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May 1, 2013

Ms. Luly Massaro, Clerk
Rhode Island Public Utilities Commission
89 Jefferson Boulevard
Warwick, RI 02888

***Re: Docket 4364 - Narragansett Bay Commission***

Dear Ms. Massaro:

Enclosed please find an original and nine (9) copies of the following:

1. Narragansett Bay Commission's Response to the Division of Public Utilities and Carriers Sixth Set of Data Requests.

Please note that an electronic copy of this filing has been sent to the service list.

Thank you for your attention to this matter.

Sincerely,

Joseph A. Keough Jr.

JAK/kf
Enclosures

The following questions pertain to NBC's March 20, 2013 filing in Docket No. 4401 seeking approval to eliminate NBC's BOD and TSS surcharges retroactive to January 1, 2013.

DIV. 6-1. Please state whether customers have been billed for BOD and TSS surcharges for January through March 2013.

a. If not, please explain why not.

b. If yes, explain whether NBC intends to refund the amounts billed.

Answer:

a. No, these charges are billed in arrears and typically calculated and billed on a quarterly basis for analytical results that were submitted for the previous three month period.

b. N/A

Prepared by: Kerry Britt, NBC's Pretreatment Manager

DIV. 6-2. Please explain how NBC proposes to recover the revenue that will be lost due to elimination of the BOD and TSS surcharges.

Answer: NBC proposes to recover this revenue through user charges and this was reflected in the Rebuttal testimony.

Prepared by: WEE

DIV. 6-3. Please provide any studies, analyses or other documents which discuss and/or quantify the potential chemical cost savings resulting from the higher incoming BOD levels to the BNR process.

Answer: The attached technical studies discuss nutrient removal and how influent BOD fits into the process. There is no quantification of the chemical cost savings.

Prepared by: Paul Nordstrom, P.E., NBC's Director of Operations and Engineering

# Implication of Using Different Carbon Sources for Denitrification in Wastewater Treatments

Carla Cherchi, Annalisa Onnis-Hayden, Ibrahim El-Shawabkeh, April Z. Gu*

**ABSTRACT:** Application of external carbon sources for denitrification becomes necessary for wastewater treatment plants that have to meet very stringent effluent nitrogen limits (e.g., 3 to 5 mgTN/L). In this study, we evaluated and compared three carbon sources—MicroC$^{TM}$ (Environmental Operating Solutions, Bourne, Massachusetts), methanol, and acetate—in terms of their denitrification rates and kinetics, effect on overall nitrogen removal performance, and microbial community structure of carbon-specific denitrifying enrichments. Denitrification rates and kinetics were determined with both acclimated and non-acclimated biomass, obtained from laboratory-scale sequencing batch reactor systems or full-scale plants. The results demonstrate the feasibility of the use of MicroC$^{TM}$ for denitrification processes, with maximum denitrification rates ($k_{dmax}$) of 6.4 mgN/gVSS·h and an observed yield of 0.36 mgVSS/mgCOD. Comparable maximum nitrate uptake rates were found with methanol, while acetate showed a maximum denitrification rate nearly twice as high as the others. The maximum growth rates measured at 20°C for MicroC$^{TM}$ and methanol were 3.7 and 1.2 day$^{-1}$, respectively. The implications resulting from the differences in the denitrification rates and kinetics of different carbon sources on the full-scale nitrogen removal performance, under various configurations and operational conditions, were assessed using Biowin (EnviroSim Associates, Ltd., Flamborough, Ontario, Canada) simulations for both pre- and post-denitrification systems. Examination of microbial population structures using Automated Ribosomal Intergenic Spacer Analysis (ARISA) throughout the study period showed dynamic temporal changes and distinct microbial community structures of different carbon-specific denitrifying cultures. The ability of a specific carbon-acclimated denitrifying population to instantly use other carbon source also was investigated, and the chemical-structure-associated behavior patterns observed suggested that the complex biochemical pathways/enzymes involved in the denitrification process depended on the carbon sources used. *Water Environ. Res.*, **81,** 788 (2009).

**KEYWORDS:** denitrification, nitrogen removal, biological nutrient removal, MicroC$^{TM}$, Biowin modeling, carbon sources, Automated Ribosomal Intergenic Spacer Analysis, and community structure.

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## Introduction

In the last decade, increasingly stringent environmental requirements have been imposed on nutrients discharge in surface waters, because excessive nutrients are considered the primary causes of eutrophication. The biological nutrient removal (BNR) process remains the most common practice for achieving nitrogen and phosphorus removal. Many wastewater treatment plants (WWTPs) are facing challenges to achieve lower effluent nutrient levels with current technology limits and available resources (Water Environment Research Foundation, 2007). The addition of external carbon sources often becomes necessary for achieving high-efficiency BNR, especially for facilities with weak influent biochemical oxygen demand (BOD) and/or those facing strict effluent limits. The addition of extra carbon in pre-denitrification anoxic zones can increase the denitrification rates and nitrogen-removal efficiencies, while external carbon addition to the post-denitrification zone often is required to reach an effluent total nitrogen concentration of less than 3 to 5 mg/L.

In the United States, methanol is the most commonly used electron donor, as a result of the higher denitrification efficiency, as indicated by the relatively lower methanol-to-nitrate ratio, lower cost, and broad availability in the market. The main disadvantage of using methanol is the safety issues associated with its transportation, handling, and storage, because it is a reactive and toxic compound. It has been estimated that an additional 25 to 31% of the capital construction cost for methanol storage, pumping, and delivery systems is required to meet the safety standards over the use of a non-flammable, non-hazardous product (CDM, 2007). The long adaptation periods required in the startup process to build the specific methanol-using denitrifying bacteria (methylotrophs) also is relevant (Christensson et al., 1994). Additionally, there have been reports of deteriorated denitrification performance under cold conditions, as a result of the potential washout of methanol-using denitrifying bacteria from the system, as the growth rates decrease at lower temperatures (Mokhayeri et al., 2006). Lastly, the prices of methanol recently have been volatile (Methanex, 2008), and, in some cases, shortages have occurred. The above concerns have motivated the investigation of other economical alternative carbon sources for denitrification.

Performances related to long-term experiences with the use of methanol and ethanol as sole electron donors and their influence on the denitrifying bacterial community have been studied and compared (Christensson et al., 1994; Nyberg et al., 1996). Alternative compounds that have been investigated for supporting denitrification are sugar (Akunna et al., 1993; Gomez et al., 2000), glycerol (Akunna et al., 1993), molasses (Quan et al., 2005), corn starch (Lee and Welander, 1996), industrial wastewater (Cappai et al., 2004), and others (Akunna et al., 1993; Lee and Welander,

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1996; Nyberg et al., 1996; Tsonis, 1997). MicroC™ is a proprietary product with an undisclosed composition, developed by Environmental Operating Solutions (EOS), and designed specifically as a nonflammable, agriculturally derived carbon source. Since 2003, MicroC™ has been distributed throughout the northeastern United States in over 200 facilities required to meet stringent effluent nitrogen limits, and its price has remained stable in the past 3 years at approximately $0.48/L ($1.81/gal). In general, plant infrastructures typically used to handle methanol or other carbon sources are compatible with MicroC™. Selection of a carbon source for denitrification must consider many aspects, including nitrogen-removal performance, cost, operational requirements and features, and possible effect on effluent quality and sludge production (Nyberg et al., 1996). The objective of this study is to evaluate the denitrification kinetics and potential of MicroC™ as a carbon source for the denitrification process and compare them with the most commonly used carbon source (methanol) and widely studied carbon source (acetate), although practical use of the latter has been limited, as a result of its higher cost. Specific goals include the following:

(1) Determine and compare the denitrification rates, kinetics, and growth rates among MicroC™, methanol, and acetate, with both acclimatized biomass and non-acclimated biomass from either laboratory-scale sequencing batch reactors (SBRs) or full-scale WWTPs.
(2) Investigate the implications and effects of using different external carbon sources on nitrogen-removal performance at full-scale facilities using Biowin model simulations. The effect of both denitrification kinetics and operational conditions are evaluated.
(3) Assess the ability and response of a specific carbon-acclimated denitrifying population to immediately use various other carbon sources.
(4) Investigate the microbial population structures and dynamics associated with various carbon-source-specific denitrifying enrichment cultures and reveal the biochemical fundamentals underlying the different denitrification behaviors observed.

## Materials and Methods

**Chemicals.** MicroC™ is a light green liquid compound with a mild alcohol odor containing agricultural products and methanol (5% w/w). Its bulk density, specific gravity, and viscosity are 1.18 g/cm$^3$, 1.18, and 0.0164 kg/m-s, respectively. The compound is soluble in water, and its pH at 25°C is 5.8. MicroC™ is stable under normal conditions and has a freezing point of −20°C, which avoids storage issues during cold seasons. Volatile organic compounds have not been detected in its composition (EOS, 2008). Stock chemicals were provided by EOS and dilutions were prepared freshly for testing and the system feeding. Methanol, sodium acetate, ethanol, and glucose were from Fisher Scientific (Springfield, New Jersey).

**Denitrifying Biomass (Sludge).** Both acclimated and non-acclimated denitrifying biomass, from laboratory-scale SBR reactors or full-scale activated sludge WWTPs, were tested for comparison.

*Full-Scale Activated Sludge.* MicroC™-acclimated sludge was provided by the municipal WWTP of Enfield, Connecticut, where the sludge had been acclimatized fully with MicroC™ added as the sole carbon source in the post-denitrification stage for 9 months; the non-acclimated sludge was from the municipal WWTP of Wareham, Massachusetts. The Wareham facility is a Modified Ludzack-Ettinger (MLE) system, with secondary clarification followed by Leopold downflow denitrification sand filters and UV disinfection. The plant did not use any external carbon source, and the sludge samples were taken from the pre-anoxic zone.

*Laboratory-Acclimated Sludge.* Three carbon-source-specific denitrifying biomasses were acclimatized fully for over 3 months before the beginning of batch testing in laboratory-scale SBRs with MicroC™, methanol, and acetate, respectively, using the same seeding sludge from the Wareham, Massachusetts, WWTP. The synthetic wastewater for the SBR feed contained MicroC™, methanol, or acetate (150 to 350 mgCOD/L), dissolved in media containing $MgSO_4 \cdot 7H_20$ (40 mg/L), $CaCl_2$ (7.5 mg/L), $Fe(SO_4) \cdot 7H_2O$ (1 mg/L), $KH_2PO_4$ (22 mg/L), $K_2HPO_4$ (56 mg/L), $NH_4Cl$ (101 mg/L), yeast extract (30 mg/L), $MnSO_4 \cdot 4H_20$ (0.2 mg/L), sodium bicarbonate (252 mg/L), and trace minerals. The SBR system had an influent flow of 9 L/d and was operated with an SRT of 15 days and 3 daily cycles, which included a 2-hours anoxic phase, 30 minutes of feeding, and 4.5-hour aerobic period. The chemical oxygen demand (COD), ammonia ($NH_4$), nitrate ($NO_3$), and nitrite ($NO_2$) were examined on a weekly basis to monitor the general functioning of the system. Total and volatile suspended solids (TSS and VSS, respectively) were maintained at concentrations between 700 and 1100 mg/L. The temperature was in the range 20 to 23°C, and the oxygen concentration during the aerobic phase was approximately 5 to 7 mg/L. The pH was kept in the optimal range of 6.5 to 7.5.

**Analytical Measurements.** Nitrate, nitrite, ammonia, volatile and total suspended solids were analyzed according to *Standard Methods* (APHA et al., 2001). A YSI 5000 dissolved oxygen meter (YSI Inc., Yellow Springs, Ohio) was used to monitor the extent of aeration, while the pH and temperature were checked using a Thermo Orion 230 meter (Thermo Fisher Scientific, Waltham, Massachusetts). Dichromate acid digestion was used to determine the total COD equivalent to MicroC™, and duplicates of different dilution series were conducted to obtain statistically confident values. The readily bio-available COD (rbCOD) value for MicroC™ was evaluated using the method proposed by Mamais et al. (1993), based on the filtered and flocculated COD (ffCOD) measurement. The principle is to determine the rbCOD as the difference between the ffCOD of the influent wastewater and the ffCOD of the final effluent in a specific treatment process. The 5-day BOD ($BOD_5$) of MicroC™ was analyzed based on *Standard Methods* (APHA et al., 2001).

**Denitrification Rates and Kinetics.** *Denitrification Batch Tests (Low Food-to-Microorganism Ratio, 0.02 to 0.05 mgCOD/mgVSS, Short Test).* A series of denitrification batch tests was conducted to determine the denitrification rates and kinetics with biomass that was acclimated with three carbon sources—MicroC™, methanol, and acetate, respectively. Denitrification rates were determined at various carbon concentrations (0 to 300 mg sCOD/L) and with an adequate initial nitrate concentration (20 to 40 mg/L), according to the method presented by Kujawa and Klapwijk (1999). The sludge was kept under a continuous nitrogen gas flow, to guarantee anoxic conditions, and the pH remained constant at 7.5. Samples were taken at intervals of 10 to 15 minutes. The denitrification rates, as a function of initial COD concentration, were then fitted to the Monod equation

using SPSS 14.0 (SPSS Inc., Chicago, Illinois) to estimate the maximum denitrification rate and half-saturation constant.

*Denitrification Tests (High Food-to-Microorganism Ratio, 2 to 3 mgCOD/mgVSS, Long Test).* The method proposed by Dold et al. (2005) was applied to estimate the maximum specific growth rates of each carbon-specific denitrifying culture and to estimate the carbon-to-nitrogen ratio (C/N) during denitrification. The tests were all run in duplicate. In these high F/M ratio tests, the biomass growth was expected to be exponential, with both the electron donor (carbon) and electron acceptor ($NO_3$) being kept at the saturation level (non-limiting condition). Kinetic parameters were determined by fitting the nitrate uptake rate versus time using the equation presented in Dold et al. (2005) using statistical software SPSS 14.0, as follows:

$$S_{NOx,t} = S_{NOx,0} - \frac{1 - Y_{HD}}{2.86} \cdot \frac{\mu_{max} \cdot X_0}{Y_{HD} \cdot (\mu_{max} - b_H)} \cdot \left( e^{(\mu_{max} - b_H)t} - 1 \right) \quad (1)$$

The method applied minimizes the sum of the squares of the residuals by adjusting the three initially estimated parameters ($\mu_{Hmax}$, $X_{N,0}$, and $S_{NO,0}$), and by fitting the assumed $Y_{HD}$ and $b_H$ to the above equation. Note that, although the yield $Y_{HD}$ in the model is assumed, it has no influence on the $\mu_{max}$ of denitrifiers (Dold et al., 2005). The decay coefficient $b_H$ for acclimated sludge was estimated to be 0.1 $day^{-1}$ by low F/M tests run at endogenous conditions, which is agreeable with the values reported in literature (Yuan et al., 2002). In cases where the accumulation of nitrite occurred during the test, the coefficient 0.6 takes into consideration the stoichiometry of the denitrification reaction from the ratio 1.71/2.86, where 1.71 and 2.86 are the oxygen equivalents of nitrite and nitrate, respectively (Kujawa and Klapwijk 1999), as shown in eq 2.

$$NOx\text{-}N = NO_3\text{-}N + 0.6 \times NO_2\text{-}N \quad (2)$$

The observed growth yield for denitrifying (mgVSS/mgCOD) was estimated from the COD/N ratio measured during the tests, using eq 3.

$$Y_{HD} = \left( 1 - \frac{2.86}{(COD/N)} \right) / 1.42 \quad (3)$$

The maximum growth rate ($\mu_{max}$) at 20 and 10°C was determined experimentally with the high F/M method, as described above.

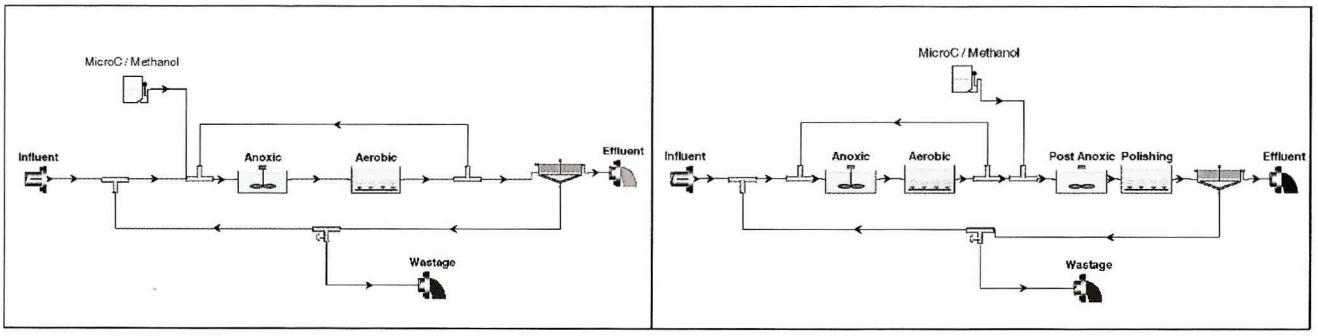
**Molecular Characterization of Carbon-Source-Specific Denitrifying Cultures.** Automated Ribosomal Intergenic Spacer Analysis (ARISA) was used in this study, to assess the dynamics of bacterial composition over time in SBR-enriched cultures. This new ecological approach differentiates the bacterial species based on the length in the intergenic region between the 16S and 23S ribosomal RNA, at a high level of resolution (Jones et al., 2007). The method was found to be highly reproducible and reliable compared with other commonly used molecular techniques, such as Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Fisher and Triplett, 1999). The bacterial community composition and dynamics then were observed and compared, to demonstrate the specificity of the community when acclimated with different carbon sources, such as MicroC™, methanol, or acetate.

Samples from the SBRs were collected every month, and DNA was extracted from activated sludge using the Ultraclean Soil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, California). DNA presence in the samples was checked using gel electrophoresis in 1% agarose Tris/Borate/EDTA (TBE) gel stained with ethidium bromide; visualization of the nucleotides fragments was done under UV light using a Bio-Rad Gel-Doc XR imaging system (Bio-Rad Laboratories, Hercules, California). Polymerase chain reaction (PCR) was performed in a Biorad IQ5 thermocycler to amplify 1 µL of extracted DNA using two primers—6FAM-labeled universal forward primer 1406F (5′-TGYACACACCGCCCGT -3′) and 23Sr bacterial specific (5′-GGGTTBCCCCATTCRG -3′) as reverse primer. The PCR consists of a preliminary DNA denaturation step for 2 minutes at 94°C followed by 30 cycles of denaturing (94°C for 35 seconds), annealing (55°C for 45 seconds), and elongation (72°C for 2 minutes) steps, ending with 2 minutes of final extension at 72°C. The ARISA was performed according to the method proposed by Fisher and Triplett (1999) using an ABI 3730 genetic analyzer. The profiles obtained by the labeled primer were analyzed using two different internal sized standards (Bioventures Inc., Murfreesboro, Tennessee)—ROX 2500 GeneScan custom sized with fragments of 50 bps in the range 100 to 2000 bps and the ROX fragile with less fragmentation within the same range. The ARISA fragments were determined using Peak Scanner software version 1.0 provided by Applied Biosystems Inc. (Foster City, California). A threshold of 100 fluorescence units was set to eliminate the background noise, and only sizes between 300 and 1550 bps were evaluated. The ARISA generally produces one peak for each bacterial isolate (Danovaro et al., 2006), and the relative abundance of each identified genotype was determined by normalizing the peak height by the total height (fluorescence units) characteristic of each electropherogram.

**Biowin Simulations.** Effects on overall nitrogen-removal performance using MicroC™ or methanol as an external carbon source were assessed by model simulation with Biowin, using the kinetics and biodegradability data determined by the batch tests. The following two commonly practiced denitrification configurations were analyzed: (1) MLE for pre-denitrification, and (2) MLE followed with a post-denitrification zone and a final aerobic polishing zone (see Figure 1). Different scenarios were simulated and compared under variant operational parameters, such as carbon dosage, anoxic hydraulic retention time (HRT), and temperature. For each scenario, effects on the final effluent total nitrogen concentration and on sludge production were the main evaluation parameters. Table 1 summarizes the conditions and operation parameters that were used for the different scenarios analyzed as well as the kinetics and stoichiometric parameters used in Biowin. The values of maximum specific growth rates and yields were the one determined in the batch tests, however for the values of the half saturation constants the default values were selected; this is because of the high standard deviation found in the batch testing for this parameter, as well as from full scale data considerations. MicroC™ utilizers are considered to growth both under aerobic and anoxic conditions due to the complexity of MicroC™ composition, whereas considered the limited availability of single-C compound in the aerated zone, the growth of methylotrophs was assumed to be limited to anoxic zones only where methanol is supplemented (default setting for Biowin).

## Results and Discussion

**Characterization of MicroC™.** The total COD of MicroC™ was found to be 663 ± 27.2 gCOD/L, which is similar to the value of 672 gCOD/L, as established by EOS in previous studies




A B

**Figure 1—Process schematic for Biowin simulations: (a) MLE configuration, and (b) MLE plus post-denitrification.**

(Ledwell, 2006). Approximately 504 gCOD/L (75% of the total COD) was determined to be rbCOD. The remaining portion of the COD (25%) seems bioavailable through hydrolysis. Soluble COD measured in the effluent from the SBR was consistently below the detection limit (5 mg/L), indicating that nearly all COD in the MicroC$^{TM}$ is used by the biomass. In addition, $BOD_5$ was evaluated and resulted in 429 g/L (65% of the total equivalent COD).

**Comparison of Denitrification Kinetics among MicroC$^{TM}$, Methanol, and Acetate.** Table 2 summarizes the denitrification rates and kinetics obtained for the three carbons sources (MicroC$^{TM}$, methanol, and acetate) using laboratory-scale SBR-acclimated sludge and full-scale WWTP sludge. The denitrification rates were determined and compared at two different temperatures (20 and 10°C), although the latter is more of a transitional rate, because the sludge was acclimated at 20°C. The maximum nitrate uptake rate found for MicroC$^{TM}$ (6.4 mgN/gVSS·h) is comparable with that obtained with methanol (6.1 mgN/gVSS·h), and therefore shows the feasibility of using MicroC$^{TM}$ as an alternative external carbon source to methanol for enhancing denitrification. The use of acetate resulted in a much higher denitrification rate (13.6 mgN/gVSS·h) than both MicroC$^{TM}$ and methanol. A wide range of values has been reported in literature for the observed specific denitrification rates for methanol, ranging from 3.3 mgN/gVSS·h (Nyberg et al., 1996) to 21 mgN/gVSS·h (Foglar et al., 2005), and, for acetate, ranging from 3.09 mgN/gVSS·h (Isaacs and Henze, 1995) to 10.6 mgN/gVSS·h (Tam et al., 1994), respectively. The variability of these rates likely was influenced by the sludge sources (acclimated versus non-acclimated) from full- or laboratory-scale systems, type of reactors, and environmental factors that generally affect biological processes (pH, temperature, etc.).

The average half-saturation constants for MicroC$^{TM}$, methanol, and acetate were found to be 38.6, 15.6 and 38.1 mgCOD/L, respectively. These values are higher than the typical value reported by Metcalf & Eddy (2003) (9 mg biodegradable COD/L). The high standard deviation of the results possibly is related mainly to the use of the soluble COD (sCOD) measurement for the estimation of this parameter. In general, for carbon sources that have a low half-saturation value, the direct measurement (e.g., gas chromatography) of the substrate, rather than the use of soluble COD measurements, might be better to estimate the half-saturation constant ($K_s$). However for a carbon source with an unknown composition, such as MicroC$^{TM}$, soluble COD would be the only measurable parameter. Half-saturation constants are important for nitrogen-removal capacity and performance at full-scale facilities, because the *in situ* specific denitrification rate in reactors typically is substrate-limiting, depending not only on the maximum specific rate, but also on the actual readily bioavailable carbon substrate concentration and the half-saturation constant.

The maximum growth rate of MicroC$^{TM}$-using denitrifiers at 20°C (3.7 $day^{-1}$) is comparable with the values previously reported for general heterotrophic denitrifying microorganisms in WWTPs (3.2 $day^{-1}$, Metcalf & Eddy, 2003), and it was nearly

**Table 1—Design and operational parameters input to Biowin simulations.**

| Design parameter | | | Kinetics and stoichiometry | | |
|---|---|---|---|---|---|
| **Configuration** | **MLE** | **MLE + post denitrification** | **Parameter** | **MicroC$^{TM}$** | **Methanol** |
| Influent flow rate, $Q$ ($m^3$/d) | 18 930 | 18 930 | $\mu_{max}$ (1/day) | 3.66 | 1.25 |
| Temperature (°C) | 13 and 20 | 13 and 20 | $K_s$ (mgCOD/L) | 20 | 5 |
| Aerobic SRT (days) | 10 | 10 | Aerobic decay (1/day) | 0.08 | 0.06 |
| Influent COD (mg/L) | 250 | 250 | Anoxic decay (1/day) | 0.08 | 0.06 |
| Anoxic volume($m^3$) | 950 to 1325 | 1325 | Yield (anoxic) (gCOD/gCOD) | 0.52 | 0.4 |
| Post-anoxic volume($m^3$) | - | 1140 to 1515 | | | |
| Aerobic volume ($m^3$) | 3445 | 3445 | Temperature coefficient (θ) | 1.1 | 1.1 |
| Polishing volume ($m^3$) | - | 378 | | | |
| Mixed-liquor recycle, MLR | 3Q | 3Q | Aerobic growth | yes | no |
| RAS | 0.5Q | 0.5Q | | | |

**Table 2—Denitrification kinetic coefficients for different carbon sources tested on laboratory-acclimated biomass in SBR systems.**

| | $K_{dmax}$ (20°C) (mgN/gVSS·h) | $K_{dmax}$ (10°C) (mgN/gVSS·h) | $K_s$ (mg sCOD/L) | COD/N (mg sCOD/mgN) | $Yield_{obs}$ (mgVSS/mgCOD) | $\mu_{max}$ (20°C) ($day^{-1}$) | $\mu_{max}$ (10°C) ($day^{-1}$) |
|---|---|---|---|---|---|---|---|
| MicroC™ | 6.4 ± 3.6 | 2.5 | 38.6 ± 29.2 | 6.5 ± 3.7 | 0.39 | 3.7 | 1.2 |
| Methanol | 6.1 ± 0.7 | 2.3 | 15.6 ± 11.2 | 4.8 ± 1.5 | 0.29 | 1.3 | 0.3 |
| Acetate | 13.6 ± 1.9 | 3.6 | 38.1 ± 16.2 | 5.7 ± 1.3 | 0.35 | - | - |

three times greater than that found for methanol (1.3 $day^{-1}$). Figure 2 shows the typical nitrate-consumption curve versus time obtained in a high F/M ratio batch test, which is used to estimate the maximum growth rate, as previously described. The maximum specific growth rate of methylotrophs reported ranged from 1 to 6.3 $day^{-1}$ (Onnis-Hayden and Gu, 2008). The most recent investigations have shown that the rate of methanol utilization under anoxic conditions may be very much slower than believed before, therefore also the default value of process simulation models such as Biowin, have been modified in the latest version. Methanol enriches for methylotrophs, a specific group of bacteria that are capable of using one-carbon ($C_1$) compounds, such as methanol, methane, and formate, as substrates for biosynthesis and energy requirements. They developed specific pathways, such as the Serine cycle, where the intermediate formation of formaldehyde occurs (Madigan and Martinko, 2006). Based on the exchange of free energy between electron donor and acceptor, the amount of biomass produced per unit of substrate removed for methylotrophs is relatively low, with respect to microorganisms grown on multi-carbon substrates (Rittmann and McCarty, 2000); therefore, it leads to a lower anoxic yield.

**Effect of Temperature on Denitrification Rates and Kinetics.** Denitrification rates decrease with declining temperature, as demonstrated in previous studies (Christensson et al., 1994; Dold et al., 2005; Mokhayeri et al., 2006; Nyberg et al., 1996). In our study, a decrease in temperature from 20 to 10°C resulted in a significant reduction in maximum denitrification rates and growth rates (Table 2) for both MicroC™- and methanol-enriched biomass. Approximately 60 and 62% decreases in the denitrification rates, and 67 and 73% decreases in the growth rates were observed at 20 and 10°C, for MicroC™ and methanol, respectively. The maximum specific denitrification rate for methanol at 10°C is comparable with those found by Dold et al. (2005). A 66% reduction of the nitrate uptake rate also was observed by Christensson et al. (1994), when the temperature was changed from 25 to 15°C. Acetate sludge seemed to be affected the most by the temperature drop, with a 73% reduction of denitrification rates from 20 to 10°C.

It is worth mentioning that significant nitrite accumulation occurred during the tests with acetate at both 20 and 10°C. Figure 3 shows the extent of nitrite accumulation with acetate-enriched biomass at 10°C, where, after 21 hours, nearly 80% of the nitrate was converted to nitrite, and the reduction of nitrite did not begin until 1.7 days later. No nitrite accumulation was observed with methanol. For MicroC™, the presence of nitrite was minimal (less than 10% of the total inorganic nitrogen) and limited to the first part of the low F/M test (when the COD/N was relatively high). The incomplete conversion of nitrate to nitrogen gas using acetate as a carbon source has been reported previously (VanRijn et al., 1996). The accumulation of nitrite has been associated with imbalanced activities of *nitrate* and *nitrite reductases*, with the inhibition of nitrite reductase by oxygen, inhibition by nitrate or nitrite, and inhibition at high COD/N ratios (>2.5) (Martienssen and Schops, 1999). The accumulation of nitrite for acetate was more pronounced at 10°C, as a result of the different temperature sensitivity of nitrite-reducer bacteria and nitrate reducers (Drysdale et al., 1999).

Note that the values measured at 10°C are "transitional" kinetics, because the sludge was not acclimated at 10°C; therefore, this test only simulates the instant population response to the temperature decrease. Because the growth rate of a population is directly related to SRT in the nitrogen-removal process, a dramatic reduction in the growth rate ($\mu_{max}$) during cold

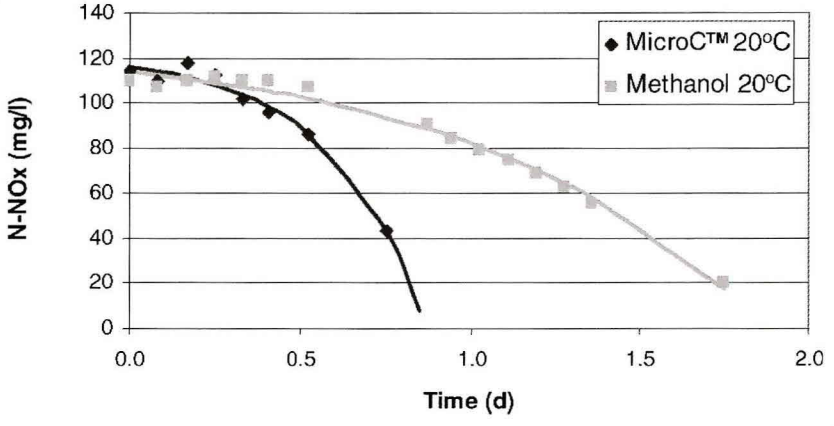



**Figure 2—Depletion of NOx during high F/M denitrification kinetic test at 20°C with MicroC™ or methanol as a carbon source.**

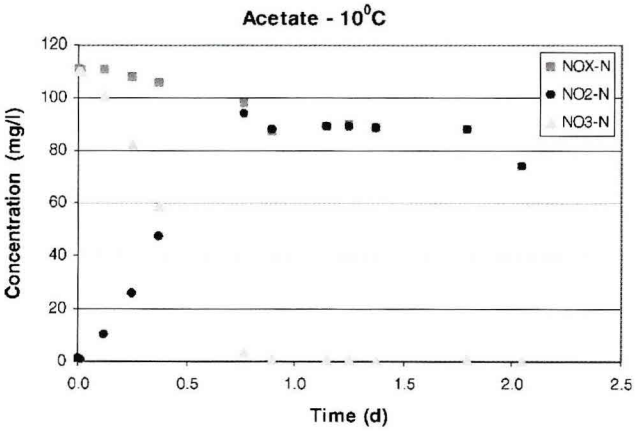



**Figure 3—Accumulation of nitrite during high F/M denitrification kinetic test with acetate as a carbon source at 10°C.**

conditions potentially could lead to the washout of the species of interest from the reactor.

**Comparison of COD/N Ratio Among MicroC™, Methanol, and Acetate.** The carbon-use-to-nitrate-consumption ratio (COD/N ratio) indicates the carbon-use efficiency for denitrification, and the values were estimated based on the depletion of COD and uptake of nitrate during the denitrification batch tests. The C/N ratio was found to be 6.5, 4.8, and 5.7 gCOD/gN, for MicroC™, methanol, and acetate, respectively. The C/N value depends not only on the theoretical yield, but also the conditional parameters (SRT, $k_d$) as shown in eq 3. For methanol and acetate, the theoretical ratio was determined to be 4.7 and 3.5 gCOD/gN, respectively (Mokhayeri et al., 2006). The observed C/N can be affected by several factors, including the possible interference of storage phenomena (luxury uptake), which can take place when a considerable amount of organic substrate is put in contact with the biomass (Majone et al., 1998); possible activity under an anoxic condition of polyphosphorus-accumulating bacteria (PAOs) if present in the sludge (Naidoo et al., 2000); possible aerobic respiration resulting from oxygen intrusion; and reliability of the COD and nitrate measurement itself. For example, the high value obtained for acetate could be associated with the abundant presence of PAOs, which was observed during an unrelated test in the acetate-fed SBR.

The determination of the correct C/N ratio is crucial for the selection of alternative carbon sources, because it is an indicator of COD usage efficiency for denitrification. High operational costs and higher biomass production can be caused by COD/N overestimation.

**Effect of Acclimation.** The comparison of denitrification rates obtained with either MicroC™-acclimated or non-acclimated sludge are presented in Table 3 and Figure 4. The results indicate that the denitrification rates and kinetics were similar for the acclimated and non-acclimated full-scale sludge (4.7 and 4.3 mgN/gVSS·h). This suggests that the denitrifying microbial population capable of using MicroC™ likely is active in typical WWTPs; therefore, acclimatization to MicroC™ may not be needed. The higher rates observed with the SBR-acclimated sludge compared with the acclimated full-scale biomass likely is the result of the higher enrichment of the denitrifying population in the SBR sludge and the higher amounts of inert solids in the biomass at WWTPs.

**Comparison of Microbial Community Structures of Denitrifying Cultures Enriched with Different Carbon Sources.** The microbial community composition of each carbon-specific denitrifying culture was monitored and compared using a molecular method (ARISA) from the startup for a period of 9 months. Figure 5 summarizes the relative abundance (assumed to be proportional to fluorescence signal intensity) of diverse bacterial species (represented by distinct peaks) present in the overall microbial community during the test period. The relative abundance of each community member identified is quantified as a percentage of total fluorescence and is shown in gray-scale for easy observation. Peaks with an abundance level of less than 1% are not shown. Figure 6 shows an example of the ARISA profile for each different denitrifying culture.

The ARISA profiles clearly show the distinct community structures of the three denitrifying cultures as the result of enrichment with three different carbon sources. Approximately 17% more ARISA peaks were found in the MicroC™-enriched culture (67) than that identified in methanol- (57) or acetate (57)-acclimated cultures, indicating larger diversity in the former culture than the other two. The community compositions are rather dynamic, and no "stable" structures were achieved, even after 9 months of acclimation. However, there were consistent trends that could be observed with some of the peaks (members) in the community.

For MicroC™-enrichment, peaks at 507 bps, 523 bps, 634 bps, 688 bps, 759 bps, 802 bps, 816 bps, 823 bps, 918 bps and 1253 bps were found recurrently, and, for some, the abundance increased over time, indicating their enrichment and potential role in the MicroC™ metabolism. For methanol-enriched culture, peaks at 483, 548, 554, 725, 758, 797 bps, 810 bps, 858 bps, 890 bps, 919 bps, 965 bps and 1262 bps were found to increase in their abundance over time, especially peaks at 548, 554, and 965 bps, which were the most dominant members in the community. However, it is not clear why peaks at 548 and 554 bps disappeared at the end of the 9th month. These predominant peaks likely represent the methanol-enriched methylotrophic organisms, which are capable of using methanol. More distinctive transition trends were observed with the acetate-enriched culture, in which only seven species with a relative abundance higher than 1% were identified, and the majority of the community (56%) consisted of

**Table 3—Denitrification kinetic coefficients for MicroC™ tested on acclimated and un-acclimated biomass.**

| | $K_{dmax}$ (20°C) mgN/gVSS·h | $K_{dmax}$ (10°C) mgN/gVSS·h | $K_s$ mg sCOD/L | COD/N mg sCOD/mgN | $Yield_{obs}$ gVSS/gCOD | $\mu_{max}$ (20°C) $day^{-1}$ | $\mu_{max}$ (10°C) $day^{-1}$ |
|---|---|---|---|---|---|---|---|
| Acclimatized SBR | 6.4 ± 3.6 | 2.5 | 38.6 ± 29.2 | 6.5 ± 3.7 | 0.39 | 3.7 | 1.2 |
| Acclimatized WWTP | 4.7 ± 0.5 | - | 28 ± 11.5 | 7.0 ± 1.4 | 0.42 | 3.9 | - |
| Unacclimatized WWTP | 4.3 ± 0.5 | - | 49.7 ± 18.8 | 4.0 | 0.20 | - | - |

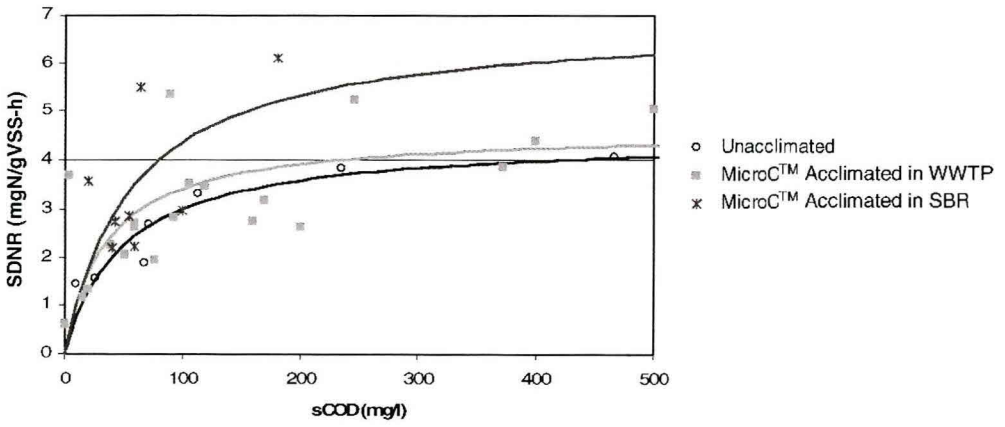



**Figure 4—Specific denitrification rates (SDNRs) at different initial soluble COD concentrations with acclimated and non-acclimated biomass.**

bacteria with intergenic space of 759 bps after 9 months of incubation.

**Ability of Specific Carbon-Acclimated Sludge to Use Other Carbon Sources.** It has been demonstrated that the quantity, quality (Lee and Welander, 1996) and combined use of external carbon sources can have various effects on denitrification. The addition of combined external carbons in post-denitrification zones can enhance the removal of nitrogen (Cho et al., 2004) or affect the metabolic properties of the established population, resulting in decreased rates. Moreover, supplemental carbon addition can reduce the capacity of denitrifiers to use internal carbon in pre-denitrification systems (Hallin and Pell, 1998). The ability and acclimatization time required of a specific population to use other carbons sources also have practical implications, affecting the easiness and adaptation time a WWTP would require when changing from one carbon source to another. In this study, we evaluated the ability of specific carbon-acclimated biomass to instantly use other carbon sources. Table 4 summarizes the response of each carbon-specific-acclimated biomass upon addition of various carbon sources. In addition, a review of the biochemical pathways for anoxic metabolism of these carbons is presented in Table 5, which highlights the key enzymes/pathways involved in the use of each specific carbon compound, and they are discussed below.

MicroC™-acclimated biomass was able to use all the carbon sources tested, including methanol, although at a relatively lower rate than the methanol-enriched biomass (data not shown). This was expected, because MicroC™ contains 5% methanol, as disclosed by EOS. The ARISA spectra indicated that the MicroC™-acclimated biomass contains a relatively large diversity of microorganisms, possibly with different metabolic pathways, as a result of the relative complexity of the MicroC™ composition. The practical implication is that, for facilities that change from using methanol as a carbon source to using MicroC™, or vice versa, lag time (acclimatization) likely is not needed. In addition, a microbial community with larger diversity typically provides more stability in the system.

Methanol-acclimated biomass could readily use all of the substrate tested for denitrification, except for glucose. Acetate is readily used by the methanol sludge, because acetate easily could enter the tricarboxylic acid (TCA)/glyoxylate cycle. Moreover, methylotrophs can use the enzymes characteristic of the glycine regeneration in the serine-glyoxylate pathway to activate the anaplerotic glyoxylate bypass of the TCA cycle when acetate is used as a carbon source. This diversion is used by organisms grown on acetate or fatty acids to provide the cells with 4- and then 3- carbons intermediates for biosynthesis. In the case of ethanol, the immediate response probably was the result of the presence of *Alcohol Dehydrogenase* enzymes, which catalyzed the conversion to acetate, and the consequent transformation into acetyl CoenzymeA. The denitrification efficiency of methanol-using bacteria seems to be affected when glucose was used as substrate, as shown previously (Mohseni-Bandpi and Elliot, 1998). Glycolysis is a multistep pathway that microorganisms use to obtain energy from glucose before entering the citric acid cycle. The high specificity of biocatalyst, perhaps not developed specifically by methylotrophs, and the complex chain reactions may be the reasons behind the reduced denitrification activity. Similar results and conclusions were found in the study of Hallin and Pell (1998). Dold et al. (2005) showed low denitrification rates using glucose in combination with mixed liquor from the nitrification stage, demonstrating that specific microorganisms and/or enzymatic systems required for glucose metabolism may not be present in methylotrophic culture.

Acetate-acclimated biomass could use only acetate efficiently, and only marginal denitrification rates were obtained with the other carbons. The specificity of carbon use seemed to be consistent with the highly selective community, which had few dominant members, as shown by the ARISA results earlier. Microorganisms grown on acetate as the only carbon and energy source require the operation of a particular anaplerotic pathway known as the *glyoxylate bypass* concomitantly with TCA. Therefore, it is likely that the acclimatization to acetate either enriches only the populations that exclusively use acetate, or simply turns off all the genes for those upstream enzymes, and therefore lacks the enzymatic activities to convert more complex multicarbon compounds into acetate (Cozzone, 1998). Specifically, metabolizing methanol must follow a reduction process to form tri-carbons or four-carbon intermediates before entering the TCA cycle. Nyberg et al. (1996) confirmed that un-specialized bacteria have difficulty in degrading methanol in systems previously acclimated with other carbons (e.g., ethanol).

**Implication of Using Various Carbon Sources for Denitrification for Full-Scale Practice.** Biowin simulations were used to compare and evaluate the effects of using different carbons

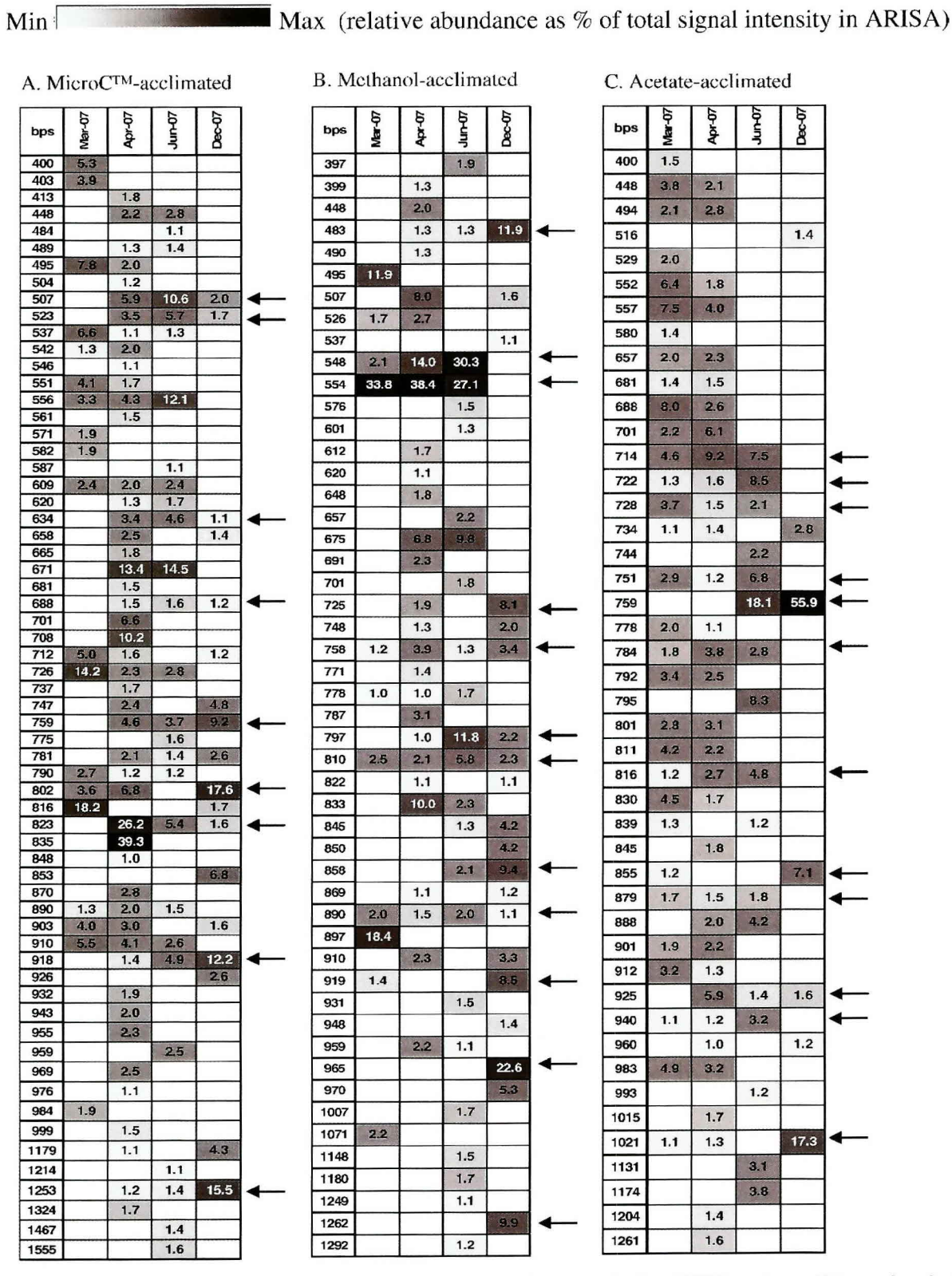



**Figure 5—Relative abundance of microbial community members (represented by ARISA peaks at different bps lengths) in each specific carbon-acclimated denitrifying culture in SBRs.**

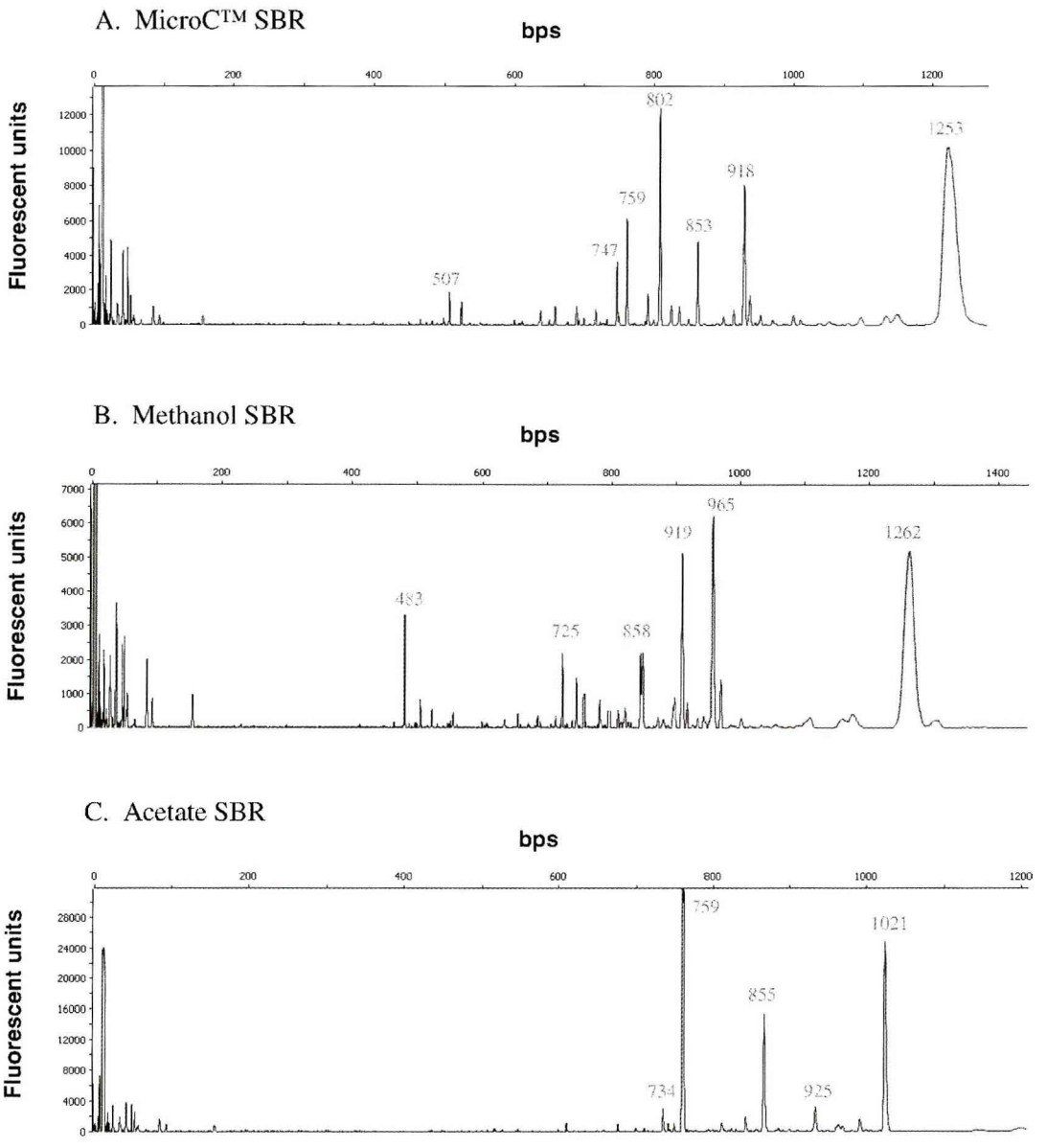



**Figure 6—Electropherograms from ARISA, characteristic of (a) MicroC™-, (b) methanol-, and (c) acetate-acclimated denitrifying bacterial community in each SBR (sample taken in December 2007).**

sources for enhancing denitrification at the full-scale level. The effects of process configuration, dosage concentrations, anoxic HRT, and temperature were assessed. Note that, in the Biowin model, MicroC™-using microorganisms are considered to be general heterotrophs, which can grow under both aerobic and anoxic conditions. This is supported by our previous observation, that MicroC™ can be consumed easily by sludges from acclimated WWTPs. Methanol use requires a special group of microorganisms, namely methylotrophs, which are capable of using single-carbon compounds for their growth. Because it remains unclear whether methylotrophs can use other COD in the aerobic zones, and considering the limited availability of single-carbon compounds in the aerated zone, the growth of methylotrophs was assumed to be limited to anoxic zones only where methanol is supplemented (default setting for Biowin). Figure 7 shows an example of the simulations results with MLE configuration with carbon addition to the pre-denitrification anoxic zone. Figure 8 shows the comparison of effluent total nitrogen with different external carbon source addition to the post-denitrification anoxic zone in a Bardenpho (4-stage) system. The results show that, for both configurations, when MicroC™, methanol, or acetate were dosed at the same concentrations (as COD), application of MicroC™ led to slightly better performance than methanol at both 13 and 20°C.

Temperature affects denitrification rates and kinetics and therefore the nitrogen-removal performance. As the temperature decreased from 20 to 10°C, the slower reaction rates led to slightly elevated effluent total nitrogen in the MLE configuration. The low temperature effect was more pronounced with MLE post-denitrification configuration, in which there was a 7% increase in the effluent total nitrogen using methanol, compared with 5% using MicroC™, as the simulation temperature dropped from 20

**Table 4—Short-term response of each specific carbon-acclimated biomass to use various carbon sources.***

| | MicroC™-acclimated sludge | Methanol-acclimated sludge | Acetate-acclimated sludge |
|---|---|---|---|
| MicroC™ | + | + | − |
| Methanol | + | + | − |
| Acetate | + | + | + |
| Ethanol | + | + | − |
| Glucose | + | − | − |

* Note: "+" = positive, able to use; "−" = negative, unable to use.

to 13°C. The denitrification in the system supplemented with MicroC™ seemed to be less sensitive to temperature drops compared with methanol supplementation. A more pronounced effect of temperature on denitrification, with MicroC™ or methanol as a carbon source, also can be illustrated by calculating and comparing the minimal SRT required to prevent washing-out at a very low temperature (5°C). The maximum growth rates at 5°C for both MicroC™ culture and methanol culture were determined based on the rates previously measured at 20°C and the temperature correction coefficient ($\theta$ = 1.1). At this low temperature, the minimal SRT required to maintain methylotrophs in the post-denitrification reactor was approximately 4.5 days. In contrast, the minimal SRT required for keeping the MicroC™-users in the system was only 1.5 days. This implies that a larger anoxic reactor volume is required for methanol compared with MicroC™ at extremely low temperature conditions. In addition, the advantage of applying a fixed-film process instead of a suspended activated sludge process for denitrification, especially at lower temperatures, is implied.

Simulations with varying anoxic HRTs show that HRT also affects the denitrification performance (data not shown); therefore, both COD dosing and HRT should be considered when adding external carbon for enhanced denitrification. Effects on sludge production with different external carbon sources also were evaluated, because the yield associated with each carbon-specific denitrifying culture affects the final sludge production from external carbon addition. Figure 9 shows the comparison of the overall sludge production in the system and the specific portion of sludge produced from MicroC™ or methanol addition. Although a higher amount of sludge was produced for the MicroC™-using biomass than for the methanol-using biomass, as a result of the higher yield, the overall sludge production at the plant level was not significantly different; the total sludge production when adding external MicroC™ was only approximately 1.5% higher than that when adding methanol for the post-denitrification scenario. The reason for the relatively small effect on overall sludge production, despite the difference in the yield values, is that the externally added COD is only a small percentage of the total amount COD fed to the system, including the raw influent COD (only <10%). The difference in the yield affects only the sludge produced from the externally added carbon, which is relatively a small percentage of the overall wastage of the plant.

The results of the simulation also show that, despite the higher COD/N for MicroC™, at equivalent COD dosages, the nitrogen removal obtained with MicroC™ addition was similar to that with methanol addition. This is because the nitrogen removal at a full-scale plant, unlike that in batch tests, depends on many other factors besides the COD/N ratio, such as HRT, limiting conditions in the anoxic zone, and abundance of specific denitrifiers in the system. In the case of MicroC™ simulations, for example, the abundance of MicroC™-users at steady state was higher than the amount of methylotrophs for equivalent COD added, as a result of higher yield, therefore resulting in a similar amount of nitrogen removal despite the higher COD/N. With adequate kinetics and stoichiometric parameters as input, the use of a simulator, such as Biowin, can help in the selection, for an existing facility, of the most effective external carbon source and selection of the optimal operational condition

**Table 5—Known biochemical pathways involved in the use of the tested carbons.**

| Carbon source | Microorganism capable of C utilization | Biochemical pathways involved | Key steps of metabolism | Enzymes involved |
|---|---|---|---|---|
| MicroC™ | Diverse community | Unknown | Diverse | Diverse |
| Methanol | Methylotrophs | Serine Pathway (Type II) | $CH_3OH$ → Formaldehyde ↓ Serine/Glyoxylate Pathway → AcetylCoA ↓ TCA cycle | *Serine transhydroxymethylase, α–ketoglutarate dehydrogenase isocitrate lyase, enzymes characteristic of TCA* |
| Acetate | Diverse community | TriCarboxylic Acid Cycle (TCA cycle) | Acetate ($CH_3COO^-$) ↓ TCA cycle/ Glyoxylate Bypass | *Citrate synthase, isocitrate dehydrogenase, isocitrate Lyase, Malate synthase, Succinyl-CoA synthetase, etc* |
| Ethanol | Diverse community | Oxidation + TCA cycle | $C_2H_5OH$ → Acetaldehyde → Acetate ↓ TCA cycle | *alcohol dehydrogenase, acetaldehyde dehydrogenase, etc* |
| Glucose | Diverse community | Glycolysis + TCA cycle | Glucose → Glyceraldehyde-3-P ↓ $Pyruvate^-$ →Acetyl CoA ↓ TCA cycle | *Hexokinase, Glyceraldehyde-3-P-dehydrogenase, Pyruvate kinase, etc* |

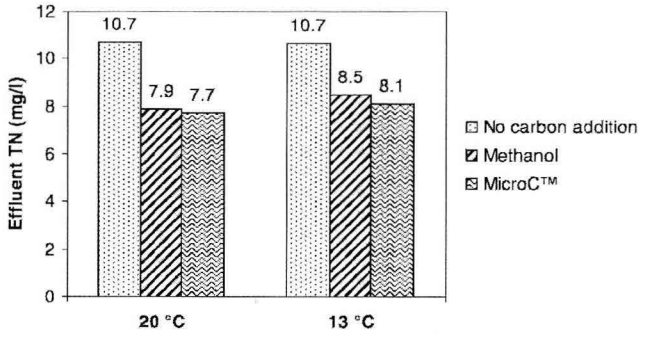



**Figure 7—Comparison of nitrogen removal in MLE configuration with methanol or MicroC™ added as an external carbon source (60 mg/L COD) at two different temperatures, using Biowin simulations.**

(carbon dosage). For a new plant, the selection of process configuration and type (suspended versus fixed-film) and anoxic volume are critical for expected nitrogen removal.

## Conclusions

Our study results have led to the following conclusions:

(1) The maximum specific denitrification rates obtained with MicroC™, at both 20 and 10°C, were comparable with those obtained with methanol, indicating that MicroC™ can effectively support denitrification. A much higher denitrification rate was observed with acetate at 20°C; however, at 10°C, significant nitrite accumulation occurred, resulting in only partial denitrification.

(2) Comparison of denitrification rates obtained with MicroC™-acclimated or non-acclimated biomass yielded similar rates, suggesting that the denitrifying microbial population capable of using MicroC™ is present in typical WWTPs; therefore, acclimatization to MicroC™ may not be needed.

(3) The maximum growth rates ($\mu_{max}$) estimated for MicroC™-acclimated culture was nearly three times greater than the one found for methanol at both 20 and 10°C. This implies that a longer anoxic SRT and larger post-denitrification reactor volume would be required using methanol than that using MicroC™, to prevent the slow-growing populations from washing-out from the system, especially at colder temperatures.

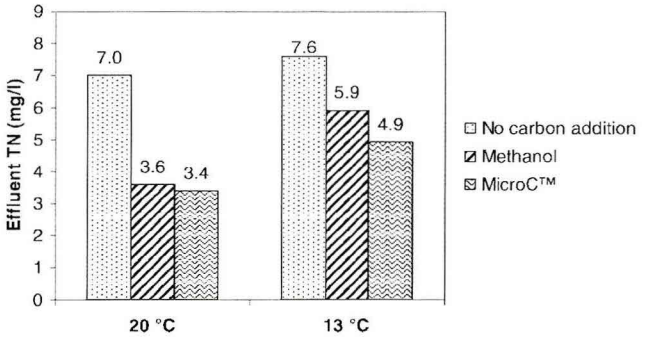



**Figure 8—Comparison of nitrogen removal in MLE + post denitrification (Bardenpho 4-stage) configuration with either methanol or MicroC™ as an external carbon source (25.4 mg/L COD) at two different temperatures, using Biowin simulations.**

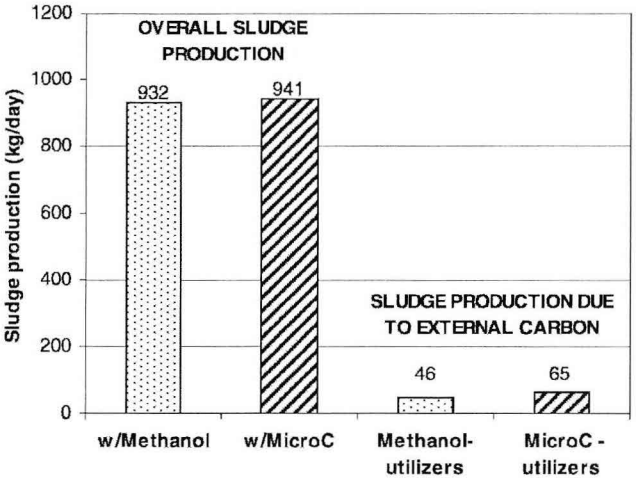



**Figure 9—Comparison of sludge production in MLE + post denitrification (Bardenpho 4-stage) configuration with methanol or MicroC™ as an external carbon source (60 mg/L COD), using Biowin simulations.**

(4) The microbial community analysis using ARISA profiles clearly shows the distinct community structures of the three denitrifying cultures, as a result of enrichment with three different carbon sources. Although the community compositions are rather dynamic over the period of acclimation, there were consistent trends that could be observed with some of the predominant members (peaks) in the community. The MicroC™ enrichment seemed to have larger diversity than methanol or acetate enrichment.

(5) Evaluation of the capability of a specific carbon-acclimated sludge to instantly use other carbon sources showed that MicroC™ sludge can readily use all the substrates tested, including MicroC™, methanol, acetate, ethanol, and glucose. Methanol-fed sludge can immediately use MicroC™, acetate, and ethanol, but not glucose. Acetate-fed sludge can only use acetate and could not use other carbons readily.

(6) Effect assessment of using different external carbon sources on the nitrogen-removal performance with typical full-scale denitrification process configurations was conducted using Biowin simulations. The results indicated that, with equivalent COD dosage, application of MicroC™ leads to slightly better performance than methanol, especially for the post-denitrification process and under lower temperature conditions. However, the results also showed that the difference in yield did not translate into a significant difference in sludge production.

## Credits

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# Alternative Carbon Sources for Achieving Biological Nutrient Removal at Municipal Wastewater Treatment Plants

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## ABSTRACT

Because of concern over eutrophication of lakes and estuaries, the US EPA and state regulatory agencies are steadily reducing the allowable levels of nitrogen (N) and phosphorus (P) in NPDES discharges. As a result, increasing numbers of municipal wastewater treatment plants (WWTP's) are having to provide on-site treatment for removal of N and P from wastewater discharges and others are having to remove additional N and P to meet increasingly stringent standards.

Normally N and P removal are performed using a biological nutrient removal (BNR) process. Both N removal and P removal require a sufficient organic carbon source as food for these microorganisms in order to make the process work, but many municipal WWTP's do not have sufficient carbon in their incoming wastewater to reliably perform BNR. In these cases, a supplemental carbon source must be added to the wastewater.

This paper explains the processes for biological removal of N and P in wastewaters and discusses available alternative carbon sources that can be used to meet BNR carbon needs. This paper also provides information on alternative commercial and non-commercial carbon sources such as the use of glycerin-based chemicals, high-fructose corn syrup (HFCS), and waste sugar water as alternative carbon sources and presents experience involving use of high-strength beverage plant wastes, acetic acid and HFCS as the BNR carbon sources at several municipal WWTP's

Keywords: Biological nutrient removal, Beneficial utilization, Sustainability

## INTRODUCTION

Because of concern over eutrophication of lakes and estuaries, the US EPA and state regulatory agencies continue to reduce the allowable levels of nitrogen (N) and phosphorus (P) in NPDES discharges. As a result, increasing numbers of municipal WWTP's are now required to provide treatment for removal of N and P from wastewater discharges, while others are now having to increase current levels of N and P removal in order to meet increasingly stringent NPDES discharge standards. One of the most extensive nutrient reduction programs in the US has been the US EPA's Chesapeake Bay Initiative. This initiative requires N and P removal to be implemented for most POTW's in Virginia, Maryland, Pennsylvania, Delaware, New York and the District of Columbia.

Normally, N and P removal are performed using a biological nutrient removal (BNR) process. P removal can also be accomplished by chemical precipitation. However, chemical precipitation substantially increases the quantity of sludge produced by a WWTP, which also increases the associated costs for dewatering and disposal. Biological removal of N is accomplished by a 2-step process of nitrification followed by denitrification. Biological phosphorus removal is accomplished in a single-sludge activated sludge system by alternating anaerobic/aerobic conditions and providing an appropriate organic food source (or carbon source) in order to selectively grow phosphate accumulating organisms (PAO's) to achieve Enhanced Biological Phosphorus Removal (EBPR).

# BIOLOGICAL NUTRIENT REMOVAL & CARBON NEEDS

Both N removal and P removal require a sufficient organic carbon source as food for the treatment microorganisms in order to make the process work. However, many municipal WWTP's do not have sufficient amounts of carbon in their incoming wastewater to reliably perform BNR so must add a supplemental carbon source to the wastewater. Supplemental carbon can come from purchased chemicals or can be produced at the WWTP by fermentation of sludge solids. Both options should be carefully considered when a treatment plant needs supplemental carbon to meet nutrient limits. As more WWTP's are upgraded to provide BNR, the demand for supplemental carbon is growing.

Traditional carbon sources for BNR include methanol for N removal and acetic acid for enhanced P removal. Both are very expensive materials and can account for a significant portion of a WWTP's operating costs. Methanol also has some significant safety issues due to its flammability and toxicity. Alternative commercial and non-commercial carbon sources include glycerin-based chemicals, high-fructose corn syrup (HFCS), corn syrup, and waste sugar water. Non-commercial carbon sources can be extremely cost-effective for WWTP's and can also benefit industries that produce such usable carbon sources as waste products. The result can be a "win-win" situation for municipal utilities wanting to assist their industrial customers while promoting beneficial utilization of a waste sugar as a Green Technology application.

BNR processes are multi-stage, single-sludge biological processes that achieve TN and TP removal by alternating conditions from anaerobic to anoxic to aerobic during treatment. By carefully controlling and alternating the process conditions in each stage, treatment organisms are selectively grown in the treatment process that are able to uptake extra amounts of phosphorus and others will remove nitrogen from the wastewater. EBPR is a relatively complicated process that is capable of effectively lowering both nitrogen and phosphorus concentrations in a single-sludge wastewater treatment process.

For either biological phosphorus removal or biological nitrogen removal (denitrification) to occur, an easily biodegradable carbon source must be present in the anaerobic and anoxic stages in order to provide energy necessary for the nutrient removing bacteria to grow and perform their work. A carbon source is simply a readily biodegradable organic energy source like acetic acid, methanol, or sugar. The amount of potential bacterial energy contained in the carbon source under anaerobic or anoxic conditions can be best measured as COD (Chemical Oxygen Demand) rather than $BOD_5$, since $BOD_5$ is measured with an aerobic test. In municipal wastewater treatment plants, biological phosphorus removal (or EBPR) consumes approximately 50 mg COD per mg of TP removed while denitrification consumes approximately 9 mg COD per mg of $NO_3$-N converted to $N_2$ (Randall). A municipal WWTP's incoming wastewater typically does not contain sufficient COD to support the entire needs for BNR treatment, so an external supplemental carbon source must frequently be added in order to provide the bacterial energy for the biological phosphorus and nitrogen removal.

Nitrogen is removed in the BNR treatment process via a two-step process that is performed by two types of bacteria in the treatment system. In the aerobic (or oxic) stage, autotrophic nitrifying bacteria oxidize the ammonia nitrogen ($NH_3$-N) first to nitrite nitrogen ($NO_2$-N) which is then oxidized to nitrate nitrogen ($NO_3$-N). This process is called nitrification. The $NO_3$-N is then transferred to an anoxic stage (zero dissolved oxygen with nitrate present) with the recycled sludge, where it is utilized by heterotrophic bacteria as an alternative oxygen source (or electron acceptor) for $BOD_5$ removal and converted to nitrogen gas ($N_2$). The nitrogen is removed from the system as the $N_2$ gas bubbles out of solution and is released into the atmosphere. This process is called denitrification.

Phosphorus is removed in the BNR treatment process by a two-step alternating anaerobic/aerobic biological process called enhanced biological phosphorus removal or EBPR. In the EBPR process, anaerobic conditions first cause certain bacteria called phosphate-accumulating organisms (PAOs) to release phosphorus. The PAOs are then sent to an aerobic stage where they uptake an even greater amount of phosphorus than was released previously. The phosphorus is removed from the system by settling out the microorganisms containing excess P and wasting the sludge from the WWTP. PAOs actually utilize volatile fatty acids (VFAs) as a food source. VFAs include organic acids such as acetic, butyric and propionic acids. Acetic acid addition can provide the necessary VFAs for the PAO function and EBPR. If a sugar solution or other alternative carbohydrates are added to the anaerobic zone as the carbon source for EBPR, the sugars are quickly fermented to produce VFAs in the process by heterotrophic bacteria called acid-forming bacteria and the VFAs are then used by the PAOs for enhanced biological phosphorus removal. The carbohydrates themselves cannot be utilized by the PAOs. However, since the sugars are quickly fermented to VFAs in a suspended growth process under anaerobic conditions, the addition of sugar instead of acetic acid does not have an observable impact on the efficiency of the EBPR process.

The most common BNR systems are 3-stage activated sludge systems which employ anaerobic, anoxic, and aerobic treatment zones in series. Several common variations of BNR processes are shown on Figure 1. Typically, these processes are designed to utilize available incoming COD as the carbon source. Another common approach being utilized for N removal in POTW's is use of the conventional aerobic activated sludge process for $BOD_5$ removal and nitrification followed by tertiary denitrification using anoxic biological filters with methanol addition for the necessary carbon source.

According to Metcalf and Eddy (2003) and Flippin (2007), the typical POTW primary clarifier effluent contains approximately 90 mg/l of readily biodegradable COD, which can be increased by another 10 mg/l to 100 mg/l through fermentation of volatile suspended solids (VSS) in the primary clarifier. At the same time, a typical POTW influent containing 31 mg/l TKN and 5 mg/l TP would require at least 152 mg/l of readily biodegradable COD in order to achieve TN and TP limits of 3 mg/l and 0.1 mg/l TP using BNR. Traditionally, POTW's have provided the required supplemental carbon for BNR through addition of methanol and/or acetic acid into the treatment process. Methanol can not be utilized by PAO's as required to achieve EBPR, so POTW's required to achieve both N and P removal must use either chemical precipitation or addition of a $2^{nd}$, PAO-compatible carbon source in order to achieve phosphorus removal. Most commonly, acetic acid has been used as the supplemental carbon source for EPBR and it can also be used as a carbon source for denitrification.

## TRADITIONAL SUPPLEMENTAL CARBON SOURCES

The most common carbon source added for denitrification is methanol since it is widely available and generally economically attractive. However, there are some significant drawbacks to methanol. Methanol is highly flammable, highly toxic, and subject to wide fluctuations in pricing due to competing uses. Approximately 90% to 95% of the methanol consumed in the US is imported- 85% of which are imported from Venezuela or Trinidad. Between 2008 and 2010, delivered methanol prices in the US have fluctuated from $1.35 to $3.25/gal. For BNR usage, this price range normalizes to $0.14 to $0.33/lb COD.

The most common chemical added as a VFA source for EPBR is acetic acid and it can also be used as a carbon source for denitrification. Acetic acid is widely available but is generally expensive for use in BNR applications. Acetic acid is highly corrosive to metals and can cause skin burns, permanent eye damage, and irritation to mucous membranes if mishandled. Most acetic acid consumed in the US is manufactured in the US for use in the chemicals industry. Although acetic acid can be produced using biological processes, most industrial grades are produced by reacting methanol with carbon monoxide. Recent pricing in the Carolinas has been

$0.73/gallon for 20% acetic acid recovered as a byproduct from acetaminophen manufacturing, which is equivalent to $0.43/lb COD.

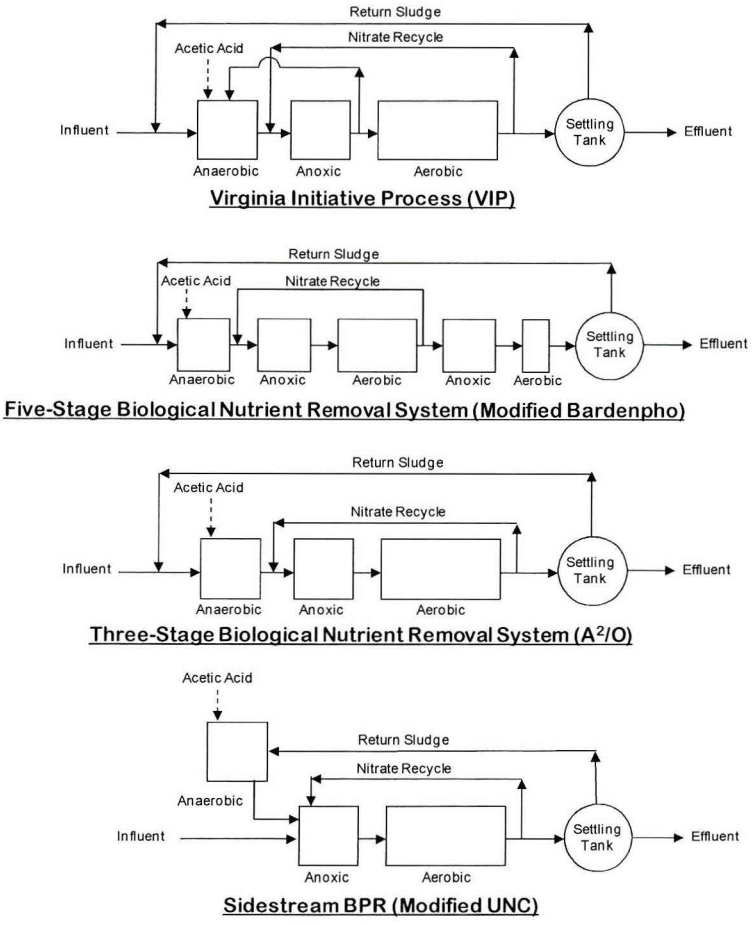


**Figure 1: Process configurations for biological nitrogen and phosphorus removal.**

# ALTERNATIVE SUPPLEMENTAL CARBON SOURCES

Alternative chemicals for use as carbon sources for denitrification and EPBR include sucrose (sugar), high fructose corn syrup (HFCS), corn syrup (dextrose or glucose), and crude glycerin. Compared to methanol and acetic acid, all of these alternatives offer increased safety as well as some opportunities for economic savings. Suffolk Sales & Service (Suffolk, VA) sells a 20 – 25% HFCS or sucrose solution for denitrification and EPBR. Environmental Operating Solutions (Bourne, MA) sells three proprietary products for denitrification: MicroCg (carbohydrate), MicroCm (carbohydrate plus 5.5% methanol) and MicroCglycerin (glycerin). Keystone Biofuels (Shiremanstown, PA) sells Unicarb, a proprietary glycerol product produced as a byproduct in biodiesel manufacturing. High sugar wastewaters from soft drink and sports drink beverage plants have also been used for BNR carbon sources and offer both safety and the opportunity for substantial economic savings. Selected information on these chemicals is shown in Table 1 below.

**Table 1**
**Comparison of Alternative BNR Carbon Sources**

| Carbon Source | COD, mg/l | Approximate cost $/gal | Equivalent Cost $/lb COD | Application |
|---|---|---|---|---|
| Methanol | 1,188,000 | $1.35 - $3.25 | $0.14 - $0.33 | DN |
| Ethanol | 1,649,000 | $3.10 - $3.20 | $0.23 - $0.23 | DN |
| Acetic Acid, 20% | 219,000 | $0.79 | $0.43 | DN, EBPR |
| Sucrose 20 Brix | 274,000 | $0.64 | $0.28 | DN, EBPR |
| Sucrose 67 Brix | 918,000 | $2.75 | $0.36 | |
| HFCS 20 Brix | 274,000 | $0.64 | $0.28 | DN, EBPR |
| MicroCg | 670,000 | NA | | DN |
| MicroCm | 670,000 | NA | | DN |
| MicroCglycerin | 1,000,000 | NA | | DN |
| Unicarb | 600,000 | NA | | DN |
| Soft Drink Waste, 5 Brix | 68,500 | $0.04 - $0.05 | $0.07 - $0.09 | DN, EBPR |

Notes:
NA- Not Available

As indicated by the costs shown above, the use of a 5 Brix soft drink waste at the normalized cost of $0.7 to $0.9 per lb COD offers the potential for significant cost savings to municipal WWTP's performing denitrification or EBPR.

# CASE EXAMPLES OF SUPPLEMENTAL CARBON ADDITION FOR BNR

## CITY OF GASTONIA WWTP'S

All wastewaters discharged to the City of Gastonia sewer system are treated by one of the two (2) municipal WWTP's operated by the City of Gastonia. The City's Long Creek WWTP has a capacity of 16 MGD and discharges to the South Fork River. The City's Crowders Creek WWTP has a capacity of 6 MGD and discharges to Crowders Creek. Both WWTP's utilize an "A2O" BNR process to reduce the concentrations of N and P contained in the wastewater before the treated effluent is discharged to the respective receiving streams.

Crowders Creek WWTP currently uses a 20% sugar water solution and Long Creek WWTP uses a 20% acetic acid solution for their respective supplemental carbon sources. The COD values for these purchased feed solutions are approximately 219,000 mg/l COD for the 20% acetic acid and 274,000 mg/l COD for the 20% sugar solution. The City paid an average of 64¢/gallon for 20% sugar solution and 73¢/gallon for 20% acetic acid during 2009. The 2009 normalized cost to the City for these purchased solutions is $0.43/lb COD for the acetic acid and $0.28/lb COD for the sugar solution.

Both Gastonia WWTP's have discharge permits that limit the concentration of total nitrogen (TN) to 6.0 mg/l TN that can be discharged into their respective receiving streams during the period of April 1 to October 31 of each year. Both WWTP's are limited to 1.0 mg/l total phosphorus (TP) concentration in their discharges on a year-round basis. As such, both plants have historically added a carbon source during warm weather months when TN limits are in effect, since both facilities have been able to provide sufficient EBPR during the winter to meet the 1.0 mg/l TP standard using only the incoming COD to the WWTP. However, the practice of discontinuing the

addition of a supplemental carbon source to the anoxic zone during the winter months has the unintended consequence of causing a gradual decline in the populations of denitrifying bacteria in the system responsible for TN removal. The population of denitrifying bacteria eventually returns once the carbon source feed is restarted, but reacclimation of the biomass normally takes 1 to 2 months to reestablish denitrification and N removal. To avoid this denitrification lag period, the Crowders Creek WWTP continued feeding their supplemental carbon source during the 2008-2009 winter months on a trial basis so that the denitrifying bacteria population would be maintained year-round, though a smaller volume of sugar solution was fed during cold weather. This year-round carbon feed successfully maintained the denitrifiers through the winter in the Crowders Creek WWTP and reduced the time required for spring restart of the nitrogen removal process.

During the April – October, 2009 period, the Crowders Creek facility purchased a 5,000 gallon tanker of sugar water an average of every 9.25 days, which equates to a COD addition of roughly 540 lbs COD per million gallons of wastewater treated or 1,200 lbs COD per day. During this same period, the Long Creek facility purchased a tanker of acetic acid every 5.5 days on average, but did not continue feeding during winter months. The Long Creek COD addition averages roughly 275 lbs COD per million gallons of wastewater treated (or 1,660 lbs/day) when TN limits are in effect.

## CHARLOTTE MECKLENBURG UTILITIES WWTP'S

Charlotte Mecklenburg Utilities (CMU) operates two WWTP's that utilize BNR for N and P removal. The McDowell Creek WWTP is a 12 MGD municipal wastewater treatment facility which employs biological phosphorus removal (BPR) and nitrogen removal. The Mallard Creek WWTP is a 12 MGD sister facility. Both WWTP's includes anaerobic, anoxic and oxic (aerated) zones in order to achieve $BOD_5$ removal, nitrogen removal (via nitrification and denitrification) and phosphorus removal using biological processes. The WWTP's include two identical treatment trains and are operated in the UCT/VIP configuration.

In order to enhance phosphorus removal, CMU began adding acetic acid in 1999 as a supplemental carbon source to the McDowell Creek WWTP at a rate of 1,400 to 2,100 gpd and a cost of approximately $400,000 per year.

Independent Beverage Corporation (IBC) is a soft drink bottler in Charlotte, NC. The IBC facility has employed a collection system for segregating and collecting high-sugar wastewaters from the facility for beneficial use as a by-product since 1995. This "reclaimed sugar water" was formerly used as a source of feed for yeast production at the Fleischmann's Yeast plant in Gastonia, NC. Capture and utilization of this sugar water produced an approximate 50% reduction in $BOD_5$ discharged to the sewer by IBC. However, when the Fleischmann's plant closed in 2000, IBC either had to find a new end-user for the sugar-water, install pretreatment, or pay increased $BOD_5$ surcharges to the municipal sewer system.

In 2000, a joint investigation by CMU and IBC was initiated to determine if the sugar water from IBC could be used to supplement or replace the acetic acid feed for EBPR at the CMU McDowell Creek WWTP. A full-scale pilot test program was initiated in November 2000, in which the sugar-water was substituted for the acetic acid in a step-wise fashion in one of the plant's two treatment trains, while the other train was operated as a control and continued to use acetic acid.

The study plan was to use a step-wise program to gradually replace the acetic acid feed in Train #2 with the sugar-water over a 10-week period, while maintaining Train #1 as a control with acetic acid continuing to be fed. The sugar-water was transported to the WWTP using 5,000 gallon tanker trucks, which also served as the storage reservoirs. A metering pump was used to feed the sugar-water at constant rate to the Train #2 anaerobic stage.

Historically, acetic acid had been fed at a minimum rate of approximately 1,400 gpd (700 gpd per train) of 20% acetic acid, contributing an average COD (and $BOD_5$) of approximately 47 mg/l (based on the average plant flow of 4.5 MGD). At least initially, the sugar-water feed rate was set to provide a similar $BOD_5$ load as the acetic acid had been providing. Analysis of the sugar content of the sugar-water loads using a refractometer indicated a sugar content of 8 to 9 brix, which corresponds to an average $BOD_5$ of approximately 85,000 mg/l. Based on this, it was estimated that roughly 2,500 gpd (1,250 gpd per train) of the sugar-water would need to be fed in order to deliver the same $BOD_5$ to the system.

Based on the success of the pilot program, by mid-February 2001, the acetic acid feed was completely phased out by CMU and replaced by the sugar-water feed in both treatment trains on a permanent basis. This program, which started out as a test program, has now operated successfully for 10 years and has demonstrated the potential beneficial use of a high sugar wastewater as a supplemental carbon source for EBPR in a full-scale application.

The substitution of sugar-water in place of the acetic acid feed at the McDowell Creek WWTP has enabled the WWTP to realize significant cost savings while maintaining a consistently high level of treatment performance. Since the initiation of the sugar-water feed, the monthly average effluent total phosphorus levels have consistently been 0.4 mg/l or less while effluent total nitrogen levels have approached 4 mg/l and have been consistently below the 10 mg/l limit without the use of the effluent denitrifying filters.

The sugar-water is transported via tanker truck to the WWTP and normally is approximately 7 Brix. The WWTP currently uses approximately 23,000 gallons per week of the sugar-water at a current cost of approximately $45,000/year and also feed some limited amount of acetic acid as needed to satisfy the BNR supplemental carbon requirements. This currently represents a cost savings of approximately $470,000 per year for CMU as compared to the potential acetic acid cost.

This cooperative effort between CMU and IBC has resulted in a win-win situation for both parties. CMU has realized significant cost savings, while maintaining phosphorus removal performance at the WWTP at levels surpassing historical performance. At the same time, this has also provided a beneficial end use of the IBC wastewater, and allowed the bottler to avoid significant costs associated with pretreatment or increased sewer surcharges. The sugar reclaim program has now been operated by CMU IBC for over 9 years. IBC collects and sells their "reclaimed sugar water" to CMU for use in their McDowell Creek WWTP for BNR. Use of the IBC sugar waste for BNR is now saving the POTW approximately $470,000 per year in acetic acid purchase costs. At the same time, IBC has both reduced its annual sewer charges and received an additional $200 payment ($47,000/year) from CMUD for each 5,000 gallon load of 5 brix (minimum) reclaimed sugar water delivered to the McDowell Creek WWTP. In 2010, the reclaimed sugar program with IBC was expanded to include the Mallard Creek WWTP and the 2nd CMU WWTP began using the IBC soft-drink reclaimed sugar to supplement the acetic acid feed to the EBPR process.

Based on the success by IBC, Choice beverage USA, a soft-drink bottler in Gastonia, NC investigated the feasibility of collecting high strength sugar waste for reclaim as a BNR carbon source in 2010. It was determined that the Choice plant could collect approximately 1,980 lbs of COD per day or 9,900 lbs COD/week for 5 day/week bottling plant operation at an approximate 5 brix strength and by doing so, could reduce its sewer $BOD_5$ surcharges by approximately $100,000 per year. Based on the 5 brix reclaim sugar solution, this meant that Choice would collect approximately 3,500 gallons per day of reclaim sugar solution and could provide sufficient product to supply one (1) full 5,000 gallon tanker every 1.4 days of operation with a COD content of approximately 2,850 lbs per load for an average 7 day per week daily average of approximately 1,400 lbs COD/day. A 5,000 gallon load of the 5 brix reclaim sugar contains approximately 2,856 lbs COD.

In July 2010, Choice began hauling reclaimed sugar water to the CMU McDowell Creek WWTP. After 2 months of sugar water capture and hauling, the results so far have been that Choice has saved approximately $10,000 per month in sewer charges and CMU has received approximately 5,000 gallons per week of 6 Brix sugar water for BNR feed. Continuation of this arrangement with Choice will result in an approximate additional savings of $70,000 per year for CMU due to further reductions in acetic acid purchases.

## SUMMARY & CONCLUSIONS

As additional municipal WWTP's are upgraded to provide N and P removal through biological processes, additional supplemental carbon sources will be needed. Alternative carbon sources, particularly soft drink wastes at the normalized cost of $0.7 per lb COD, offer the potential for significant cost savings to municipal WWTP's performing denitrification or EBPR.

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# True Confessions of the Biological Nutrient Removal Process

*Sam Jeyanayagam*

Nitrogen and phosphorus are essential growth elements for microorganisms used in wastewater treatment; therefore, during all biological treatment, some level of nutrient removal occurs. The resulting cell mass contains about 12 percent nitrogen and 2 percent phosphorus by weight. When a treatment system is engineered to remove nutrients greater than these metabolic amounts, it is called biological nutrient removal (BNR). In essence, BNR is comprised of two processes: biological nitrogen removal and enhanced biological phosphorus removal (EBPR).

## Biological Nitrogen Removal

Key biological nitrogen removal reactions are nitrification and denitrification (**Figure 1**). Other related reactions include ammonification (conversion of organic nitrogen to ammonia nitrogen) and nitrogen uptake for cell growth.

### Nitrification

Nitrification is the oxidation of ammonia to nitrite and nitrate. The key organisms involved are thought to be *Nitrosomonas* and *Nitrobacter*. These are autotrophs that oxidize inorganic nitrogen compounds for energy:

*Nitrosomonas*

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{New cells}$$

*Nitrobacter*

$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{New cells}$$

Carbon for cell growth is obtained from carbon dioxide. Consequently, organic substrate (BOD) is not a prerequisite for the growth of nitrifiers. Nitrite accumulation is typically not encountered in a fully nitrifying system because *Nitrosomonas* is slower growing; however, there is some indication that at wastewater temperatures of above 25 oC to 30 oC, nitrite-to-nitrate conversion may become rate-limiting, resulting in increased chlorine demand for disinfection.

It is now known that organisms other than *Nitrosomonas* and *Nitrobacter* can also mediate the nitrification process; therefore, the term ammonia oxidizing bacteria (AOB) is used to refer to them collectively.

In BNR systems, nitrification is the controlling process for two reasons: (1) AOBs lack functional diversity. They represent about 2 percent of the microbial mass. (2) AOBs have stringent growth requirements and are sensitive to environmental conditions.

Nitrification is strongly impacted by the following factors:

- ***Solids Retention Time (SRT)***: Since the growth rate of nitrifiers is slow compared to heterotrophs (BOD-removing organisms), longer SRTs are required for reliable nitrification. The nitrification SRT is a direct function of the wastewater temperature.
- ***Temperature***: The nitrification rate increases with temperature up to a certain point (30° C to 35° C), and then it decreases. A rule of thumb is that a temperature change from 20° C to 10° C will decrease the nitrification rate to approximately 30 percent, requiring about three times the mass of MLSS to produce an equivalent effluent ammonia concentration. Consequently, a system designed for winter nitrification can generally meet year-round ammonia nitrogen limits.
- ***Dissolved Oxygen (DO)***: The nitrification oxygen demand is approximately 4.6 mg of oxygen per mg of $NH_4$-N oxidized. When the DO drops to significantly below 2 mg/L for an extended period, nitrification would be inhibited.
- ***Alkalinity and pH***: Nitrification results in the destruction of 7.1 mg of alkalinity ($CaCO_3$) per mg of $NH_4$-N oxidized. If the influent contains inadequate alkalinity, nitrification would be compromised. As alkalinity is destroyed, pH is decreased and this could potentially reduce the nitrification rate. Most WWTPs operate in a pH range of 6.8 to 7.4.
- ***Inhibitory Compounds***: Nitrifiers are inhibited by certain heavy metals and organic compounds. Some polymers used in sludge conditioning are also inhibitory. Typically, inhibition is a concern if significant industrial discharges are present.

Nitrification results in the conversion of nitrogen from a reduced form (ammonia) to an oxidized form (nitrate). It is not in itself a significant nitrogen removal mechanism.

### Denitrification

Denitrification must follow nitrification to achieve significant total nitrogen removal. Denitrification is the reduction of nitrate to nitrogen gas by certain heterotrophic bacteria. The process requirements are anoxic conditions and a source of rapidly biodegradable organic matter (RBOM). Anoxic refers to the presence of combined oxygen (nitrate and nitrite) and the absence of free or dissolved oxygen (DO). The simplified reaction is:

$$NO_3^- + \text{RBOM} \rightarrow (N_2\,(\text{gas}) + CO_2 + H_2O + OH^- + \text{New cells})$$

Denitrification results in the recovery of 3.6 mg of alkalinity as $CaCO_3$ and 2.9 mg of oxygen per mg of $NO_3$-N reduced; therefore, by combining nitrification (aerobic) and den-

*Continued on page 38*

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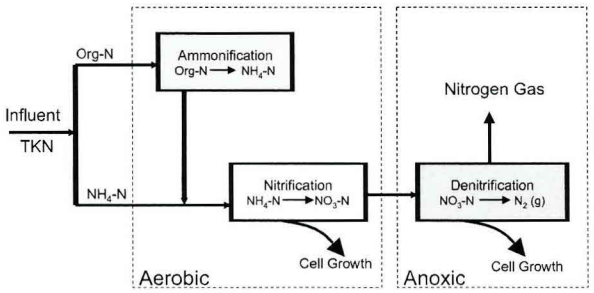
Figure 1: Biological Nitrogen Removal



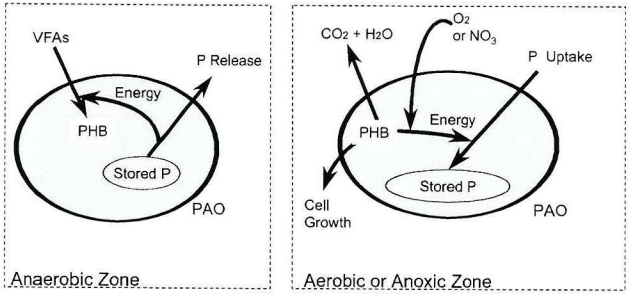



Figure 2: Biological Phosphorus Removal

*Continued from page 37*

itrification (anoxic), partial alkalinity recovery and oxygen credit can be attained. An additional benefit of incorporating an anoxic selector is improved sludge settleability.

The denitrification rate (g $NO_x$-N reduced/g MLVSS.d), which determines the amount of nitrate denitrified, is primarily a function of: (1) availability of RBOM, and (2) temperature.

• ***Availability of RBOM***: Denitrifiers, being heterotrophs, use organic matter as the energy and carbon source. As a first approximation, a minimum BOD:TKN ratio of about 3:1 is required in the bioreactor influent for reliable denitrification. The actual ratio will depend on operating conditions and substrate biodegradability. Within limits, higher F:M ratios in the anoxic zone achieve higher denitrification rates due to the presence of increased RBOM. Likewise, the type of substrate also impacts the denitrification rate. Significantly higher denitrification rates are possible with methanol and fermentation end-products, such as volatile fatty acids (VFAs) present in the influent wastewater. Denitrification supported by endogenous decay is associated with slow denitrification rates.

• ***Temperature***: Higher wastewater temperatures trigger increased microbial activity, leading to higher denitrification rates. For a given substrate (BOD) concentration, a temperature change from 20°C to 10°C will decrease the denitrification rate to approximately 75 percent.

## *Enhanced Biological Phosphorus Removal (EBPR)*

As noted previously, the typical phosphorus content of MLSS in conventional secondary treatment is approximately 2 percent by weight. Enhanced biological phosphorus removal (EBPR) refers to phosphorus uptake greater than these metabolic requirements by specialized aerobic heterotrophs called Phosphorus Accumulating Organisms (PAOs).

*Acinetobacter* is the most widely recognized PAO. The phosphorus content of the biomass can be as high as 10 percent by weight, but is typically in the range of 3 to 5 percent; hence, the biological phosphorus removal capability of a system is directly related to the fraction of PAOs in the MLSS. Key process features that favor the selection of PAOs include:

- Anaerobic zone with adequate RBOM—in particular, volatile fatty acids (VFAs).
- Subsequent aerobic zone.
- Recycling of the phosphorus-rich return sludge to the anaerobic zone

In the anaerobic zone (**Figure 2**), the PAOs take up and store VFAs as carbon compounds such as poly-b-hydroxybutyrate (PHB). Note that PAOs, being aerobes, can not use the VFAs for cell growth in the anaerobic zone. Instead, the VFAs are used to replenish the cell's stored PHB for subsequent utilization in the aerobic zone. In other words, in the anaerobic zone the PAOs do not multiply, but get fat! The energy required for PHB accumulation is provided by the cleavage of another storage product, the inorganic polyphosphate granules. This splitting of energy-rich polyphosphate bonds results in the release of phosphorus and may be likened to a battery discharging.

In the subsequent aerobic zone, the PAOs use the internally stored PHB as a carbon and energy source and take up all the phosphate released in the anaerobic zone and additional phosphate present in the influent wastewater to renew the stored polyphosphate pool (recharging of the battery). This is because 24 to 36 times more energy is released by PHB oxidation in the aerobic zone than is used to store PHB in anaerobic zone; hence, the phosphorus uptake is significantly more than the phosphorus release. Net phosphorus removal is realized when sludge is wasted. When the phosphorus-rich return sludge is recycled to the anaerobic zone, the process is repeated (**Figure 3**).

In short, the complex biochemical reactions of the EBPR process are fueled by the cyclical formation and degradation of stored organic compounds (e.g. PHB), in concert with the degradation and formation of inorganic polyphosphate granules.

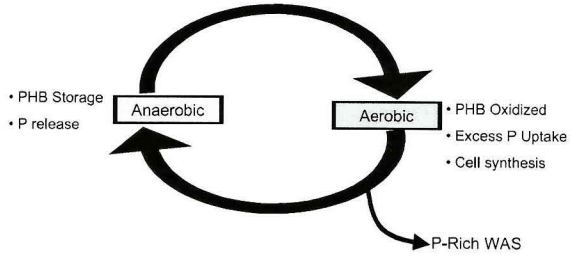
Some PAOs have the capability to denitrify. Denitrifying PAOs (DePAO) use nitrate instead of free oxygen to oxidize their internally stored PHB and effect phosphorus uptake in the anoxic zone.

The PAOs require higher energy than other heterotrophs (non-PAOs) to accomplish the cyclical reactions associated with the EBPR process. The two most critical factors that favor the proliferation of PAOs, and therefore the reliability of EBPR are: (1) the integrity of the anaerobic zone and (2) the availability of VFAs.

• ***Integrity of the Anaerobic Zone***: Strict anaerobic conditions must be maintained to provide the PAOs the first opportunity to take up the substrate. This means that the anaerobic zone should be protected from dissolved oxygen (DO) and nitrate sources, which eliminate anaerobic conditions and place the PAOs at a competitive disadvantage with other heterotrophs. Screw pumps and free fall over weirs

*Continued on page 40*

Figure 3: Anaerobic-Aerobic Cycling for EBPR



| In-line Sources | Off-line Sources |
|---|---|
| ➢ Fermentation in:<br>• Collection system<br>• Anaerobic zone of the bioreactor<br>• Primary clarifiers | ➢ Fermentation in:<br>• Primary sludge fermentor<br>• Gravity thickener<br>• First stage of a two-phase anaerobic digester<br><br>➢ Purchased acetic acid |

Table 1: Potential Sources of VFAs at a Municipal WWTP

| Zone | Process | Mediating Organism |
|---|---|---|
| Anaerobic | Phosphorus release and PHB storage | Heterotrophs (PAOs) |
| | Fermentation: Complex organics converted to VFAs | Heterotrophs (non-PAOs) |
| Pre-Anoxic | Denitrification: Nitrate to nitrogen gas via the:<br>• Use of influent substrate - BOD removal, and | Heterotrophs (non-PAOs) |
| | • Use of stored substrate (PHB) - Phosphorus uptake | Heterotrophs (DePAOs) |
| Post-Anoxic (if provided) | Denitrification: Nitrate to nitrogen gas via the:<br>• Use of cellular substrate (endogenous reactions). or<br>• Use of methanol | Heterotrophs (non-PAOs) |
| Aerobic | BOD removal | Heterotrophs (non-PAOs) |
| | Ammonification: Organic nitrogen to ammonia nitrogen. | Heterotrophs (non-PAOs) |
| | Nitrification: Ammonia nitrogen to nitrate nitrogen | Autotrophs (*Nitrosomonas & Nitrobacter*) |
| | PHB degradation and excess phosphorus uptake | Heterotrophs (PAOs) |

PAO Phosphorus accumulating organism PHB Polyhydroxybutyrate
DePAO Denitrifying PAO VFA Volatile Fatty Acids

Table 2: BNR Process Reactions

*Continued from page 38*

introduce DO into the influent. Likewise, the internal mixed-liquor recycle used in total nitrogen removal processes is a significant source of DO and nitrates, and the return sludge in nitrifying systems can also recycle nitrates. Unlike nitrification, the desirable SRT for EBPR is relatively low. When no nitrification is required, maintaining an SRT of about two to four days would prevent nitrate formation and its impact on the anaerobic zone.

• ***The Importance of Volatile Fatty Acids***: The presence of adequate VFAs in the anaerobic zone is pivotal to achieving reliable EBPR. They have also been shown to enhance denitrification rates. All VFAs are not equally efficient in achieving EBPR. Acetic acid is thought to be the preferred VFA, while formic acid does not appear to be on the menu of PAOs. Recent studies have indicated that sustained and reliable EBPR is favored by a mixture of VFAs. Methanol, a rapidly biodegradable organic compound commonly used for enhancing denitrification, has not been implicated in EBPR. Volatile fatty acids can be generated by in-line sources within the main process stream or off-line sources (**Table 1**). The benefits and drawbacks associated with each of these options should be evaluated in detail before the preferred source of VFAs is selected.

## *Process Selection*

The biochemical processes and microbial interactions associated with the BNR process are fairly complex. A working understanding of the various biological reactions, summarized in **Table 2**, is essential for designing, optimizing, controlling, and troubleshooting the BNR process.

The challenge facing designers and operators of BNR systems is to expose the microbial consortium to the required environmental conditions (i.e. anaerobic, anoxic, and aerobic) in the optimum sequence for the appropriate length of time. Considering the variations in influent flow and loadings (BOD and nutrients), this is easier said than done.

The selection of the most appropriate BNR process is generally based on influent characteristics and target effluent quality.

<u>Influent Characteristics</u>

The BNR process is very sensitive to influent characteristics. In particular, VFAs play a central role in enhancing phosphorus removal and denitrification rates. The BOD:TP and BOD:TKN ratios of the bioreactor influent are commonly used as indicators of wastewater's amenability to BNR. The minimum acceptable ratios are:

| | |
|---|---|
| BOD:TP | 20:1 to 25:1 |
| BOD:TKN | 2:1 to 3:1 |

If the influent BOD:TP is low (BOD limited), adequate VFAs may not be available and phosphorus removal could be compromised. Likewise, low BOD:TKN ratio could result in poor denitrification. Dilute influent, excessive BOD removal in the primary clarifiers, or significant recycled phosphorus and nitrogen loads from sludge processing operations may cause BOD limited conditions. A note of caution: The nitrogen and phosphorus loads in recycle streams from sludge handling and processing operations should be included in determining these ratios.

<u>Target Effluent Quality</u>

The target effluent quality used for process design should generally be lower than the permit requirements. As shown in **Figure 4**, the effluent TN and TP are comprised of the following components:

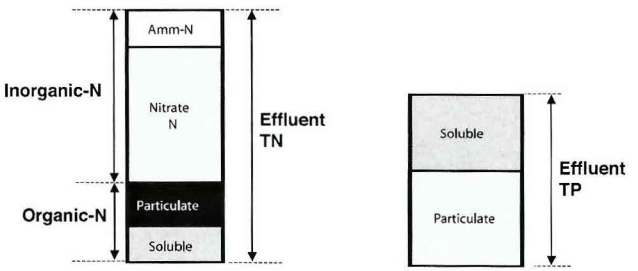



Figure 4: Components of Effluent TN and TP



| Form | Common Removal Mechanism | Technology Limit, mg/L |
|---|---|---|
| Total Nitrogen<br>Ammonia-N | Nitrification | <0.5 |
| Nitrate-N | Denitrification | 1-2 |
| Particulate Organic-N | Solids separation | <1.0 |
| Soluble Organic-N (non-biodegradable) | None | 0.5- 1.5 |
| Total Phosphorus<br>Soluble P | Microbial uptake and/or chemical precipitation | 0.1 |
| Particulate | Solids removal | <0.05 |

Table 3: Effluent TN and TP Components and Achievable Limits

Effluent TN = (Ammonia-N) + (Nitrate-N) + (Particulate Organic-N) + (Soluble Organic-N)

Effluent TP = (Soluble-P) + (Particulate-P)

The various effluent TN and TP factions, the removal mechanisms involved, and the respective technology limits are shown in **Table 3**.

Soluble P removal can be accomplished by biological or chemical means. In biological phosphorus removal, the amount of VFAs available to the bugs will determine the effluent soluble P. In the case of chemical phosphorus removal, the chemical dose used will dictate the amount of soluble P precipitated; however, reaching very low effluent soluble P would require proportionally more chemical (surpassing the stoichiometric requirement), which would result in increased sludge production.

The lowest effluent TN limit that can consistently be achieved by technologies commonly used in municipal wastewater treatment is about 3 mg/L. Further reduction in TN may be achieved by targeting the larger nitrogen fractions, namely Nitrate-N and non-biodegradable soluble Organic-N. These can be removed by reverse osmosis (RO). However, doing so would prove cost-prohibitive and may not provide an overall sustainable environmental benefit, considering the need to dispose highly concentrated reject water from the RO system.

Particulate P removal is dependent on the solids capture effectiveness of the final clarifiers and effluent filters (if provided). In the absence of effluent filtration, an effluent TP of less than 0.7 mg/L can be achieved by enhanced biological phosphorus removal (EBPR) followed by good clarification.

Good solids control becomes increasingly important as the target effluent TP is lowered. The effluent solids from an EBPR system have an average phosphorus content of around 4 to 7 percent (dry weight basis) and can contribute significantly to the effluent total phosphorus levels. For example, as shown in **Figure 5**, 10 mg/L effluent TSS corresponds to about 0.4 mg/L effluent particulate phosphorus (assuming phosphorus content of 6 percent and VSS of 75 percent). Consequently, the higher the phosphorus content of the sludge, the lower the effluent soluble phosphorus will need to be for a given effluent TP.

Reaching less than 0.2 to 0.3 mg/L effluent TP would require granular filtration. Still lower TP levels (<0.05 mg/L) can be achieved with membrane filtration or ballasted flocculation, which increase solids capture capability. This means that the effluent TP permit limit may require the plant to achieve an effluent TSS that is lower than the permitted TSS value.

## *Process Configurations*

The tank in which all the biological reactions take place is referred to as the bioreactor. Over the years, several bioreactor configurations have been developed to achieve TN and TP removal. All of them incorporate the anaerobic, anoxic, and aerobic zones. The differentiating features are the zone sequence and location of the recycle streams. Some of the common configurations are discussed below.

<u>Nitrogen Removal Process Configurations</u>

In the Modified Ludzack-Ettinger (MLE) process (**Figure 6**), the anoxic zone is placed ahead of the aerobic zone to provide the denitrification reaction the first opportunity to use the influent substrate. An internal mixed-liquor recycle (IMLR) is used to increase denitrification.

Typically, IMLR rates higher than 4Q (Q = Influent Flow) provide marginal benefits. Higher IMLR rates also increase the potential for DO recycle to the anoxic zone. Effluent TN level achievable with the MLE process is in the range of 6-8 mg/L.

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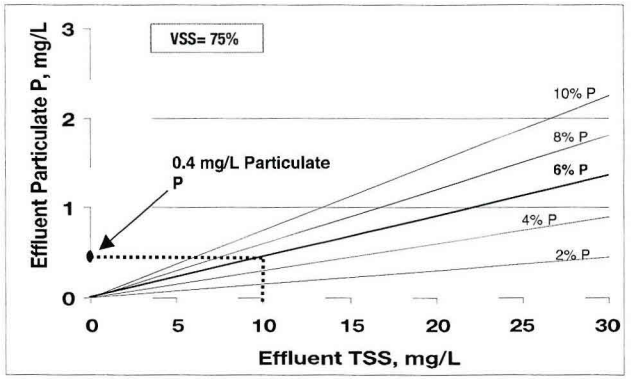


Figure 5: Impact of Effluent TSS on Effluent Particulate P

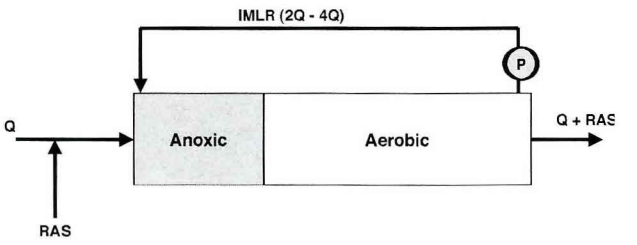


Figure 6: Modified Ludzack-Ettinger Process

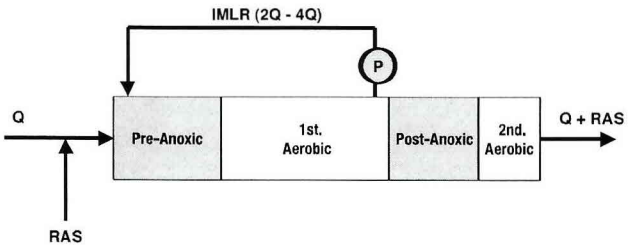


Figure 7: Four-Stage Bardenpho Process

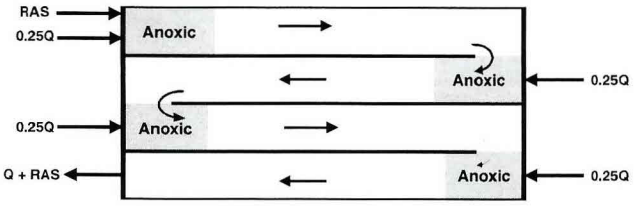


Figure 8: Step-Feed Configuration

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The four-stage Bardenpho configuration (**Figure 7**), includes a second anoxic zone for post-denitrification (endogenous or methanol-induced). This represents the Limit of Technology (LOT) for nitrogen allowing 3 mg/L TN to be reached consistently. The final aeration step is provided to drive out any remaining nitrogen gas so that it does not contribute to poor clarification in the final clarifiers.

Another LOT process configuration entails the use of denitrification filters following a nitrification system. Methanol addition would be required to sustain a viable nitrifier population in the filters. Both deep-bed and continuous backwash filters have been used for the purpose.

As illustrated in **Figure 8**, the step-feed system can be operated with an anoxic zone in each pass to produce 6-8 mg/L TN. Step-feed also offers other advantages, such as lower solids loading to the final clarifiers, higher SRT for the same tank volume, and prevention of solids washout during high-flow conditions by using the first pass for sludge reaeration.

Sequencing batch reactors (SBR) are capable of producing 6-8 mg/L TN with proper cycle times. The use of SBRs eliminates the need for final clarifiers; however, effluent equalization would be required to avoid sizing the downstream disinfection system for peak decant flow rates.

Combined Nitrogen and Phosphorus Removal Process Configurations

Biological phosphorus removal can be accomplished by placing an adequately sized anaerobic zone ahead of the aerobic zone to favor the growth of phosphorus-removing organisms. Facilities that have turned off the air supply in an effort to create an anaerobic selector at the beginning of the bioreactor have accomplished fairly good phosphorus removal.

Several potential configurations are available for combined nitrogen and phosphorus removal. These include A²O (**Figure 9**), Modified University of Cape Town (**Figure 10**), Five-Stage Bardenpho (**Figure 11**), and the Johannesburg process configurations. Oxidation ditches have also been used to attain reliable BNR.

The typical configuration encompasses an anaerobic tank followed by the completely mixed oxidation ditch. Tight DO control allows simultaneous nitrification-denitrification to be achieved in the ditch. **Table 4** compares some of the commonly used BNR processes.

Other proprietary and non-proprietary processes that have been used for achieving various levels of nitrogen and phosphorus removal include Phased Isolation Ditch, Biolac, integrated fixed film activated sludge (IFAS) systems, biological aerated filters, trickling filters, and membrane bioreactors.

### *Design Considerations*

Optimizing the complex BNR process entails maintaining a dynamic equilibrium among the functional groups and their interactions. System design should incorporate adequate flexibility to allow plant operators to respond to adverse operating conditions and influent variability. Here are some of the key design considerations for reliable BNR performance:

- Characterize the bioreactor influent using a minimum of two years of plant data. Unlike the secondary system, nutrient removal processes are extremely sensitive to influent characteristics and their variability. Recycle loads from sludge operations can modify the influent characteristics significantly and should be accounted for.
- Optimize nitrification first, since it is the controlling process and a prerequisite for denitrification. Next, optimize denitrification to achieve TN removal. Finally, maximize the biological phosphorus removal capability and consider chemical addition to accomplish additional phosphorus removal, if required.

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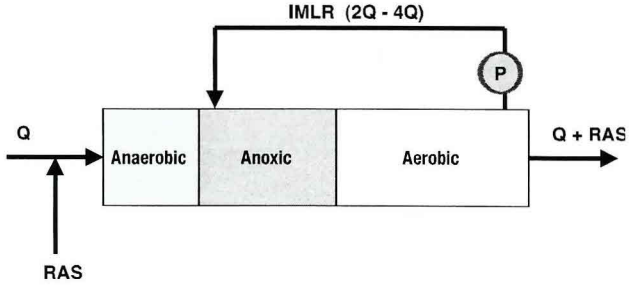


Figure 9: A²O Process

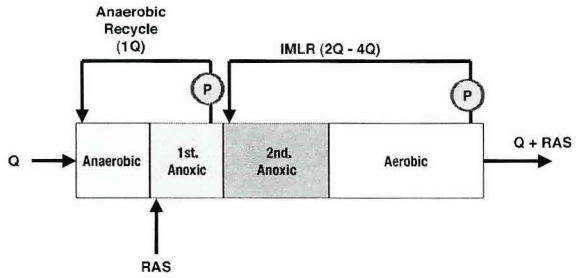


Figure 10: Modified University of Cape Town Process

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• Temperature is the single most important factor in the design of nitrogen removal systems. Use the lowest monthly average temperature for nitrification design (see discussion on temperature impacts).
• Apply an adequate safety factor (1.5 to 2.5) to determine the design nitrification SRT. The safety factor provides a necessary margin of error and accounts for influent variability, MLSS fluctuations, and other unexpected operating conditions.
• Use a realistic denitrification rate to size anoxic volume to handle IMLR nitrate load. If the recycled DO in the IMLR is significant, the anoxic volume should be large enough to deplete this as well. For municipal WWTPs with primary clarification, the anoxic volume is typically 25 to 40 percent of the total bioreactor volume.
• Design structures to achieve even flow split to bioreactors and final clarifiers. Uneven flow distribution causes operational challenges and prevents the full treatment potential of the system from being realized.
• Ensure proper mixing of the bioreactor influent and return sludge, which have different densities. If they are not mixed well, BNR organisms will not be in contact with the substrate for the entire contact time, diminishing the nutrient removal efficiency of the system.
• Size the anaerobic zone to produce adequate VFAs for phosphorus removal and to remove nitrates in the RAS flow (if applicable). Substrate uptake and storage is normally a rapid reaction and not rate limiting.
• Anoxic and anaerobic mixers should be sized for proper mixing without entraining air. Submersible mixers are commonly used in modern BNR plants.
• Consider including primary clarifiers to remove "junk" solids. Primary clarification will increase the active biomass fraction of the MLSS and reduce the bioreactor volume.
• Use inter-zone baffles to preserve the integrity of the anoxic and anaerobic zones. Baffles should be designed to prevent backmixing by considering the density differential between aerated and unaerated zones, adequate forward velocities, and water-level drop between zones. Provide free passage for scum and foam.
• Provide selective surface wasting of scum and foam to avoid accumulation in the bioreactor.
• Consider providing intra-zone baffles to promote plug flow within a zone and achieve higher reaction rates by maintaining a concentration gradient.
• Control IMLR rate to minimize DO recycle. Consider a DO exhauster zone prior to IMLR withdrawal.
• Provide variable-speed IMLR and return sludge pumps.
• Provide flexibility to vary DO spatially within the aerobic zone to match demand. DO probes, on-line ammonia-nitrogen analyzers, ORP probes, or NADH measurements may be used to achieve tight DO control.
• Incorporate anoxic/aerobic swing cells if significant influent load fluctuations are anticipated.
• Avoid conditions that entrain air upstream of the bioreactor, such as screw pumps, free-fall weirs, turbulence, etc.
• Provide flexibility to waste sludge from the aeration zone. This practice will keep the sludge fresh and prevent secondary phosphorus release.
• Use state point analysis to examine final clarifier performance. Site-specific sludge settleability data should be used for this purpose.
• Avoid using a common suction header to withdraw sludge from multiple final clarifiers. Such a design prevents independent control of the sludge pumping rate from the various clarifiers.
• Incorporate strategies for managing recycle streams (see discussion below).

## *Operational Considerations*

No matter how well designed a BNR system may be, proper operation is central to achieving its full nutrient removal potential. Some of the key operational considerations are discussed below.

Temperature

Biological reaction rates are temperature-dependent. The typical response is an increase in biological activity with temperature until a maximum rate is reached. Beyond this optimum temperature, biological reaction rates are inhibited as the temperature rises.

As a rule of thumb, a temperature change from 20° C to 10° C will decrease the nitrification rate to about 30 percent, requiring three times the mass of MLSS to produce an equivalent effluent ammonia concentration. Aerobic volume or MLSS should be increased in the colder months to compensate for reduced growth rates. Typically, nitrification inhibition sets in at around 40° C.

With respect to phosphorus removal, temperatures above 30° C appear to decrease the EBPR capability. This may be attributed to lower anaerobic VFA production rates and aerobic phosphorus uptake rates. Also at higher temperatures, PAOs are at a competitive disadvantage and are unable to compete effectively for the available VFAs in the anaerobic zone with organisms that do not accumulate PHBs, such as Glycogen Accumulating Organisms (GAOs).

DO Control

Avoid over-aeration. Controlling aera-

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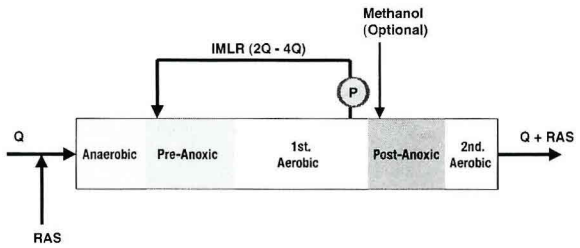



Figure 11: Five Stage Bardenpho Process

| Process | Nitrogen Removal | Phosphorus Removal | Other Features |
|---|---|---|---|
| MLE | Good | None | • Moderate basin volume |
| Four-Stage Bardenpho | Excellent | None | • Larger basin volume<br>• Potential for adding methanol |
| Step-Feed | Moderate | None | • No IMLR<br>• Smaller Final Clarifiers<br>• Potential for preventing solids washout |
| SBR | Moderate | Inconsistent | • No IMLR<br>• No Final Clarifiers<br>• May require effluent flow equalization |
| $A^2O$ | Good | Good | • Moderate basin volume<br>• Sensitive to RAS nitrate and DO recycle |
| Modified UCT | Good | Excellent | • Separate anoxic zone for RAS denitrification - protects anaerobic zone<br>• Larger anaerobic volume<br>• Two internal recycles<br>• Increased process complexity |
| Five-Stage Bardenpho | Excellent | Good | • Larger reactor volume<br>• Potential for adding methanol |
| Oxidation Ditch | Excellent | Good | • No IMLR<br>• Long HRT (larger tank volume)<br>• Tight DO control essential |

Table 4: Comparison of Common BNR Process Configurations

*Continued from page 44*

tion zone DO is crucial to BNR performance. Air supply should be just sufficient to meet the carbonaceous and nitrogenous demands and achieve good mixing. Detrimental impacts of over-aeration include:

- Secondary phosphorus release due to cell lysis
- High DO in the IMLR flow
- High O&M cost

By maintaining low DO levels (0.5-1.0 mg/L) at the tail end of aeration zone, these problems may be avoided.

Tight DO control is also essential for promoting simultaneous nitrification/denitrification (SND), which occurs in the aerobic zone when regions low in DO are established within the floc. If sufficiently long SRTs are maintained, the low DO conditions can achieve significant denitrification without impacting nitrification. Complete mix systems (e.g. oxidation ditch process) rely on SND to achieve reliable TN removal without the use of baffled anoxic and aerobic zones.

Filamentous Growth

Conditions necessary for BNR are also favorable to filamentous growth, which could potentially cause poor settling in the final clarifiers. Filamentous growth may be controlled by:

- Creating anaerobic or anoxic selector zones to allow only floc-formers to access the food. By placing the filaments at a disadvantage, they are prevented from proliferating. It should be noted that selectors have not been found to be effective against organisms such as *Microthrix parvicella* and Type 0092.
- Chlorinating the RAS to kill filaments; however, overfeeding chlorine can be detrimental to the BNR process.
- Eliminating or controlling the operating conditions (low DO, low F:M, SRT, complete mix, etc.) that cause filamentous growth. Identifying the dominant filament would be helpful in determining the conditions that favor its growth. Consider using emerging and more accurate methods of filament identification, such as molecular fingerprinting. Using this technique, researchers at the University of Cincinnati were able to isolate *Paenibacillus* spp., a non-filamentous organism that traditional methods failed to identify. Their work indicated that this organism represented up to 30 percent of the biomass in the system investigated and contributed to the complete failure of the clarifier.
- Adding polymers to final clarifiers to enhance sludge settleability. Care should be exercised in selecting a polymer that neither inhibits nitrification nor contributes to effluent toxicity.

Scum and Foam

The most effective way to deal with scum and foam is to remove them from the biological system as quickly and completely as possible. Clarifiers should be designed with good scum removal facilities. Foam may be removed directly from the bioreactor by selective wasting from the surface. Accumulation in the bioreactor and re-inoculation of the influent stream should be avoided. Although the preferred method is to handle scum and foam separately, many facilities find it convenient to process them in the solids handling system.

Recycle Loads

Recycle streams from sludge processing operations could potentially impose significant additional nutrient loadings to the BNR bioreactor, surpassing the system's nutrient removal capability. The magnitude of the problem is dependent on the type of sludge processing and handling operations. The impact of recycle streams could be minimized by:

- Equalizing recycle flows
- Scheduling sludge processing/conditioning operations
- Treating the sidestreams

Secondary Release

Although VFA uptake is always associated with P release, P release could occur without concomitant uptake of VFAs. This is termed secondary release. Because there is no energy (VFA) storage, subsequent aerobic uptake of the released phosphorus may not be possible and elevated effluent phosphorus levels could result. Potential causes of secondary release include:

- Long anaerobic, anoxic, or aerobic retention times
- Co-settling EBPR sludge in the primary clarifier
- Septic conditions in final clarifiers due to deep sludge blanket
- Anaerobic digestion of primary and EBPR waste sludge
- Unaerated storage of the EBPR sludge
- Blending and storing primary and EBPR sludge

## Conclusion

It is anticipated that an increasing number of WWTPs would be required to achieve nutrient removal in order to protect the aquatic ecosystem. The BNR process is a proven method of removing nutrients using naturally occurring microorganisms.

The primary objective of BNR plant operations is to achieve regulatory compliance consistently. Other objectives often include operational cost savings; process optimization; and a safe, clean workplace. Meeting these objectives demands proper design, operation, and management. Designers should incorporate features that would provide maximum process flexibility and ease of operation and maintenance. The plant staff, in turn, is responsible for operating the facility as intended and achieving the effluent goals.

The BNR process is mediated by several functional groups and is more complex than a secondary system. More than ever before, we are getting closer to understanding the competing and complimenting reactions at a microbial level. It behooves designers and operators of BNR systems to keep abreast of developments in the field, while contributing to the pool of knowledge by sharing their experiences and lessons learned.


# Considering an alternative of

## BNR plants share lessons learned and unexpected observations made during full-scale supplemental carbon pilot tests

*Katya Bilyk, Theresa Bruton, Joe Rohrbacher, Ron Latimer, Paul Pitt, Robert Dodson, and John Dodson*

Wastewater treatment plants (WWTPs) that discharge into nutrient-sensitive watersheds face strict new regulations requiring enhanced removal of total nitrogen (TN) and total phosphorus (TP), often with levels of TN at or below 3 mg/L. To provide sufficient denitrification to reduce to these levels, many of these facilities will require the addition of supplemental carbon to the second anoxic zones of their biological nutrient removal (BNR) tanks and denitrification filters. Methanol historically has been used for denitrification at WWTPs, but for various reasons, many utilities are considering alternative carbon sources. Two mid-Atlantic municipal WWTPs conducted full-scale evaluations of supplemental carbon alternatives to evaluate whether these products were effective in meeting their low-effluent nitrogen discharge limits. Bench-scale experiments also were conducted.

## The denitrification process

Denitrification is a two-step reaction, which includes the reduction of nitrate ($NO_3^-$) to nitrite, then the reduction of nitrite to nitrogen gas ($N_2$). In general, the half reaction for complete denitrification is:

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2\,(g) + 6H_2O$$

In the biological wastewater treatment process, facultative heterotrophic bacteria are responsible for denitrification. Although aerobic respiration is favored, these bacteria will use nitrate and nitrite when oxygen is limited or absent, and sufficient organic substrate exists. Because the majority of biodegradable organic material is oxidized during the aerobic process, limited carbon is available in the secondary anoxic zones of the BNR processes and/or denitrification filters. In addition, many WWTPs have unfavorable biochemical oxygen demand to total Kjeldahl nitrogen ratios for enhanced nitrogen removal. Therefore, it often is necessary to add supplemental carbon in the secondary anoxic zones and/or denitrification filters to aid the denitrification process and comply with low TN limits.

At WWTPs, methanol historically has been the most prevalent carbon donor for the denitrification process. However, methanol has serious safety concerns, significant cost volatility, problematic supply due to offshore production, and questionable long-term sustainability in the wastewater industry due to competing needs for methanol. In addition, using methanol as a carbon source requires the development of a specialized bacteria population – methylotrophs – because ordinary heterotrophic bacteria cannot use methanol to denitrify. Methylotrophs have lower growth rates than ordinary heterotrophs and are more sensitive to low temperatures. The biomass must become acclimated to the methanol feed before denitrification is successful, and the acclimation period can take several weeks, particularly at low temperatures, and methylotroph washout can occur at these low temperatures if retention times are too short or methanol is fed only intermittently.

## Alternative carbon sources

Because of the many challenges associated with methanol, many wastewater utilities use alternative sources of carbon for denitrification. The sources that have come onto the market recently include corn syrup, glycerin-based products, acetic acid, ethanol, and monopropylene glycol. Glycerin was used in these full-scale investigations.

Glycerin is a byproduct of biodiesel production, and the increase in the production of biofuels has led to interest in glycerin recovery and the establishment of a market for glycerin byproducts used in wastewater treatment as a carbon source for denitrification. Glycerin-based products are available in refined proprietary form, as well as in a crude and potentially more variable form. The characteristics of these products can vary significantly between suppliers, and even within the same product the characteristics can vary from shipment to shipment, particularly for unrefined products. Also, the quality of waste glycerin byproducts depends on the biodiesel feedstock, which varies according to raw material availability and market conditions. Some glycerin products have sufficient residual methanol concentration to require the same safety precautions as needed with methanol or ethanol.

## Testing the waters

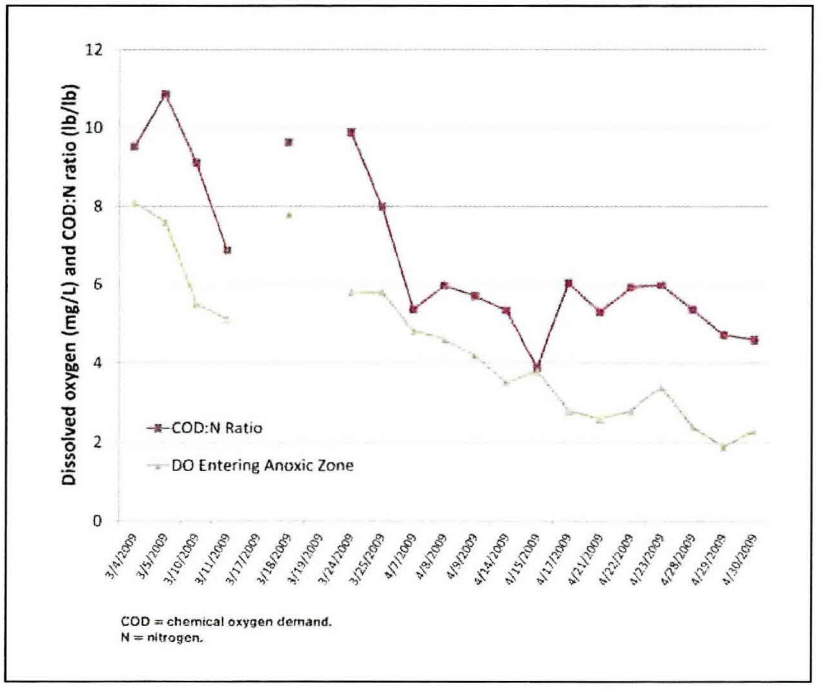
The mid-Atlantic municipal WWTPs that conducted full-scale evaluations with supplemental carbon alternatives, plants A and B, were two 76,000-$m^3$/d (20-mgd) facilities in North Carolina. Both plants are expecting lower TN and TP limits in the next 5 to 7 years, and are being proactive in their compliance approach by gaining experience with these products. Plant A has to meet TN 3 mg/L and TP 0.23 mg/L, and Plant B must meet 2.2 mg/L TN and 0.23 mg/L TP in the near term. Each of these facilities added supplemental carbon to the influent to the second anoxic zone of a five-stage BNR process. The supplemental carbon was introduced to test basins at plants A and B through totes and temporary piping.

To quantify the performance of the supplemental carbon sources, process monitoring at each facility was conducted several times during the course of each full-scale pilot test. The monitoring generally included anoxic zone performance profiles that involved collecting grab samples of the second anoxic zone influent, midpoint, and effluent locations for each of the basins being monitored. Treatment process profiles such as ammonia, nitrate, nitrite, and dissolved oxygen (DO) concentrations, were quantified. The supplemental carbon products also were analyzed to evaluate their properties and verify that the chemical oxygen demand (COD) content of the product was comparable to the manufacturers' claims. Neither plant had any on-line nutrient instrumentation prior to this study.

Bench-scale experiments also were performed to determine the carbon utilization/carbon to nitrate ratio and the specific denitrification rates (SDNR) in a controlled environment. The tests were carried out in a 4-L reactor filled with undiluted mixed liquor from the downstream end of the aerobic zone. Nitrogen

**During the full-scale evaluations, supplemental carbon sources were dosed into the biological nutrient removal system from totes and temporary piping.**
Hazen and Sawyer

**Figure 1. Post-anoxic zone influent dissolved oxygen versus observed COD:N ratio**




gas was bubbled through the liquid to remove the residual DO and maintain anoxic conditions. A low-speed mixer was used to keep the liquid in suspension and avoid vortex formation. COD and nitrate were added in excess at the start of each batch test to avoid rate-limiting conditions. Temperature, pH, and DO were monitored continuously. A control reactor with no supplemental carbon also was run at each facility to assess the impacts of endogenous carbon utilization for denitrification.

The reactors – both test and control – were sampled every 10 to 15 minutes for the first hour and then every 15 to 30 minutes for the duration of the experiment, which was typically 2 to 3 hours. The samples were filtered immediately through a 0.45-µm filter and analyzed for nitrate, nitrite, ammonia, and COD. Total and volatile suspended solids were measured at the beginning and end of each experiment.

The various full-scale pilot tests indicated that glycerin is an effective supplemental carbon source when applied to second anoxic zones of biological nutrient removal. These facilities are proceeding with design of full-scale facilities for carbon addition. Several lessons were learned from the full-scale application and bench-scale experiments of these products, and are consistent with results seen at several other mid-Atlantic wastewater treatment plants that conducted full-scale evaluations with glycerin and corn syrup.

## Lesson 1: Controlling DO upstream of the carbon addition point is critical for optimizing carbon use efficiency and minimizing operating costs

Excessive DO concentrations entering the second anoxic zone resulted in increased carbon to nitrogen requirements and decreased denitrification efficiency. The heterotrophic bacteria responsible for denitrification are facultative aerobes when using glycerin, corn syrup, or other non-methanol substances as carbon sources. Therefore, in the presence of DO, they prefer to use oxygen as an electron acceptor instead of nitrate or nitrite. This results in a biomass that preferentially metabolizes the supplemental carbon source aerobically, thereby decreasing the carbon available for denitrification and reducing effective residence time under anoxic conditions.

In order to manage DO concentrations entering the second anoxic zone, two DO control zones can be provided in the aerobic zone: one to regulate airflows to the majority of the aerobic zone, and the other to regulate airflows to the downstream end of the aerobic zone in order to maintain minimum mixing conditions and minimize the oxygen mass entering the anoxic zone.

Figure 1 (left) shows a plot of aerobic zone effluent DO concentrations data points versus the calculated full-scale carbon to nitrogen requirement for glycerin application at Plant A. Manual butterfly valves that served the last grid of diffusers in the aerobic zone were throttled as the pilot progressed in order to decrease oxygen entering the second anoxic zone. Subsequently, the required carbon-to-nitrogen ratio decreased, and the denitrification performance was enhanced.

Proper DO management is paramount to avoid wasting supplemental carbon as well as aeration energy, both of which are significant operational expenditures at WWTPs. Also, limiting DO to the second anoxic zone will result in greater use of the tank volume dedicated to denitrification.

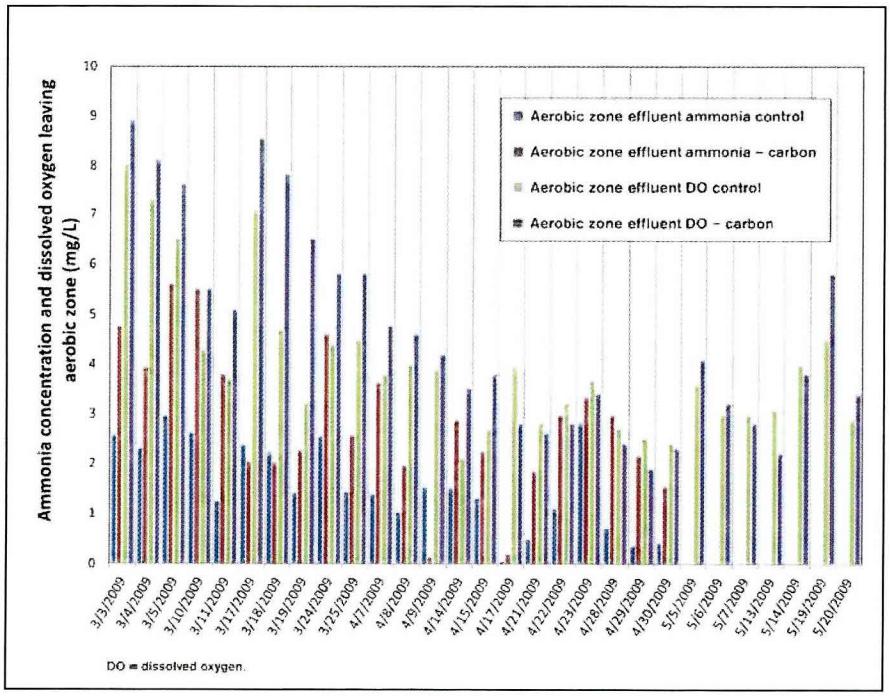
## Lesson 2: Denitrification rates increased with acclimation to glycerin

A comparison of temperature-adjusted SDNRs from anoxic batch tests performed before and after full-scale glycerin addition revealed significantly faster denitrification activity. The results suggest that although glycerin does not require a specialized bacteria population, denitrification rates may increase after prolonged glycerin addition due to buildup of a glycerin denitrifying population.

The anoxic batch tests included collecting mixed liquor suspended solids (MLSS) from the end of the aerobic zone of the BNR process, and then adding COD, in the form of glycerin, and nitrate to the MLSS in the anoxic batch reactors. The anoxic batch tests were conducted prior to any carbon addition in the full-scale plant, and then repeated after a month of full-scale addition. An acclimated biomass more adept at denitrifying glycerin was not expected. The temperature-adjusted SDNRs for both the control and test reactor increased considerably from the initial, not acclimated, batch tests.

The SDNR of the control reactor also increased, although this reactor was not fed carbon. It is hypothesized that some carbon was stored in the acclimated biomass in the full-scale reactor that was used as the seed for the batch tests. This carbon then could have been released in the acclimated control reactor to enhance the SDNR when compared with the earlier temperature-

**Figure 2. Aerobic zone effluent ammonia and dissolved oxygen at Plant B**




adjusted control SDNR. Bench-scale testing with glycerol at other facilities also has demonstrated this phenomenon. More research into this topic is warranted.

It also should be noted that different glycerin products were used for the acclimated and non-acclimated batch tests. However, it was not expected that the different source of glycerin would have much of an impact on the SDNR because there are fairly consistent SDNRs between various glycerin products, according the findings presented in the WEFTEC 2009 paper, "Evaluation of Alternative Supplemental Carbon Sources at Four BNR Facilities."

## Lesson 3: Carbon addition enhanced biological phosphorus removal and first anoxic zone performance

Data gathered at Plant B showed improved biological phosphorus removal (BPR) performance immediately after supplemental carbon was added. Similar results have been observed at other facilities as well. When carbon is added to enhance denitrification, the nitrate loading to the anaerobic zone (and in the effluent) is reduced because the return activated sludge (RAS) has a lower nitrate concentration. This concentration reduction is due to the increased nitrogen removal in the second anoxic zone associated with the supplemental carbon. As a result, the polyphosphate accumulating organisms responsible for BPR have more volatile fatty acids available for biological phosphorus release because of decreased carbon competition with the denitrifying heterotrophs. Therefore, the addition of a supplemental carbon source led to enhanced BPR. (It should be noted that a similar phenomenon would occur if methanol had been added to the second anoxic zone.)

In addition to enhanced BPR performance, supplemental carbon also has been observed to impact first anoxic zone denitrification. Due to the increased removal of nitrate in the second anoxic zone and subsequent reduction of nitrate returned during the nitrified recycle and RAS flows, the first anoxic zones may become underutilized without an adjustment to the nitrified recycle rate. This adjustment will counteract the decreased nitrate concentration. Maximizing the use of the first anoxic zone and the influent carbon available for nitrification in this zone minimizes the supplemental carbon necessary to feed to the second anoxic zone. Analyzing samples collected downstream of the first anoxic zone for nitrate and increasing the nitrified recycle rate when nitrates are not found improves the overall efficiency of nitrogen removal in an activated sludge process. It also can reduce operating costs associated with supplemental carbon addition.

## Lesson 4: Incomplete nitrification in the aerobic zone prior to the second anoxic zone can

**Figure 3. Nitrate removal performance at Plant B**

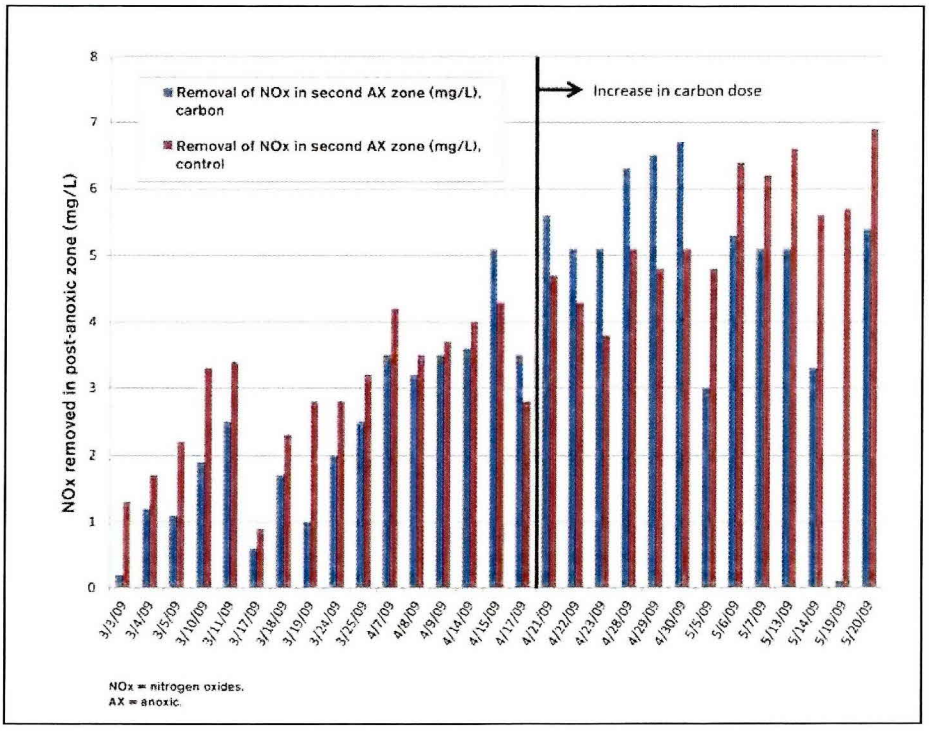
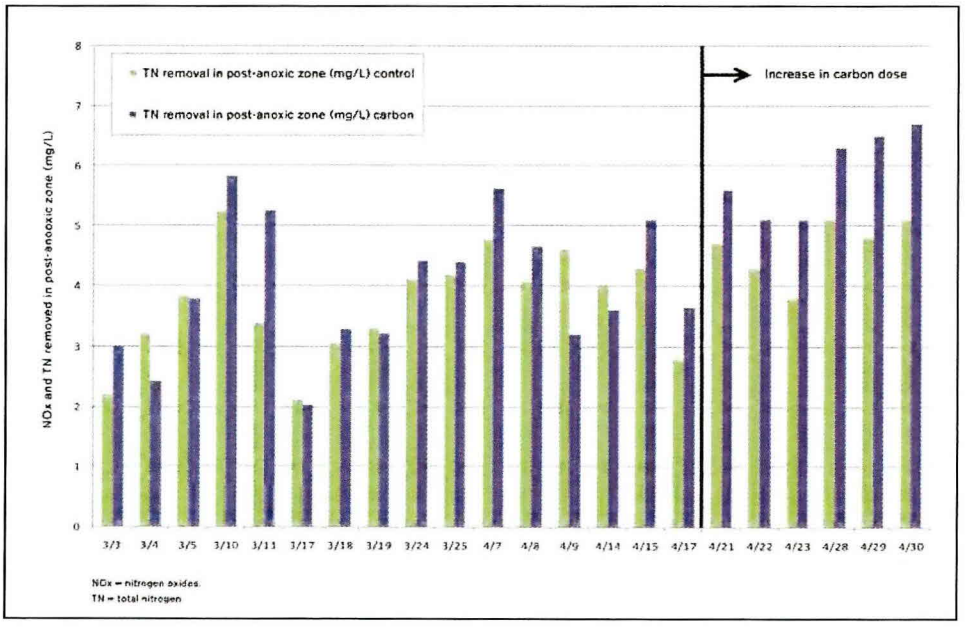


**Figure 4. Total nitrogen removed across post-anoxic zone at Plant B**




## mask the effectiveness of supplemental carbon addition

During its full-scale pilot test, Plant A added carbon to the second anoxic zone of a single test basin. Denitrification in the test basin was compared to the control basin where no carbon was fed. Nutrient profiles were conducted in both basins.

After the glycerin is added, the results of the first 6 weeks of pilot testing show no improvement in denitrification. In fact, considering the nitrate removal data alone, there was greater denitrification performance in the control basin.

During this 6-week period, the ammonia and DO concentrations entering the second anoxic zone of the test basin were elevated compared to the control basin (See Figure 2, p. 59). The carbon feed rate was increased starting the week of April 21 to overcome residual oxygen demand in the anoxic zone. In the test basin, 1 to 2 mg/L more $NO_x$–N was removed than in the control basin once the feed rate was increased. The amount of $NO_x$–N removed in the second anoxic zone of the test and control basins is shown in Figure 3 (p. 59).

TN removal in the test basin typically was greater than or equal to TN removal in the control basin, even during the early phase of the pilot (See Figure 4, above). In the early phase, the test basin removed more TN because nitrification occurred in the anoxic zone due to the high DO. The test basin and control basin also achieved a similar amount of denitrification. This underscores the importance of monitoring DO and ammonia, in addition to nitrate and nitrite, in and out of the second anoxic zone when assessing denitrification performance. These observations illustrate the importance of on-line nutrient analyzers at ensuring that nitrification has been completed prior to carbon addition, as nitrate concentrations would increase across the re-aeration zone when residual ammonia is nitrified, partially reversing the benefits of the supplemental carbon addition.

## A good alternative

Alternative carbon sources such as glycerin are promising alternatives to the use of methanol for denitrification at WWTPs. Not only were they very effective in denitrification, but they also can be cost-competitive to methanol depending on the market conditions, particularly when the safety issues with methanol are taken into account.

Full-scale pilot tests with these products underscore the need for complete nitrification and proper management of DO downstream of the aerobic zone, as this affects the efficiency of the first and second anoxic zones. A holistic approach to nitrogen and phosphorus removal is recommended. This involves monitoring orthophosphate, ammonia, nitrite, and nitrate to maintain a nutrient mass balance throughout the process, and making the appropriate operational adjustments to maximize treatment efficiency and limit supplemental carbon costs. For example, after supplemental carbon is added, adjustments to the nitrified recycle rates may be necessary to take full advantage of the influent carbon available for denitrification in the first anoxic zone and minimize energy costs for aeration.

Compliance with low nutrient limits necessitates full nitrification in the aerobic zone and careful operation to limit ammonia release in the anoxic zones. On-line nutrient analyzers for nitrate are useful at the end of the aerobic zone and end of anoxic zones to aid in dosing carbon and monitoring the denitrification process. This information also can be used to make other operational changes such as adjustments to the nitrified recycle rate. Proper mixing and baffle design that prevents back-mixing also are necessary to improve denitrification and carbon utilization efficiency.

The batch tests suggests that denitrification occurs more quickly with prolonged use of the glycerin products, possibly due to a shift in the microbial population and carbon storage. Also, supplemental carbon addition can improve biological phosphorus removal efficiency. Further research into the exact mechanisms behind these observations is needed to understand fully the reasons behind them.

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DIV. 6-4. Please provide any studies, analyses or other documents which address the potential benefits of higher TSS levels and the cost savings that may result.

Answer: See answer to DIV. 6-3.