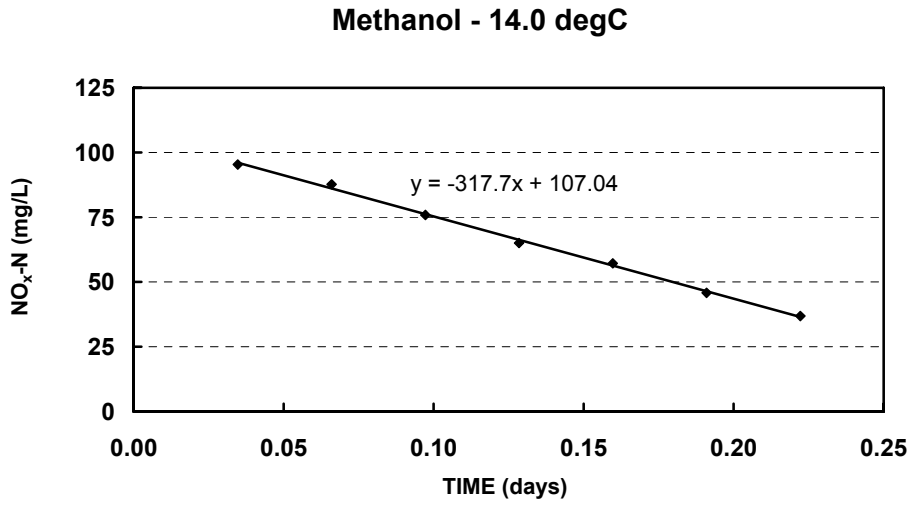
A series of specific denitrification rate (SDNR) batch tests were also performed. The tests were conducted in a similar manner to the μ_{METH} batch tests excepting that undiluted plant mixed liquor was used so that the initial concentration of mixed liquor was much higher (approximately 2,000 mg/L). As a result, the decrease in nitrate concentration is linear, and far more rapid than in the MuMax tests, so tests were conducted over a period of approximately 6 hours. The SDNR is reported with units of mg NO_3-N/gVSS/hour as per Eq. 8. SDNR tests were conducted with methanol, ethanol, acetate and sugar as the substrate. Figure 7 shows an example of the linear nitrate response observed in an SDNR test with methanol addition.

$$SDNR(mgNO_3 - N/gVSS/hour) = \frac{Slope\ of\ NO_3 - N\ response}{VSS} \quad (8)$$

where

VSS = VSS concentration in SDNR test (g/L)

Figure 7 – Nitrate Response in an SDNR Test at 14.0°C – Methanol as Carbon Source



RESULTS AND DISCUSSION

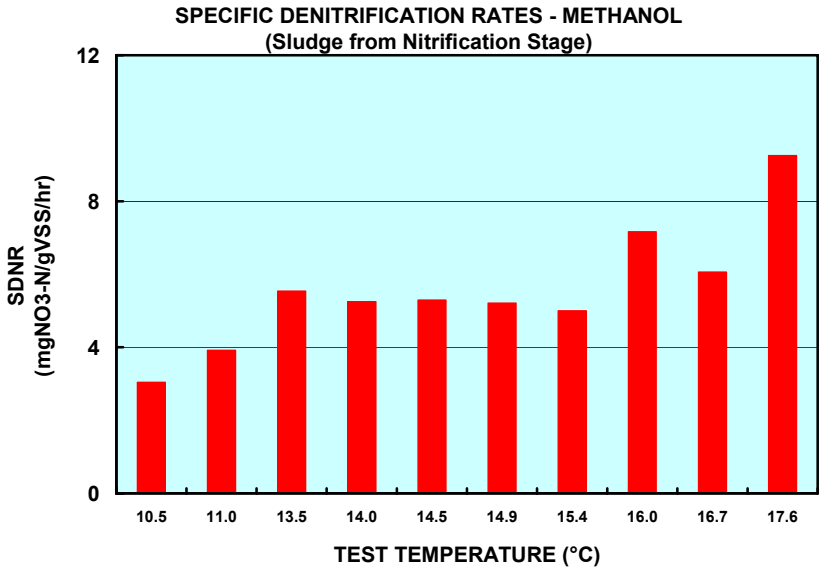
Specific Denitrification Rate (SDNR) Tests

Specific denitrification rate (SDNR) batch tests were performed using mixed liquor from the Blue Plains Nitrification Stage (i.e. denitrifying biomass generated from growth on methanol) over a period when the plant mixed liquor temperature varied between 13 and 20°C. Tests were conducted in a laboratory incubator at the same temperature as that in the full-scale plant at the time of sampling. An example of a test response with methanol as the added substrate is shown

in Fig. 7.

Figure 8 shows the results of ten SDNR tests (units of mg NO_3-N/gVSS/hour) where the added substrate was methanol. The temperature in the test is recorded along the x-axis; however, note that this is not a linear temperature scale. Also, it should be recognized that the data were collected over a period of months (as the plant temperature changed) so changes in factors such as plant SRT, amount of methanol added to the plant, and hence the methanol-utilizer fraction of the VSS may also have changed, obscuring the true temperature dependency. Nevertheless, the limited data set indicates a relatively strong temperature dependency. The temperature dependency was investigated more definitively through the MuMax batch tests.

Figure 8 – SDNR Test at 14.0°C – Methanol as Carbon Source



A limited number of SDNR batch tests were performed using mixed liquor from the Blue Plains Nitrification Stage but with substrates other than methanol: ethanol (two), acetate (three) and sugar (two). Also, two tests were conducted with a mix of equal amounts (COD basis) of methanol and ethanol. The objective here was to investigate whether adding a second soluble substrate together with methanol, will contribute substantially to denitrification by the 'ordinary' heterotrophs in this denitrifying biomass generated from growth on methanol. If so, this could be a strategy to reduce the effluent nitrate during winter months at Blue Plains.

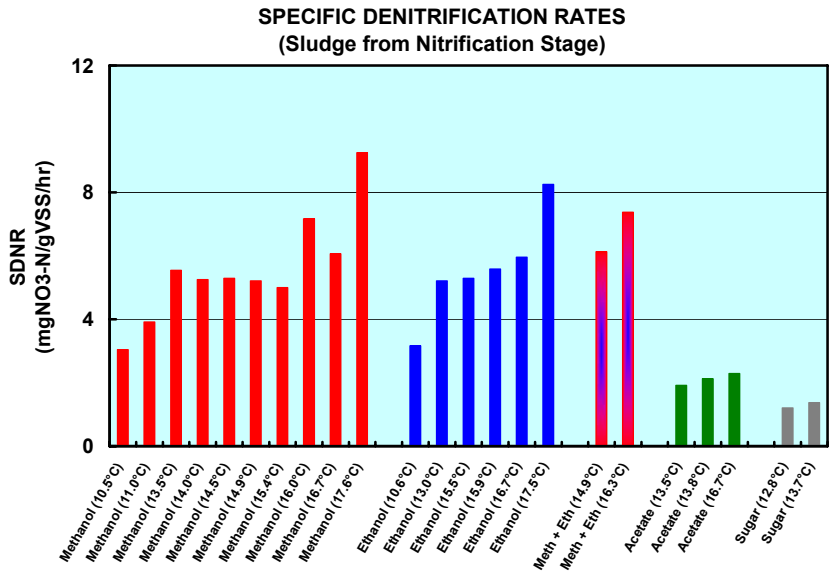
Figure 9 shows the SDNR results for these tests together with the methanol data from Fig. 8. The substrate and test temperature are recorded along the x-axis; again, note that this is not a linear temperature scale. Two significant results are evident from the data in Fig. 9:

- It appears that ethanol essentially is used as easily and at a similar rate to methanol (by the methanol-adapted sludge) at a given temperature. This observation appears in conflict with the 'methylotroph concept'; that is, that biomass grown on methanol can

only utilize methanol as substrate. Evidently the organisms grown on one-carbon methanol are able to adapt rapidly to two-carbon ethanol. This observation is only of academic interest in terms of a winter strategy at Blue Plains. The similar rates with methanol and ethanol, and a mix of the two, will not result in an immediate improvement in denitrification performance during winter.

- The SDNR tests with acetate and sugar showed very low values compared to methanol. This confirms the idea that the methanol-grown organisms cannot utilize acetate or sugar (at least not immediately). Apparently acetate and sugar are utilized by the limited number of 'ordinary' heterotrophs in the mixed liquor of the Nitrification Stage at Blue Plains; hence the low SDNR values. Adding acetate/sugar to the full-scale plant will not result in an immediate increase in denitrification. Therefore, adding acetate or sugar to the plant in winter will not result in an immediate improvement in denitrification performance.

Figure 9 – SDNR data for different carbon substrates



MuMax Batch Tests with Methanol (for estimating μ_{METH})

Twenty two MuMax batch tests for estimating the maximum specific growth rate of methanol-utilizing heterotrophs (μ_{METH}) where conducted over a period when the Nitrification Stage mixed liquor temperature varied between 10 and 25°C. A limited number of tests were conducted on sludge drawn from two other plants in the area: (a) the Alexandria Sanitation Authority (ASA) WWTP, a single-sludge plant with methanol addition to a secondary anoxic zone, and (b) the Western Branch WWTP, a three-sludge configuration where methanol is added to a third stage following BOD removal and nitrification stages.

Figure 10 shows an example of the nitrate response in a batch test conducted at 12.8°C (the temperature of the mixed liquor in the Nitrification Stage at the time of the test). In this test the initial TSS (from adding a small volume of Nitrification Stage mixed liquor) was approximately 300 mg/L. Figure 11 shows the pH response in the test. Periodically a few drops of 5M sulfuric acid was added to reduce pH.

There is limited curvature in the nitrate response data in Figure 7. This makes for difficulties in estimating the μ_{METH} value using the non-linear least squares method. However, at the start of the test there is a significant rate of decrease in nitrate concentration; this indicates that a significant portion of the biomass is methanol -utilizer biomass. In the example shown earlier (Fig. 5 for the simulated test) the nitrate response is far more exponential, and therefore the μ_{METH} value can be estimated with greater confidence. The μ_{METH} value estimated from the data in Fig. 10 (12.8°C) was only 0.56 /day. This low value was a surprising result, even when accounting for the likely temperature dependency.

More curvature in the nitrate response (for improved μ_{METH} estimation) could be induced by decreasing the amount of seed sludge. Figure 12 shows the nitrate response in a test where the initial seed concentration was approximately 100 mgTSS/L (and the temperature was higher - 25°C). With the low seed concentration the initial decrease in nitrate is very slow, but the overall curvature is far more significant.

Figure 10 – Nitrate Response in a Batch Test at 12.8°C – Methanol as Carbon Source

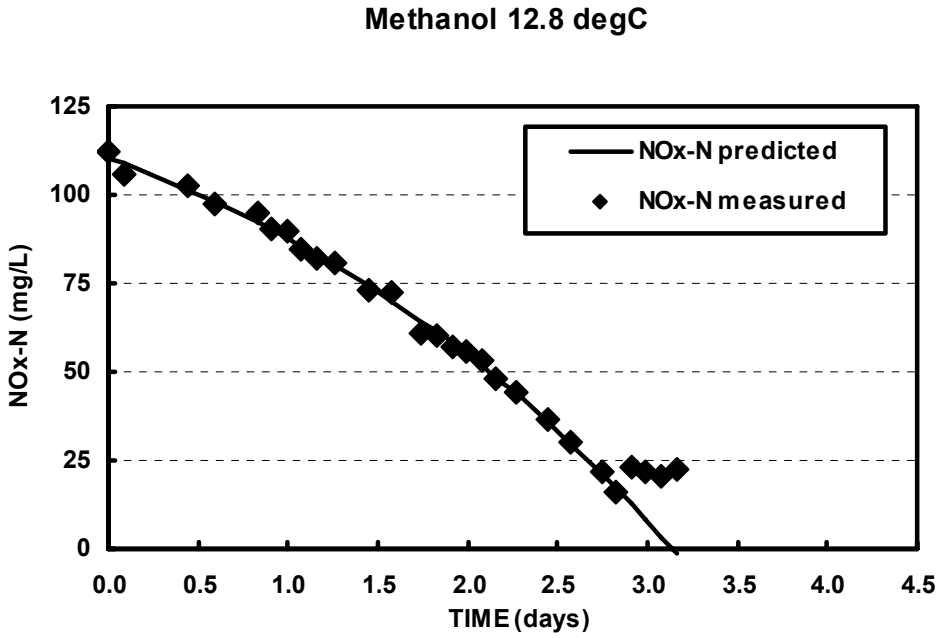


Figure 11 – pH Response in the Batch Test at 12.8°C – Methanol as Carbon Source

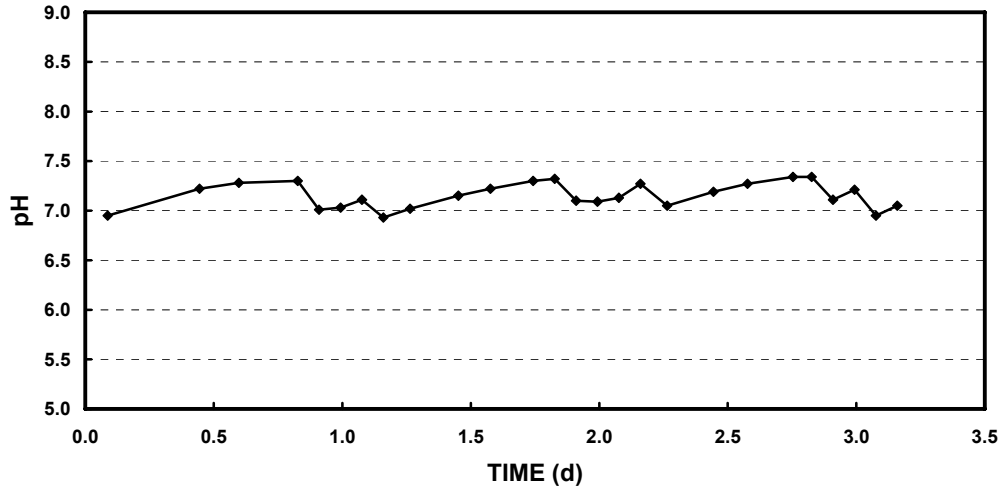


Figure 12 – Nitrate Response in a Batch Test at 25°C – Methanol as Carbon Source

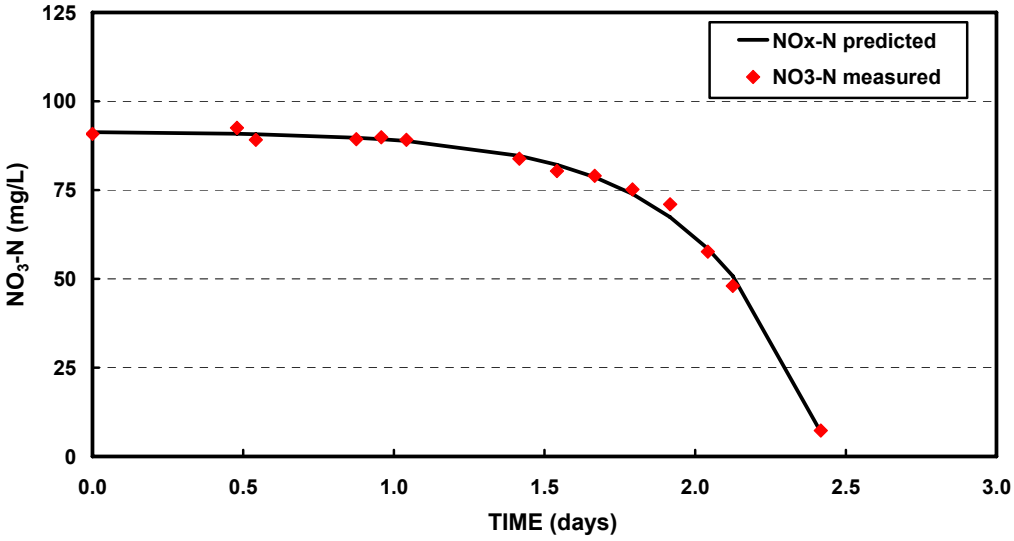
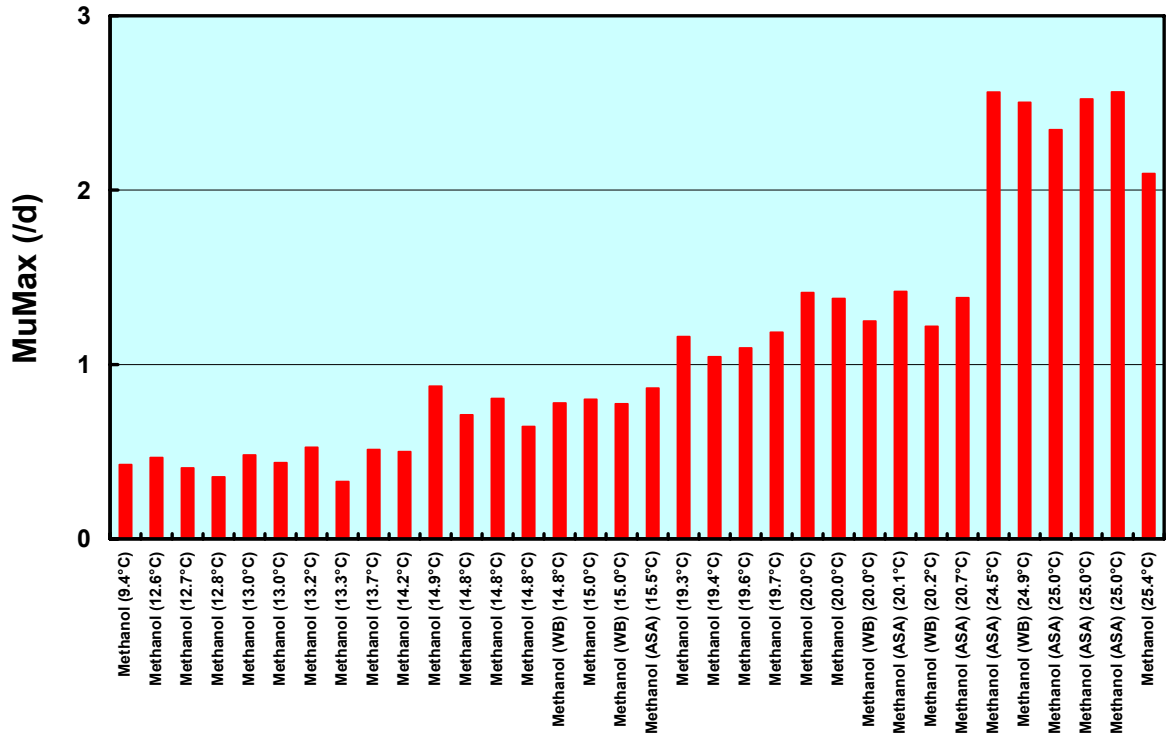


Figure 13 shows the results of thirty four MuMax batch tests conducted with methanol over a range of temperatures from approximately 10 and 25°C. Again, it should be noted that this is not a linear temperature scale. The data include 22 tests conducted with Blue Plains Nitrification stage sludge, 7 tests with ASA sludge, and 5 tests with WB sludge. The data are shown in one set to illustrate that the maximum specific growth rates from tests with sludge from the other two plants with methanol addition can be grouped together with the Blue Plains data. Presumably this implies that the same methanol-utilizing biomass is growing in each plant.

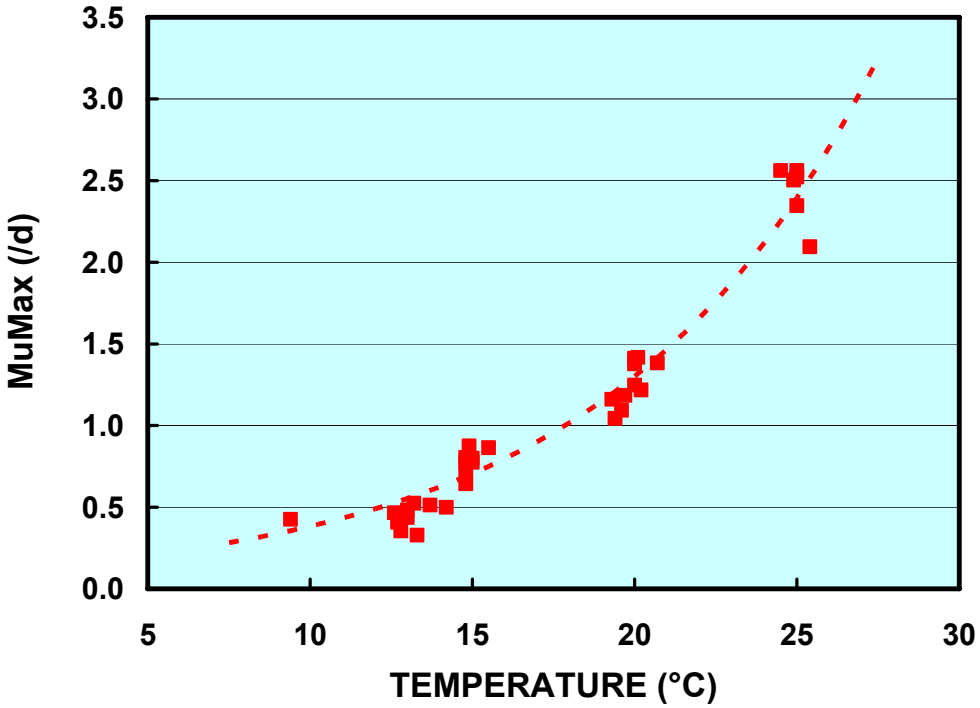
Figure 13 – Summary of MuMax Batch Test Results – Methanol as Carbon Source



igure 14 shows a plot of the μ_{METH} data from the MuMax tests with methanol plotted *versus* temperature on a linear scale. The significant impact of temperature on the maximum specific growth rate is very evident. The dashed line in Fig. 14 is a plot of the van't Hoff-Arrhenius expression typically used to quantify temperature dependency in the models for activated sludge systems. The μ_{METH} at a temperature T ($\mu_{METH,T}$) is referenced to the value at 20°C ($\mu_{METH,20}$), and adjusted via the Arrhenius coefficient, θ, according to Eq. 9. The data indicates a $\mu_{METH,20}$ value of 1.3 /day with an Arrhenius coefficient, θ, of 1.13. The temperature dependency for the methanol-utilizers evidently is more significant than for many other organism groups in activated sludge.

$$\mu_{METH,T} = \mu_{METH,20} \cdot \theta^{(T-20)} \qquad (9)$$

Figure 14 –MuMax Batch Test Results *versus* Temperature – Methanol as Carbon Source

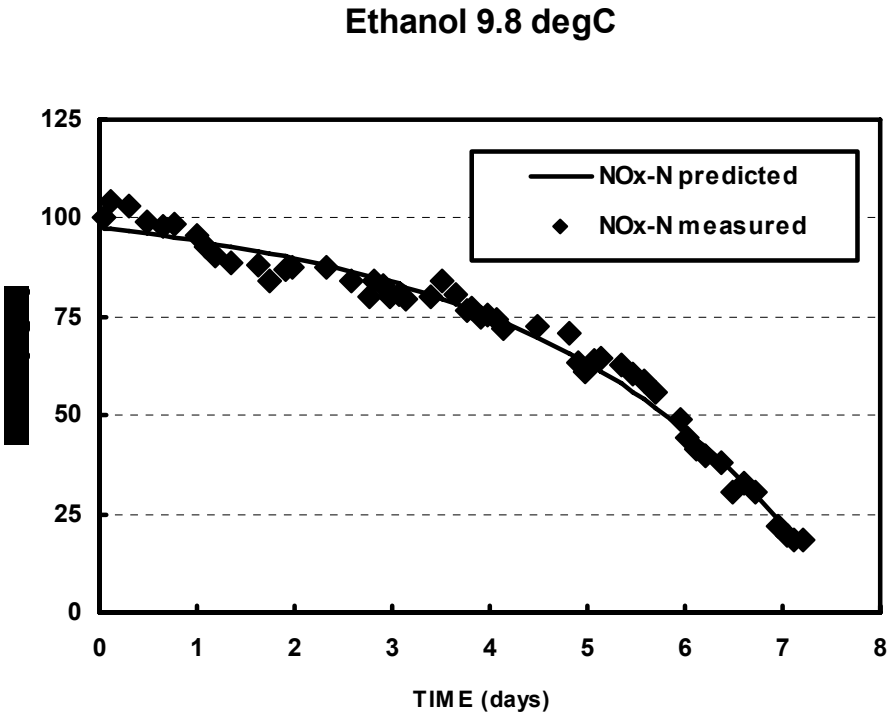


MuMax Batch Tests with Ethanol

Figure 15 shows an example of the nitrate response in a MuMax batch test conducted at 9.8°C (the temperature of the mixed liquor in the Nitrification Stage at the time of the test), but with ethanol substituted for methanol as the carbon source for denitrification. In this test the initial TSS (from adding a small volume of Nitrification Stage mixed liquor) was approximately 300 mg/L. In this case the maximum specific growth rate of organisms utilizing ethanol was 0.37 /day.

The test conditions for the data in Figure 15 (ethanol) and Figure 10 (methanol) were very similar, aside from the ethanol test being at a slightly lower temperature. The similarity in the responses again appears to indicate that the organism in the Nitrification Stage sludge can utilize ethanol as easily as methanol (without any acclimation), and at a similar rate.

Figure 15 – Nitrate Response in a Batch Test at 9.8°C – Ethanol as Carbon Source



MuMax Batch Tests with Acetate and Sugar

A limited number of MuMax batch tests were conducted with acetate (7 tests) or sugar (6 tests) as the carbon substrate. All of these tests were carried out with the Nitrification Stage mixed liquor as the seed, also over a range of temperatures from approximately 10 to 20°C. On the presumption that methanol-utilizing biomass from the seed does not use acetate or sugar, the maximum specific growth rate values estimated from these tests presumably indicate growth rates for the 'ordinary' heterotrophs. Figures 16 and 17 show examples of nitrate responses in tests with acetate and sugar, respectively.

The data from these tests indicate maximum specific growth rate values at 20°C with both acetate and sugar of approximately 4.0 /day, compared to the value of 1.3 /day for the methanol-utilizer organisms. Therefore, denitrification rates in systems where acetate or sugar are used continuously for denitrification (i.e. with sludges grown on these substrates) should be significantly higher than in methanol addition systems.

Figure 16 – Nitrate Response in a Batch Test at 12.8°C – Acetate as Carbon Source

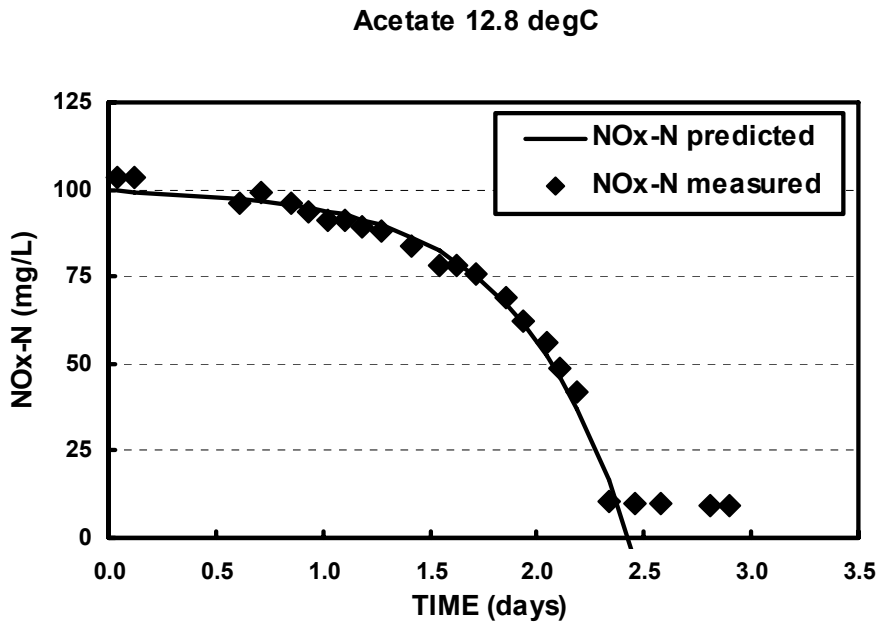
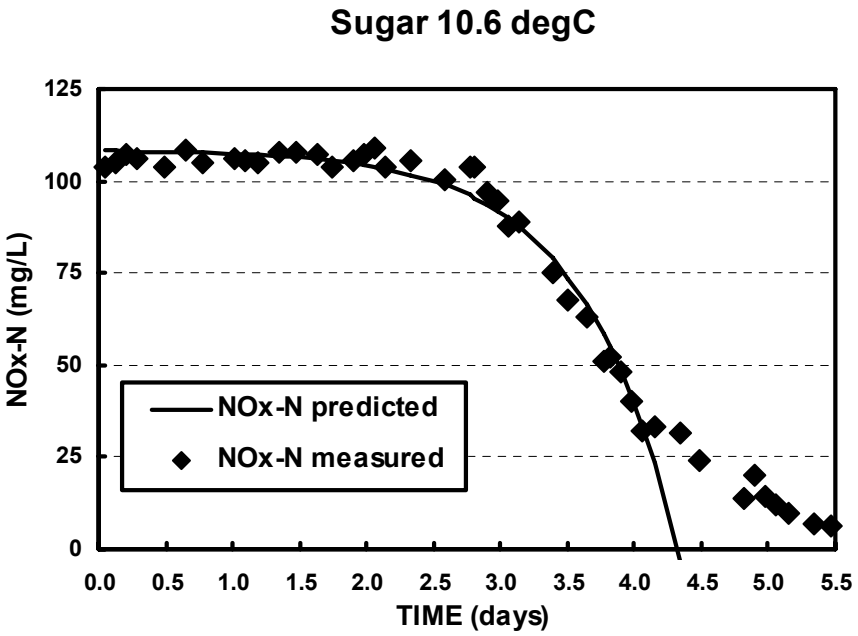


Figure 17 – Nitrate Response in a Batch Test at 10.6°C – Sugar as Carbon Source



CONCLUSIONS

Data from the two types of batch test conducted in this study provide useful insights into denitrification behavior. The testing program was designed primarily to address specific concerns regarding denitrification performance at the Blue Plains AWTP. Nevertheless, the information will be useful to other nitrogen removal plants with external carbon addition or plants evaluating options to reduce effluent TN loads. Quantifying the maximum specific growth rate and the temperature dependency of methanol-utilizers will be very useful where computer simulation models are applied to evaluate and optimize the capacity of nitrogen removal plants.

There were a number of specific conclusions from the study:

- The maximum specific growth rate of methanol-utilizing organisms is quite low; approximately 1.3 /day at 20°C.
- A strong temperature dependency was observed for the maximum specific growth rate of the methanol-utilizers (Arrhenius coefficient of 1.13).
- The low methanol-utilizer growth rate, compounded by the strong temperature dependency, highlights the importance of providing sufficient anoxic SRT to avoid washout in methanol addition systems.
- Ethanol is used as easily and at a similar rate to methanol (by the methanol-adapted sludge); this appears in conflict with the 'methylotroph concept'.
- MuMax tests with methanol and ethanol show similar slow responses (i.e. little curvature) indicating similar, low μ_{MAX} values for methanol- and ethanol-utilizing denitrifiers.
- Methanol-utilizing organisms cannot utilize acetate or sugar (at least not immediately). Adding acetate/sugar to the Blue Plains AWTP together with methanol will not result in an immediate improvement in denitrification.
- The maximum specific growth rates of organisms utilizing acetate or sugar for denitrification are significantly higher than the rate for methanol-utilizers; approximately 4.0 /day at 20°C *versus* the 1.3 /day for methanol-utilizers.

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Authors. Peter Dold and Imre Takács are president and manager of model development, respectively, EnviroSim Associates Ltd. Walt Bailey is Director, Department of Wastewater Treatment at DCWASA and Sudhir Murthy is Manager, Process Development, in the Department of Wastewater Treatment. Much of the experimental work was conducted by three graduate students from George Washington University under the supervision of Dr. Rumana Riffat: Yalda Mokhayeri, Adam Nichols and Jeneva Hinojosa.

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