Examining the influence of substrates and temperature on maximum specific growth rate of denitrifiers

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Abstract Facilities across North America are designing plants to meet stringent limits of technology (LOT) treatment for nitrogen removal (3–5 mg/L total effluent nitrogen). The anoxic capacity requirements for meeting LOT treatment are dependent on the growth rates of the denitrifying organisms. The Blue Plains Advanced Wastewater Treatment Plant (AWTP) is one of many facilities in the Chesapeake Bay region that is evaluating its ability to meet LOT treatment capability. The plant uses methanol as an external carbon source in a post-denitrification process. The process is very sensitive to denitrification in the winter. One approach to improve anoxic capacity utilization is to use an alternative substrate for denitrification in the winter to promote the growth of organisms that denitrify at higher rates. The aim of this study was to evaluate denitrification maximum specific growth rates for three substrates, acetate, corn syrup and methanol, at two temperatures (13 °C and 19 °C). These temperatures approximately reflect the minimum monthly and average annual wastewater temperature at the Blue Plains AWTP. The results suggest that the maximum specific growth rate (μ_{max}) for corn syrup (1.3 d^{-1}) and acetate (1.2 d^{-1}) are higher than that for methanol (0.5 d^{-1}) at low temperature of 13 °C. A similar trend was observed at 19 °C.

Keywords Acetate; corn syrup; denitrification; growth rate; methanol; temperature

Introduction

An important step for nitrogen elimination in wastewater treatment is heterotrophic denitrification. In this process, organic substrates are utilized as electron donors by denitrifying bacteria, and the electron acceptor (nitrate and/or nitrite) in the anoxic reactor is converted to nitrogen gas. Sufficient addition of exogenous carbon source enables nitrate reduction during heterotrophic denitrification (Flere and Zhang, 1999; Zhang and Lampe, 1999; Oh *et al.*, 2002). External sources of organic carbon are often added to the reactor to facilitate the denitrification process by providing energy and carbon to the heterotrophic bacteria. External carbon addition typically is needed to attain low effluent nitrate concentrations because of a deficiency of carbon in the wastewater. The main disadvantage of adding organic substrates is the high cost (Bandpi *et al.*, 1999).

Blue Plains AWTP in Washington, DC has a high rate activated sludge system followed by a suspended growth nitrification–denitrification system. Methanol is used for denitrification, because it is the least expensive synthetic compound available that does not leave a residual biological oxygen demand (BOD) in the process effluent. The process achieves low total nitrogen values in summer, while during winter (January, February and March), the process is more difficult to operate due to increased flows and low temperature, as shown in Figures 1 and 2.

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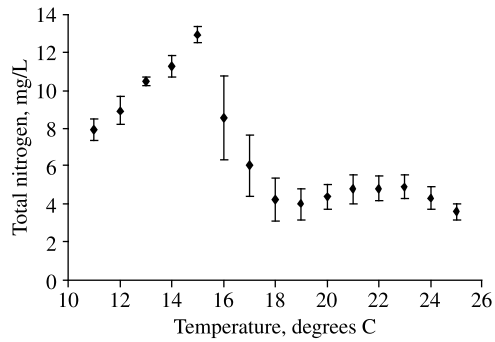

Figure 1 Relationship between temperature and total nitrogen for Blue Plains' effluent (2004 data – 30 day rolling average)

It has been estimated that double the denitrification process volume would be required to achieve the same nitrate levels in winter as in summer. This is mainly a consequence of the impact of temperature on μ_{max}, of the methanol-utilizing organisms. A decrease in μ_{max} decreases the extent of nitrate removal as shown in Figure 3. This results in a gradual wash-out of methanol utilizing heterotrophs. Subsequent reestablishment of these heterotrophic organisms in the spring (March–May) also takes time. Due to the decrease in nitrate removal, the time during winter becomes a point of concern to utilities which are required by regulators to achieve LOT treatment for nitrogen removal (3–5 mg/L total effluent nitrogen). A review of the literature did not reveal any other studies that focused on evaluating denitrifier growth rates for domestic wastewater using methanol or other substrates.

The overall objective of this research was to evaluate the denitrification rates of heterotrophic bacteria using different substrates, e.g. methanol, corn syrup, and acetate. Experiments were performed at warm and cold temperatures to evaluate the impact of temperature on each substrate. A batch test method developed by Dold *et al.* (2005) was employed for estimating μ_{max}. This method is analogous to the High F/M Method for estimating the maximum specific growth rate of nitrifiers (μ_{AUT}) (WERF, 2003). The resulting discussion is based on the kinetic parameters derived from the data obtained through operation of batch reactors.

Methods

Batch reactor set-up

A relatively low concentration of mixed liquor from the denitrification process at the Blue Plains AWTP was used as a seed (50–150 ml) for the batch tests. The seed was

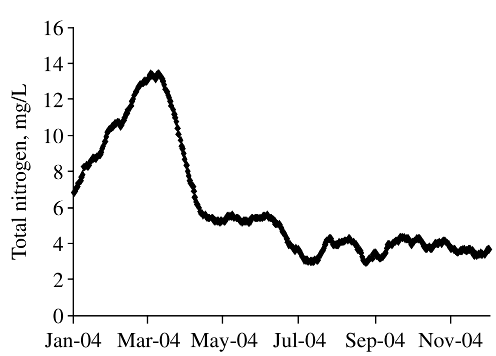

Figure 2 Total nitrogen for Blue Plains' effluent (2004 data – 30 day rolling average)

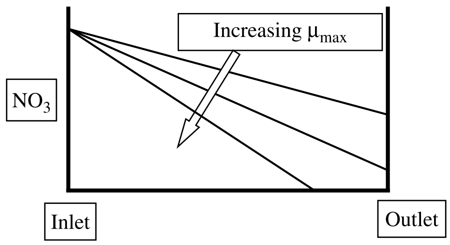

Figure 3 Maximum specific growth rate effects on nitrate removal

drawn from the anoxic zone and diluted with a large volume of clear treated effluent (1,500–1,700 ml). The diluted seed was subsequently spiked with 100 mgN/L of potassium nitrate (KNO_3), 600 mgCOD/L of substrate (methanol, acetate or corn syrup) and nutrients [20 mgN/L of ammonium chloride (NH_4Cl) and 20 mgP/L of potassium dihydrogen phosphate (KH_2PO_4)]. The substrate corn syrup consisted of 78% glucose, 16% fructose, 4% maltose and 2% other sugars. The optimal pH for denitrification falls in the same range as for most heterotrophic bacteria (between 6.5 and 7.5), with the rate decreasing to about 80% of maximum when the pH is lowered to 6.1 or raised to 7.9 (Viessman and Hammer, 1998). Therefore, the reactor pH was manually controlled using aliquots of 93% sulphuric acid and 25% sodium hydroxide to simulate field conditions (pH 6.6–7.0) to maximize the heterotrophic growth rate. pH, DO and temperature were recorded at regular intervals throughout the experiment (2 to 4 days typically). This study was performed in the laboratory reactor illustrated in Figure 4.

Maintenance of anoxic conditions

To maintain anoxic conditions the liquid was stirred at a low intensity to prevent vortexing. A floating Styrofoam lid was used as a barrier for oxygen transfer into the liquid. Nitrogen gas was bubbled in the head-space above the Styrofoam or directly into the liquid, to reduce oxygen transfer when required. Cylinders of 99.9% pure nitrogen gas manufactured by AirGas were used. Data analysis was only conducted for a data range where no DO was recorded. A DO concentration of 0.1 mg/L or above has been reported to inhibit denitrification in dispersed cells (Focht and Chang, 1975; Krul, 1976).

Sampling

Sampling commenced 30 minutes after initiating the experiments. Sampling frequency was maintained at 2–3 hour time intervals. Samples were filtered prior to analysis for nitrate and COD.

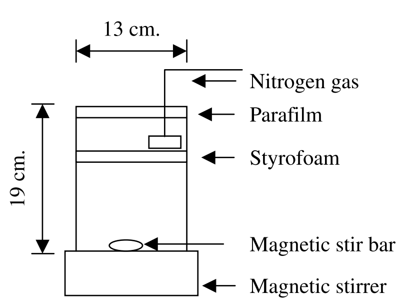

Figure 4 Diagram of reactor

Analyses

Monitored parameters included pH, DO, temperature, nitrate, chemical oxygen demand (COD), initial total suspended solids (TSS), and initial volatile suspended solids (VSS). All parameters were measured according to Standard Methods (1998). The initial mixed liquor suspended solids (MLSS) concentration ranged from 99 to 480 mg/L. The mixed liquor volatile suspended solids (MLVSS) concentration ranged from 82 to 397 mg/L.

Soluble nitrate and COD were tested using the Hach DR4000 Spectrophotometer. All analyses were conducted at Blue Plains Advanced Wastewater Treatment Plant Laboratory in Washington DC.

Maximum specific growth rate

The specific growth rate of heterotrophic biomass in the IWA-type activated sludge models is based on the amount of soluble readily biodegradable substrate concentration according to Monod kinetics. The heterotrophic growth rate (μ_{DENIT}) approaches a maximum value (μ_{max}) when the substrate concentration is considerably larger than the half-saturation coefficient.

$$\mu_{DENIT} \approx \mu_{max} \tag{1}$$

Applying this concept to the experiments, the amount of substrate added to each reactor was in excess of the amount required to denitrify the nitrate.

At the start of the batch test, with a non-limiting substrate concentration, the nitrate (S_{NO_3}) utilization rate is (Dold *et al.*, 2005):

$$\frac{dS_{NO_3}}{dt} = -\frac{1-Y_M}{2.86}\cdot\frac{\mu_{MAX}}{Y_M}\cdot X_{DENIT} \tag{2}$$

where Y_M = yield coefficient, mg biomass COD/mg$NO_3^- - N$, X_{DENIT} = biomass concentration, mgCOD/L, 2.86 = oxygen: nitrate equivalence factor, mgO_2/mg$NO_3^- - N$

The change in biomass concentration is the result of growth and decay of the denitrifiers:

$$\frac{dX_{DENIT}}{dt} = \mu_{MAX}\cdot X_{DENIT} - b_{DENIT}\cdot X_{DENIT} \tag{3}$$

where b_{DENIT} = endogenous decay rate of denitrifiers, d^{-1}

Using equations 2 and 3, and integrating from time zero to time t, equation 4 is obtained (Dold *et al.*, 2005).

$$S_{NO_3,t} = S_{NO_3,0} - \frac{1-Y_M}{2.86}\cdot\frac{\mu_{MAX}\cdot X_{DENIT,0}}{Y_M\cdot(\mu_{MAX}-b_{DENIT})}\left\{e^{(\mu MAX - b_{DENIT})t} - 1\right\} \tag{4}$$

where S_{NO_3},0 = initial nitrate concentration, mg/L, S_{NO_3},t = nitrate concentration after time t, mg/L

A non-linear regression technique was used to fit equation 4 to the observed nitrate response data. For temperature adjustment of decay coefficient, the van't Hoff–Arrhenius model was used with a value of 1.029 for θ. The values of b_{DENIT} and Y_M were selected from the literature. Term b_{DENIT} was estimated to be 0.12 d^{-1} at 20 °C. Y_M was calculated from the relationship with the substrate required for denitrification. This requirement can be considered on the basis of a consumptive ratio, which is the amount of soluble carbon used per unit mass of nitrate reduced. For methanol, the consumptive ratio is 4.7 mgCOD/mg$NO_3^- - N$. The mg COD required/mg NO_3^-N removed for acetate and corn syrup are approximately 3.5 and 4.5, respectively. Using those values, Y_M for methanol utilizing heterotrophs is 0.40 mg biomass COD/mg NO_3^-N. Similarly, the yield coefficient for acetate and corn syrup are considered to be approximately 0.18 and 0.37 mg biomass COD/mgNO_3^--N, respectively. In the analysis procedure the estimate for μ_{max} is independent of the assumed Y value.

Results and discussion

Approximately 50 batch tests were conducted in this study. Only data from experiments at temperatures of 13 °C and 19 °C were selected for analysis here. These temperatures reflect the minimum monthly low temperature and the average annual temperature of Blue Plains AWTP. A total of six experiments were performed at 13 °C using three different substrates. In certain tests, low concentrations of DO were measured at the start of the experiments. In these cases parameter estimation was only applied to data after DO was depleted.

Figure 5 shows results from pairs of tests for each of the three substrates. The shape of the nitrate response curve is primarily influenced by the type of substrate used and the

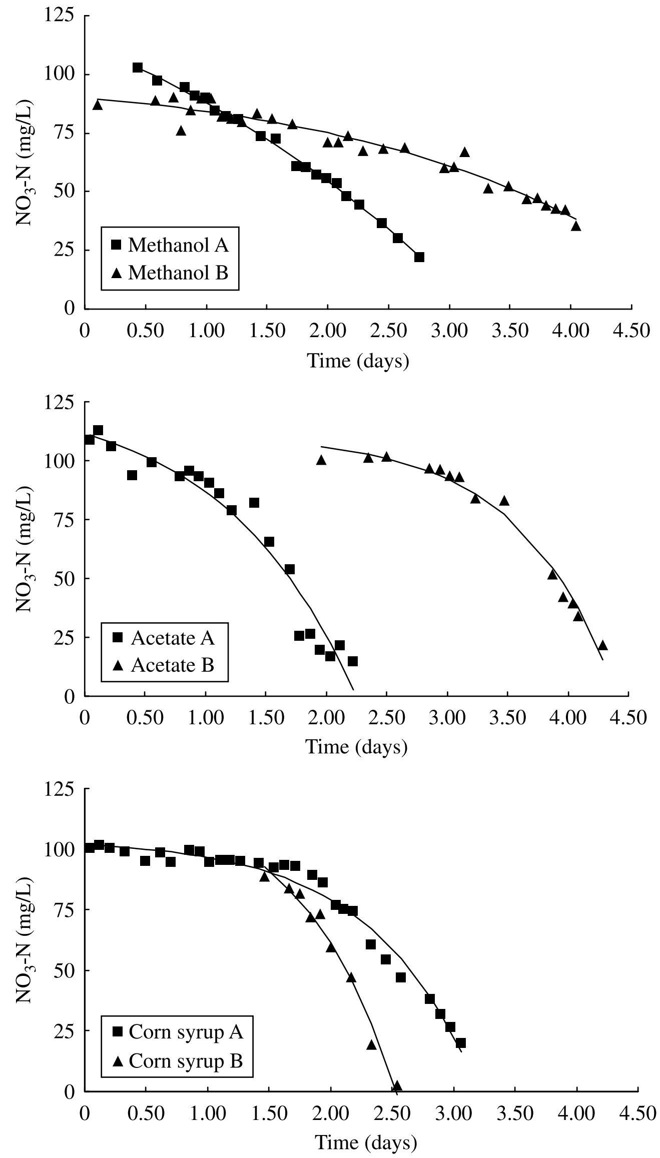

Figure 5 Temporal decrease of nitrate concentration with different substrates at 13 °C

active fraction of the microorganisms utilizing the substrate. Therefore the shape of the curve is influenced by the amount of initial seed. As shown in Figure 5, methanol A has a shorter lag phase, and more rapid decline in nitrate, and consequently a shorter completion time ($\sim$3 d) compared to methanol B. This is directly related to the initial seed MLVSS concentration which was 300 mg/L for methanol A and 117 mg/L for methanol B. The calculated μ_{max} was very similar, 0.4 d^{-1} for methanol A and 0.5 d^{-1} for methanol B despite the differences in curve shapes. The COD/N ratios for methanol A and B were 4.9 and 4.5 mg COD/mgNO_3^--N respectively, close to the theoretical value of 4.7 mg COD/mg NO_3^--N.

Analysis of data from the pair of tests with acetate as substrate resulted in μ_{max} estimates of 1.0 d^{-1} for acetate A and 1.3 d^{-1} for acetate B; COD/N ratios were 3.7 and 3.2 mg COD/mgNO_3^--N, respectively.

Experiments with corn syrup yielded a μ_{max} of 1.3 d^{-1} for corn syrup A and 1.4 d^{-1} for corn syrup B. There were no COD results recorded for corn syrup A. The COD/N ratio for corn syrup B was 4.6 mg COD/mg NO_3^--N.

It is interesting to compare the results of experiments conducted at the same temperature, and with the same amount of initial biomass seed, but with different substrates. Figure 6 compares three experiments performed at 19 °C with methanol, acetate and corn syrup with the same initial seed volume (90 mg/L). It should be noted that the seed was drawn from the full-scale plant with methanol addition. The lag phases for acetate and corn syrup were longer, indicating that very few of the seed organisms can use these substrates. However, the nitrate depletion profiles were much more rapid (more exponential), confirming the higher growth rates compared to methanol.

The μ_{max} and COD/N values obtained for methanol were 1.0 d^{-1} and 4.6 mg COD/mgNO_3^--N, respectively. Results for acetate were 3.7 d^{-1} for μ_{max} and 3.5 mg COD/mg NO_3^--N for COD/N. Corn syrup provided 3.5 d^{-1} for μ_{max} and 4.7 mg COD/mg NO_3^--N for COD/N.

Comparison of μ_{max} for all substrates at 13 °C and 19 °C is shown in Figure 7. Use of acetate and corn syrup generated similar maximum specific growth rates (19 °C) of 3.7 d^{-1} and 3.5 d^{-1}, respectively. Methanol produced a lower μ_{max} of 1.0 d^{-1} at 19 °C. Similarly, at 13 °C, use of acetate and corn syrup generated μ_{max} of 1.3 d^{-1} and 1.2 d^{-1}, respectively. In comparison, the use of methanol generated a μ_{max} of 0.5 d^{-1}. The results show that the use of acetate and corn syrup generate μ_{max} values 2–3 times greater than that for methanol.

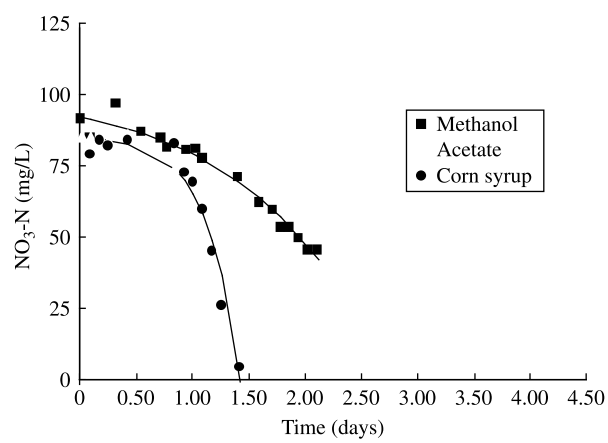

Figure 6 Temporal decrease of nitrate concentration with different substrates at 19 °C

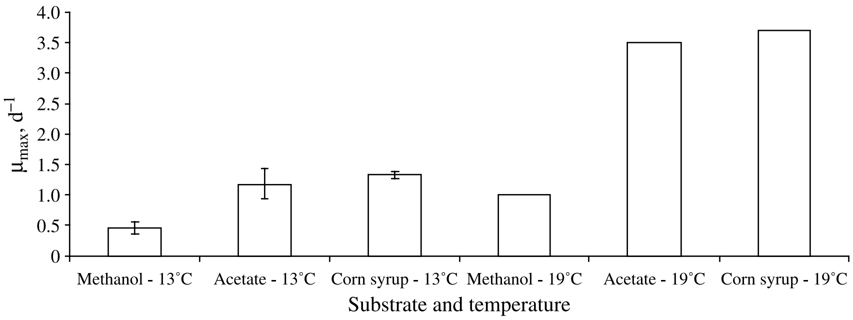

Figure 7 Comparison of μ_{max} for different substrates at 13 °C and 19 °C

Conclusions

- Anoxic capacity requirements substantially depend on maximum specific growth rate of substrate-specific heterotrophic bacteria and the corresponding active fraction of these organisms in the mixed liquor. For winter wastewater temperatures of 13 °C, the μ_{max} for methanol utilizers was found to be approximately 0.5 d^{-1}, the μ_{max} for acetate utilizers was about 1.2 d^{-1} and the μ_{max} for corn syrup utilizers was about 1.3 d^{-1}.
- For wastewater temperature of 19 °C, the μ_{max} for methanol utilizers was found to be approximately 1 d^{-1}, the μ_{max} for acetate utilizers was about 3.7 d^{-1} and the μ_{max} for corn syrup utilizers was about 3.5 d^{-1}.
- The current testing suggests that denitrification rates using alternate substrates, such as acetate and corn syrup, can be doubled or tripled relative to methanol. This has implications for upgrades of plants designing for LOT treatment, to achieve low nitrogen levels in the winter.
- In comparison to the cost of methanol per unit mass of nitrate removed, acetate is approximately 3–4 times and corn syrup is approximately 1.5–2.5 times more expensive than methanol. A cost/benefit assessment would be needed to determine the option of building additional reactors versus using the more expensive alternative substrates.
- Additional testing is being conducted to confirm these growth rate estimates and to estimate the acclimation time required for intermittent use of alternative substrates.

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