Evaluation of Glycerol and Waste Alcohol as Supplemental Carbon Sources for Denitrification

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ABSTRACT

Supplemental carbon has been successfully added and implemented at biological nutrient removal treatment plants all around the world in order to reach low nitrogen discharge limits. Although, methanol has been the most prevalent external electron donor used due to its low cost and effectiveness, many utilities are moving away from it due to cost volatility, safety issues, and hindered performance in cold weather conditions. Many sustainable and alternative sources are being researched, such as glycerin-based products (Rohrbacher et al., 2009), sugar-based waste products (Pretorius et al., 2007), and effluents from food and beverage industries (Swinarski et al., 2009).

Four 22-L sequencing batch reactors (SBRs) were utilized to investigate four different supplemental carbon sources: 100% reagent grade methanol, 100% reagent grade glycerol, bio-diesel glycerol waste, and an industrial waste alcohol. These reactors were operated at 20°C with a 15 day solids retention time. Intensive profiles were carried out three times a week to monitor performance and collect data to calculate COD consumption: nitrate-nitrogen denitrified (C: N) ratios. The glycerol and bio-diesel glycerol waste reactors performed similarly as they both exhibited significant and consistent nitrite accumulation during the entire experiment. Based on reactor restart, nitrite accumulation was evident and significant within two days after startup and consistent for all further operation. Rapid nitrate to nitrite reduction coincident with COD uptake was also observed. The two glycerol reactors demonstrated an increased carbon demand over time. The commonly reported hypothesis that activated sludge transitions from a generalist population of ordinary heterotrophic organisms (OHO) that use substrate, glycerol in this case, less efficiently, producing low yields and slow growth rates, to a specialist population that use glycerol more efficiently, with higher yields and slightly faster growth rates, was verified. This is known as the generalist-specialist theory. While this hypothesis appears to be supported from an overall analysis of the

data, the actual mechanism seems to be intracellular glycerol storage coincident with rapid nitrate to nitrite denitrification, followed by slow nitrite reduction to nitrogen gas. This can possibly lead to degradation of the internally stored glycerol in the aerobic zones of the following cycle, implying a significant economic impact with glycerin addition. Although this has not been investigated further, it is believed that the presence of glycogen-accumulating organisms (GAOs) could be responsible for this intracellular storage of glycerol resulting in partial denitrification and accumulation of nitrite.

The methanol and waste alcohol reactors also performed similarly to each other and neither of these reactors exhibited any nitrite accumulation upon carbon addition. The specific denitrification rate (SDNR) of the waste alcohol was slightly higher and increased more rapidly than for the methanol reactor. The C: N for these two reactors was comparable, and methanol was close to the expected value of 4.8 g COD utilized/ g nitrate-N denitrified. The C: N for the waste alcohol during steady state operation was somewhat higher than expected. The waste alcohol exhibited an "alcoholic" odor upon addition to the reactors during startup, but this issue diminished as the biomass became acclimated to the waste alcohol.

Both industrial waste alcohol and glycerol can be considered viable alternatives to methanol; however, glycerol supplementation for denitrification can be problematic. If the glycerol dose is not optimized, then partial denitrification is observed and will lead to nitrite in the effluent, causing an increased chlorine demand for plants applying chlorine for disinfection. This is thought to occur due to energy limitations resulting from carbon storage and thus, using glycerol at treatment plants performing biological phosphorus removal (BPR) or enhanced biological phosphorus removal (EBPR) might see inefficient removal due to selective carbon utilization by polyphosphate-accumulating organisms (PAOs), or due to competition between PAOs and GAOs. Although denitrification of nitrate to nitrite occurs more quickly with prolonged glycerol addition, it also results in an increased carbon demand which causes a significant impact economically.

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1: INTRODUCTION AND PROJECT OBJECTIVES

Increasing stringency on the limits of total nitrogen (TN) and total phosphorus (TP) discharged by wastewater treatment plants has led to investigations to enhance biological nutrient removal (BNR) processes. The focus is on achieving complete denitrification through the use of external organic carbon, which is readily biodegradable and added to BNR processes to enhance the rate and amount of denitrification within the existing capacity of the activated sludge system (Swinarki et al., 2011). Discharging nitrogen and phosphorus into receiving water bodies has a negative environmental impact, as these nutrients encourage algae and plant growth. Thus, limiting the amount of nutrients released in the effluent will help to amend the problem of eutrophication, especially in areas around the Chesapeake Bay, where restoration effects are underway. Many municipal facilities will face stringent limits in the future; hence, knowledge on external carbon sources will be highly valued. When supplemental carbon sources are researched, they are generally compared to methanol, in terms of denitrification kinetics, sludge production and carbon usage (biomass yield), safety, availability, and cost.

1.1 Project Objectives

There are multiple objectives this research aims to meet. The first includes determining the feasibility of using glycerol as a supplemental carbon source for denitrification. Two unusual observations associated for glycerol supplementation for denitrification have been observed: the generalist-specialist theory and partial denitrification to nitrite. It is hypothesized that the biomass transitions from generalist heterotrophs capable of glycerol degradation with a somewhat lower yield to a specialist population of heterotrophs, specifically adapted for glycerol degradation and do so with an increased yield (Omari et al., 2011). Generalists are capable of using a wide range of substrates and utilize the carbon in the influent wastewater as well as the external organic carbon source. Investigations have concluded that the yield and C: N ratio increase over time, starting with biomass previously not exposed to glycerol (Selock et al., 2008). It is expected that a greater amount of supplemental carbon will be required to remove the same nitrate load after several SRTs of glycerol addition. Faster SDNRs have also been observed with prolonged glycerol use (Bilyk et al., 2011)

Another hypothesis linked with adding glycerol is the occurrence of partial denitrification, in which nitrate to nitrite denitrification occurs rapidly and coincidently with

carbon uptake, followed by slower nitrite reduction. This can lead to nitrite presence in the effluent. The presence of nitrite in the effluent is a significant problem for plants employing chlorine for disinfection as nitrite will exert a chlorine demand and reduce the efficiency of disinfection. Chen et al (2001) found the breakpoint curves were consistent with high chlorine demand, when nitrite was present in the effluent. Nitrite accumulation also signifies a waste of supplemental carbon, as nitrate is reduced to nitrite, but no overall reduction in effluent total nitrogen. This rapid nitrate to nitrite reduction was observed in a study by Bodík et al (2009). Nitrite accumulation is often not considered when designing biological nitrogen removal systems, and this may lead to measurement errors as it is likely to yield higher rates than the real nitrogen utilization rate (NUR) values in denitrification (Guven, 2009). Therefore, it is imperative to look at SDNRs for both nitrate and nitrite, when designing supplemental carbon addition systems and selecting carbon sources.

Another objective was to determine the feasibility of a proprietary industrial waste alcohol product (MicroC 3000^{TM}, Environmental Operating Solutions, Inc.) as an alternative carbon source to methanol. The hypothesis is that this product will be able to perform as well as methanol, if not better, due to the ethanol that is present in the product. Since this product is priced at a discount to pure methanol on a \$/lb NO_X-N denitrified (EOS Communications, 2011), it is appealing to determine its capability for denitrification. Reported odor issues with this product were also investigated.

1.2 References

Bilyk, K., Bruton, T., Rohrbacher, J., Latimer, R., Pitt, P. (2011) Faster SDNRs with Prolonged Glycerin Addition, Carbon Storing Bugs, and the Enemy (Dissolved Oxygen), and Other Things Encountered While Piloting Supplemental Carbon at Several BNR Plants. *Proceedings of the Water Environment Federation (WEF) Nutrient Recovery and Management 2011 Conference*

Bodík, I., Blštáková, A., Sedláček, S., Hutňan, M. (2009). Biodiesel waste as source of organic carbon for municipal WWTP denitrification. *Bioresource Technology* 100, 2452-2456

Chen, W., Jensen, J. (2001). Effect of chlorine demand on the ammonia breakpoint curve: model development, validation with nitrite, and application to municipal wastewater. *Water Environ. Res. Nov-Dec,* ***76****(6); 721 31*

EOS Communications (Personal communications, July 15, 2011)

Güven, D. (2009) Effect of Different Carbon Sources on Denitrification Efficiency Associated with Culture Adaptation and C/N Ratio. *Clean Journal*, **37** (7), 565-573

Omari, A., Murthy, S., Takacs, I., Mokhayeri, Y., Riffat, R., Nopens, I. (2011) Modelling the use of External Carbon Substrate for Denitrification by Generalists and Specialists. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

Selock, K., Burton, W., Bott, C., Cutting, N., Wimmer, B., Neethling, J.B., Amad, S., Hinjosa, J., Murthy, S.(2008) Glycerin 101- Lessons Learned While Pilot Testing Glycerin to Enhance Denitrification. Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC –National Conference of the Water Environment Federation). 6583-6602.

Swinarski, M., Makinia J., Stensel, H., Czerwionka, K., Drewowski, J. (2011) Modeling External Carbon Addition in Combined N-P Activated Sludge Systems with Extension of the IWA Activated Sludge Models. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

2: LITERATURE REVIEW

In wastewater treatment, nutrients, such as nitrogen (N) and phosphorus (P), are removed biologically through a process known as BNR. It is important to remove these nutrients as they have been deemed accountable for eutrophication, or excessive plant growth (algae), in receiving bodies of water. Due to this, wastewater treatment plants (WWTPs) around the country have started upgrading to include both nitrogen and phosphorus removal. Phosphorus can be removed by chemical precipitation or by biological means whereas nitrogen is removed by biological nitrification and denitrification processes. In most activated sludge plants, both nitrogen and phosphorus are removed simultaneously by biological means (Williams et al., 1994).

The EPA has developed increasingly stringent limits on discharges, especially by WWTPs discharging into the Chesapeake Bay. Treatment facilities in this watershed are focusing their attention on nitrogen removal to meet current and future effluent limitations of about 3-5 mg N/L (Hinojosa et al., 2008). This is a result of the 2000 Chesapeake Bay Agreement.

2.1 Biological Nitrogen Removal

In raw sewage, total kjeldahl nitrogen (TKN) is the measure of the availability of nitrogen for building microbial cells, as well as the potential nitrogenous oxygen demand that will have to be satisfied (Davis, 2011). This also quantifies the amount of total organic and ammonia nitrogen in the wastewater.

2.1.1 Nitrification

In order to remove ammonia (NH_4-N), a processes known as nitrification occurs. This is a two-step process in which ammonia (NH_4^+) is oxidized to nitrite (NO_2^-), which is then oxidized to nitrate (NO_3^-). This aerobic oxidation process occurs by two different types of bacteria, ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (Tchobanoglous et al., 2003). In order to accomplish nitrification, aerobic autotrophic bacteria must predominate, of which two genera are commonly recognized, Nitrosomonas and Nitrobacter (Davis, 2011). Nitrification occurs by the following:

1^{ST} step – by *Nitroso*-bacteria (AOB)

$$NH_4^+ + 1.5\ O2 \longrightarrow NO_2^- + 2H^+ + H_2O \quad (1)$$

2^{ND} step – by *Nitro*-bacteria (NOB)

$$NO_2^- + 0.5\ O_2 \longrightarrow NO_3^- \quad (2)$$

This entire oxidation reaction can be written as:

$$NH_4^+ + 2\ O_2 \longrightarrow NO_3^- + 2H^+ + H_2O \quad (3)$$

From equation 3, the oxygen required for the total oxidation of ammonia is 4.57 g O_2/g N. Seventy-five percent of the oxygen (3.43 g of O_2) is used for nitrite production and the rest (1.14 g O_2) is used for oxidation of nitrite (Davis, 2011).

In addition to sufficient DO, pH also plays a role. Nitrification will occur in the range of 6.8 to 8 (Davis, 2011), but is usually controlled to 7.0-7.2 (Tchoblongous et al., 2003).

Nitrification also consumes alkalinity. Thus, it is important for the sewage influent to have sufficient alkalinity, or it will need to be added in the form of lime, soda ash, sodium bicarbonate, or magnesium hydroxide. It is stated that for each gram of ammonia nitrogen (as N) that is converted, 7.14 grams of alkalinity as $CaCO_3$ is required (Davis, 2011).

The growth rate of AOBs controls the overall conversion reaction, especially below 28 °C. The maximum specific growth rate for nitrifying organisms is much lower than the specific growth rate for heterotrophic bacteria. Maximum specific growth rates for AOBs vary from 0.25 to 0.77 g VSS/g VSS-day (Randall et al, 1992), and thus, site specific rates need to be determined.

2.1.2 Denitrification

Since releasing any nitrogen into a receiving body of water is not ideal, nitrate can be reduced to nitric oxide (NO), nitrous oxide (N_2O), or nitrogen gas (N_2), to reduce the potential for eutrophication (Davis, 2011). Nitrate removal occurs biologically, in two modes: assimilating nitrate reduction and dissimilating nitrate reduction (Tchobanoglous et al., 2003).

The most common process in biological nitrogen removal is the Modified Ludzak-Ettinger (MLE) process, which consists of an anoxic tank followed by an aerobic tank where nitrification takes place. Nitrate produced in the aerobic tank is recycled back to the anoxic tank

where it is reduced to nitrogen gas. This occurs because the organic substrate in the influent wastewater acts as the electron donor for nitrate reduction. In this process, the anoxic tank precedes the aeration process, and is known as preanoxic denitrification (Tchobanoglous et al., 2003). In another process, deemed postanoxic denitrification, where denitrification occurs after nitrification, BOD removal has already occurred and thus is not available to drive nitrate reduction. These post anoxic processes depend on endogenous respiration for energy, and is associated with a much slower rate of reaction than for the preanoxic processes using wastewater BOD (Tchobanoglous et al., 2003). Therefore, in most postanoxic processes for denitrification, an exogenous carbon source is generally added to provide sufficient BOD. The reactions for two common electron donors are shown in equation (4) and (5), as influent wastewater and methanol, respectively:

Influent Wastewater

$$C_{10}H_{19}O_3N + 10\ NO_3^- \rightarrow 5\ N_2 + 10\ CO_2 + 3\ H_2O + NH_3 + 10\ OH^- \quad (4)$$

Methanol

$$5\ CH_3OH + 6\ NO_3^- \rightarrow 3\ N_2 + 5\ CO_2 + 7\ H_2O + 6\ OH^- \quad (5)$$

In both equations 4 and 5, cell growth required for denitrification is not taken into account, and thus, the actual value of electron donor needed to remove nitrate will be higher depending on the actual yield of the biomass.

2.2 Biological Phosphorus Removal

Biological phosphorus removal (BPR) entails the phosphorus in the influent wastewater to be integrated into the cell biomass, which is then removed through sludge wasting. Polyphosphate-accumulating organisms (PAOs) grow and store phosphorus in systems that use reactor configurations that provide PAOs with a competitive advantage over ordinary heterotrophic bacteria (Tchobanoglous et al., 2003). The removal of phosphorus generally occurs through a two-stage process

In the first stage (anaerobic), using energy from stored polyphosphates, PAOs assimilate acetate and store the carbon in the form of poly-β-hydroxybutyrate (PHB), which is a type of polyhydroxyalkanoate (PHA). The energy for PHB production comes from the hydrolysis of

previously stored polyphosphate compounds, which in turn, causes orthophosphate to be released (Tchobanoglous et al., 2003).

The second stage (aerobic), where the stored PHB is metabolized, can produce some glycogen. The energy yielded in this process is used by the poly-P bacteria to replenish the polyphosphate by absorbing soluble orthophosphate from solution. The phosphorus is then reabsorbed and removed from the system (Williams et al., 1994). It is important that the anaerobic zone does not contain any nitrate or dissolved oxygen, as energy will be utilized from the oxidation pathways rather than from the hydrolysis of polyphosphate.

A potential reason for the failure of enhanced biological phosphorus removal (EBPR) is due to the unfavorable growth of glycogen accumulating organisms (GAOs), which can compete with PAOs for carbon sources. These microorganisms are also capable of volatile fatty acids (VFAs) uptake and conversion to PHA (Oehman et al., 2006). However, GAOs do not contribute to phosphorus removal as they do not release P anaerobically, nor do they take up P aerobically. Instead, GAOs hydrolyze glycogen as their sole source of energy for anaerobic VFA uptake (Oehman et al., 2006) and thus consume and regenerate more glycogen under anaerobic and aerobic conditions, respectively (Zhou et al., 2008). Under anaerobic conditions, GAOs degrade stored glycogen to provide the energy required for VFA uptake. Under aerobic conditions, GAOs oxidize the PHAs synthesized anaerobically to provide energy for cell growth and glycogen replenishment (Zhou et al., 2008). This is how GAOs can survive and grow in EBPR systems. Since these GAOs consume valuable VFAs without removing phosphorus, they are unwanted in EBPR systems. Therefore, it is desirable to minimize or eliminate the growth of GAOs in EBPR systems. It has been hypothesized that pH could be used as a control parameter to reduce the undesirable proliferation of GAOs and improve phosphorus removal in EBPR systems (Oehman et al., 2005).

2.3 Supplemental Carbon

Since nutrient limits are becoming more stringent, the exploration for alternative external carbon sources is on the rise. The goal is to use as much of the influent wastewater carbon as effectively as possible. However, the organic carbon present naturally in influent wastewaters is usually limited, and thus, in order to achieve complete removal of nitrogen, a carbon source needs to be added for denitrification (Van Dongen et al., 2001). Sometimes process

configurations can have an impact on the amount of carbon that can be utilized, which can also vary level of treatment (Ledwell, 2008). An external carbon source is most likely supplied to the post-denitrification processes, as the organic matter in the influent wastewater is consumed by the preceding aerobic processes (Christensson et al., 1994).

In order to effectively select a supplemental carbon source, certain criteria should be determined, such as availability, safety and handling, and performance. It is popular to choose a low cost and low yielding product that will work well in cold temperatures. When a carbon source is introduced, each unit of carbon goes towards new cell synthesis (yield) or is oxidized to produce energy for denitrification. If the yield is low, then that means the energy produced is high, which results in efficient denitrification and less sludge production. Yield is directly proportional to sludge production. The yield coefficient for the heterotrophs utilizing glycerol can be estimated from the C:N ratio, using the equation below:

$$C{:}N = \frac{2.86}{1 - Y}$$

Where 2.86 = O_2 equivalent of NO_3-N removed, g O_2/g NO_3-N (Tchobanoglous et al., 2003)

Of the many commercially available sources, such as methanol and ethanol, and alternative sources such as waste materials and hydrolyzed sludge, methanol has been the main external carbon source used for denitrification and is generally added to the anoxic zone of a single-sludge system as supplement to the influent COD (Peng et al., 2007), or directly into the denitrification tank of a two-sludge system or a fixed film post denitrification process.

2.3.1 Methanol

Methanol has been the most commonly used external carbon source used for denitrification, based on cost and availability (Christensson et al., 1994), as well as being known to support a lower biomass yield (Hallin et al., 2006). Methanol has been known to be used by the bacteria of the genus *Hyphomicrobium* (Sperl and Hoare., 1971; Carrera et al., 2003).

However, utilities are attempting to move away from using methanol, due to some concerns associated with its use: (i.) methanol toxicity and flammability, (ii.) slow denitrification

kinetics at cold wastewater temperatures (Dold et al., 2008), and (iii.) the need for special C1 degrading bacteria required for denitrification (Kang et al., 1992).

Methanol burns without a visible flame and is produced in energy intensive process using methane, a natural gas; thus, the cost of methanol is tied to the cost of the fossil fuel market, which is volatile. Due to it being a reactive and toxic compound, another disadvantage of using methanol is the safety issues associated with its transportation, handling, and storage (Cherchi et al., 2009).

It is thought that denitrification processes are retarded in the winter due to declines in microbial growth rates at lower temperatures. Mokhayeri et al (2006) concluded that the maximum specific growth rate (μ_M) for methanol using biomass at 19°C are double that of the μ_M at 13°C, and denitrification with methanol addition does not achieve low nitrogen values during colder temperatures. Although there has been an abundance of research on methanol bacterial growth rates, Dold et al (2008) have estimated the anoxic yield coefficient of methanol utilizing heterotrophs to be 0.4 g COD/g COD. This value reported by Dold et al (2008) results in a C:N of 4.8 g substrate/g nitrate, which is considered to be the observed value for methanol. Dold et al (2008) have also reported the μ_M of methanol-utilizing organisms to be 1.3 d^{-1} at 20°C. A study by Mokhayeri et al (2009) found methanol's yield to be 0.45 $\pm$ 0.05, at 13°C.

The lag period before denitrification is fully efficient can be attributed to establishment of a specialist population of methylotrophic bacteria known as methylotrophs. This specialist population is capable of using degrading C1 compounds and using these substrates for biosynthesis and energy requirements. The lag period depends on the solids retention time (SRT) of the reactor. It is common to allow 4-8 weeks for the growth of a new population of bacteria before significant methanol uptake and denitrification result (Selock et al., 2008).

2.3.2 Ethanol

According to Christensson et al (1994), ethanol is another possible alternative carbon source, as it is available as inexpensive waste products from chemical and pharmaceutical industries. The price of ethanol is linked with the methanol market and is commonly more expensive, which is why the availability of waste rich in ethanol can potentially provide a source of readily available carbon (Onnis-Hayden et al., 2008). In the study by Christensson et al (1994), results indicated that ethanol was a more readily available carbon source for

denitrification than methanol, as the maximum growth rate of denitrifiers were 2-3 times higher with ethanol than with methanol. Christensson et al (1994) observed growth rates of 1.9 d^{-1} and 4.8 d^{-1} at 15°C and 25°C, respectively, with ethanol use. Peng et al (2007) found that ethanol had higher SDNRs than did methanol, as 9.6 and 3.2 mg N/ (g VSS·h), respectively, in batch tests at 20-23°C. Mycielsky et al (1983) reported a C: N of 4.16 g COD/g nitrate for ethanol use, which is lower than the observed value for methanol (4.8). Mokhayeri et al (2009) found that the yield, in g COD/g COD for ethanol at 13°C, as 0.53 $\pm$ 0.06. Peng et al (2007) found ethanol's sludge yield to be 0.42 g MLSS/g COD, which was higher than observed for methanol. The lag period, in which methanol selects for a highly specialized denitrifying population, is not observed with ethanol use. This is because ethanol is converted by the bacterial cell to acetyl-ScoA, similar to acetate, before entering the tricarboxylic acid cycle (Peng et al., 2007). Since acetate may account for 10% of the total COD in sewage (Henze et al., 1994), suitable denitrifying populations with the appropriate enzymes for ethanol degradation must therefore already exist in activated sludge (Peng et al., 2007).

2.3.3 Glycerol

Glycerol, also referred to as glycerin, has the chemical composition $C_3H_8O_3$, and is a three carbon trihydroxyl alcohol. This compound is miscible in water, biodegradable by ordinary heterotrophic organisms, and nontoxic (Selock et al., 2008). It is also noncorrosive and most importantly, it is nonflammable, which makes it an appealing alternative to replace methanol. Akuna et al (1993) and Grabinska-Loniewska et al (1985) have both reported that glycerol is effective as a carbon source.

Pure glycerol is too expensive to be applied at full-scale, so there has been research that focuses on glycerol waste, which is produced as a by-product from biodiesel fuel production (Hinojosa et al., 2008). This by-product, referred to as crude glycerin, typically contains glycerol, methanol, and high molecular weight hydrocarbons, all of which are considered biodegradable and therefore, a potential supplemental carbon source (Tsuchihashi et al., 2008). Tsuchihashi et al (2008) state several benefits of using crude glycerin: (i.) the byproduct is essentially a mix of biodegradable hydrocarbons; (ii.) it can be purchased at prices below methanol and ethanol commodity prices, and (iii.) it is noncorrosive and nonflammable, which simplifies full-scale storage requirements.

A study by Hinojosa et al (2008) using a biodiesel-based glycerol estimated a μ_M of 3.4 d^{-1} and a C:N of 4.2 mg COD/mg NO_X-N. This C: N is determined based on NO_X-N as opposed to NO_3-N, which will result in slightly lower values due to the presence of nitrite. Tsuchihashi et al (2008) ran crude glycerol bench scale tests and found average SDNRs to be 6 mg-NO_X-N/g VSS·h at temperatures between 22-27°C. A study by Ramalingam et al (2007) used biodiesel waste acclimated sludge in 12 batch experiments at 21.4°C, which resulted in an average SDNR of 3.2 mg NO_X-N/g VSS·h. A study by Fillos et al (2007) used biodiesel waste in a SBR at 19°C and intermittently checked kinetics for a study period of 10 days. This study resulted in increasing SDNRs with glycerol use and it was stated that biodiesel waste has a potential for higher SDNRs than both methanol and ethanol, and should be evaluated further in pilot studies. A study by Selock et al (2008) reported that although glycerin addition enhanced denitrification almost immediately, the pilot study experienced a higher than expected glycerin utilization rate.

A study by Docket (2011) showed that C:N for glycerol fed sludge increased as time went by with glycerol addition in two batch reactors, seeded with generalist biomass and methanol acclimated biomass, respectively. Upon start up, the observed C:N was measured around 5 g substrate COD removed/ g NO_3-N removed, but increased to above 10 g substrate COD removed/ g NO3-N removed over a period of 60 days. For the methanol acclimated biomass, the starting C:N was closer to 8 g substrate COD/ g NO_3-N removed, as nitrogen removal was not occurring efficiently. After acclimation to glycerol, the C: N for this reactor was measured to be 11 g substrate COD/ g NO_3-N removed. There was also nitrate to nitrite reduction coincident with COD consumption, followed by slow nitrite reduction, suggesting storage of glycerol was occurring. This study confirmed the observation that when glycerol is used for denitrification, there is an observed increase carbon demand.

Nitrite accumulation was also an issue for Tsuchihashi et al (2008), where crude glycerol was used in a pilot study. They noted that a fraction of NO_X-N in the form of nitrite was increasing in the anoxic tank indicating that the rate of denitrification from nitrate to nitrite is higher than denitrification from nitrite to nitrogen gas. The study by Docket (2011) also experienced severe nitrite accumulation when glycerol was fed into SBRs. This study concluded that nitrite accumulated when carbon was slug fed into the reactors at the beginning of the anoxic phase. This was thought to have created a population of organisms that flourish on “feast-famine” conditions, allowing them to store carbon substrate. This problem of nitrite

accumulation was corrected after the glycerol feed was spread out over the first half of the anoxic phase. Nitrite accumulation has received considerable attention, as this inorganic form of nitrogen is toxic to aquatic life (Barak et al., 1998) and increases costs significantly of disinfection if found in the effluent of plants that utilize chlorine. Wilderer et al (1987) has stated that there are at least two mechanisms responsible for this phenomenon: (i) repression of the synthesis of nitrite reductase, and (ii) selection and enrichment in favor of microorganisms capable of reducing nitrate, but only to nitrite. They also stated that carbon limitations need to be avoided in order to diminish the effects of nitrite buildup. Martiensson et al (1999) also stated that one important factor on the amount of nitrite accumulation seems to be the type and the amount of the organic substrate used as the carbon source. This study also claimed that the estimation of the nitrite reduction capacity may be used as an indicator for the denitrification efficiency of a given sludge.

2.3.4 Waste Materials

Waste materials are becoming increasingly more popular to use as supplemental carbon sources for denitrification. Swinarski et al (2009) proved that food industry effluents are considered a potential alternative for methanol when seeking external carbon sources to enhance denitrification in municipal wastewater treatment plants. In the Swinarski et al (2009) study, effluents from three industries were tested (distillery, brewery, and fish-pickling process) and deemed viable by comparing nitrate uptake rates. The reason these effluents appear to be a good choice is because of the high C: N ratios and content of readily biodegradable organic fraction (Cappai et al., 2004; Sage et al., 2006). The research on the use of various industrial byproducts or waste materials for denitrification has been carried out for over 20 years (Sage et al., 2006). When choosing waste materials as carbon sources for denitrification, these materials should be free of metals and other contaminants; stable, in terms of composition and content of readily biodegradable organic compounds; and available in the required quantity on a consistent schedule (WEF, 2005). Another example of a waste material that has been tested is dairy process water (Bodík et al., 2009).

2.4 Generalist-Specialist Theory

Omari et al (2011) suggested that activated sludge consists of generalists and specialists. The generalist population of ordinary heterotrophic organisms (OHO) uses a specific substrate less efficiently, thus producing much lower yields and slightly slower growth rates. The specialists, on the other hand, use the substrates more efficiently with much higher yields and somewhat faster growth rates. Omari et al (2011) also states that the efficiency of substrate use is broadly based on the concept that there are lower overall maintenance requirements for degrading a single substrate versus producing a multitude of enzymes to degrade complex substrates in wastewater. Thus, Omari et al (2011) hypothesized that the development of specialists does not necessarily require a shift in microbial species, but perhaps constitutes a mere reallocation of resources within generalists.

When glycerol addition as a supplemental carbon source is initiated, it is hypothesized that the biomass transitions from generalist ordinary heterotrophs capable of glycerol degradation with somewhat lower yield to a specialist population of heterotrophs, specifically adapted for glycerol degradation and does so with increased yield. A study by Katehis et al (2011) determined that when modeling glycerin utilization for denitrification, it should be carried out using specialized population approach (similar to modeling of denitrification with methanol), because glycerin utilization was found to exhibit a lower overall denitrification rate (complete reduction from nitrate to nitrogen gas) than readily biodegradable organics found in typical municipal wastewater.

In order to keep costs relatively low, design and operation should focus on maintaining a majority population of generalists rather than specialists (Omari et al, 2011). However, to maintain winter denitrification, the improved kinetics of the specialist population might also be advantageous.

2.5 Sequencing Batch Reactor

Sequencing Batch Reactors (SBRs) are used around the world and have been around since the 1920s (Al-Rekabi et al., 2007). It is a system employing activated sludge process and is an alternative wastewater treatment technology (Leung et al., 1994). SBRs are suspended growth biological wastewater treatment reactors, in which all the metabolic reaction and solid-liquid separation takes place in one tank and in a well-defined and continuously repeated time

sequence. They are reliable, cost-effective, and highly efficient in tackling the challenge of wastewater treatment due to varying influent characteristics and stringent effluent regulations (Singh et al., 2011). An SBR with suspended biomass configuration can perform relatively better in terms of carbon removal over conventional suspended growth systems (Mohan et al., 2005). This process is known to save more than 60% of the expenses required for conventional activated sludge process and achieve high quality effluent at the same time. In fact, more than 90% biochemical oxygen demand (BOD) removal has been reported while the conventional processes are capable of removing 60-95% of BOD (Tchobanoglous et al., 2003). Generally, these reactors are applied in small communities where space is limited and/or treatment requirements do not permit the use of oxidation ponds (Davis, 2011). Denitrification as well as BOD reduction can be achieved in this system.

The SBR treatment process generally involves five stages in a timed sequence (Davis, 2011):

(1) Fill: during the fill process, influent wastewater is added to the settled biomass from the previous reactor cycle. The fill mode can be aerated or unaerated and varies in time, depending on the volume of the tanks and flow rates (Singh et al., 2011).
(2) React: influent wastewater is restricted from entering the tank and aeration and mixing occurs during this phase. The time dedicated to this react time can exceed 50% of the total cycle time. Treatment is controlled by controlling air, either on or off, creating anaerobic, anoxic, and aerobic conditions (Singh et al., 2011). This cycling of air on and off results in nitrification, denitrification, and phosphorus removal.
(3) Settle: mixing and aeration is stopped allowing the activated to settle. The entire tank acts as a clarifier. These SBRs generally give better clarification than conventional clarifiers due to quiescent conditions (Singh et al., 2011).
(4) Draw (Decant): a decanter is used to remove the clear supernatant that will be discharged (Davis, 2011). It is imperative to make sure not to collect the floating material (Singh et al., 2011). During this time, excess waste activated sludge is also removed.
(5) Idle: This is the period between draw and fill. This may be long or short depending on the flow rate, and may be eliminated if the flow is high (Davis, 2011).

2.6 References

J. C. Akuna, C. Bizeau and R. Moletta, "Nitrate and Nitrite Reductions with Anaerobic Sludge Using Various Carbon Sources: Glucose, Glycerol, Acetic Acid, Lactic Acid and Methanol," *Water Research*, **(27)** 2, April 1993, pp. 1303-1312

Al-Rekabi, W., Qiang, H., Qiang, W. (2007). Review on Sequencing Batch Reactors. *Pakistan Journal of Nutrition*. **6**(1); 11-19

Barak, Y., Tal Y., Rijn T. (1998) Light-Mediated Nitrite Accumulation during Denitrification by *Pseudomonas* sp. Strain JR12. *Appl. Envrion Microbiol.*1998 March; **64**(3); 813-817

Cappai, G.; Carucci, A.; Onnis, A. (2004) Use of Industrial Wastewaters for the Optimization and Control of Nitrogen Removal Processes. Water Sci. Technol., 50 (6), 17–24

Carrera, J., Baeza J., Vincent, T., Lafuente, J. (2003). Biological Nitrogen Removal of High-Strength Ammonium Industrial Wastewater with Two-Sludge System. *Water Res.,* **37** (17), 4211-4221

Cherchi, C., Onnis-Hayden, A., El-Shawabkeh, I., Gu, A. (2009). Implication of using different carbon sources for denitrification in wastewater treatments. *Water Environ Res. **81**:788-99*

Christensson, M,. Lie, E., Welander, T. (1994). A comparison between ethanol and methanol as carbon sources for denitrification [J]. *Water Science and Technology*, 30(6): 83–90

Davis, Mackenzie Leo. *Water and Wastewater Engineering: Design Principles and Practice*. New York: McGraw Hill, 2011. Print

Docket, N. (2011) Anoxic Carbon Yield Investigation Using Glycerol as the Carbon Source for Denitrification in Sequencing Batch Reactors. *M.Eng Thesis*. Howard University

EOS Communications (Personal communications, July 15, 2011)

Fillos, J., Ramalingam, K., Jezek, R., Deur, A., Beckmann, K. (2007) Specific Denitrification Rates with Alternative External Sources of Carbon. *Proceedings from the 10th International Conference on Environmental Science and Technology*. Sept. 2007. Kos island, Greece

Grabinska-Loniewska, A., Slomczynski, T., and Kanska, Z. (1985). Denitrification Studies with Glycerol as a Carbon Source. *Water Research*, **19** (12) 1471-1477

Guerrero, J., Guisasola, A., Baeza, J. (2011) The nature of the carbon source rules the competition between PAO and denitrifiers in systems for simultaneous biological nitrogen and phosphorus removal. *Water Res. 2011; **45**: 4793-4802*

Guerrero, J., Tayà, C., Guisasola, A., Baeza, J. (2012) Glycerol as a sole carbon source for enhanced biological phosphorus removal. *Water Res. 2012; **46**: 2893-91*

Hallin, S., Rothman, M., Pell, M. (1996). Adaptation of denitrifying bacteria to acetate and methanol in activated sludge. *Water Res.* **32**:13-18

Hallin, S., Throback IN., Dicksved, J., Pell, M. (2006). Metabolic profiles and genetic diversity of denitrifying communities in activated sludge afer addition of methanol or ethanol. *Appl. Environ. Microbiol.* **7 2**:5445 5452

Hinojosa, J., Riffat, R., Fink, S., Murthy, S., Selock, K., Bott, C., Takacs, I., Dold, P., and Wimmer, (2008). Estimating the Kinetics and Stoichiometry of Heterotrophic Denitrifying Bacteriawith Glycerol as an External Carbon Source. *Proceedings of the 81th Annual Water Environment Federation Technical*

Exhibition and Conference (WEFTEC - National Conference of the Water Environment Federation), Chicago, Illinois USA.

Kang, S.J., Bailey, W.F., and Jenkins, D. (1992). Biological nutrient removal at the Blue Plains Wastewater Treatment Plant in Washington, D.C. *Water Science and Technology*, **26** (9-11) 2233-2236

Katehis, D., Deur, A., Sharp R. (2011) Usage of Glycerin for Denitrification in High Rate Activated Sludge Processes: Benefits and Limitations. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

Ledwell, Samuel (March 19, 2008). Carbon Sources for Biological Denitrification. *MWPCA Quarterly Meeting*. Bourne, MA0000

Leung, G., Tam, N. (1994). Operation strategy of a sequencing batch reactor for simultaneous removal of wastewater organic matter and nutrients. *Resources, Conservation and Recycling.* **11**, 209-223

McCarty, P., Beck, L., Amant P. (1969). Biological denitrification of wastewaters by addition of organic materials. 24th Ind. Waste Conf., Purdue Univ., Lafayette, Ind., pp. 1271-1285

Mokhayeri, Y., Nichols, A., Murthy, S., Riffat, R., Dold, P., Takacs, I. (2006) Examining the influence of substrates and temperate on maximum specific growth rate of denitrifiers. *Water Sci. Technol.* **54**(8), 155-162

Mokhayeri, Y., Riffat, R., Murthy, S., Bailey, W., Takacs, I., Bott, C. (2009) Balancing yield, kinetics and cost for three external carbon sources used for suspended growth post-denitrification. *Water Science & Technology.* **60**(10), 2485-2491

Mycielski, R., Blaszczyk, M., Jackowska, A. & Olkowska, H. (1983) Denitrification of high concentrations of nitrites and nitrates in synthetic medium with different sources of organic carbon. *Acta Microbiol. Polon.* 32(4), 381–388

Nyberg U., Aspegren H., Andersson, B., Jansen, J. (1992). Full-scale application of nitrogen removal with methanol as carbon source. *Wat. Sci. Technol.* **26,** 1077-1086

Oehman, A., Saunders, A., Vives, M., Yuan, Z., Keller, J. (2006) Competition between polyphosphate and glycogen accumulating organisms in enhanced biological phosphorus removal systems with acetate and propionate as carbon sources. *Journal of Biotechnology.* 123, 22-32

Oehman, A., Vives, M., Lu, H., Yuan, Z., Keller, J. (2005) The effect of pH on the competition between polyphosphate-accumulating organisms and glycogen-accumulating organisms. *Water Research* 39, 3727 3737

Omari, A., Murthy, S., Takacs, I., Mokhayeri, Y., Riffat, R., Nopens, I. (2011) Modelling the use of External Carbon Substrate for Denitrification by Generalists and Specialists. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

Onnis-Hayden, A., Gu, A., (2008) Comparisons of Organic Sources for Denitrification: Biodegradability, Denitrification Rates, Kinetic Constants and Practical Implication for Their Application in WWTPs. Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation). 252-273.

Peng, Y., Yong, M., Wang, S. (2007). Denitrification potential enhancement by addition of external carbon sources in a pre-denitrification process. Journal of Environmental Sciences, 19 (3), 284-289, 2007

Ramalington, K., Filos, J., Deur, A., and Beckmann K., Specific Denitrification Rates with Alternate Sources of Organic Carbon Proceedings of 2nd External Carbon Source Water Environment Research Federation Workshop, 2007, Washington, D.C.

Randall, C., Barnard, J., Stensel, H. (1992). Design and Retrofit of Wastewater Treatment Plants for Biological Nutrient Removal. *Water Quality Management Library*. Vol. 5, Technomic Publishing Co., Lancaster, PA.

Sage, M.; Daufin, G.; Gesan-Guiziou, G. (2006) Denitrification Potential and Rates of Complex Carbon Source from Dairy Effluents in Activated Sludge System. Water Res., 40, 2747–2755

Selock, K., Burton, W., Bott, C., Cutting, N., Wimmer, B., Neethling, J.B., Amad, S., Hinjosa, J., Murthy, S.(2008) Glycerin 101- Lessons Learned While Pilot Testing Glycerin to Enhance Denitrification. Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC –National Conference of the Water Environment Federation). 6583-6602

Singh, M., Srivastava, R. (2011) Sequencing batch reactor technology for biological wastewater treatment: a review. *Asia-Pacific Journal of Chemical Engineering*. Vol. 6, 3-13

Skrinde, J., Bhagat, S. (1982). Industrial Wastes as Carbon Sources in Biological Denitrification. *Journal (Water Pollution Control Federation)*. Vol. 54, No. 4; 370-377

Sperl, GT., Hoare, DS. (1971). Denitrification with methanol: a selective enrichment for Hyphomicrobium species. *Journal of Bacteriology.* 1971, Nov. **108** (2): 733-6

Swinarski, M., Makina, J., Czerwionka, K., Chrzanowska, M., Drewnowski, J. (2009) Comparison of the Effects of Conventional and Alternative External Carbon Sources on Enhancing the Denitrification Process. *Water Environ. Res.*, **81**(9); 896-906

Tchobanoglous, George, Franklin L. Burton, and H. David. Stensel. (2003) Metcalf & Eddy *Wastewater Engineering: Treatment and Reuse*. Boston: McGraw-Hill. Print

Tsuchihashi, R., Bowden, G., Beckmann, K., Deur, A., Bodniewicz, B. (2008). Evaluation of Crude Glycerin as a Supplemental Carbon Source for a High Rate Step-Feed BNR Process. *Proceedings of the 81st Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC–National Conference of the Water Environment Federation)* Chicago, Illinois, USA

Water Environment Federation (2005) Biological Nutrient Removal (BNR) Operation in Wastewater Treatment Plants, Manual of Practice No. 30; Water Environment Federation: Alexandria, Virginia

Wilderer, P., Jones, W., Dau, U. (1987) Competition in Denitrification Systems Affecting Reduction Rate and Accumulation of Nitrite. *Wat. Res.* **21**(2); 239-245

Williams, S., Wilson, A. (1994). Beckton Demonstration Biological Nutrient Removal Plant. *Water and Environment Journal*. Vol. 8 (6); 664-670

Van Dongen U, Jetten MSM, van Loosdrecht MCM. The SHARON-ANAMMOX process for treatment of ammonium rich wastewater. *Water Sci. Technol* 2001; 44:153– 60

Venkatamohan, S., N.C. Rao, K.K. Prasad, B.T.V. Madhavi, P.N. Sharma.Treatment of complex chemical wastewater in sequencing batch reactor (SBR) with an aerobic suspended growth configuration. *Process Biochem.*, **2005;** *40*(5), 1501–1508.

Zhou, Y., Pijuan, M., Zeng, R., Lu, H., Yuan, Z. (2008) Could polyphosphate-accumulating organisms (PAOs) be glycogen-accumulating organisms (GAOs)? *Water Research* 10-11, 2361-2368

3. Manuscript 1: Glycerol-Driven Denitrification: Evaluating the Specialist-Generalist Theory and Partial Denitrification to Nitrite

NOTE: An early draft of this manuscript was submitted to and accepted for the 85TH Annual Water Environment Federation Technical Exhibition and Conference in New Orleans, Louisiana September 29-October 4, 2012.
NOTE 2: This manuscript will be submitted to Water Environment Research.

ABSTRACT

In order to comply with strict total nitrogen (TN) limits, supplemental carbon must be added to improve biological nutrient removal (BNR) processes. This research investigated alternatives to methanol that can be used in full-scale applications, such as glycerol (also known as glycerin). The objective was to attempt to carefully profile glycerol alongside methanol in order to verify two unusual observations reported for glycerol supplementation for denitrification: increasing COD demand over time (yield) and partial denitrification resulting in nitrite buildup. Three sequencing batch reactors (SBRs) were operated to assess 100% reagent grade methanol, 100% reagent grade glycerol, and a biodiesel glycerol waste. The methanol reactor performed as expected. Over-dosing carbon led to very low levels of residual nitrogen in all the reactors, while under-dosing resulted in incomplete removal of TN. Nitrite accumulation in the glycerol reactors was consistent and significant, whereas the methanol reactor did not exhibit this phenomenon. Increased yield over time after startup and anoxic storage of COD were apparent for both glycerol reactors.

KEYWORDS: supplemental carbon, methanol, glycerin, bio-diesel glycerol waste, generalist, specialist, nitrite, denitrification, sequencing batch reactor

3.1 Introduction

Denitrification is the reduction of nitrate (NO_3^-) to nitrite (NO_2^-) to nitrogen gas (N_2) and is the second major step of the BNR process. The addition of supplemental carbon is necessary in order to meet strict limits (below 12 mg N/L) of total nitrogen discharge. External carbon addition is being utilized in several states in the mid-Atlantic region, such as in North Carolina, Virginia, and Maryland (Bilyk et al., 2009) and is added during denitrification to facilitate the removal of nitrate (Hinojosa et al., 2008). Currently, methanol is the most prevalent supplemental carbon source, but alternatives currently being considered include glycerol.

Supplemental carbon, which serves as the electron donor, is used to encourage complete denitrification to achieve low levels of total nitrogen in the effluent. Heterotrophic microorganisms use carbon to reduce nitrate, the electron acceptor, to nitrogen gas. Since most organic material from the influent wastewater is oxidized during aerobic phases (nitrification), there is limited carbon in the second anoxic zone of a four or five stage system. Therefore, these bacteria will use nitrate as the electron donor when oxygen is absent. Hence, full-scale experience with supplemental carbon products underscores the need for complete nitrification upstream of the post-anoxic zone was well as proper management of dissolved oxygen in the zone upstream of the carbon addition point (Bilyk et al., 2009; Rohrbacher et al., 2009).

3.1.1 Methanol

Methanol is the most prevalent and has been a popular supplemental carbon source for denitrification in wastewater treatment facilities. The addition of methanol can also improve biological phosphorus removal by creating anaerobic conditions and increasing the availability of organic carbon in wastewater for polyphosphate-accumulating organisms (PAOs) (Ginige et al., 2009). However, many utilities avoid using methanol due to: (i.) methanol toxicity and flammability, (ii.) slow denitrification kinetics particularly at cold wastewater temperatures (Dold et al., 2008), and (iii.) need for special C1 degrading bacteria (methylotrophs) required for denitrification (Kang et al., 1992). There has also been evidence that methanol is known to support lower biomass yield, thus reducing carbon feed requirements as well as sludge production (Hallin et al., 2006). It has also been observed that there is a lag period before maximum denitrification rates are achieved, supporting the theory that special C1 degrading methylotrophs need to be established before full denitrification can begin (Hallin et al., 2006;

Selock et al., 2008). Methanol is commonly used for denitrification because of its low cost and ability to closely control the denitrification process for nitrate elimination (Mokhayeri et al., 2008). In addition, recent price and supply volatility has created uncertainty in the long-term availability of methanol (Katehis et al., 2010). A study by Ginige et al (2009) proposed that reactor configuration has a major impact on the effectiveness of methanol as an external carbon source for enhancing denitrification. When a large amount of methanol is required, it should be added to the secondary anoxic zone. On the other hand, when a small amount is needed, methanol can be added in the primary anoxic zone. When a small amount of methanol is added to the secondary anoxic zone, nitrite accumulation may occur, which does not improve overall nitrogen removal. In this study, Ginige noticed that when added at a low level (under-dose), methanol is used to reduce nitrate to nitrite, which leads to nitrite accumulation and hence no N removal. The underlying reason for this was not clear. The nitrate to nitrite reduction for methanol is not as rapid as has been observed for other carbon sources (i.e. glycerol).

3.1.2 Glycerol

Glycerol, also known as glycerin, has the chemical composition $C_3H_8O_3$, and is a three carbon trihydroxy alcohol. This compound is biodegradable by ordinary heterotrophic organisms, nonflammable and nontoxic (Selock et al., 2008). Implementing a purified form of this compound for external carbon addition is unlikely given its high cost; however, glycerol is the principal byproduct of biodiesel fuel production, which, sources indicate, is a growing industry (Hinjosa et al., 2008). The literature has a variety of reported Specific Denitrification Rates (SDNR) regarding glycerin and thus more work on this subject is needed to better understand the kinetics.

Glycerin is produced by combining virgin or waste vegetable oil, or animal fat, with 10-20% methanol and sodium or potassium hydroxide. This reaction will yield a product that is 80-90% biodiesel fuel, and 10-20% glycerin waste. This waste is typically contaminated with <5% methanol, residual sodium salts, long chain fatty acids, and others (Selock et al., 2008). It is generally observed that there is little to no acclimation time needed for this substrate.

Enhanced biological phosphorus removal (EBPR) technology based on the enrichment of activated sludge with polyphosphate accumulating organisms (PAO) has been successfully incorporated. The effectiveness of the enrichment is dependent on the nature of the electron

donor as a high percentage of volatile fatty acids (VFA) in the influent COD is critical to obtain a population of microorganisms that have a high P-removal efficiency (Guerrero et al., 2012b). Guerrero et al (2012a) state that wastewaters with low organic matter content are one of the major causes of enhanced biological phosphorus removal (EBPR) failures in full-scale WWTPs.

The availability of nitrate in the anaerobic reactor is one of the most reported causes of EBPR failure (Guerrero et al., 2011), signifying the importance to optimize the amount of COD, as substrate, for nutrient removal. The nitrate must be depleted (< 1 mg/L) before anaerobic P-release can occur. The presence of nitrate activates the activity of OHOs which will reduce nitrate using COD resulting in less COD for PAO growth, hindering phosphorus removal. A study by Guerrero et al (2011) challenged the idea that denitrifying OHOs outcompete PAOs when competing for the electron donor as EBPR was still observed after switching to MLE configuration, using a carbon source that combined propionic acid, acetic acid, and sucrose. The literature results vary as different carbon sources are used. It has seen that P-release occurs only when denitrification is completed (NO_3-N < 1 mg/L) with most carbon sources except acetic acid. However, there have also been reported results of simulatenous nitrate reduction and P-release (Guerrero et al., 2011). This study concluded that the capacity of PAO to outcompete OHO for the carbon source is fundamentally linked to the nature of the organic matter and to the population distribution in the sludge.

Glycogen accumulating organisms (GAOs) accumulate PHA anaerobically, similar to PAOs. However, the main difference is that GAOs use only glycogen for energy under anaerobic conditions, whereas PAOs use phosphate (Erdal et al., 2007). This allows the GAOs to uptake organic material without releasing P. Erdal et al (2007) concluded that reduced performance of EBPR facilities at warm temperatures (> 20°C) wasn't a cause of GAO proliferation, but rather may be related to the efficient use of the glycolytic pathway by PAOs which results in more glycogen storage and less P uptake, thus, reducing EBPR.

It has been reported that interactions between PAOs and GAOs in EBPR systems is one of the major causes of EBPR failure. The GAOs appear to use the remaining VFA after the PAOs have run out of stored phosphorus (Phillips et al., 2009; Neethling, 2005). It was thought that the proliferation of GAOs was the cause of the reduced EBPR. However, a study by Machado et al (2007) contradicted reports that GAO presence is typically related to EBPR failure

as they found a greater amount of GAO bacterium than PAO bacterium at a full-scale EBPR plant.

There is has been evidence that GAOs co-exist with phosphorus accumulating organisms (PAO) in BNR plants where phosphorus is removed to very low levels (Filipe et al., 2001). Filipe et al (2001) also stated that minimizing the accumulation of GAOs and increasing the reliability of BPR can be done through by controlling the pH of the anaerobic zone. They determined that when the pH of the anaerobic zone is less than 7.25, then the GAOs seemed to dominate the PAOs, as GAOs took up acetate faster than PAOs. When the pH increased to 7.5 and above, the PAOs were more dominant.

Another key factor influencing the competition between GAOs and PAOs is the phosphorus/carbon (P/C) feeding ratio (Kiss et al., 2011)). Liu et al (1997) determined that the P/C feeding ratio was a key factor influencing "internal energy-based" competition between PAOs and GAOs. In the Liu study, when excess P was provided (P/C = 20/100), PAOs outcompeted GAOs. However, the reduction of P/C ratio (2/100) resulted in the PAOs to be outcompeted by the GAOs.

A study by Yuan et al (2009) witnessed that when acetate was replaced by glycerol as the primary carbon source and fed directly into the anaerobic zone, the system failed to maintain EBPR. This suggested that glycerol may not be a suitable direct carbon source, as there was very little phosphorus release in this anaerobic zone. This implied that an improper carbon source may cause a shift in the microbial community population away from the PAOs.

For this work, it is believed that during the feast phase (when glycerol is taken up) substrate is converted to storage compounds rather than used for growth (Gàrate et al., 2011). When the glycerol is depleted, the famine stage begins. Stored polymers are the only carbon and energy source available during the rest of the cycle. Gàrate et al (2011) determined that the use of a feast-famine SBR for community selection was successful to give a stable community with high PHA-accumulating capacity. Bilyk et al (2011) also witnessed glycerol's storage capability. EBPR was observed and thought to be a result of COD storage by OHO in the process of carbon assisted denitrification. The stored COD was fermented to produce VFAs in the anaerobic cells, ensuing EBPR. Using glycerol for enhanced biological phosphorus removal (EBPR) seems promising, but due to scarcity of data, no formal conclusions can be made (Guerrero et al., 2012a). There have been reports that anaerobic fermentation of glycerin leads to the production

of relatively equal amounts of acetate and propionate, an important consideration for biological phosphorus removal (Selock et al., 2008). Zhang et al (2009) have stated that anaerobic degradation of glycerol under certain conditions can yield significant propionate production, which is necessary for EBPR. Pijuan et al (2004) stated that PAOs could selectively consume propionate against glycogen accumulation organisms (GAOs), who are the main competitors of PAOs. This study showed that in situ production of propionate from glycerol would be a possible solution to achieve EBPR with glycerol use.

3.2 Hypothesis

As discussed by Omari et al (2011), current models do not describe the necessary dose (a consequence of yield) and the adaptation time (a consequence of active population and kinetics) for many specific carbon sources, particularly during start-up or transitioning to a new substrate.

3.2.1 Generalist-Specialist Theory

When glycerol is added to activated sludge previously unexposed to supplemental carbon, the generalist population of ordinary heterotrophic organisms that utilize glycerol inefficiently will select for a specialist population of heterotrophic organisms that can utilize the glycerol more efficiently (higher rate) and do so with an increased yield. The efficiency can increase with adaptation or population shift. (Omari et al., 2011)

3.2.2 Partial Denitrification

It has been observed that adding glycerol results in rapid nitrate to nitrite denitrification, followed by slower nitrite denitrification, leading to nitrite accumulation which is a significant issue for plants with chlorine disinfection. This rapid nitrate to nitrite reduction was observed in a study (Bodík et al., 2009), where bio-diesel glycerol waste was used as a source of external organic carbon.

3.3 Project Objectives

The first objective is to examine the generalist-specialist theory for glycerol through the use of SBRs. Full-scale and laboratory investigations have concluded that the yield and C: N ratio seem to increase over time, starting with biomass not previously exposed to glycerol. It is expected that roughly 20-40% more COD is required to remove the same nitrate load after several SRTs of glycerol addition.

A second purpose for this investigation is to confirm and characterize the rapid nitrate to nitrite denitrification for glycerol, followed by much slower nitrite denitrification. An under-dose of glycerol would be expected to result in low effluent nitrate, but high nitrite, which is a problem for plants employing chlorine for disinfection. The reactors should demonstrate previous field observations of nearly complete COD removal at the same time as rapid nitrate to nitrite denitrification, suggesting that carbon storage is occurring. An overdose of carbon should lead to low effluent levels of total nitrogen.

In order to examine these two observations, reactor operation and sampling were conducted in different phases: To evaluate the specialist-generalist hypothesis, the reactors were operated as follows: (i.) startup and operation to steady-state, (ii.) careful sampling steady-state conditions with carbon added to denitrify 7, 15, and 4 mg/L NO_3-N representing the approximate carbon demand, an overdose, and an underdose, respectively, (iii.) reactor shut down, (iv.) restart and reseed with fresh mixed liquor not exposed to glycerol or methanol with carbon dosing based on 7 mg/L NO_3-N, and (v.) careful sampling to determine COD/NO_3 ratio as the reactors proceeded from startup to steady-state.

3.4 Methodology

Three 22.0 Liter Sequencing Batch Reactors (SBRs) were run concurrently having the same solids retention time (SRT) and hydraulic retention time (HRT) and set up to roughly simulate a 5-stage Bardenpho process. These reactors operated with three 8-hour cycles per day regulated automatically through programmable timers (ChronTrol Corporation San Diego, CA). These three reactors had thick styrofoam floating covers to minimize oxygen transfer through the surface during anoxic and anaerobic periods. Figure 3 shows the typical layout of the experiment.

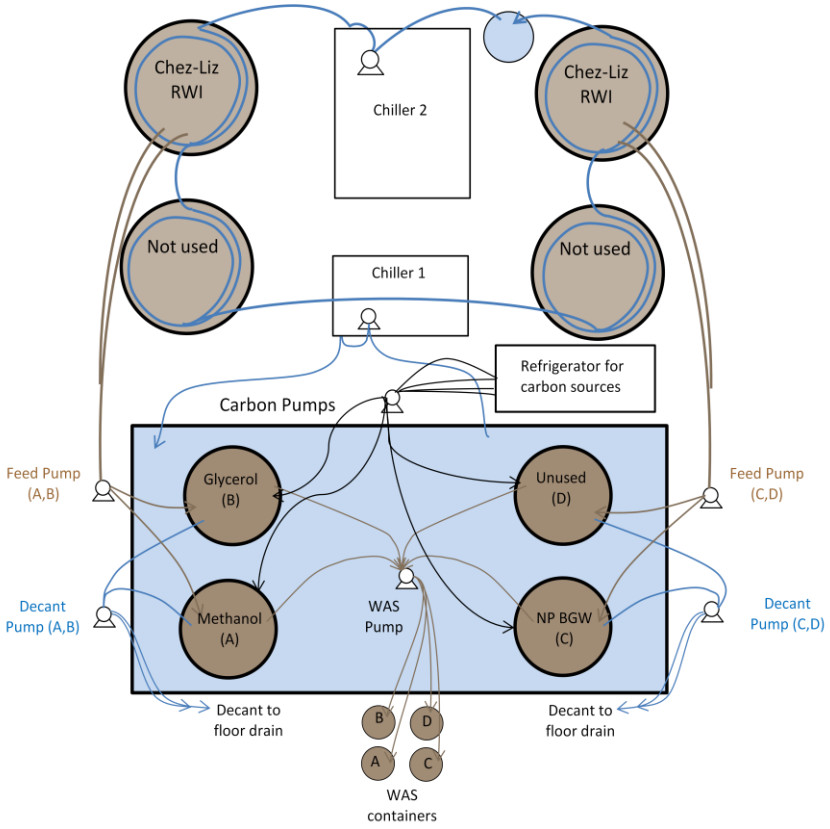


Figure 3: Schematic of SBR Set-up

3.4.1 SBR Feed

Raw wastewater influent was collected from the Hampton Roads Sanitation District (HRSD) Chesapeake-Elizabeth Wastewater Treatment Plant in Virginia Beach, Virginia. Three 50 gallons drums were filled with raw wastewater every Monday, Wednesday, and Friday between 9:00 – 10:00 a.m., which was considered to be a peak load period when influent ammonia concentrations were highest. The raw wastewater was transferred into drums that fed directly to the SBRs. Submersible pumps in these drums turned on five minutes before each feed to ensure mixing of the influent before being fed into the reactors. Attempting to simulate a 5-stage BNR process in an SBR is generally not feasible because there is no direct sludge return to imitate nitrate recycle (NRCY). However, in order to overcome this obstacle, half the raw wastewater was pumped in the beginning of the cycle (before anaerobic period) and the other half was fed before the first anoxic period. Figure 3.1 shows the 8 hour cycle, roughly simulating a 5-stage Bardenpho process. Another purpose of splitting the feed was to have a relevant distribution of heterotrophic bacteria that could utilize carbon from the influent as well as from

supplemental addition.

3.4.2 SBR Temperature Control

The drums that held the raw wastewater influent that fed directly to the reactors contained vinyl coated copper tubing connected to a chiller (Aquatic Eco-Systems, Inc., Apopka, FL). These chillers were regulated by temperature controllers which circulated tap water through the copper tubing and helped cool the raw wastewater to 20 $\pm$ 0.5 °C before being fed into the SBRs. A chiller was used to control the temperature of the SBRs through a water bath regulated to 20 $\pm$ 0.5 °C.

3.4.3 SBR Cycle & Sampling Schedule

In order to calculate rates, careful intensive sampling for nutrient analysis was performed at different points throughout the cycle. Figure 3.1 shows the sampling schedule for a representative 8-hour cycle beginning at 0800 and ending at 1500, followed by settling for one hour. There was a 10 minute deoxygenation period in the start of the second anoxic zone, prior to carbon addition. This allowed for the residual oxygen to be reduced to zero as it was necessary to ensure that the reactor was completely anoxic before any carbon was added. The ten minute post aeration is to help boost DO and strip N_2 gas, prior to settling.

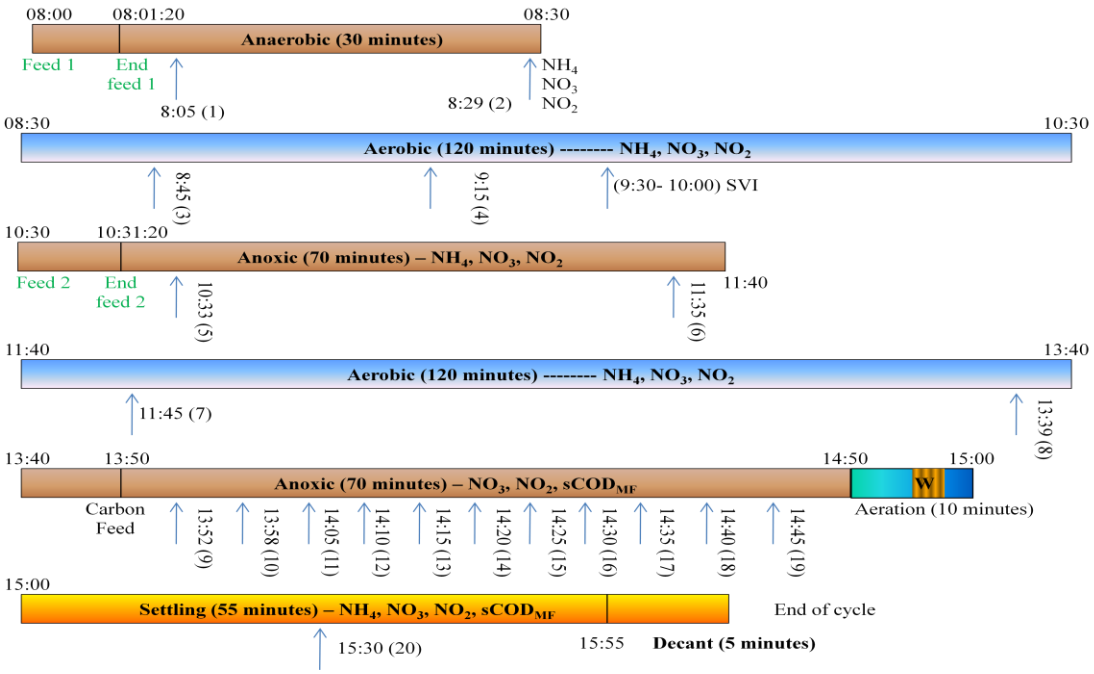


Figure 3.1: Eight-hour SBR Cycle Schedule and Sampling Points during Cycle

3.4.4 Sampling and Nutrient Analysis

Samples for nutrient analysis (NH_3-N, NO_3-N, NO_2-N) were taken in 15 mL aliquots and immediately vacuum filtered using 0.45 µm membrane filters and stored at 4°C until analysis. These nutrients were analyzed by Hach tubes (Loveland, CO). These kits were then analyzed through a Hach DR2800 spectrophotometer.

Ammonia was analyzed through Hach Test N' Tube Plus (TNT plus) 830 or 831, ultra-low range and low range, respectively. These kits used the salicylate method (method 10205) for analysis where ammonium ions react with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenols. The amount of color formed was directly proportional to the ammonia nitrogen present in the sample and results were measured at 690 nm.

Nitrate was analyzed using TNT plus 835 (low range). This kit employs the dimethylphenol method (10206), where nitrate ions in solution containing sulfuric and phosphoric acids react with 2, 6-dimethylphenol to form 4-nitro-2, 6-dimethylphenol. These results were measured at 345 nm.

Nitrite was analyzed using Test 'N Tube NitriVer3 Reagent sets, which uses the diazotization method (10019) in which nitrite in the sample reacts with sulfanilic acid to form an intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present.

For MLSS/MLVSS and effluent TSS/VSS, 240 mL and 1L, respectively, were removed from the reactors and analyzed per *Standard Methods*. COD, TKN, TP, and sludge volume index were also measured per *Standard Methods*. Soluble COD (sCOD) and soluble TKN (stTKN) were measured by filtering samples through a 0.45 µm membrane filter and measured per *Standard Methods*.

3.4.5 Solids Retention Time

The SBRs were designed to run in Garrett configuration, where biomass was wasted directly from the reactor, thus achieving and maintaining a desired SRT. Solids wasting occurred during each cycle, during the reaeration period prior to settling. Due to slight differences in peristaltic waste pump flow rates among the three reactors, the waste activated sludge (WAS) was stored and weighed every 8-11 cycles to determine the exact volume of waste per cycle.

This, in conjunction with effluent total suspended solids (TSS) and mixed liquor suspended solids (MLSS), helped to determine the actual SRT in each reactor. During sampling days, the volume of MLSS removed for nutrient analysis was recorded and this volume was returned to the reactor. Waste sludge was also returned to the reactor to correct for differences in effluent TSS and waste pump flow rates. Thus, by monitoring wastage, biomass concentrations, sampling volume extracted, and effluent TSS, all three reactor SRTs were maintained at exactly 15 days.

3.4.6 Dissolved Oxygen

Continuous mixing was provided in the SBRs by top entry paddle mixers. DO control was provided by aeration ON/OFF control using solenoid valves. The low and high set points were set at 2 and 3 mg/L, respectively. If the measured DO was below 2 mg/L, the solenoid values would be turned on, allowing dissolved oxygen into the reactor. Once the DO reached 3.0 mg/L, the solenoid valves would shut the air off. These solenoid valves were connected to the programmable timer to allow aeration during aerobic cycle periods only. DO in the reactors was measured using conventional galvanic membrane probes (Royce Technologies, College Station, TX). Aeration was provided using aquarium style air stones, and DO data was logged every 20 seconds (Telog, Victor, NY) to allow oxygen uptake rate (OUR) calculations.

3.4.7 pH

The pH was not controlled during this research since it was within reasonable range. pH readings were also logged and stored every two minutes (Telog). The pH was monitored using Foxboro probes. Peristaltic pumps were available to add alkalinity but the pumps were turned off during the duration of this experiment.

3.4.8 Supplemental Carbon Addition

Supplemental carbon addition in the anoxic period was done through the use of peristaltic pumps. Carbon sources were kept in collapsible sealed LDPE containers and stored in a refrigerator. The carbon fed from the LDPE containers to the reactors directly through viton tubing to prevent evaporation and tubing diffusion between cycles. 100% reagent grade methanol and 100% reagent grade glycerol were used in reactors A and B, respectively. Reactor C used a

bio-diesel glycerol waste (BGW), which comprised of a mixture of MicroCglycerin (Environmental Operating Solutions) and BiocarbDN (Suffolk Sales). During normal reactor operation, COD was added based on assumed C: N ratios and 7 mg/L NO_3-N. During over-dose and under-dose reactor operation, carbon was added based on 15 mg/L NO_3-N and 4 mg/L NO_3-N, respectively.

Methanol and glycerol were added based on the theoretical COD of the pure chemicals, while the measured COD of the BGW was 1020 g/L (Table 3.1). The methanol was based on an assumed yield of 0.4 g COD/g COD. The glycerol anoxic yield was determined by assuming a C: N value of 6 and directly using equation 1 from section 3.4.9. All carbon sources were diluted, as shown in Table 3.2 before being placed in LDPE containers and stored in the refrigerator.

Table 3.1: Determination of Carbon Source Solutions

Electron Donor	Assumed Anoxic Yield (g COD/g COD)	Formula	Molecular Weight (g/mol)	Theoretical COD (g COD/g Chem)	COD of Product (g/L)	NO_3-N add (mg/L)	Reactor Vol. Basis (L)
Methanol	0.40	CH_4O	32	1.50	-	7	22
Pure Glycerol	0.52	$C_3H_8O_3$	92	1.22	-	7	22
BGW	0.52	-	-	-	1020	7	22

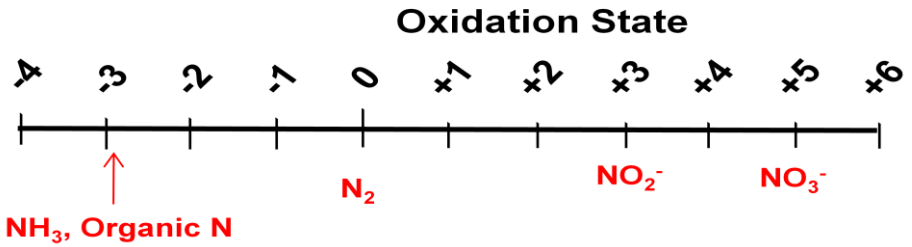
Mass NO_3-N (g/cycle)	Carbon (g Chem/g NO_3-N)	Carbon (g COD/g NO_3-N)	COD to add (g/cycle)	Mass Chem (g/cycle)	Vol. to add (mL/cycle)
0.15	3.2	**4.8**	0.73	0.49	-
0.15	4.9	**6.0**	0.92	0.75	-
0.15	-	**6.0**	0.92	-	0.91

Table 3.2: Diluted Concentrations of Carbon Source

	Typical dose (7 mg/L NO_3-N)	Over-dose (15 mg/L NO_3-N)	Under-dose (4 mg/L NO_3-N)
Carbon Source	**Solution Concentration (%)**		
Methanol	20.6	44.2	11.8
Glycerol	9.9	21.4	5.7
BGW	15.1	32.4	8.6

3.4.9 Carbon to Nitrogen Ratios

In order to determine actual supplemental carbon to nitrogen ratios (C: N in g COD consumed/g NO_3-N fully denitrified) during the second anoxic period, an electron equivalence technique was used to take residual DO consumption and accumulation of nitrite into consideration.



$$NO_3^- \rightarrow NO_2^-: \quad \frac{5\ e^-}{mole\ NO_3} \times \frac{mole\ NO_3}{14\ g} = \frac{0.357 e^-}{g\ NO_3\text{-}N}$$

$$NO_2^- \rightarrow N_2: \quad \frac{3\ e^-}{mole\ NO_2} \times \frac{mole\ NO_2}{14\ g} = \frac{0.214 e^-}{g\ NO_2\text{-}N}$$

$$O_2 \rightarrow 2\ H_2O: \quad \frac{4\ e^-}{mole\ O_2} \times \frac{mole\ O_2}{32\ g} = \frac{0.125 e^-}{g\ O_2}$$

Figure 3.2: Determination of Electron Transfer

After determining specifically how nitrate reduced (to NO_2, N_2) as well as DO removal, the total electrons transferred was summed. The amount of sCOD consumed was divided by the amount of total electrons consumed, and then converted back to an equivalent NO_3-N fully denitrified basis. The true anoxic yield was estimated as:

$$\text{Equation 1: } C:N = \frac{2.86}{(1-Y)}$$

Since NO_2^- is stoichiometrically oxidized to NO_3^- during dichromate COD analysis representing a positive interference, all COD data were corrected for the NO_2^- concentration present in each filtered sample.

3.5. Results and Discussion

3.5.1 Reactor Operation

Table 3.3: Effluent Nutrient Concentrations (mg/L)

	Methanol				**Glycerol**				**BGW**			
	START-UP											
	NH_3-N	**NO_3-N**	**NO_2-N**	**OP-P**	**NH_3-N**	**NO_3-N**	**NO_2-N**	**OP-P**	**NH_3-N**	**NO_3-N**	**NO_2-N**	**OP-P**
AVG.	0.04	2.1	0.1	1.5	0.03	1.1	0.5	1.2	0.04	1.04	0.8	1.34
±	0.02	0.92	0.24	1.44	0.01	0.81	0.52	1.16	0.02	0.86	0.95	1.18
	STEADY-STATE (SS)											
AVG.	0.03	1.98	0.05	1.12	0.04	1.23	0.33	0.86	0.04	1.23	0.79	0.68
±	0.01	0.84	0.03	0.67	0.01	0.99	0.49	0.27	0.02	0.97	1.05	0.29
	OVER-DOSE (OD)											
AVG.	0.03	0.38	0.04	1.60	0.03	0.18	0.02	2.23	0.04	0.19	0.03	1.60
±	0.01	0.37	0.02	0.95	0.01	0.03	0.02	1.70	0.02	0.01	0.03	1.99
	UNDER-DOSE (UD)											
AVG.	0.02	1.47	0.06	1.00	0.03	0.99	0.90	1.20	0.05	1.05	0.75	2.57
±	0.00	0.53	0.01	0.50	0.01	0.09	0.43	1.21	0.03	0.13	0.68	2.22
	RE-START											
AVG.	0.04	1.19	0.06	0.61	0.04	0.60	0.08	0.56	0.03	1.02	0.24	0.59
±	0.00	0.90	0.02	0.38	0.01	0.42	0.04	0.28	0.01	0.83	0.51	0.27

Table 3.3 shows the persistence of effluent nitrite accumulation for the glycerol fed SBRs. Since denitrification was not complete at the end of the second anoxic cycle, the post-aeration resulted in the conversion of some residual nitrite to nitrate, resulting in higher effluent nitrate values and less nitrite. This signifies inefficiency with glycerin addition. A majority of the

readily biodegradable COD (rbCOD) is consumed coincident with nitrate to nitrite reduction. There is a lag in nitrite reduction, as there is limited rbCOD remaining to fuel reduction of nitrite to nitrogen gas. The nitrite gets oxidized back to nitrate in the aeration stage prior to settling, thus resulting in "wasted" carbon as there is not a reduction in effluent total nitrogen. Importantly, there was residual nitrite in the effluent of all glycerol reactor experiments except when glycerol was overdosed. This finding indicated that control of effluent nitrite was strongly linked to the amount of electron donor that was provided during anoxic operation.

The concentrations recorded for orthophosphate were not reliable measurements as there were problems associated with data collection. It was determined that contamination from the membrane filters gave skewed results. Influent and effluent total phosphorus concentrations can be seen in figure 3.3 below.

Figure 3.3 reveals that all three reactors were doing reasonable biological phosphorus removal, as the effluent concentrations of phosphorus averaged around 0.2 mg/L for all reactors. A profile of orthophosphate during a reactor cycle can be seen in figure 3.3a below.

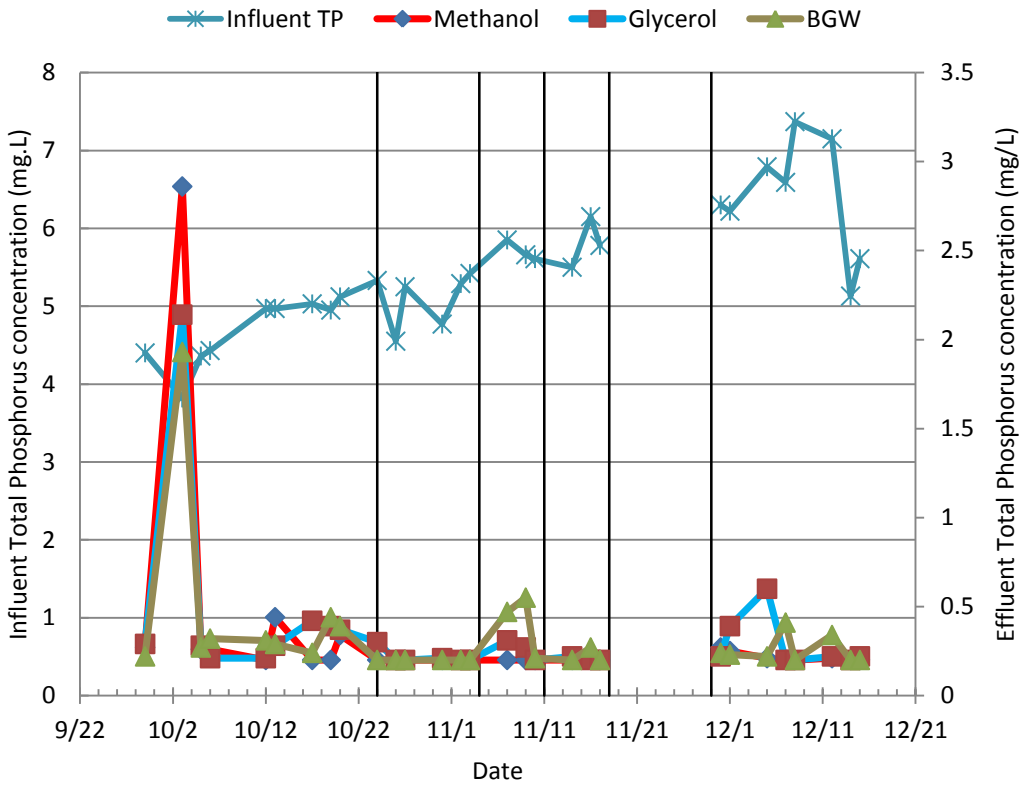


Figure 3.3: Influent and Effluent Concentrations of Total Phosphorus

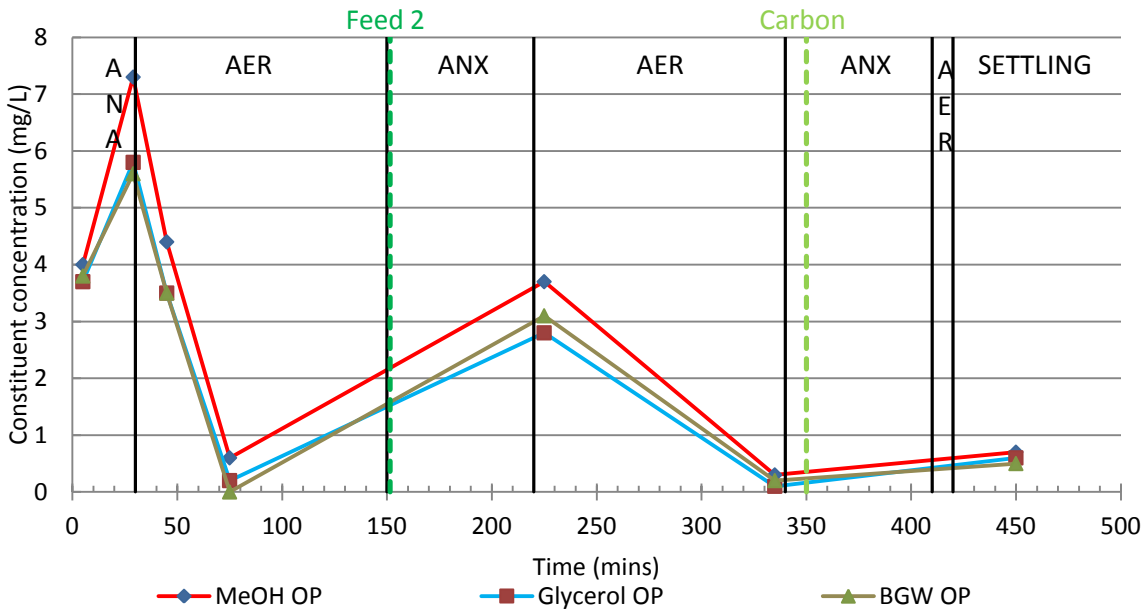


Figure 3.3a: Typical Profile of Orthophosphate during Reactor Steady State Operation

From figures 3.3 and 3.3a, it can be seen that when supplemental carbon was added, there was no significant release of phosphorus during the second anoxic zone, even after the nitrate was depleted. The importance of this is further discussed in section 3.5.3.

3.5.2 Start-up

The SBRs were seeded with biomass from an existing BNR plant (Virginia Initiative Plant-VIP), which had not been exposed to any supplemental carbon. The SBRs were profiled for nutrients to examine start-up characteristics. The first day of intensive profiling to analyze reactor operation occurred two cycles after initial seeding. Upon start-up, it was evident that the biomass was not yet acclimated to the carbon sources, thus denitrification was incomplete, resulting in high TN in the effluent. There was no significant accumulation of nitrite in any of the reactors during the first two days of startup. The first anoxic zone was denitrified with raw wastewater. Ammonia was completely removed before the second anoxic zone in all reactors, suggesting complete nitrification. Figure 3.4 shows the startup profile, one day after the initial seed.

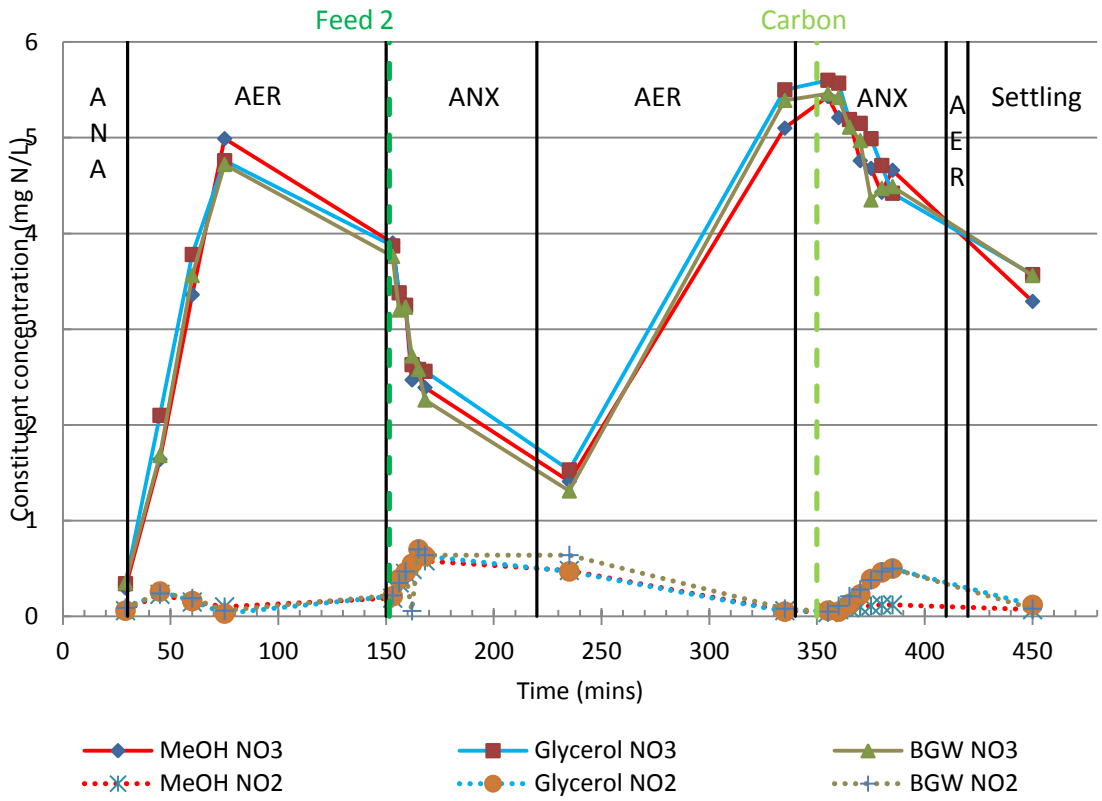


Figure 3.4: Start-up SBR profile

Figure 3.5 shows typical dissolved oxygen (DO) concentrations during a reactor cycle. The first ten minutes of the second anoxic period was intended for deoxygenation prior to carbon addition. If there is DO present in the reactors after the ten minute deoxygenation period, it is removed first prior to nitrate reduction. The ten minute post-aeration was required to boost DO and strip N_2 gas prior to the settling phase.

Figure 3.6 demonstrates the behavior of DO during carbon addition for the start-up period. The oxygen uptake rate for all three reactors was similar. After carbon addition, the oxygen uptake rates for all three reactors increased and were similar in rate. In Figure 3.6, roughly 1.5 mg/L of DO is in the reactors when carbon is introduced. It is important to get the reactor to complete anoxia conditions prior to carbon addition for maximum denitrification and efficient use of carbon. In addition to impacting the carbon dose, residual oxygen could also impact denitrification rates (Oh et al., 1999).

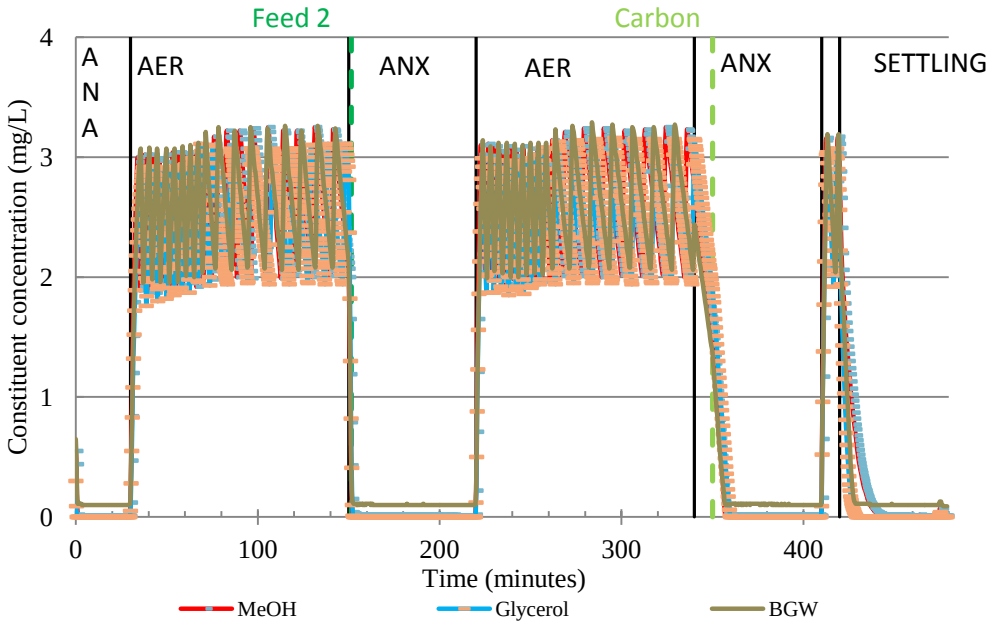


Figure 3.5: Representative DO Profile during Entire SBR Cycle

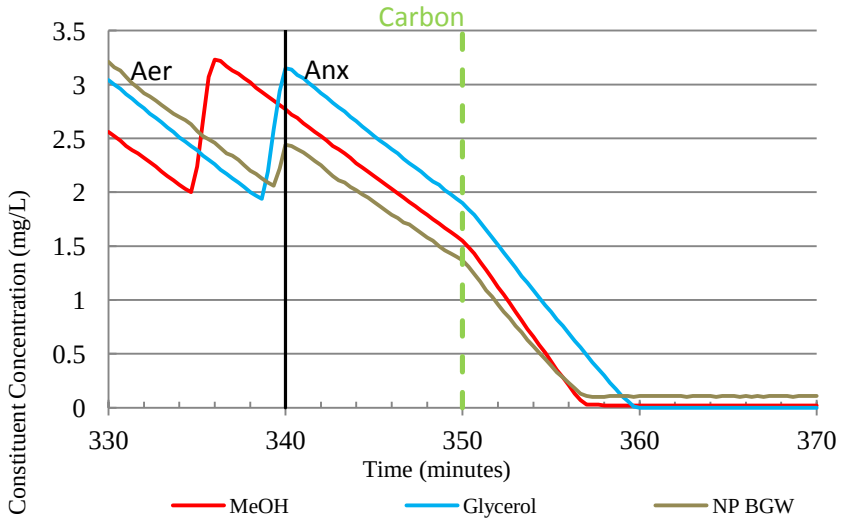


Figure 3.6: Representative DO profile during Supplemental Carbon Addition at Start-up

The pH was monitored closely in order to determine the necessity to add alkalinity. Figure 3.7 shows the typical pH profile during the SBR cycle. pH profiles in the reactors were consistent through each cycle during the entire study. During reactor operation, the highest and

lowest measured pH was deemed sufficient for operation, thus acid and caustic were not added at any point of the experiment.

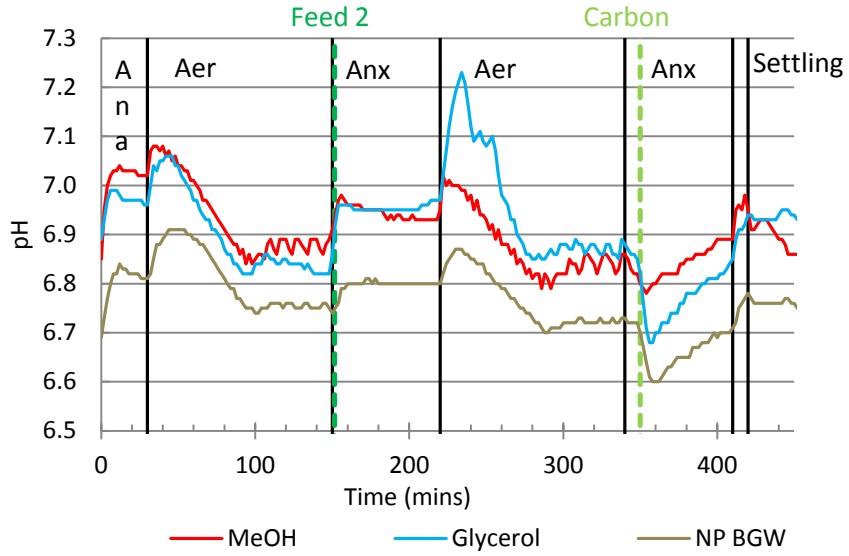


Figure 3.7: Representative Profile of pH during SBR Cycle

3.5.3 Steady-State Operation

After a period of 26 days of continuous SBR operation, steady state conditions were assumed to be reached on 10/24/11. Intensive profiling was conducted for nutrients as well as COD starting 10/24/11 and repeated three days a week. During this period, carbon was dosed assuming a NO_3-N concentration of 7 mg/L in the reactors. Figure 3.8 shows the profile of the reactors at the start of assumed steady state conditions.

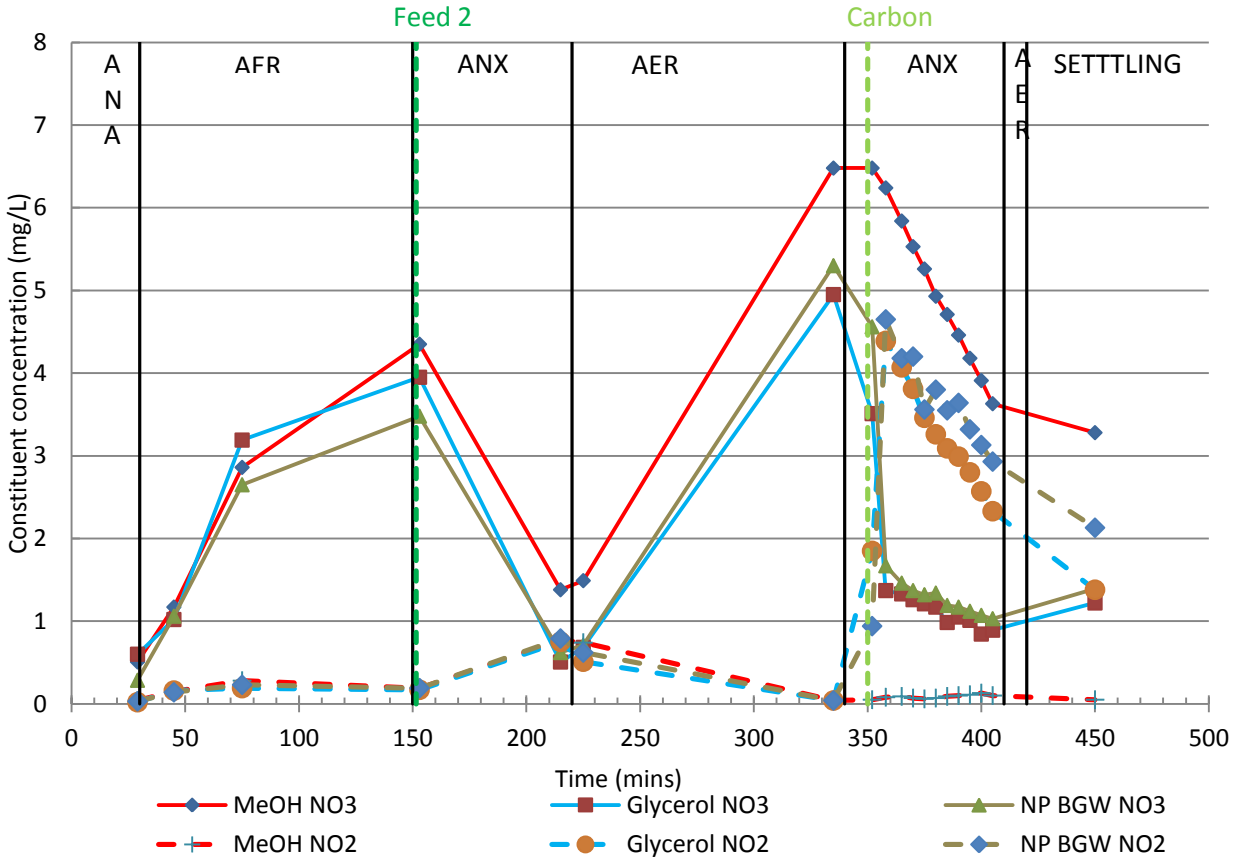


Figure 3.8: Representative Intensive Nutrient Profiling after Achieving Steady-State Reactor Operation

As shown in Figure 3.8, the rate of nitrate to nitrite denitrification in the glycerol reactors was much more rapid than in the methanol reactor. This led to significant nitrite accumulation in these glycerol reactors once the carbon was added, producing high levels of nitrite at the end of the second anoxic cycle. A portion of the nitrite was then oxidized to nitrate in the reaeration stage. Nitrite accumulation was not observed in the methanol reactor. Since the reactors were dosed for 7 mg/L NO_3-N, the methanol should have completely denitrified, since there was less than 7 mg/L of nitrate-nitrogen in the reactor at the time of carbon addition. This is suggestive that the second anoxic period time was inadequate. Figure 3.9 shows the second anoxic zone for all three reactors during the first day of assumed steady state operation more clearly.

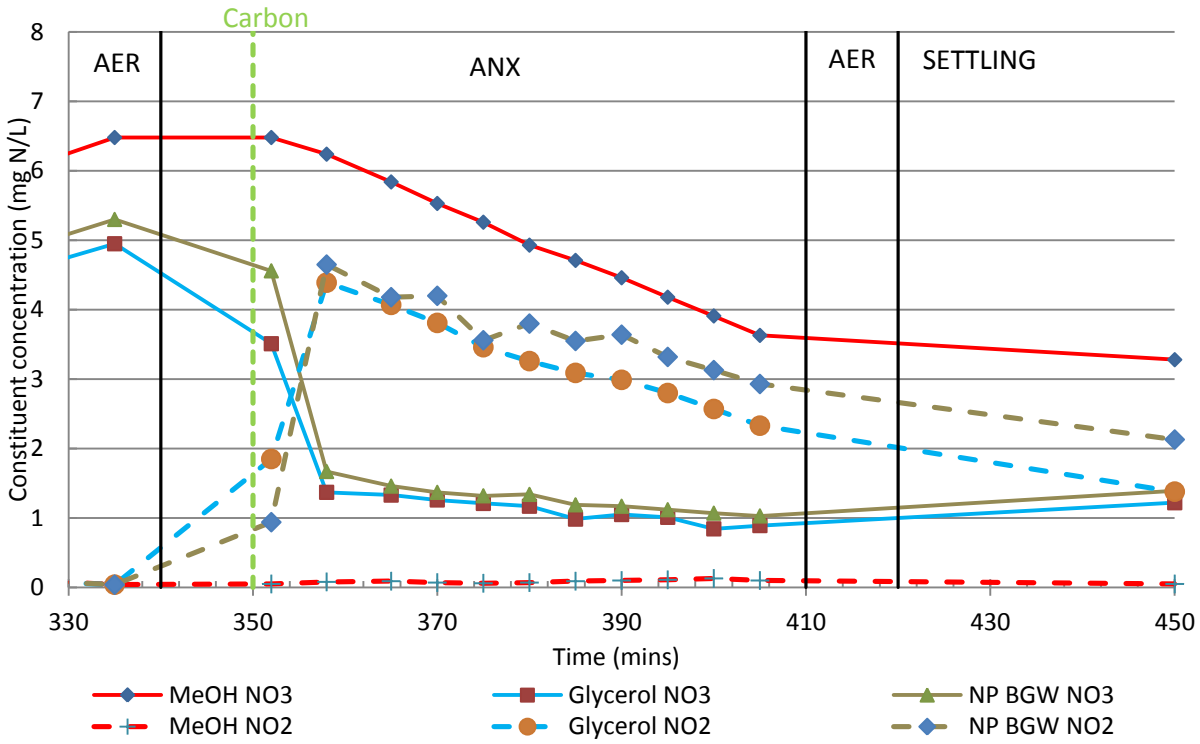


Figure 3.9: Second Anoxic Zone Profile for All Reactors during First Day of Steady State Operation

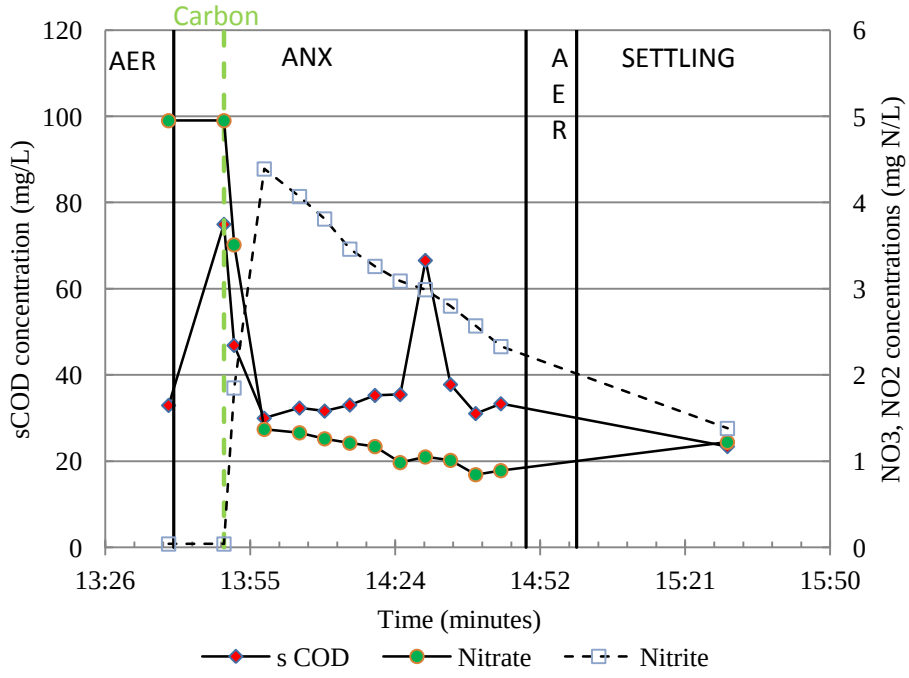


Figure 3.10: Glycerol Intensive Profile Emphasizing Carbon Addition in Second Anoxic Zone during Steady State Reactor Operation

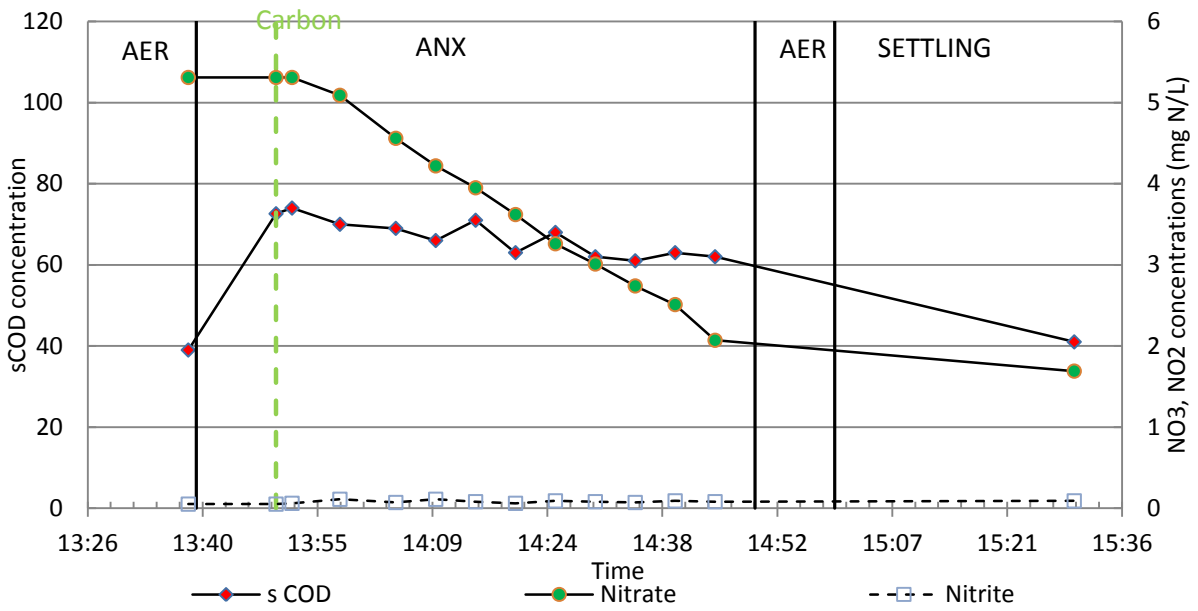


Figure 3.11: Methanol Intensive Profile Emphasizing Carbon Addition in Second Anoxic Zone during Steady State Reactor Operation

Figure 3.10 highlights the intensive nutrient and COD profile for glycerol during the second anoxic phase in order to emphasize the nitrite accumulation. Intensive profiling with COD was necessary to calculate C: N ratios and true anoxic yield. COD was removed coincidentally with rapid nitrate to nitrite reduction. This trend suggests that the specialist community has a pre-disposition for carbon storage and nitrate to nitrite reduction. Both nitrate and COD were removed within ten minutes from the time carbon was added. The nitrate SDNR for this ten minute period is much higher than any textbook. The spike of COD in figure 3.10 in the anoxic phase is likely an outlier and inconsistent with other profiles. Figure 3.11 shows the same plot as figure 3.10, except strictly for methanol. There was no significant nitrite accumulation in the methanol reactor at any point during the second anoxic zone. It is clear that there is rapid COD uptake as nitrate is reduced very rapidly to nitrite. The rate of COD consumed per nitrate denitrified to nitrite must suggest carbon storage. The absence of phosphorus release in the second anoxic zone after carbon addition (as seen in figure 3.3 and 3.3a), especially in the glycerol reactors, signifies that PAOs are not responsible for the intracellular carbon storage. This rapid nitrate to nitrite reduction coincident with rapid COD uptake and storage must not be dependent on glycerol fermentation to acetic acid or propionic acid, as previously thought. The study by Gárate et al (2011) concluded that no fermentation, at

least for glycerol, is needed to produce PHA. Therefore, the organisms that are responsible for using and storing the glycerol must be using it directly and since there was no significant phosphorus release in the second anoxic zone, then it must not have been the PAOs. Although the literature is scarce and more research is needed for confirmation, it was believed that GAOs, the competitors of PAOs, were responsible for this storage.

There have been few studies on the use of glycerol as a carbon source for EBPR. Guerrero et al (2012) concluded that glycerol can be an alternative external carbon source, and could be applied in full-scale WWTPs if sufficient hydraulic retention time was provided. They also stated that development of a microbial community able to use glycerol for EBPR is possible and opens a new range of possibilities.

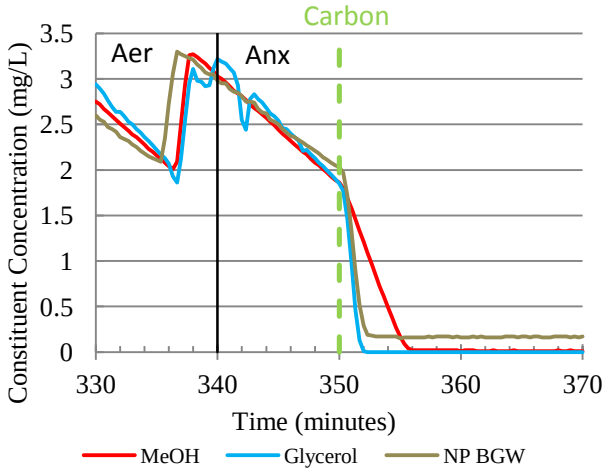


Figure 3.12: Dissolved Oxygen during Carbon Addition during Steady State Reactor Operation

Figure 3.12 shows the rate of DO depletion after carbon addition during steady-state conditions. As shown, with acclimation, the glycerol reactors consumed DO more rapidly than during startup, and more rapidly than the methanol reactor, as compared in Figure 3.6. This new trend started to occur approximately eight days after reactor start-up. This result was expected as aerobic degradation rates for acclimated biomass are typically higher for compounds that release more energy (such as C2 or C3 compounds vs. C1 compounds) during oxidation (Atkinson et al., 2007). The ten minute deoyxgenation period was not sufficient to achieve near zero values of dissolved oxygen prior to carbon addition. This ten minute period intended for oxygen stripping was ineffective in lowering the DO. This could be explained by the limited amount of bio-available electron donor that is present in the reactor at the end of the aerobic stage. This

emphasizes the need to find an optimal supplemental carbon dose in order to account for aerobic respiration requirements.

3.5.4 Over-dose and Under-dose of Supplemental Carbon

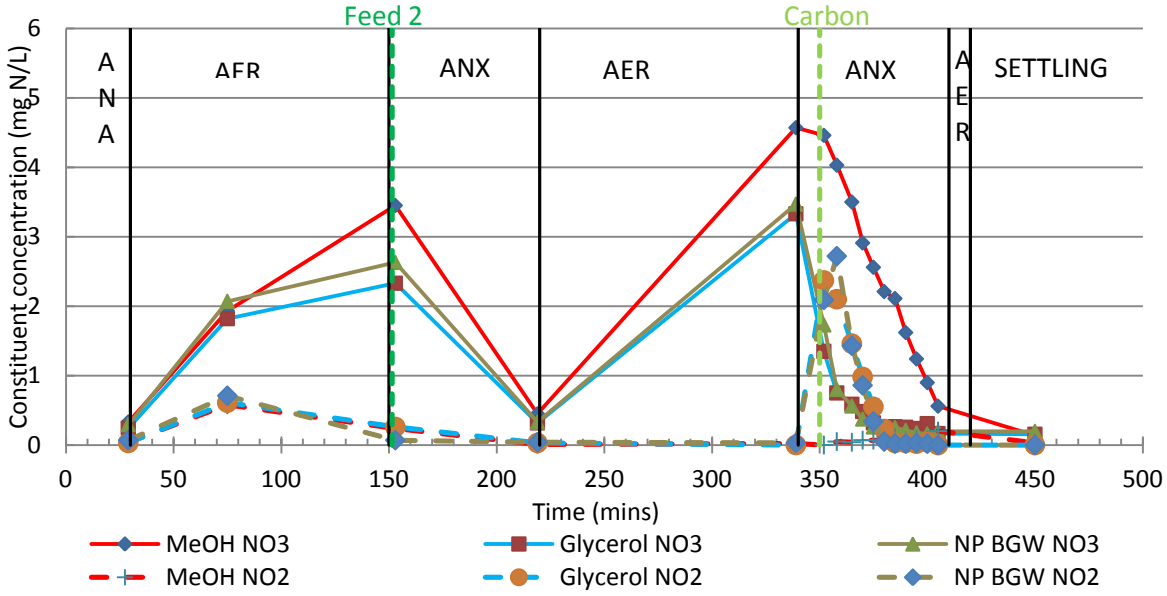


Figure 3.13: Intensive Nutrient Profiling with Carbon Overdose

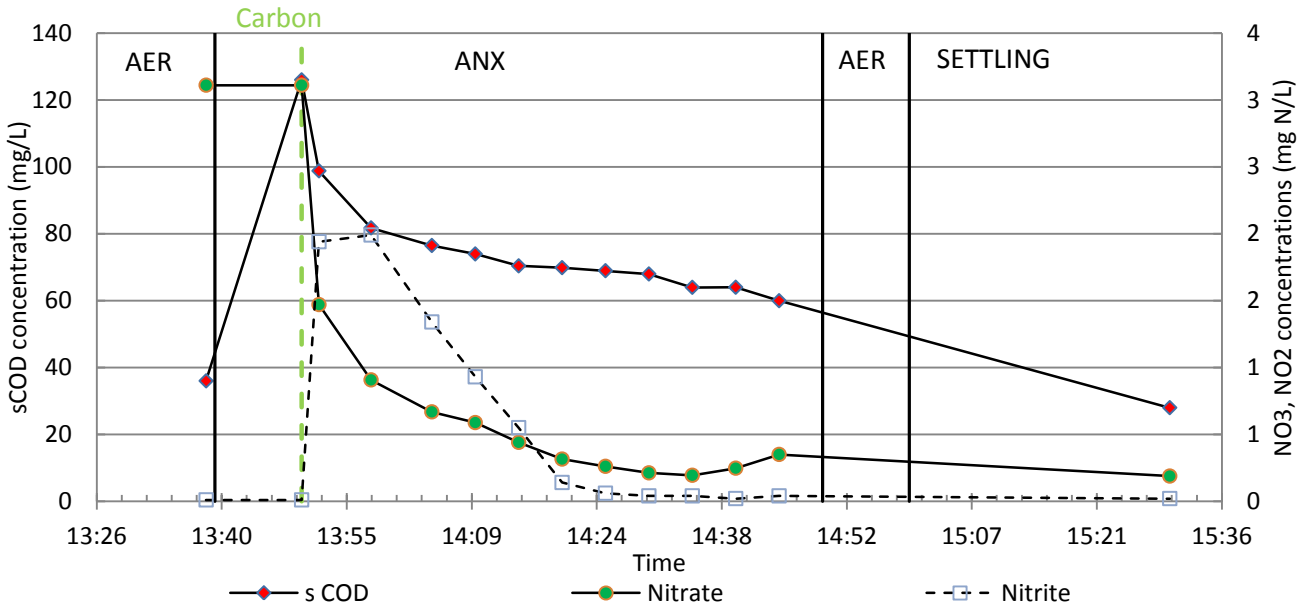


Figure 3.14: Glycerol Intensive Profile Emphasizing Carbon Addition in Second Anoxic Zone during Overdose

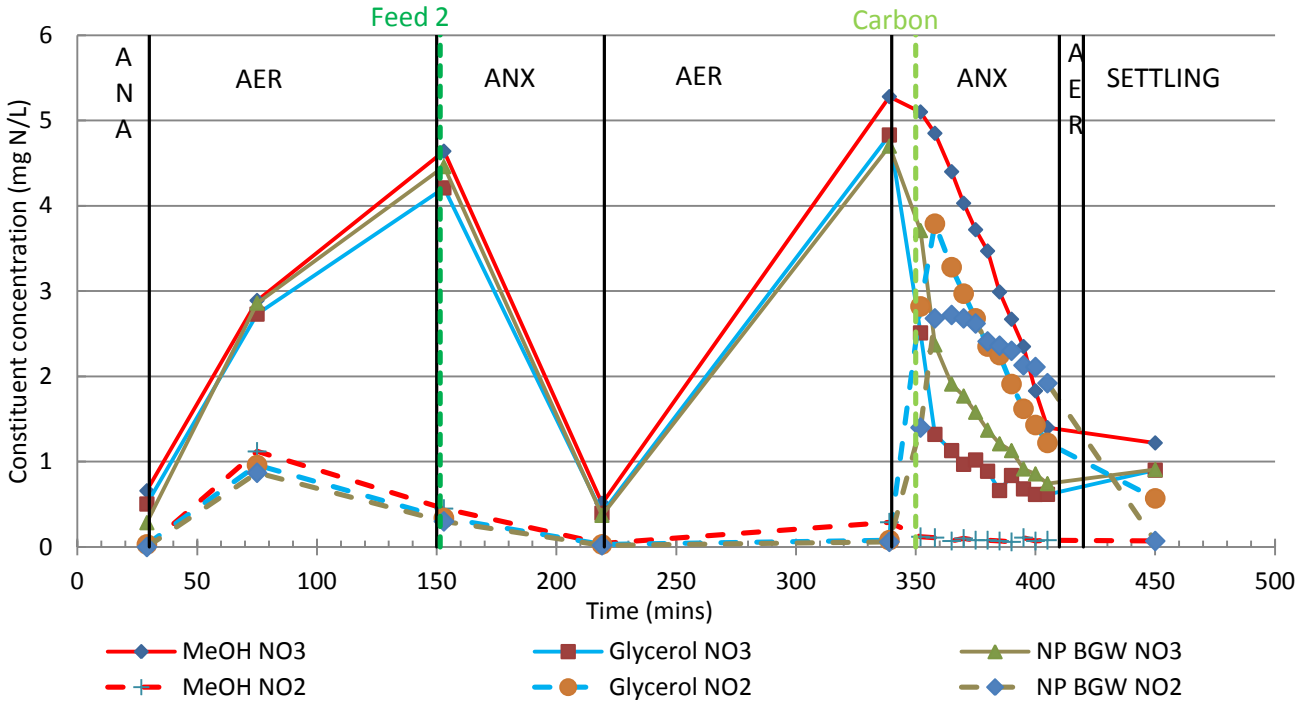


Figure 3.15: Intensive Nutrient Profiling with Carbon Under-dose

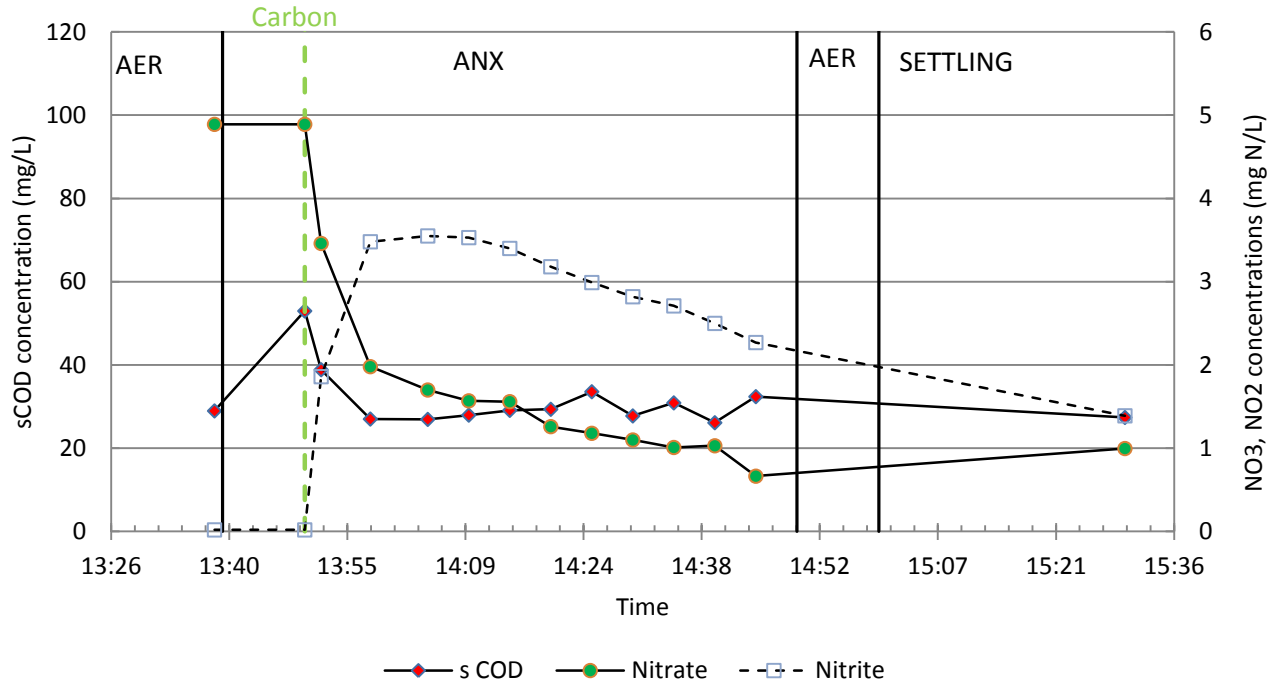


Figure 3.16: Glycerol Intensive Profile Emphasizing Carbon Addition in Second Anoxic Zone during Under-dose

Figures 3.13 and 3.14 reveal that when carbon was over-dosed, complete nitrate removal was observed in the glycerol reactors, as there was very low residual nitrogen in the effluent. Rapid nitrate to nitrite denitrification in these reactors was still visible. However, the degree of nitrite accumulation was greater with an over-dose, which is evident via comparisons of Figures 3.15 and 3.16 to 3.13 and 3.14. Nitrite reduction was induced more rapidly when carbon was fed in excess. There was still COD to be consumed during an overdose, thus the nitrite could be reduced and have no residuals in the effluent.

Figure 3.15 reveals that when carbon was under-dosed, the nitrate consumption for the glycerol was still rapid and coincident to COD uptake. The nitrite accumulation coincided with nitrate reduction and neither was completely removed. Reduction of nitrite was slow because the COD had already been consumed for nitrate reduction and there wasn't enough biodegradable carbon available to reduce the nitrite. There was no nitrite accumulation in the methanol reactor for either an over-dose or under-dose, although the observed average nitrite concentration in the effluent for methanol during an under-dose was higher than for any of the other doses. An under-dose could have resulted in some accumulation, as observed by Ginige et al (2009). Methanol however, similar to glycerol, resulted in complete nitrate removal when excess carbon was added, whereas incomplete removal was observed with a carbon under-dose. There was also no nitrite accumulation in any of the reactors during the first anoxic period when the raw influent wastewater served as the source of organic carbon.

3.5.5 Reactor Restart

Restarting the reactors and intensively sampling for COD and nutrients was necessary to evaluate the original hypotheses. Figure 3.17 shows start up characteristics for the reactors. This figure shows that the lag period preceding nitrate reduction decreased over time. The longer the reactor was fed glycerol, the more rapid and more efficient the nitrate to nitrite reduction began to occur. The nitrite accumulation still coincided well with nitrate consumption, as well as COD uptake. This nitrite accumulation became more rapid and significant with "acclimation" of the biomass to the substrate. However, over time, the nitrite was eventually completely removed by the end of the cycle. As the C: N and yield increased, nitrate to nitrite denitrification became more rapid and efficient, causing increased and significant nitrite accumulation. This specialist

population is more efficient and has a higher disposition to carbon storage than the generalist population.

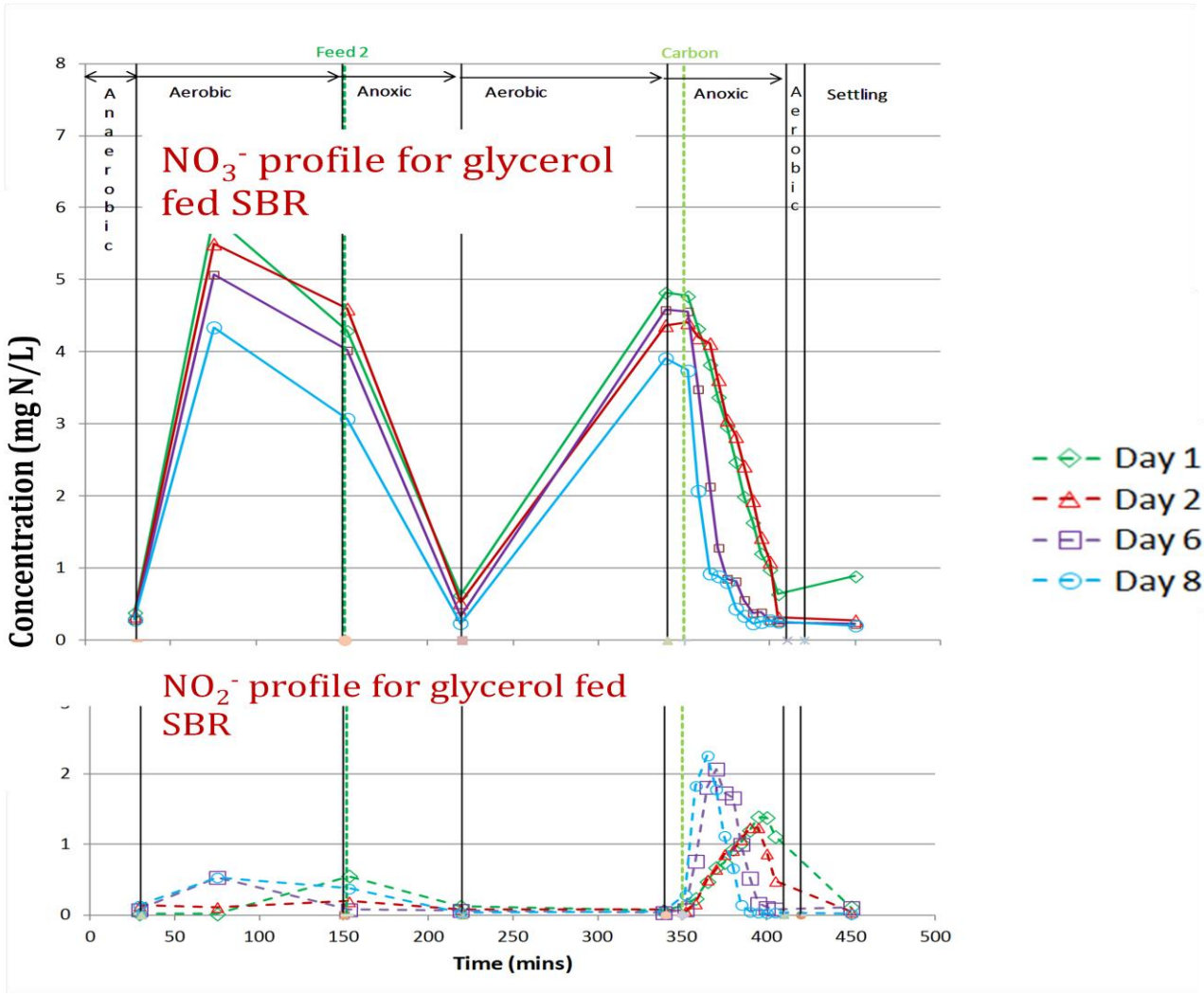


Figure 3.17: Reactor Restart Characteristics during Second Anoxic Zone

3.5.6 Validation of Hypotheses

Figure 3.18 demonstrates the significance of nitrite accumulation after carbon addition throughout this research. As expected, and noted previously, there was no significant nitrite accumulation for methanol. However, evidence of significant buildup for both glycerol and BGW was clear. The accumulation shown in figure 3.18 is not the nitrite that was measured in the effluent, but rather, what the initial peak values of nitrite reached as the nitrate was rapidly reduced. Martiensson et al (1999) concluded that nitrite accumulation may be caused by different mechanisms: (1) imbalanced activities of nitrate and nitrite reductases, (2) inhibition of nitrite

reductase by oxygen, nitrate, or nitrite, and (3) selection and enrichment in favor of microorganisms accumulating nitrite.

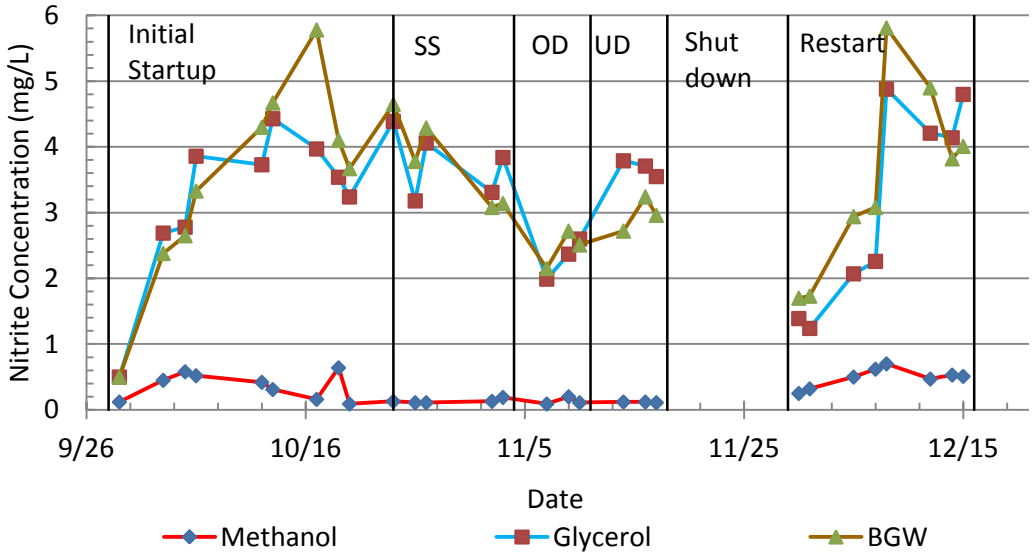


Figure 3.18: Maximum NO_2-N Accumulation during Second Anoxic Zone

After shutting the reactors down and re-seeding with fresh biomass previously not exposed to carbon, intensive COD sampling occurred and start-up C: N ratios were examined.

Table 3.4: Average Observed C/N ± Std. Dev. (g COD consumed / g equivalent NO_3-N removed)

	SS	OD	UD	Restart	All conditions
Methanol	4.46 ± 0.4	5.6 ± 0.5	4.67 ± 0.2	4.7 ± 0.5	4.86 ± 0.4
Glycerol	12.9 ± 1.8	19.9 ± 0.9	6.8 ± 0.5	8.5 + 1.9	12.0 ± 1.3
BGW	11.5 ± 0.4	18.5 ± 0.7	7.7 ± 0.6	9.9 ± 1.6	11.9 ± 0.83

From Table 3.4, the original hypothesis regarding generalists-specialists was confirmed. The C: N ratio for the glycerol reactors was much higher in the steady-state phase, after selection of a higher yield specialist community, than during reactor start-up with a generalist population. This proved that the specialists utilizing glycerol increased demand for carbon over time. When carbon was fed in excess, the ratio in both glycerol reactors increased drastically. The high C: N ratio during glycerol overdose is indicative of glycerin storage, as it translates to unrealistically high yields. On the contrary, when carbon was under-dosed, the C:N ratio dropped considerably. Thus, it can be stated that significant nitrite accumulation will occur when the glycerol dose is not optimized. Both reagent grade glycerol and bio-diesel glycerol waste exhibited the same trends, suggesting that slow NO_2 denitrification was not related to other constituents in the bio-diesel glycerol waste (e.g. contaminants, unreacted long chain fatty acids, methanol concentration).

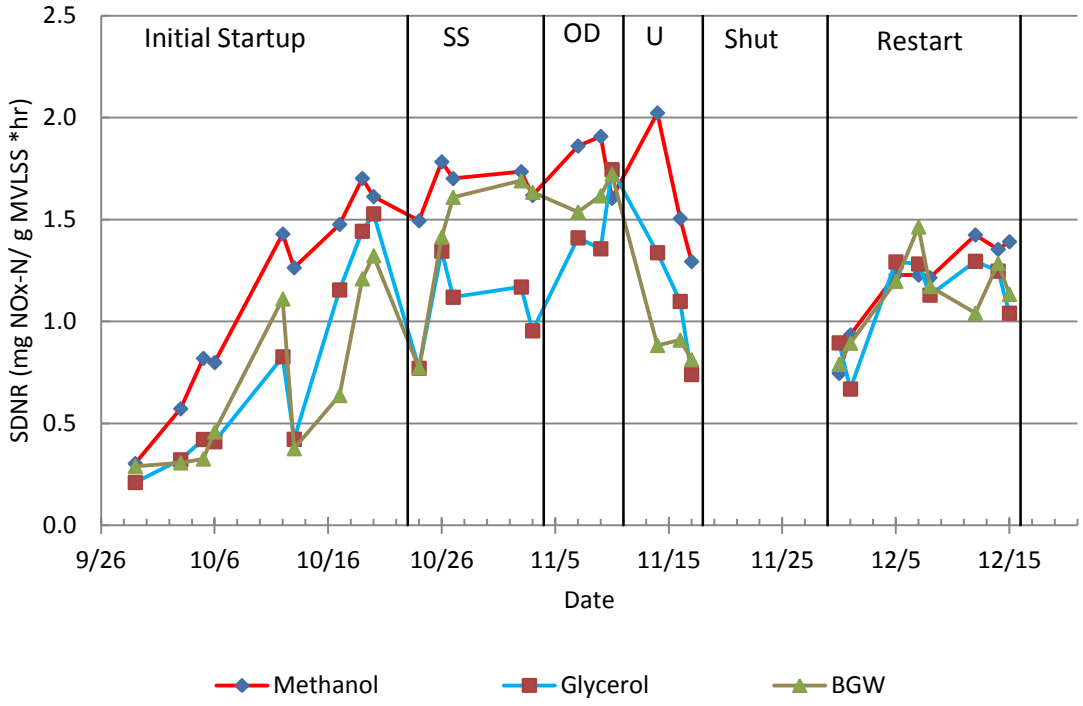


Figure 3.19: SDNR of NO_x-N during Entire Second Anoxic Zone Profiles

Denitrification of nitrate to nitrite was much faster in the glycerol reactors than in the methanol reactor, however, when nitrite was accounted for (Figure 3.19), the methanol reactor SDNR was greater than that of the glycerol reactors. This finding signified the need to account for both nitrate and nitrite reduction steps, when designing and implementing supplemental carbon addition in full-scale applications. The average SDNR values are listed in Table 3.5.

As time passed and the activated sludge adapted to the carbon source, the observed SDNR for all reactors increased. However, when carbon was under-dosed, the SDNR decreased. This SDNR value was highest when carbon was over-dosed, for all reactors. This was sensible as more carbon was able to be used for denitrification. Both glycerol and BGW reactors were similar in performance, which was expected. These SDNR values, shown in Table 3.5, were similar and comparable to the values in a study comparing different glycerin products at full-scale applications (Bilyk et al., 2009). The increasing SDNRs in this experiment agrees with another study by Bilyk et al (2011) which also revealed significantly faster SDNRs after an extended period of glycerin addition.

The increasing of C: N and SDNR through time supports previous theories of generalists being wide spectrum, inefficient substrate users by exhibiting a low yield and lower rates during the start-up phase. This efficiency increases by time, during adaptation (Omari et al., 2011).

Table 3.5: Average SDNRs based on NO_3-N and NO_x-N

	Average SDNR NO_3-N					Average SDNR NO_x-N				
	mg NO_3-N/ g MLVSS*hr					mg NO_x-N/ g MLVSS*hr				
	Start	**SS**	**OD**	**UD**	**Restart**	**Start**	**SS**	**OD**	**UD**	**Restart**
Methanol	1.2	1.7	1.8	1.6	1.3	1.1	1.7	1.8	1.6	1.2
Glycerol	2.0	2.5	3.0	1.8	2.0	0.7	1.1	1.5	1.1	1.1
BGW	1.9	2.9	3.0	1.5	2.1	0.7	1.4	1.6	0.9	1.1
	Standard Deviation SDNR NO_3-N					Standard Deviation SDNR NO_x-N				
Methanol	0.45	0.10	0.19	0.35	0.30	0.50	0.11	0.16	0.37	0.24
Glycerol	0.65	0.63	0.37	0.64	0.65	0.51	0.22	0.21	0.30	0.23
BGW	0.90	0.80	0.20	0.11	0.55	0.42	0.38	0.09	0.05	0.21

3.6 Conclusion

Evidence gathered and observations made in this research showed that the specialist-generalist theory for glycerol, both reagent grade and biodiesel waste, was supported. The yield

increased as the unacclimated biomass began to grow on the supplemental carbon, proven in both reactors using pure glycerol and bio-diesel glycerol waste, supporting the notion that the population of OHO transitioned from generalist to specialists after several SRTs of reactor operation. The OHO in the glycerol reactors acclimated to the carbon faster when compared to the organisms in the methanol reactor, which was expected. The increase in yield for glycerol justifies evolution into a specialist population. This transition from low efficiency carbon utilization organisms to highly efficient organisms was due to adaptation or population shift (Omari et al., 2011).

The research has also shown that adding methanol results in no significant nitrite accumulation, similar to a study done by Cherchi et al (2009) and the C: N is consistent with the expected theoretical value (4.8 g COD/ g NO_3-N).

The evidence of partial denitrification is also substantial. Nitrate to nitrite conversion occurs rapidly as the glycerol is added, but the reduction of nitrite varies depending on the carbon dosage. In this system, it is believed that the biomass uses glycerol to denitrify nitrate to nitrite, but also stores a large fraction of the added glycerol. Since COD is taken up coincidentally with NO_3-N reduction, it is assumed that the carbon storage is occurring with the specialist population. Since there was no obvious release of phosphorus in the second anoxic zone after carbon addition, then it was assumed that GAOs rather than PAOs were responsible for this carbon storage and direct utilization, without the need for glycerol fermentation. Carbon storage, not completely utilized in the anoxic zone for denitrification, may be used in subsequent aerobic zones, representing a potential significant economic impact on glycerin addition. As a result of this storage phenomenon, insufficient soluble electron donor is present to fuel nitrite reduction to nitrogen gas. Instead, nitrite reduction becomes dependent on solubilization of internal storage products. This can partly explain the lag period that is observed in all glycerol reactor experiments.

In order to continue this work, future work includes explicitly measuring the rates of nitrite reduction in the presence of different supplemental carbon sources to determine whether specific carbon sources can select for more rapid nitrite reduction. Another recommendation is to minimize carbon storage by implementing different feed strategies geared toward reducing the magnitude of feast/famine in the second anoxic zone.

3.8 References

American Public Health Association, American Water Works Association, Water Environment Federation. (2005). *Standard Methods for the Examination of Water and Wastewater*; 21st ed.: Washington D.C.

Atkinson, J., Bowden, G., Esposito, K., Mahoney, K. (2007) Estimation of Air Emissions Resulting from Implementation of Supplemental Carbon to Enhance Denitrification for the NYC Stepfeed BNR WPCPs. *Proceedings of the Water Environment Federation (WEF) Technical Exhibition and Conference.* SanDiego, California, USA. 7655-7675

Bilyk, K., Bruton, T., Rohrbacher, J., Latimer, R., Pitt, P. (2011) Faster SDNRs with Prolonged Glycerin Addition, Carbon Storing Bugs, and the Enemy (Dissolved Oxygen), and Other Things Encountered While Piloting Supplemental Carbon at Several BNR Plants. *Proceedings of the Water Environment Federation (WEF) Nutrient Recovery and Management 2011 Conference*

Bilyk, K., Rohrbacher, J., Bruton, T., Pitt, P., Latimer, R. (2009) Evaluating the Cost and Process Performance of Carbon Alternatives to Methanol. *Proceedings of the Water Environment Federation (WEF) Nutrient Removal 2009 Specialty Conference*. 754-779. Washington D.C.

Bodík, I., Blštáková, A., Sedláček, S., Hutňan, M. (2009). Biodiesel waste as source of organic carbon for municipal WWTP defnitrification. *Bioresource Technology* 100, 2452-2456

Cherchi, C., Onnis-Hayden, A., El-Shawabkeh, I., Gu, A. (2009). Implication of using different carbon sources for denitrification in wastewater treatments. *Water Environ Res.* ***81****:788-99*

Dold, P., Takacs, I., Mokhayeri, Y., Nichols, A., Hinojosa, J., Riffat, R., Bott, C., Bailey, W., Murthy, S. (2008). Denitrification with Carbon Addition – Kinetic Considerations. *Water Environment Research*, **80** (5), 417 427

Erdal, U., Erdal Z., Daigger,G., Randall, C. (2007) Is it PAO-GAO Competition of Metabolic Shift in EBPR System? Evidence From An Experimental Study. *Proceedings from the Water Environment Federation (WEF) Technical Exhibition and Conference.* San Diego, CA, USA

Filipe, C., Daigger, G., Grady, C. (2001) Ph as a Key Factor in the Competition between Glycogen-Accumulating Organisms and Phosphorus-Accumulating Organisms. *Water Environment Research*. **(73)** 2; 223-232

Gárate, H., Mar'atusalihat, E., Kleerebezem, R., van Loosdrecht, M. (2011) Microbial community engineering for biopolymer production from glycerol. *Appl. Microbiol Biotechnol, 2011* ***92****:631-639*

Ginige, M., Boywer, J., Foley, L., Keller, J., Zhiguo, Y., (2009) A comparative study of methanol as a supplementary carbon source for enhancing denitrification in primary and secondary anoxic zones. *Biodegradation* 2009, **20**: 221-234

Guerrero, J., Guisasola, A., Baeza, J. (2011) The nature of the carbon source rules the competition between PAO and denitrifiers in systems for simultaneous biological nitrogen and phosphorus removal. *Water Res. 2011;* ***45****: 4793-4802*

Guerrero, J., Tayà, C., Guisasola, A., Baeza, J. (2012a) Glycerol as a sole carbon source for enhanced biological phosphorus removal. *Water Res. 2012;* ***46****: 2893-91*

Guerrero, J., Tayà, Carlota., Guisasola, A., Baeza, J. (2012b) Understanding the detrimental effect of nitrate presence on EBPR systems: effect of the plant configuration. *J Chem Technol Biotechnol 2012;* ***87****: 1508-1511*

Hallin, S., Rothman, M., Pell, M., (1996). Adaptation of Denitrifying Bacteria to Acetate and Methanol in Activated Sludge. *Water Research Vol.* 30, 1445-1450

Hinojosa, J., Riffat, R., Fink, S., Murthy, S., Selock, K., Bott, C., Takacs, I., Dold, P., and Wimmer, R. (2008). Estimating the Kinetics and Stoichiometry of Heterotrophic Denitrifying Bacteria with Glycerol as an External Carbon Source. *Proceedings of the 81th Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation)*, Chicago, Illinois USA. 274-288

Kang, S.J., Bailey, W.F., and Jenkins, D. (1992). Biological nutrient removal at the Blue Plains Wastewater Treatment Plant in Washington, D.C. *Water Science and Technology*, **26** (9-11) 2233-2236

Katehis, D., Sharp, R., Deur, A., Young, P., Rubino, V., Beckman, K. (2010) Deploying Alternative Supplemental Carbon for Denitrification at Full Scale: Glycerol from Biodiesel Production. *Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation). New Orleans, Louisiana, USA.* 2422-2429

Kiss, B., Bakos, V., Liu, W., Jobbágy, A. (2011) Full-Scale use of Glycogen-Accumulating Organisms for Excess Biological Carbon Removal. *Water Environment Research* **(83)** 9; 855-864

Lopez-Vasquez, C., Oehmen, A., Hooijmans, C., Brdhanovic, D., Gijzen, H., Yuan, Z., van Loosdrecht, M. (2009). Modeling the PAO-GAO competition: Effects of carbon source, pH, and temperature. *Water Research*, 43**(2)**, February 2009, 450-462

Machado, J., Batista, J. (2007) Abundance of PAO and GAO bacteria in a well performing enhanced biological phosphorus removal system. *Proceedings of the Water Environment Federation (WEF) 2007 Technical Exhibition and Conference*. San Diego, CA, USA

Martiensson, M., Schöps, R. (1999) Population Dynamics of Denitrifying Bacteria in a Model Biocommunity. *Water Res.* **(33)** 3, 639-646

Mokhayeri, Y., Riffat, R., Takacs, I., Dold, P., Bott, C., Hinjosa, J., Bailey, W., Murthy, S. (2008) Characterizing denitrification kinetics at cold temperatures using various carbon sources in lab-scale sequencing batch reactors. *Water Sci Technol*. 2008; **58** (1): 233-8

Neethling, J. (2005) WERF Project 01-CTS-3 *Biological Phosphorus Removal Survey and Investigations.*

Oehman, A., Saunders A., Vives, M., Yuan, Z., Keller J. (2006). Competition between polyphosphate and glycogen accumulating organisms in enhance biological phosphorus removal systems with acetate and propionate as carbon sources. *Journal of Biotechnology* 123, 22-32

Oh, J., Silverstein, J. (1999). Oxygen inhibition of activated sludge denitrification. Water Research. 1999; **33**; 1925-1937

Omari, A., Murthy, S., Takacs, I., Mokhayeri, Y., Riffat, R., Nopens, I. (2011) Modelling the use of External Carbon Substrate for Denitrification by Generalists and Specialists. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

Phillips, H., Barnard, J., deBarbadillo, C., Shaw, A., Steichen, M., Lage, C. (2009) Denitrification Rates,: Sampling, Modeling, and Design Considerations. *Proceedings from the Water Environment Federation Nutrient Removal Conference.* Washington D.C., USA

Pijuan, M., Saunders, A.,M., Guisasola, A., Baeza, J.A., Casas, C., Blackall L.L. (2004). Enhanced biological phosphorus removal in a sequencing batch reactor using propionate as the sole carbon source. *Biotechnology Bioengineering*, 85 **(1)**, 56-67

Rohrbacher, J., Bilyk, K., Bruton, T., Pitt, P., Latimer, R., (2009) Evaluation of Alternative Supplemental Carbon Sources at Four BNR Facilities. *Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation)* Orlando, Florida, USA. 4464-4483

Selock, K., Burton, W., Bott, C., Cutting, N., Wimmer, B., Neethling, J.B., Amad, S., Hinjosa, J., Murthy, S., (2008) Glycerin 101- Lessons Learned While Pilot Testing Glycerin to Enhance Denitrification. *Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation).* 6583-6602

Yuan, Q., Sparling, R., Oleszkieqicz, J. (2009) Enhanced Biological Phosphorus Removal by Glycerol Fermentation. *Proceedings of the Water Environment Federation Nutrient Removal Conference.* Washington D.C., USA

Zhang, A., Yang, S.T. (2009). Propionic acid production from glycerol by metabolically engineered Propionibacterium acidipropionici. *Process Biochemistry*, 44 **(12)**, 1346-1351

4. Manuscript 2 - Supplemental Carbon for Denitrification: Investigating Waste Alcohol as a Replacement Electron Donor to Methanol

ABSTRACT

Biomass, previously unexposed to any supplemental carbon, was seeded in sequencing batch reactors (SBRs) to test the efficiency of two different organic carbon sources, methanol and MicroC 3000^{TM}, an industrial waste alcohol containing mostly methanol and ethanol, for denitrification. Specific denitrification rates (SDNR) and C:N ratios were determined to investigate if MicroC 3000^{TM} provided the same capability for denitrification as methanol. Results proved that MicroC 3000^{TM} provided slightly higher SDNRs and C:N ratios than methanol. This can be explained by the other constituents, such as ethanol, found in the product. An over-dose of carbon resulted in complete nitrogen removal while an under-dose led to incomplete removal, with relatively similar effluent characteristics as when the appropriate carbon demand was dosed. Odor causing compounds (1, 4 and 2, 3-butanediol) associated with waste alcohol use were considered and not found to be in the effluent. It can be implied that these compounds were degraded by the biomass.

4.1 Introduction

Because of concern over eutrophication, a process where excess nutrients encourage plant growth in water bodies, the United States Environmental Protection Agency (US EPA) and state regulatory agencies are progressively reducing the maximum levels of N and P in National Pollutant Discharge Elimination System (NPDES) discharges. Point sources, such as wastewater treatment plants (WWTPs), discharging into the Chesapeake Bay region are required to comply with very stringent regulations. These increasingly stringent limits on discharges by WWTPs have led to investigations to better enhance nitrogen removal.

Biological nutrient removal (BNR) processes are implemented at WWTPs in order to reach the recognized nitrogen regulations. An important step for nitrogen elimination in wastewater treatment is heterotrophic denitrification. Denitrification is the process of converting nitrate to nitrogen gas. In this anoxic process, heterotrophic bacteria use nitrate in lieu of oxygen as an electron acceptor while organic substrates serve as electron donors to denitrifying bacteria. Energy sources for denitrification have been categorized as internal (present in influent wastewater), endogenous (self-generated within the system, as a result of organism decay), and external (not present in wastewater) (Van Haandel et al, 1981). However, due to carbon limitations in the influent wastewater, an external carbon source is sometimes required and added to attain the desired low nitrogen levels.

The denitrification potential of wastewater is primarily a function of the available organic carbon, which is expressed as the chemical oxygen demand (COD) to nitrogen ratio, also labeled as COD/N (Peng et al., 2007), which is the carbon-use-to-nitrate-consumption ratio (Cherchi et al., 2009). Cherchi et al (2009) state that the determination of the correct C: N ratio is crucial when choosing an external carbon source as high operational costs and higher biomass production can be caused by overestimation of C: N. They also affirm that 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 3 to 5 mg/L.

As more WWTPs are being upgraded to provide BNR, the demand for supplemental carbon is growing (Fiss et al., 2010). Supplemental carbon sources are categorized into two groups: conventional and alternative. Conventional includes sources such as methanol, ethanol, acetic acid, and glucose. However, due to the high cost of these compounds, alternative carbon

sources have received more attention. Acetate is found to give the highest rates in almost all cases, but methanol is often the substrate of choice in practice (Hallin et al., 1998). Practical use of acetate has been limited as a result of its higher cost (Cherchi et al., 2009).

4.1.1 Methanol

Methanol, sometimes referred to as methyl alcohol, is the most commonly used organic substrate because of its low cost and ability to denitrify without leaving a residual biochemical oxygen demand (BOD) in the process effluent (Mokhayeri et al., 2009). However, there are also some unpleasant characteristics of methanol use such as: (i.) toxicity and flammability, (ii.) slow denitrification kinetics particularly at cold wastewater temperatures (Dold et al., 2008), and (iii.) need for special C1 (one-carbon) degrading bacteria (methylotrophs) required for denitrification (Kang et al., 1992). Methylotrophs are a specific group of bacteria that are capable of using C1 compounds as substrates for biosynthesis and energy requirements. Due to it being a reactive and toxic compound, a main disadvantage of using methanol is the safety issues associated with its transportation, handling, and storage (Cherchi et al., 2009). Although methanol removes nitrogen very well during the summer months, the process is inhibited during the winter, as microbial growth rates decline due to lower mixed liquor (Dold et al., 2008). This lowered growth rate is likely due to potential washout of methanol-using denitrifying bacteria from the system (Mokhayeri et al., 2006). Thus, switching to a different carbon source to promote growth of organisms and denitrification rates during the winter months seems practical (Mokhayeri et al., 2009). There has been an abundance of research conducted determining the rates and kinetics of methanol use. Common C/ N ratios reported with methanol are 4.45 g COD/ g NO_3-N in continuous experiments; 4.0 g COD/ g NO_3-N at 15°C and 4.16 g COD/ g NO_3-N at 25°C in pure batch cultivations (Christensson et al., 1994). The yield is also an important parameter to consider. The C: N ratio can be directly used to calculate the yield through the following equation:

$$C{:}N = \frac{2.86}{(1 - Y)}$$

Dold et al (2008) have estimated the yield coefficient of methanol utilizing heterotrophs to be 0.4 mg COD/ mg COD. This value reported by Dold et al (2008) results in a C: N of 4.8, which is considered to be the observed value for methanol. Studies have also reported the

maximum growth rates of methanol in various conditions, such as (i.) 1.2 d^{-1} at 20°C (Cherchi et al., 2009); (ii) 1.3 d^{-1} at 20°C (Dold et al., 2008); and (iii) 0.8 d^{-1} and 2.1 d^{-1} at 15°C and 25°C, respectively (Christensson et al., 1994). Cherchi et al (2009) found that a decrease in temperature from 20°C to 10°C results in methanol denitrification rates and growth rates to be reduced by 63% and 73%, respectively. It has also been observed that there is a lag period for adaptation before maximum denitrification rates are achieved, supporting the theory that special C1 degrading methylotrophs need to be established before full denitrification can begin (Hallin et al., 2006; Selock et al., 2008). The addition of methanol can improve biological phosphorus removal by creating anaerobic conditions and increasing the availability of organic carbon in wastewater for polyphosphate-accumulating organisms (Ginige et al., 2009). This study has also stated that depending on the amount of methanol required, the point of addition (1^{st} vs. 2^{nd} anoxic zone) will have an effect on denitrification efficiency. This investigation has also seen nitrite accumulation if methanol is dosed at a low level (under-dose) since the limited available carbon is primarily used to reduce nitrate to nitrite.

4.1.2 Ethanol

Ethanol, also referred to as ethyl alcohol, has also been researched and deemed a viable carbon source for denitrification. Although ethanol is more expensive than methanol in pure form, it can be obtained as an inexpensive waste product from chemical and pharmaceutical industries (Peng et al., 2007; Christenssen et al., 1994). Christensson et al (1994) found that ethanol was more readily available as a carbon source for denitrification than methanol. They observed that denitrification was established in a shorter time and with better stability. The lag period, in which methanol selects for a highly specialized denitrifying population, is not observed with ethanol use. This is because ethanol is converted by the bacterial cell to acetyl-ScoA, similar to acetate, before entering the tricarboxylic acid cycle (Peng et al., 2007). Since acetate may account for 10% of the total COD in sewage (Henze et al., 1994), suitable denitrifying populations with the appropriate enzymes for ethanol degradation must therefore already exist in activated sludge (Peng et al., 2007). When using ethanol as a carbon source, Christensson et al (1994) observed growth rates of 1.9 d^{-1} and 4.8 d^{-1} at 15°C and 25°C, respectively. When comparing this to the values obtained using methanol at the same temperatures, the growth rate with ethanol was higher than that of methanol. Peng et al (2007)

concluded that between methanol, ethanol, and acetate, ethanol was found to be the best carbon source for denitrification based on denitrification potential, sludge yield, adaptation time, response time, and price. Mokhayeri et al (2008) also determined that ethanol was an effective carbon source. They summarized that both methanol and ethanol are interchangeable substrates. Thus, a switch from methanol to ethanol in late fall should not result in a lag in denitrification rates and a switch back to methanol in early spring may also not produce a substantial lag period. The price of ethanol is linked with the methanol market and is commonly more expensive, which is why the availability of waste rich in ethanol can potentially provide a source of readily available carbon (Onnis-Hayden et al., 2008).

4.1.3 Waste Materials

Due to the volatility of cost for some of the conventional sources, alternative carbon sources have received more attention, such as glycerol and various industrial by-products or waste materials (Czerwionka et al., 2012). However, implementing a purified form of glycerol for external carbon addition at full-scale is unlikely given its high cost; however, glycerol is the principal byproduct of biodiesel fuel production, which, sources indicate, is a growing industry (Hinojosa et al., 2008). Other emerging alternative carbon sources emerging include agriculture or industrial wastes (Onnis-Hayden et al., 2008). Swinarski et al (2009) have concluded that food industry effluents, such as brewery and distillery wastewater, are potential alternatives to methanol. Although these effluents may have a variation in quality and quantity from production cycles, they do hold high carbon-to-nitrogen ratios and high contents of readily biodegradable organic matter (Cappai et al., 2004). Pretorius et al (2007) determined that high-fructose corn syrup (HFCS) is a cost-effective alternative for methanol in a tertiary denitrification system.

4.1.4 Butanediol

Poldrugo et al (1984) determined that 1, 4-Butanediol (BD) is a naturally occurring aliphatic alcohol that does not produce behavior analogous to intoxicating doses of other alcohols. Instead, they concluded that this compound induces a state of sedation and akinesia at one tenth the dose of ethanol. This substance can be absorbed into the body by inhalation of its vapor and by ingestion (Ishikawa, 1999). 1, 4-BD is the most widely used of all the four carbon-

based diols in industry today (Haas et al., 2005). The isomers of BD of interest in this study are 1, 4-BD, which is also referred to as 1, 4-Butelyene glycol, and 2, 3-butanediol. 1, 4 is colorless industrial chemical used primarily as an intermediate in the manufacture of other organic chemicals (Irwin et al., 1996).

The Littleton/Englewood WWTP in Englewood, Colorado preliminarily tested this industrial waste alcohol comprised primarily of methanol and ethanol in a pilot study on denitrification filters. Observed C:N values ranged from 4.15 to 4.25, which is slightly lower than what is observed with methanol use. Also promising was the absence of nitrite breakthrough to the effluent, specifically for WWTPs that use chlorine disinfection. However, during this trial, a negative observation was the presence of an "alcohol odor" at the time this feed was initiated. This odor was detected in the chlorine contact tank, which indicates that something was breaking through the filter. It was hypothesized that the butanediol compounds present in the product were the reason for the odor (EOS Communications, 2011).

4.2 Project Objectives

The objective of this research was to investigate alternative carbon sources to methanol, specifically MicroC 3000^{TM}, an industrial waste alcohol product from Environmental Operating Solutions, Inc (EOSi). MicroC 3000^{TM} is comprised of different alcohols, and the chemical composition of this product can be found in Table 4.1 (EOS Communications, 2011):

Table 4.1: MicroC 3000^{TM} Chemical Composition

Chemical Name	Weight (%)
Methyl Alcohol	65%
Ethyl Alcohol	7.5%
2-Propanol	4.9%
1-Propanol	0.95%
Butanols & other alcohols	2%
Water	18.5%

The majority of this product is comprised of methanol and ethanol, which are both known to be sufficient for the purposes of denitrification. Therefore, the hypothesis is that this product will be able to perform at the same level as methanol, if not better, due to the presence of ethanol. This waste alcohol product has the same rating as methanol on the National Fire Protection Agency (NFPA) hazard diamond for flammability.

The objectives of this research were to: (1) compare specific denitrification rates (SDNR) between methanol and MicroC 3000^{TM} at 20°C; (2) determine C: N ratios for the two substrates, which can help choose the more appropriate and efficient product by comparing cost with efficiency. MicroC 3000^{TM}, on a $/lb NOx-N denitrified, is a discount to pure methanol (EOS Communications, 2011); and (3) to verify the degradation of 1, 4- and 2, 3- butanediol by the activated sludge upon carbon addition. The hypothesis is that these two compounds were present and are responsible for the "alcohol odor" when initially added into the reactors, since butanediol compounds have a low odor threshold compared to methanol, ethanol, and propanol (EOS communications, 2011). It was necessary to prove that these butanediol compounds are readily degradable and will be degraded by the biomass in the reactors and thus, be absent in the effluent. If the compounds are not degradable, then an alcohol odor will linger in the around WWTPs that utilize this product.

In order to complete the objectives, reactor operation and sampling were conducted in different phases: The reactors were operated as follows: (i.) startup and operation to steady-state (SS), (ii.) careful sampling steady-state conditions with carbon added to denitrify 7, 15, and 4 mg/L NO_3-N representing the approximate carbon demand, an over-dose (OD), and an under-dose (UD), respectively, (iii.) reactor shut down, (iv.) restart and reseed with fresh mixed liquor not exposed to MicroC 3000^{TM} or methanol with carbon dosing based on 7 mg/L NO_3-N, and (v.) careful sampling to determine COD/NO_3 ratio as the reactors proceeded from startup to steady-state.

4.3 Methodology

Two 22.0 Liter Sequencing Batch Reactors (SBRs) were run concurrently having the same solids retention time (SRT) and hydraulic retention time (HRT). These reactors were configured to roughly simulate a 5-stage Bardenpho process. These reactors were temperature and dissolved oxygen controlled and also had the capability to control pH. These reactors operated with three 8-hour cycles per day regulated automatically through programmable timers (ChronTrol Corporation San Diego, California). Figure 4.1 shows a schematic of the experiment setup. Floating styrofoam covers to minimize oxygen transfer through the surface were placed on the surface of these reactors (Figure 4.2).

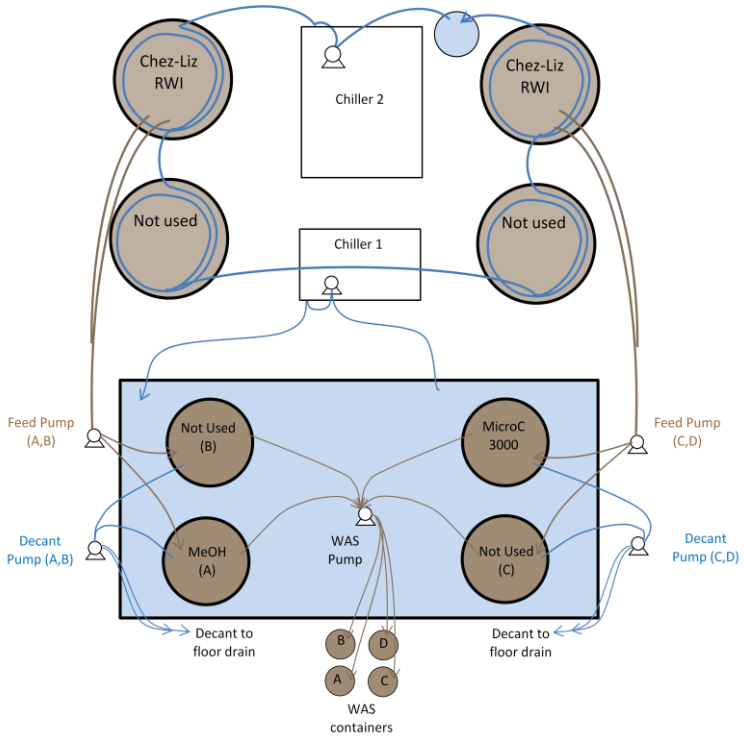


Figure 4.1: SBR Set-up

Figure 4.2: Floating Styrofoam Covers

4.3.1 SBR Feed

Raw wastewater influent was collected from the Hampton Roads Sanitation District Chesapeake-Elizabeth Wastewater Treatment Plant in Virginia Beach, Virginia. Three 50 gallons drums were filled with raw wastewater every Monday, Wednesday, and Friday between 9:00 – 10:00 a.m., which was considered to be a peak load period when influent ammonia concentrations were highest. The raw wastewater was transferred into drums that fed directly

into the reactors. Submersible pumps in these drums turned on five minutes before each feed to ensure mixing of the influent before being fed into the reactors. Simulating a 5-stage Bardenpho process in an SBR is generally not feasible because there is no possibility for nitrate to be recycled (NRCY). Thus, half the raw influent was pumped in the beginning of the cycle (before anaerobic period) and the other half was fed before the first anoxic period. Figure 4.3 shows this 8 hour cycle, roughly simulating a 5-stage Bardenpho process. Another purpose of splitting the feed was to have a relevant distribution of heterotrophic bacteria that utilized carbon from the influent as well as carbon from supplemental addition.

4.3.2 SBR Temperature Control

The drums that held the raw wastewater influent that fed directly to the reactors contained vinyl coated copper tubing connected to a chiller (Aquatic Eco-Systems, Inc., Apopka, Florida). These chillers were regulated by temperature controllers which circulated tap water through the copper tubing and helped cool the raw wastewater to 20 $\pm$ 0.5 °C before being fed into the SBRs. A chiller was used to control the temperature of the SBRs through a water bath regulated to 20 $\pm$ 0.5 °C.

4.3.3 SBR Cycle & Sampling Schedule

In order to determine different rates, careful intensive sampling for nutrient analysis was performed at different points throughout the cycle. Figure 4.3 shows the schedule for a representative 8-hour cycle beginning at 0800 and ending at 1500, followed by settling for one hour. There was a 10 minute deoxygenation period at the start of the second anoxic zone, prior to carbon addition. This allowed for the residual oxygen to be reduced to zero, prior to carbon addition, so it was necessary to ensure that the reactor was completely anoxic before any carbon was added. The ten minute post aeration is to help boost DO and strip N_2 gas, prior to the settling phase.

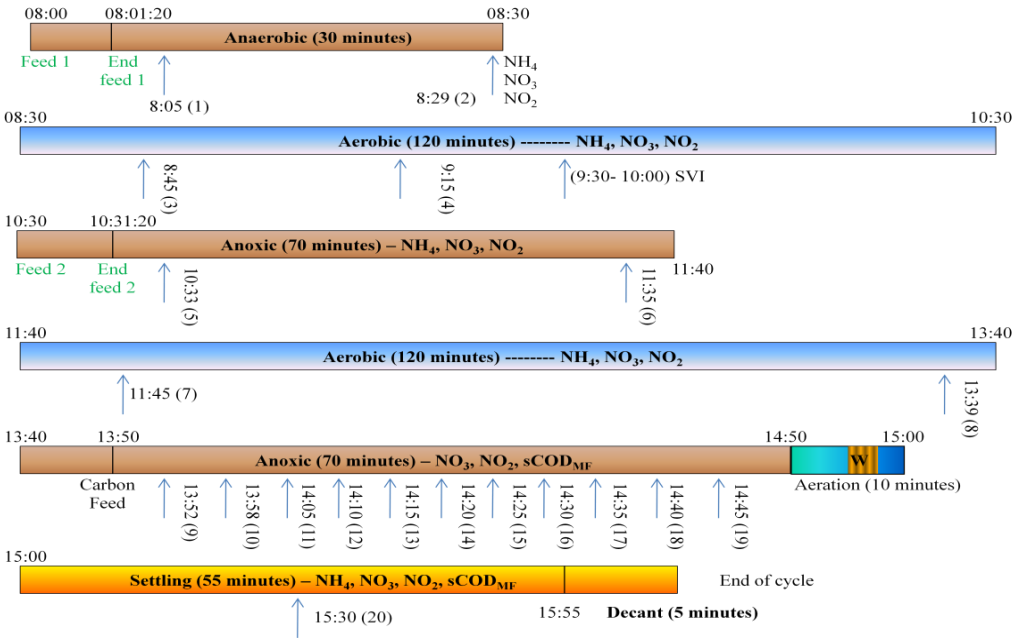


Figure 4.3: Eight-hour SBR Cycle Schedule and Sampling Points during Cycle

4.3.4 Sampling and Nutrient Analysis

Samples for nutrient analysis (NH_3-N, NO_3-N, and NO_2-N) were taken in 15 mL aliquots and immediately vacuum filtered using 0.45 µm hydrophilic mixed cellular ester membrane filters by Pall Corporation (Ann Arbor, MI) and stored at 4°C until analysis. These nutrients were analyzed by Hach tubes (Loveland, CO). These kits were then analyzed through a Hach DR2800 spectrophotometer.

Ammonia was analyzed through Hach Test N' Tube Plus (TNT plus) 830 or 831, ultra-low range and low range, respectively. These Hach kits used the salicylate method (method 10205) for analysis where ammonium ions react with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside as a catalyst to form indophenols. The amount of color formed was directly proportional to the ammonia nitrogen present in the sample and results were measured at 690 nm.

Nitrate was analyzed using TNT plus 835 (low range). This kit employs the dimethylphenol method (10206), where nitrate ions in solution containing sulfuric and phosphoric acids react with 2, 6-dimethylphenol to form 4-nitro-2, 6-dimethylphenol. These results were measured at 345 nm.

Nitrite was analyzed using Test 'N Tube NitriVer3 Reagent sets, which uses the diazotization method (10019) in which nitrite in the sample reacts with sulfanilic acid to form an

intermediate diazonium salt. This couples with chromotropic acid to produce a pink colored complex directly proportional to the amount of nitrite present.

MLSS/MLVSS and effluent TSS/TVSS, 240 mL and 1L, respectively, were removed from the reactors and analyzed per *Standard Methods*. Fifty milliliters of sample were also gathered for COD, TKN, and TP testing and were also measured per *Standard Methods*. Sludge volume index (SVI) were also measured according to *Standard Methods*. Soluble COD and soluble TKN were measured by vacuum filtering samples through a 0.45 µm membrane filter.

4.3.5 Solids Retention Time

The SBRs were designed to run in Garrett configuration, where biomass was wasted directly from the reactor, helping to achieve and maintain a desired SRT. Solids wasting occurred during each cycle, during the post-aeration period prior to settling/decant (Figure 4.3). Due to slight differences in peristaltic waste pump flow rates between the two reactors, the waste activated sludge (WAS) was stored and weighed every 8-11 cycles to determine the exact volume of waste per cycle. The collected WAS, in conjunction with effluent total suspended solids (TSS) and mixed liquor suspended solids (MLSS), helped to determine the actual SRT in each reactor. During sampling days, the volume of MLSS removed for nutrient analysis was recorded and this volume was returned to each reactor. Waste sludge was also returned to the reactor to correct for differences in effluent TSS and variability in waste pump flow rates. Thus, by monitoring wastage, biomass concentrations, sampling volume extracted, and effluent TSS, reactor SRT was maintained at exactly 15 days, notwithstanding the error of the measurements involved.

4.3.6 Dissolved Oxygen (DO)

Continuous mixing was provided in the SBRs by top entry paddle mixers. DO control was provided by aeration ON/OFF control using solenoid valves. The low and high set points were set at 2 and 3 mg/L, respectively. If the measured DO was below 2 mg/L, the solenoid values would be turned on, allowing dissolved oxygen into the reactor. Once the DO reached 3.0 mg/L, the solenoid valves would shut the air off. These solenoid valves were connected to the programmable timer to allow aeration during aerobic cycle periods only. DO in the reactors was measured using conventional galvanic membrane probes (Royce Technologies, College Station,

Texas). Aeration was provided using aquarium style air stones, and DO data was logged every 20 seconds (Telog, Victor, NY) to allow oxygen uptake rate (OUR) calculations.

4.3.7 pH

The pH was not controlled during this research since it was within reasonable range. pH readings were also logged and stored every two minutes (Telog). The pH was monitored using Foxboro probes. Peristaltic pumps were available to add alkalinity but the pumps were turned off during the duration of this experiment.

4.3.8 Supplemental Carbon Addition

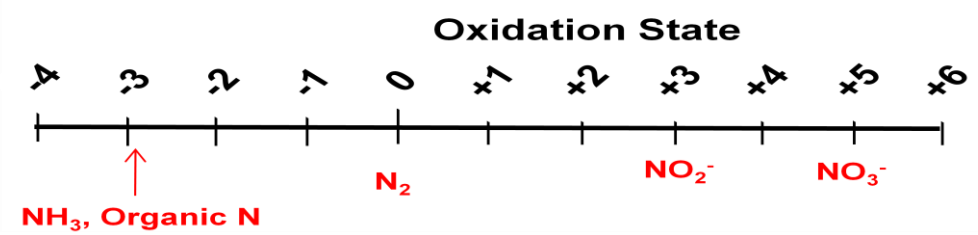
Supplemental carbon addition in the second anoxic period was done through the use of peristaltic pumps. Carbon sources were kept in collapsible sealed LDPE containers and stored in a refrigerator. The carbon was fed from the LDPE containers to the reactors directly through viton tubing to prevent evaporation and tubing diffusion between cycles. The two carbon sources evaluated were 100% reagent grade methanol and MicroC 3000^{TM} by Environmental Operating Solutions, Inc. During normal reactor operation, COD was added based on assumed COD/NO_3-N ratios and 7 mg/L NO_3-N. During over-dose (OD) and under-dose (UD) reactor operation, carbon was added based on 15 mg/L NO_3-N and 4 mg/L NO_3-N, respectively. Methanol was added based on the theoretical COD of the pure chemical, while the measured COD of MicroC 3000^{TM} was 1070 g/L (Table 2). The yield of MicroC 3000^{TM} was assumed to be 0.40 as well due to the dominance of methanol in the product.

Table 4.2: Determination of Supplemental Carbon Addition

Electron Donor	Yield (g COD/ g COD)	Formula	MW (g/mol)	COD (g COD/ g Chemical)	COD of Product (g/L)	NO3-N in reactor (mg)	Reactor Volume (L)
Methanol	0.40	CH_4O	32	1.50	-	7	22
MicroC 3000™	0.40	-	-	-	1070	7	22
Electron Donor	**Mass NO_3-N (g/cycle)**	**Theoretical Carbon (g Chemical/ g NO_3-N)**	**Actual Carbon (g Chemical/ g NO_3-N)**	**Actual Carbon (g COD/ g NO_3-N)**	**COD to add (g/cycle)**	**Mass of Chemical (g/cycle)**	**Vol. to add (mL/cycle)**
Methanol	0.154	1.91	3.18	4.8	0.74	0.49	-
MicroC 3000™	0.154	-	-	4.8	0.74	-	0.69

4.3.9 Carbon to Nitrogen Ratios

In order to determine actual supplemental carbon to nitrogen ratios (C/ N in g COD consumed/g NO_3-N fully denitrified) during the second anoxic period, an electron equivalence technique was used to take residual DO consumption and nitrite accumulation into consideration.



$$NO_3^- \rightarrow NO_2^-: \quad \frac{5\ e^-}{\text{mole } NO_3} \times \frac{\text{mole } NO_3}{14\ \text{g}} = \frac{0.357 e^-}{\text{g } NO_3\text{-N}}$$

$$NO_2^- \rightarrow N_2: \quad \frac{3\ e^-}{\text{mole } NO_2} \times \frac{\text{mole } NO_2}{14\ \text{g}} = \frac{0.214 e^-}{\text{g } NO_2\text{-N}}$$

$$O_2 \rightarrow 2\ H_2O: \quad \frac{4\ e^-}{\text{mole } O_2} \times \frac{\text{mole } O_2}{32\ \text{g}} = \frac{0.125 e^-}{\text{g } O_2}$$

Figure 4.4: Determination of Electron Transfer

After determining specifically how nitrate reduced (to NO_2, N_2) as well as DO removal, the total electrons transferred was summed. The amount of sCOD consumed was divided by the amount of total electrons consumed, and then converted back to an equivalent NO_3-N fully denitrified basis. The true anoxic yield was estimated as:

$$C:N = \frac{2.86}{(1-Y)}$$

Since NO_2^- is stoichiometrically oxidized to NO_3^- during dichromate COD analysis representing a positive interference, all COD data were corrected for the NO_2^- concentration present in each filtered sample.

4.3.10 Butanediol Degradation

Two samples were taken per day for butanediol analysis in 15 mL aliquots, for a total of three days. One sample is directly from the reactor after the carbon fed and the second sample is from the reactor effluent. These collected samples were vacuum filtered through 0.45 µm membrane filters and frozen until analysis to prevent degradation. The samples were taken on the last day of the under-dose (11/17/11), the sixth day of reactor restart (12/05/11), and the 10th day of reactor restart (12/9/11). Samples were collected using syringes and stored in clear glass VOA vials and frozen until analysis.

The Central Environmental Lab (CEL) of Hampton Roads Sanitation District (HRSD) analyzed samples these samples to see if 1, 4- and 2, 3-BD was present. An Agilent Tech 6890N Gas chromatography (GC) consisting of a J&W Scientific DB-Wax column coupled with a flame ionization detector (FID) was used to analyze these samples in order to detect BDO. Half a µL was direct water injected with a 100:1 split.

4.4 Results and Discussion

The behavior of activated sludge differs with substrate type. However, in this research, both systems were believed to have generated methylotrophs in order to degrade the C1 compounds of methanol. Since MicroC 3000^{TM} is comprised of 65% methanol, the hypothesis is that both reactors' biomass will behave similar. Figure 4.5 shows the growth and decay of methanol and MicroC 3000^{TM} during this research. The patterns exhibited by these two reactors were extremely similar, confirming the hypothesis. Steady-state operation was determined to be

achieved when the biomass concentrations appeared to be stable and consistent (10/24). Figures 4.6, 4.7, and 4.8 show effluent ammonia, effluent nitrate, and sludge volume index, respectively.

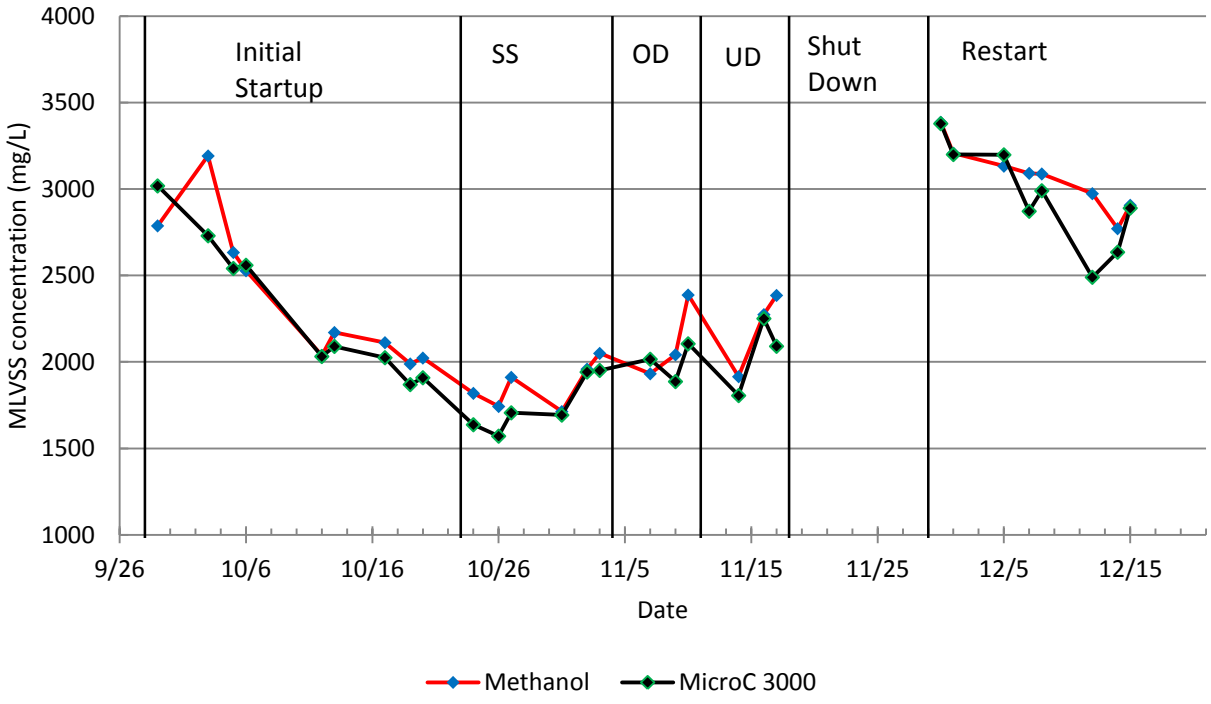


Figure 4.5: Mixed Liquor Volatile Suspended Solids

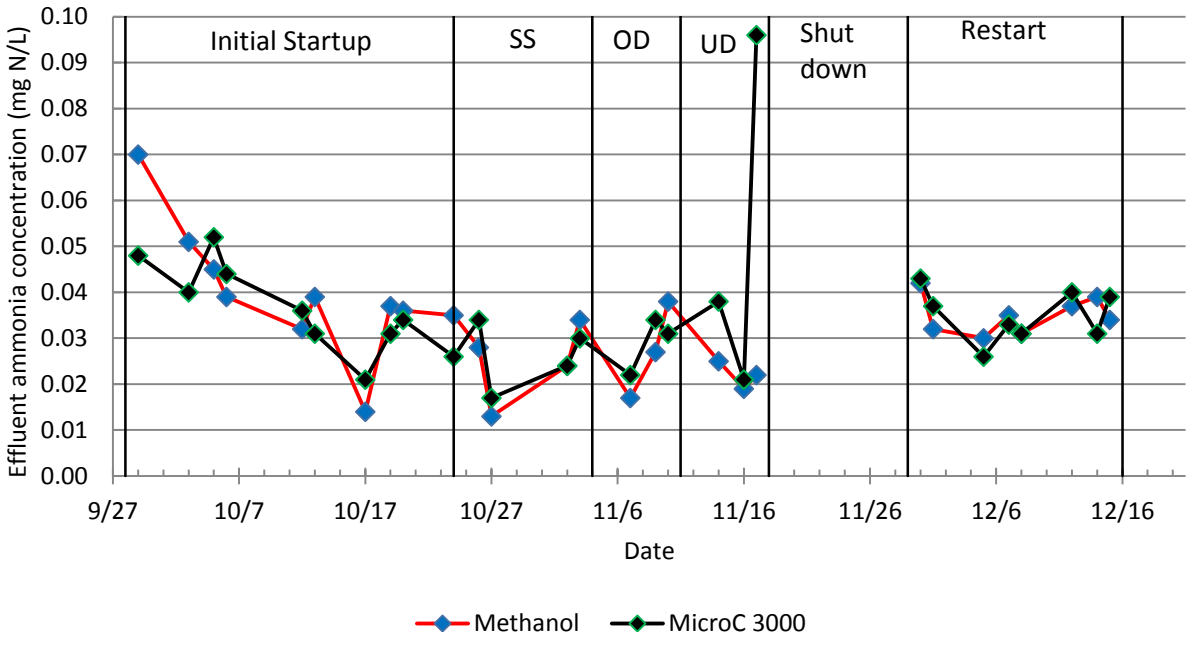


Figure 4.6: Effluent Ammonia Concentrations

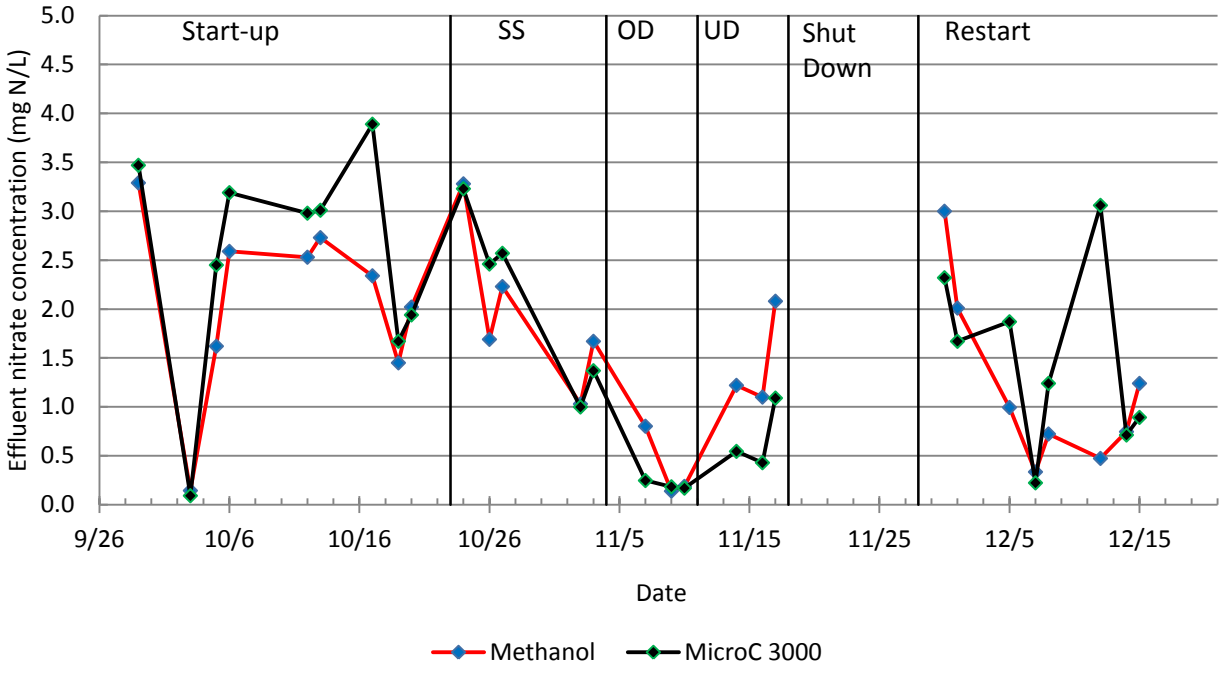


Figure 4.7: Effluent Nitrate Concentrations

Figure 4.7 illustrates that nitrate removal improved as the biomass became selective for a specialist population. During the initial start-up period, both reactors performed similarly in terms of nitrate removal. MicroC 3000™ became more efficient than methanol when carbon was provided in abundance (over-dose) than when it was insufficient (under-dose). Until the system reached steady-state, levels of nitrate removal for both reactors varied.

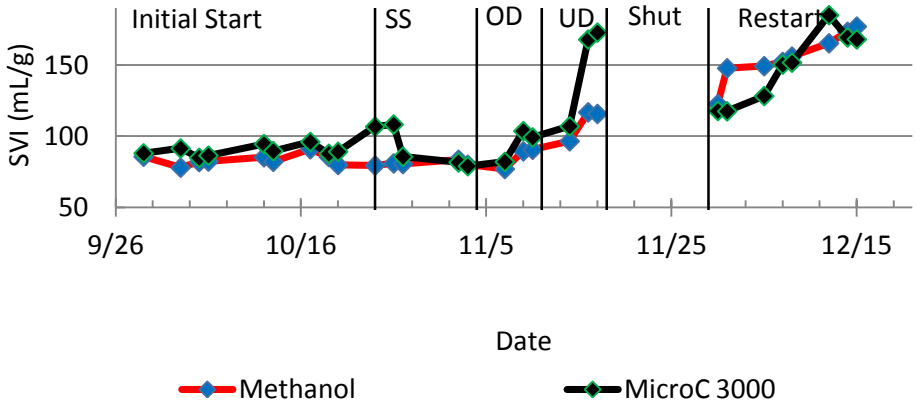


Figure 4.8: Sludge Volume Index

From figures 4.6 and 4.8, it is even more apparent that both reactors utilizing methanol and MicroC 3000™ acted similar in nature. Both reactors do a good job of completely nitrifying

as effluent ammonia concentrations were always less than 0.1 mg/L. The SVI's were comparable during the experiment; however, both reactors exhibited less desirable settling characteristics during an under-dose of external carbon, as the SVI's increased drastically. During the reactor restart, the sludge volume index for both reactors started high and increased over time. This cause of this increase in both reactors is unknown.

Influent TP concentrations in the influent raw wastewater ranged from 5-7 mg/L and effluent TP concentrations were measured close to 0.2 mg/L. Effluent TP concentrations can be found in figure 4.9 This suggests that biological phosphorus removal was occurring at all times during this research.

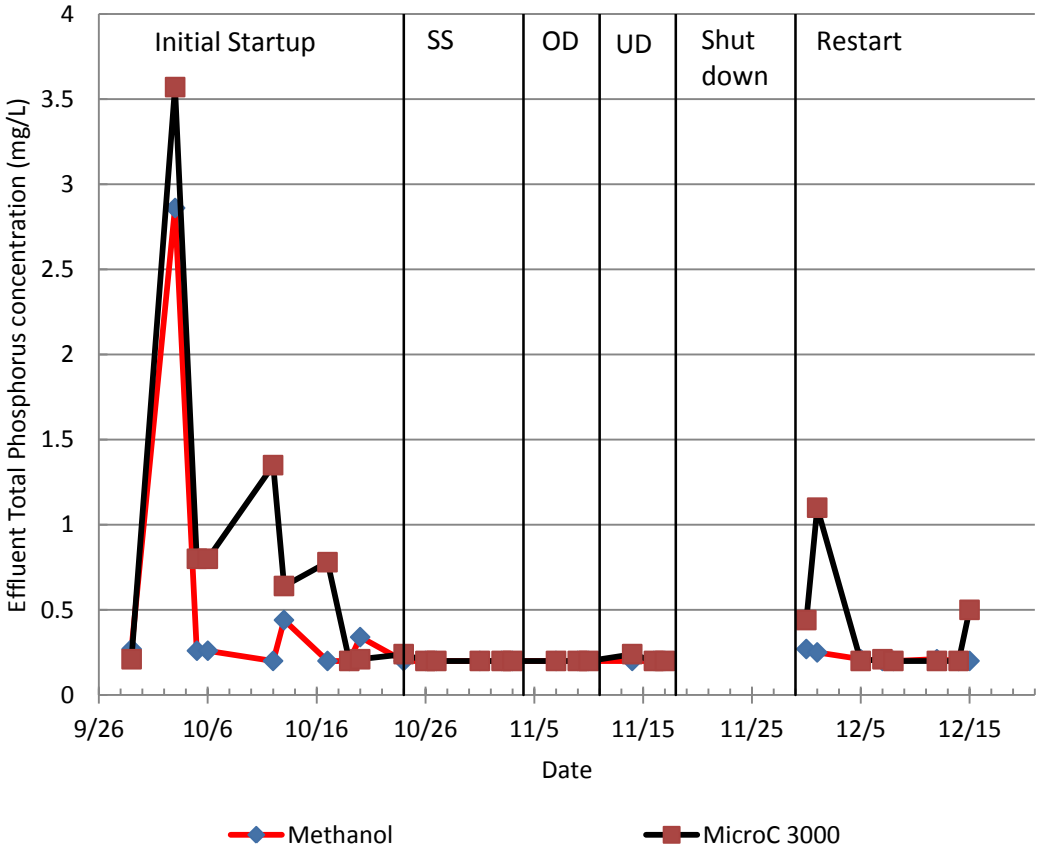


Figure 4.9: Effluent Total Phosphorus Concentration (mg/L)

SBR nutrient profiles for both substrates were completed three times a week. Figure 4.10 and 4.11 show the calculated SDNR for each substrate, as NO_3-N and NO_X-N, respectively.

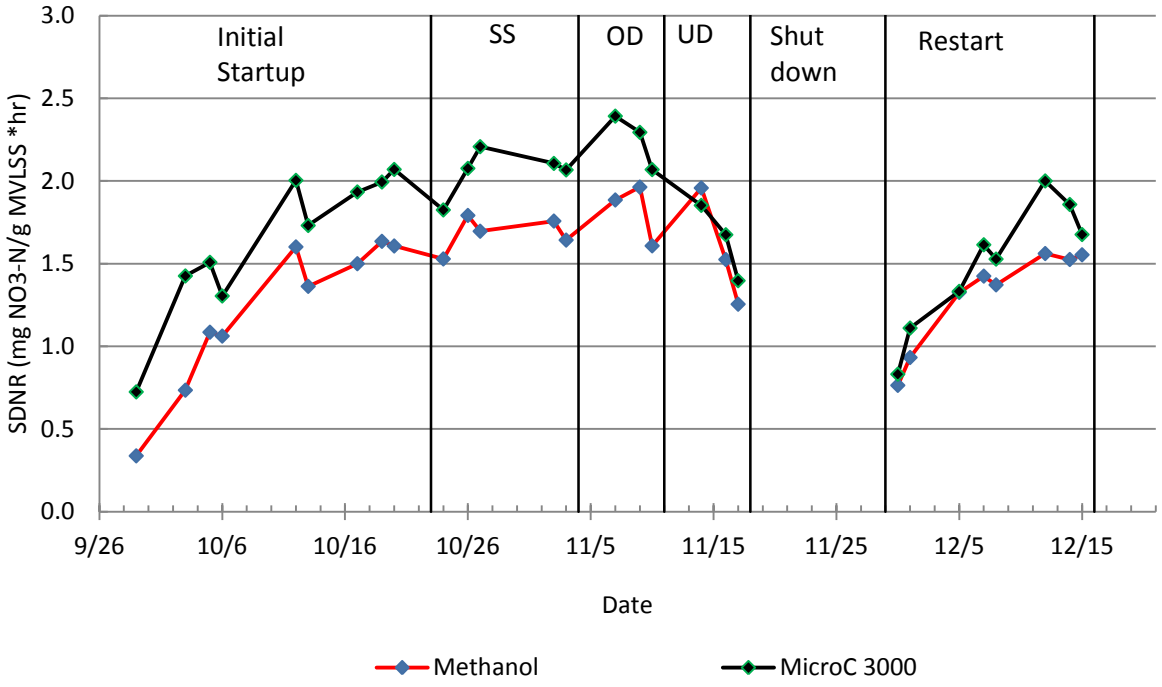


Figure 4.10: SDNR of NO_3-N during Second Anoxic Zone

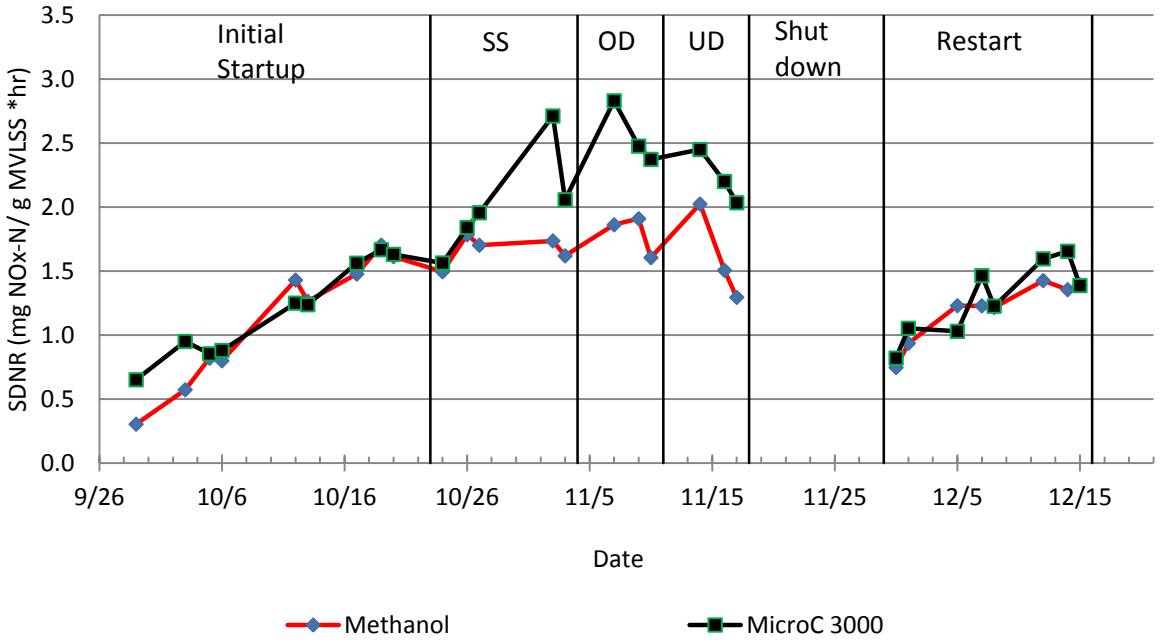


Figure 4.11: SDNR of NO_X-N during Second Anoxic Zone

The SDNR increased during both start-up phases suggesting that during this acclimation period, the biomass was establishing a specialist methylotrophic population to efficiently reduce the C1 compounds of methanol. Although the denitrification rates started around the same value, the MicroC 3000^{TM} rate increased more rapidly and was higher than that of methanol during

acclimation. This evidence supported the idea that methanol degrading organisms (methylotrophs) were selected and established before the maximum rate of denitrification was reached. This faster increase for MicroC 3000™ was likely due to the contribution from the ethanol present in the product, which is known to be a faster, more readily biodegradable substrate compared to methanol When SDNR of NO_X-N was calculated (Figure 4.11), patterns in both reactors were similar in nature. The SDNR in figure 4.11 was lower than the SDNR in figure 4.10 for MicroC 3000™ indicating that there was some nitrite accumulation associated with MicroC 3000™, suggesting the reduction from nitrate to nitrite was more rapid with MicroC 3000™ than with methanol. The SDNR was higher for both reactors when carbon was over-dosed and decreased when carbon addition was insufficient (under-dose), as compared to the typical carbon dose. This was expected as the addition of carbon first reduced nitrate to nitrite. If an adequate amount of supplemental carbon was not provided, nitrite would accumulate and nitrite reduction occurred slowly, resulting in a hindered overall TN reduction. Average SDNR for NO_3-N and NO_X-N are shown in table 4.3 below.

Table 4.3: Average SDNR (mg NO_3-N or NO_X-N / g MVLSS *hr) During Reactor Operation

	Start Up	
	NO_3-N	**NO_X-N**
Methanol	1.2 ± 0.5	1.1 ± 0.5
MicroC 3000™	1.6 ± 0.4	1.2 ± 0.4
	SS	
	NO_3-N	**NO_X-N**
Methanol	1.7 ± 0.1	1.7 ± 0.1
MicroC 3000™	2.1 ± 0.1	2.0 ± 0.4
	OD	
	NO_3-N	**NO_X-N**
Methanol	1.8 ± 0.2	1.8 ± 0.2
MicroC 3000™	2.3 ± 0.2	2.6 ± 0.2
	UD	
	NO_3-N	**NO_X-N**
Methanol	1.6 ± 0.4	1.6 ± 0.4
MicroC 3000™	1.6 ± 0.2	2.2 ± 0.2
	Restart	
	NO_3-N	**NO_X-N**
Methanol	1.3 ± 0.3	1.2 ± 0.2
MicroC 3000™	1.5 ± 0.4	1.3 ± 0.3

Typical three-days-a-week SBR profiles are shown in Figures 4.12, 4.13, 4.14 as typical carbon addition, carbon over-dose, and carbon under-dose, respectively.

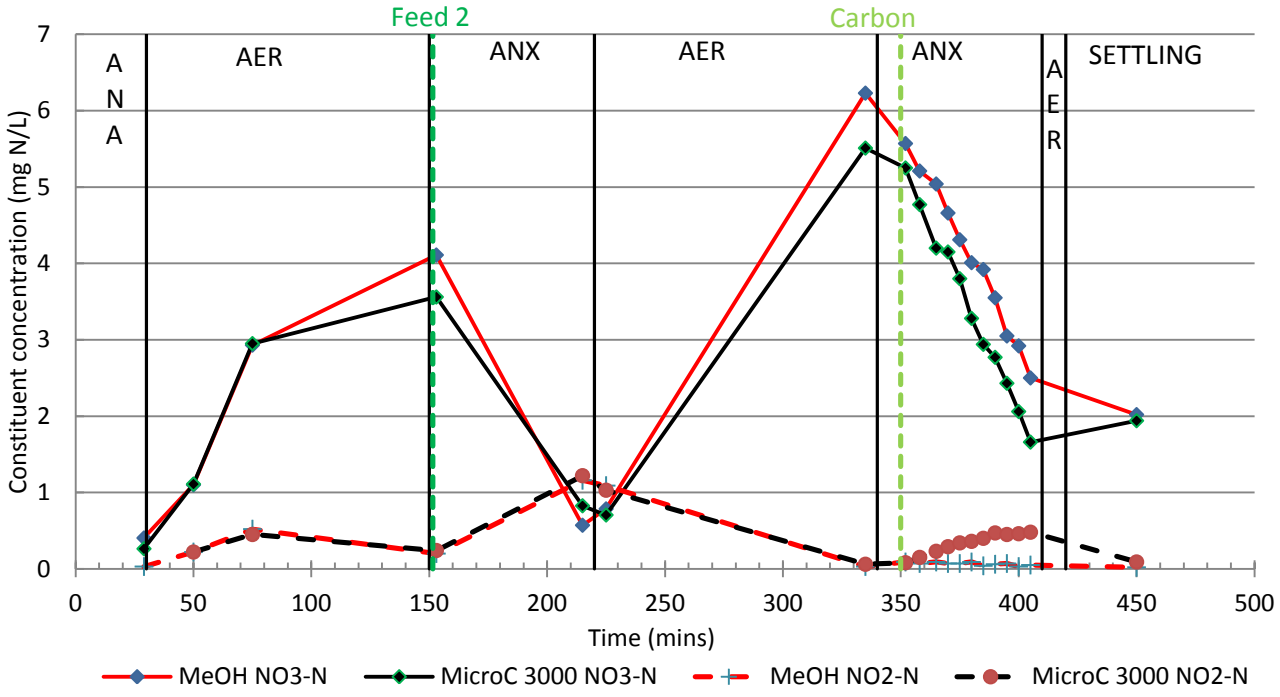


Figure 4.12: Intensive Nutrient Profiling with Typical Carbon Dose

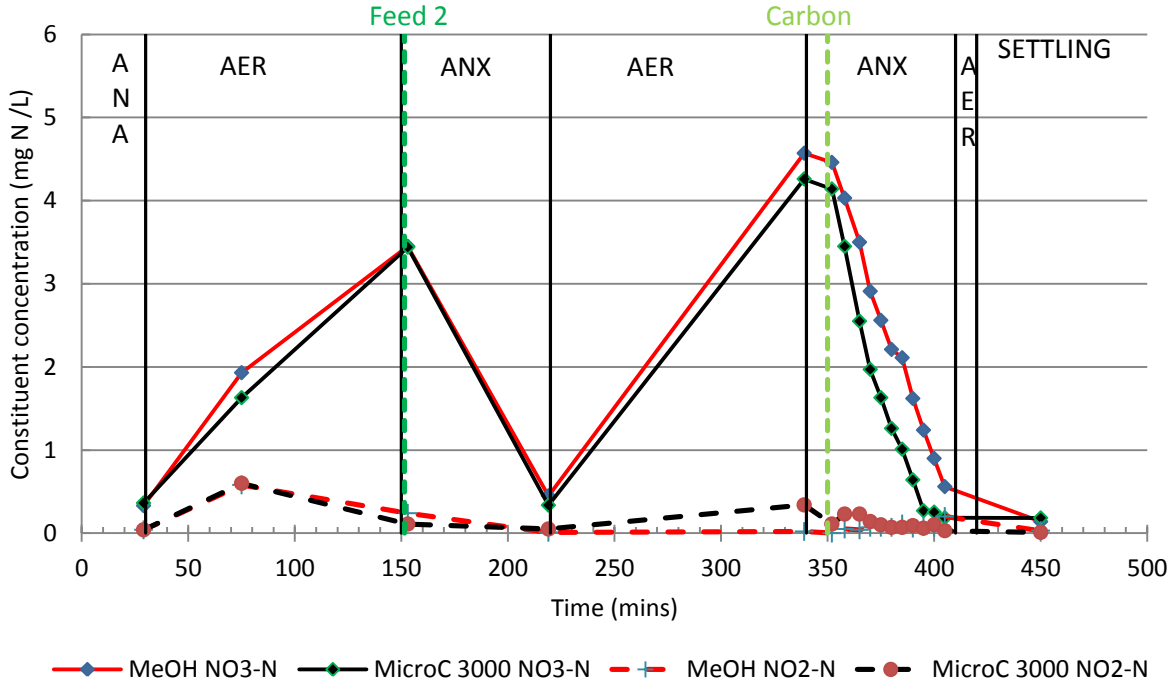


Figure 4.13: Intensive Nutrient Profiling with Carbon Overdose

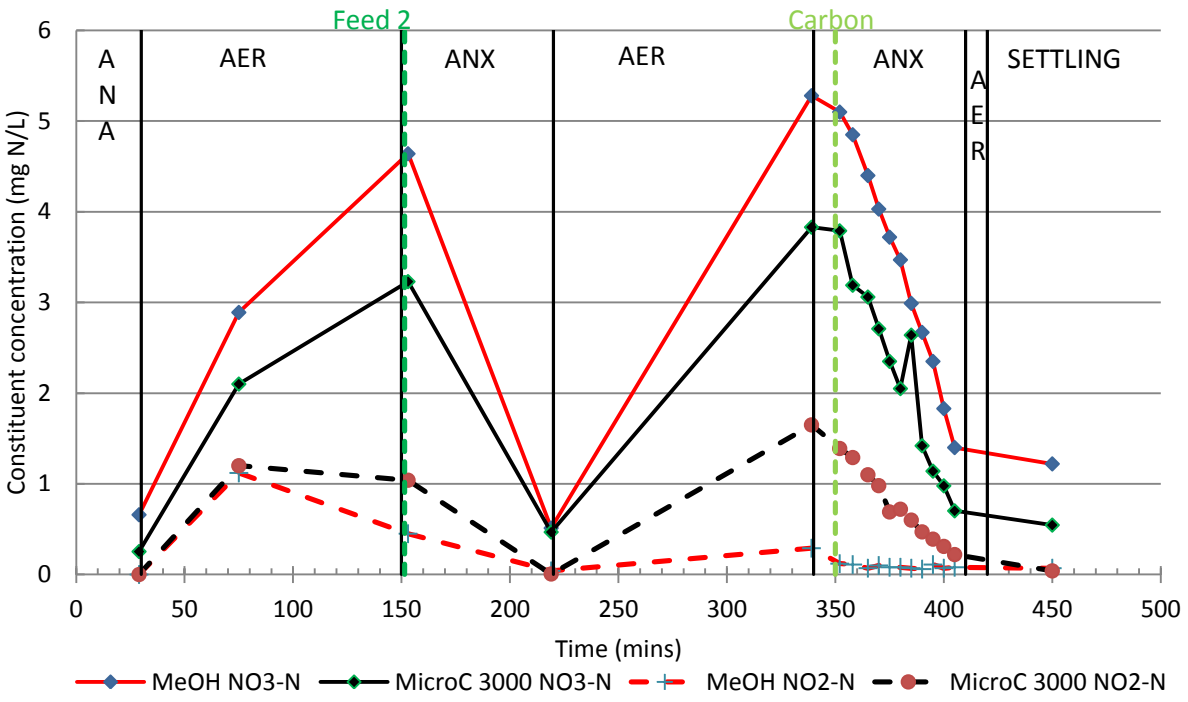


Figure 4.14: Intensive Nutrient Profiling with Carbon Under-dose

Both reactors performed similarly to each other, when the typical carbon dose was fed into the reactors, as seen in figure 4.12. There was incomplete TN removal and nitrate was present at the end of the second anoxic period. When carbon was fed in excess, as shown in Figure 4.13, there was complete nitrogen removal in both reactors, as effluent nitrogen values were close to zero. During an under-dose, TN removal was incomplete, similar to when carbon was appropriately dosed. These results demonstrate the importance of optimizing the carbon dose to ensure complete nitrogen removal. In figure 4.14, nitrite in the MicroC 3000^{TM} reactor at the beginning of the second anoxic phase was high, suggesting that the reactor biomass may have had problems with nitrification. There was no significant nitrite accumulation in the methanol reactor at any point of reactor operation, specifically with a carbon under-dose, as previously suggested by Ginige et al (2009).

The study by Cherchi et al (2009) also observed no nitrite accumulation with methanol. However, in this research, there was some nitrite accumulation associated with MicroC 3000^{TM} during the initial start-up as compared to methanol but was resolved as reactor operation approached steady-state conditions, seen in figure 4.15. This nitrite accumulation was still minor.

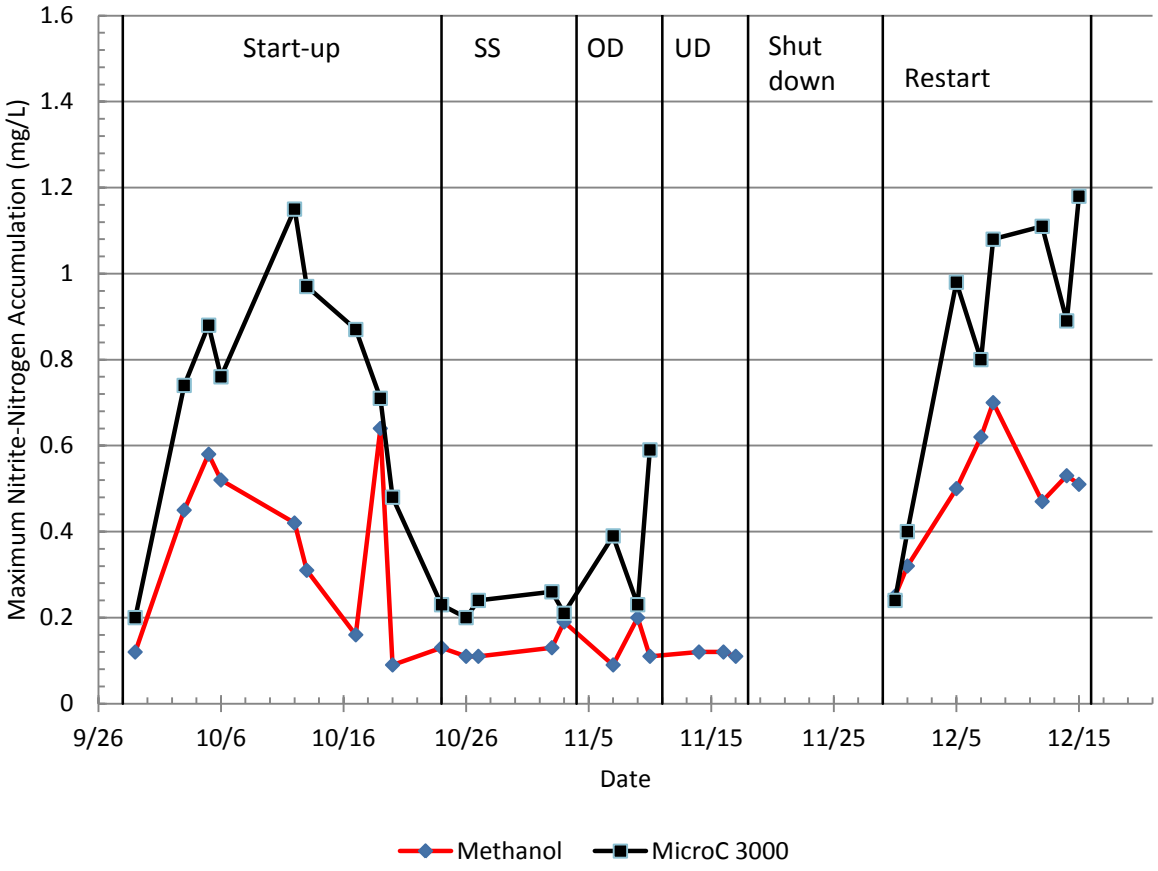


Figure 4.15: Maximum NO_2-N Accumulation during Second Anoxic Period

After taking soluble COD measurements alongside nitrate and nitrite in the second anoxic zone, the C: N ratio was calculated. Figure 4.16 demonstrates a profile for these two reactors, during the second anoxic phase and it is clear that there were no spikes in nitrite measurements, implying no nitrite accumulation. Nitrate was reduced in a linear pattern, relatively similar to COD uptake.

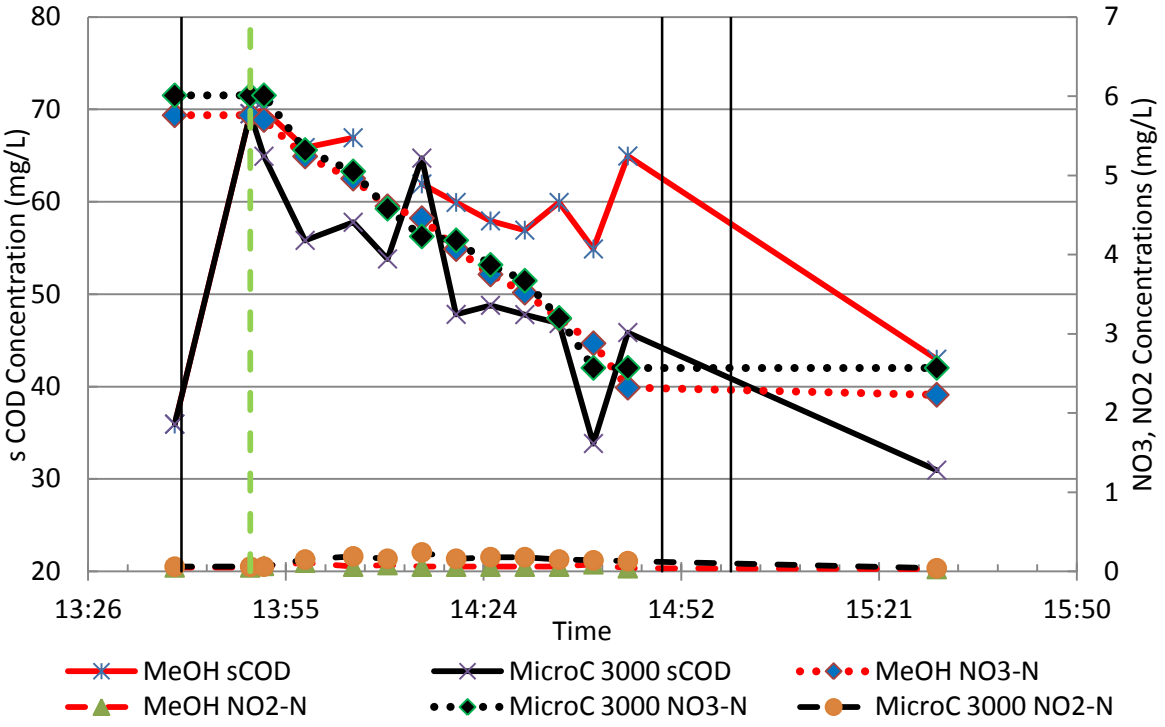


Figure 4.16: Representative Intensive Profile during Second Anoxic Zone

Based on the measurements for COD, nitrate, and nitrite in this second anoxic zone, the carbon-to-nitrogen ratio can be calculated.

Table 4.4: Average Observed C: N ± Std. Dev. (g COD consumed / g equivalent NO_3-N removed)

	SS	OD	UD	Restart	All conditions
Methanol	4.46 ± 0.4	5.6 ± 0.5	4.67 ± 0.2	4.7 ± 0.5	4.86 ± 0.4
MicroC 3000™	6.7 ± 0.9	4.7 ± 0.1	4.9 ± 1.0	5.1 ± 0.4	5.35 ± 0.6

Table 4.4 illustrates that the C: N ratio for both substrates was similar, which was expected. The ratio for methanol during steady-state, under-dose, and the restart was close to the expected value of 4.8 g COD/g NO_3-N, whereas the MicroC 3000^{TM} ratio during steady-state was closer to 5.4, which was a bit higher than expected. Since MicroC 3000^{TM} is comprised of mostly methanol, the C: N ratio should have been similar to that of methanol. This higher obtained value can be attributed to the ethanol and other constituents that are present in the product, as ethanol is known to support a higher yield than methanol. This suggests that the use of MicroC 3000^{TM} is as efficient as using methanol, but more carbon is needed to denitrify the same amount of nitrate, causing economic impacts. C: N ratios during reactor restart were similar to the ratios calculated during steady state operation for both reactors. However, for the methanol reactor, the C:N ratio increased when carbon was over-dosed, which was the opposite result for MicroC 3000^{TM}, which decreased. This is peculiar as they both should have exhibited the same pattern. Both ratios decreased when substrate was under-dosed, which is logical due to the limited energy present and able to be used by the biomass. The C: N determination can be affected by several factors, including the interference of storage phenomena (presence of PAOs and GAOs), which can take place when a considerable amount of organic substrate is put in contact with the biomass (Majone et al., 1998).

The sampling to test the degradation of 1,4- and 2,3-Butanediol was completed and analyzed through GC/FID. The results verified that it was completely degraded. Lab analysis found no traces of BD in the effluent, nor in the biomass sample taken two minutes after carbon addition. This suggests that 1,4-Butanediol was completely degraded by the biomass, as expected. During the Littleton/Englewood WWTP study, the odor subsided in the after the first week of use leading to the notion that there was insufficient biomass in the filter during rampup or there was an acclimation period required to degrade one of the alcohols in the mixture (EOS Communications). In this research, the same patterns were observed. During the first week of sampling, the “odor” was noticeable during addition of MicroC 3000^{TM} into the reactor. However, this odor became less and less noticeable as it approached steady-state conditions.

4.5 Conclusion

It can be stated with confidence that MicroC 3000^{TM} performed relatively similar to methanol and can be a viable substitute for methanol for denitrification. These reactors all achieved full nitrification as all ammonia was removed before carbon addition. The higher SDNRs of MicroC 3000^{TM} compared to SDNRs for methanol makes it a more logical choice, but the higher C: N ratio suggested that more electron donor is needed for denitrification, making cost of the chemical a factor to consider. The C: N for MicroC 3000^{TM} was higher than for methanol, due to the presence of ethanol in the product. The 1, 4- and 2, 3- butanediol was also found to be degraded upon carbon addition, with subsiding odor issues after a week of use. GC/FID found no traces in collected samples and thus, is not expected to be an issue after the biomass has time for adaptation to the supplemental carbon sources.

4.6 References

American Public Health Association, American Water Works Association, Water Environment Federation. (2005). *Standard Methods for the Examination of Water and Wastewater*; 21st ed.: Washington D.C.

Cappai, G., Carucci, A., Onnis, A. (2004) Use of Industrial Wastewaters for the Optimization and Control of Nitrogen Removal Processes. *Water Sci. Technol.*, **50** (6); 14-24

Cherchi, C., Onnis-Hayden, A., El-Shawabkeh, I., Gu, A. (2009). Implication of using different carbon sources for denitrification in wastewater treatments. *Water Environment Research. 81:788-99*

Christensson, M,. Lie, E., Welander, T. (1994). A comparison between ethanol and methanol as carbon sources for denitrification [J]. *Water Science and Technology*, 30(6): 83–90

Czerwionka, K., Makinia, J., Kaszubowska, M., Majtacz , J., Angowski, M. (2012) Distillery wastes as external carbon sources for denitrification in municipal wastewater treatment plants. *Water Science and Technology*, Vol 65 (9), 1583-1590

Dold, P., Takacs, I., Mokhayeri, Y., Nichols, A., Hinojosa, J., Riffat, R., Bott, C., Bailey, W., and Murthy, S. (2008). Denitrification with Carbon Addition – Kinetic Considerations. *Water Environment Research*, 80 (5), 417-427

EOS Communications (Personal communications, July 15, 2011)

Fiss, E., Fiss, E.M., Rebodos, R., (2010) Alternative Carbon Sources for Achieving Biological Nutrient Removal at Municipal Wastewater Treatment Plants. Technical Paper for NC AWWA-WEA 90th Annual Conference, Winston-Salem, NC, USA (2010)

Ginige, M., Boywer, J., Foley, L., Keller, J., Zhiguo, Y., (2009) A comparative study of methanol as a supplementary carbon source for enhancing denitrification in primary and secondary anoxic zones. *Biodegradation* 2009, **20**: 221-234

Hallin, S. & Pell, M. (1998) Metabolic properties of denitrifying bacteria adapting to methanol and ethanol in activated sludge. *Water Research*, 32 (1), 13-18

Hallin, S., Throbäck, IN., Dicksved, J., Pell, M. (2006) Metabolic profiles and genetic diversity of denitrifying communities in activated sludge after addition of methanol or ethanol. *Appl Environ Microbiol*, 2006 Aug; 72 (8) 5445-52

Haas, T., Jaeger, B., Weber, R., Mitchell, S., King, C. (2005). New diol processes: 1,3-propanediol and 1,4 butanediol. *Applied Catalysis A: General* 280 (2005) 83–88

Henze, M., Krisetnsen, G.H., Strube, R., (1994) Rate-Capacity Characterization of Wastewater for Nutrient Removal Processes. *Water Science and Technology*, 29 (7), 101-107

Hinojosa, J., Riffat, R., Fink, S., Murthy, S., Selock, K., Bott, C., Takacs, I., Dold, P., and Wimmer, R. (2008). Estimating the Kinetics and Stoichiometry of Heterotrophic Denitrifying Bacteria with Glycerol as an External Carbon Source. *Proceedings of the 81th Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation)*, Chicago, Illinois USA. 274-288

Irwin, Richard D. "NTP Summary Report on the Metabolism, Disposition, and Toxicity of 1,4 Butanediol." *National Toxicology Program* 54th ser. (1996), United States Department of Health and Human Services

Ishikawa, Kazuhide. "1,4-Butanediol." *SIDS Initial Assessment Report for 10th SIAM*. UNEP Publications, 16 Dec. 1999

Kang, S.J., Bailey, W.F., and Jenkins, D. (1992). Biological nutrient removal at the Blue Plains Wastewater Treatment Plant in Washington, D.C. *Water Science and Technology*, 26 (9-11) 2233-2236

Lopez-Vasquez, C., Oehmen, A., Hooijmans, C., Brdhanovic, D., Gijzen, H., Yuan, Z., van Loosdrecht, M. (2009). Modeling the PAO-GAO competition: Effects of carbon source, pH, and temperature. *Water Research*, 43**(2)**, February 2009, 450-462

Majone, M., P. Massanisso & R. Ramadori (1998) Comparison of carbon storage under aerobic and anoxic conditions. *Water Science and Technology*, 38(8-9), 77-84

Mokhayeri, Y.; Nichols, A.; Murthy, S.; Riffat, R.; Dold, P.; Takacs, I. (2006) Examining the Influence of Substrates and Temperature on Maximum Specific Growth Rate of Denitrifiers. *Water Science and Technology*, 54 (8), 155–162

Mokhayeri, Y., Riffat, R., Murthy, S., Bailey, W., Takacs, I., Bott, C. (2009) Balancing yield, kinetics and cost for three external carbon sources used for suspended growth post-denitrification. *Water Science and Technology*, 60 (10), 2485-2491

Mokhayeri, Y., Riffat, R., Takacs, I., Dold, P., Bott, C., Hinojosa, J., Bailey, W., Murthy, S. (2008) Characterizing denitrification kinetics at cold temperatures using various carbon sources in lab-scale sequencing batch reactors. Water Science and Technology, 58 (1), 233-238

Oehman, A., Saunders A., Vives, M., Yuan, Z., Keller J. (2006). Competition between polyphosphate and glycogen accumulating organisms in enhance biological phosphorus removal systems with acetate and propionate as carbon sources. *Journal of Biotechnology* 123, 22-32

Onnis-Hayden, A., Gu, A., (2008) Comparisons of Organic Sources for Denitrification: Biodegradability, Denitrification Rates, Kinetic Constants and Practical Implication for Their Application in WWTPs. Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation). 252-273

Peng, Y., Yong, M., Wang, S. (2007). Denitrification potential enhancement by addition of external carbon sources in a pre-denitrification process. Journal of Environmental Sciences, 19 (3), 284-289, 2007

Pijuan, M., Saunders, A.,M., Guisasola, A., Baeza, J.A., Casas, C., Blackall L.L. (2004). Enhanced biological phosphorus removal in a sequencing batch reactor using propionate as the sole carbon source. *Biotechnology Bioengineering*, 85 **(1)**, 56-67

Poldrugo, F., Snead III, O., (1984). 1,4 Butanediol, γ-Hydroxybutric Acid And Ethanol: Relationships and Interactions. Neuropharmacology, Vol. 23, No. 1, 109-113

Pretorius, C.; Kilian, R.; Jannone, J. (2007) High Fructose Corn Syrup as a Substrate for Denitrification. Proceedings of the WEF-IWA Specialty Conference ‘‘Nutrient Removal 2007: State of the Art’’ [CDROM], Baltimore, Maryland, March 4–6; Water Environment Federation: Alexandria, Virginia

Selock, K., Burton, W., Bott, C., Cutting, N., Wimmer, B., Neethling, J.B., Amad, S., Hinjosa, J., Murthy, S. (2008) Glycerin 101- Lessons Learned While Pilot Testing Glycerin to Enhance Denitrification. Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation). 6583-6602

Swinarski, M., Makina, J., Czerwionka, K., Chrzanowska, M., Drewnowski, J. (2009) Comparison of the Effects of Conventional and Alternative External Carbon Sources on Enhancing the Denitrification Process. *Water Environ. Res.*, **81**(9); 896-90

Van Haandel, A. C.; Ekama, G. A.; Marais, G. v. R. (1981) The Activated Sludge Process 3—Single Sludge Denitrification. Water Research., 15, 1135–1152

Zhang, A., Yang, S.T. (2009). Propionic acid production from glycerol by metabolically engineered Propionibacterium acidipropionici. *Process Biochemistry*, 44 **(12)**, 1346-1351

Chapter 5: ENGINEERING SIGNIFICANCE

This research focused on determining the feasibility of implementing glycerol and an industrial waste alcohol as supplemental carbon sources for wastewater treatment. As utilities desire to comply with increasingly stringent effluent nutrient limits, methanol is slowly becoming replaced as an electron donor as new alternatives are being discovered.

The observations associated with glycerol supplementation for denitrification (specialist-generalist theory and partial denitrification to nitrite) need to be verified as these occurrences can have significant impacts on biological nutrient removal processes. This research has concluded that the generalist-specialist theory is valid for glycerol, either due to adaptation or population shift, as well as the significant occurrence of nitrite accumulation due to rapid nitrate to nitrite reduction coincident with COD uptake and storage. The phenomena of partial denitrification resulting in nitrite accumulation and shift to carbon storage are most likely linked and seem to be the clear mechanisms for the reported increase in C: N by full-scale plants utilizing glycerol.

The verification of the generalist-specialist theory for glycerol is important as this will have to be considered when designing a treatment process. A review of the literature concludes that pilot studies using glycerol have observed little to no time for acclimation, increasing denitrification rates as steady state conditions are approached, and a higher demand for carbon than the stoichiometric expectations.

Little to no acclimation is crucial especially when starting up a treatment plant or transitioning to a new substrate. If full denitrification can occur almost immediately, then annual effluent limits can be met with greater ease. The increasingly faster denitrification rates and increase in anoxic yield (higher C: N) prove the activated sludge transitions from generalists to specialists. Activated sludge having selected for a specialist population will ensure more efficient nitrate removal rather than with generalists. The higher yield of specialists will result in greater stress on solids handling. These specialists do something unusual in order to account for the increase in yield. Over time, the reduction of nitrate to nitrite coinciding with COD uptake and storage becomes more rapid, resulting in higher a higher carbon demand in order for complete denitrification.

Nitrite accumulation examined in this study was significant. The rapid nitrate to nitrite reduction coincident with rapid COD uptake and subsequent carbon storage resulted in a slow nitrite reduction. This can result in having nitrite in the effluent, especially when the carbon dose

is not optimized. If a plant utilizes chlorine for disinfection, then this will have a significant impact on the disinfection dose demand. Most treatment plants don't have nitrite analyzers in the disinfection stage and thus the only way to know if nitrite is present is when the chlorine demand increases.

If the nitrite is not reduced to nitrogen gas by the end of the post anoxic zone, then most likely it will be oxidized back to nitrate by NOB in the re-aeration tank. This results in wasted carbon because the supplemental carbon is added based on reducing nitrate to nitrogen gas. If this carbon is only used for nitrate to nitrite reduction, and then stored, there is excess carbon added that won't result in low effluent TN concentrations (mg/L).

Having nitrite in the effluent will significantly affect SDNR calculations. The nitrate to nitrite reduction is very rapid, thus calculating nitrate SDNR measurements might be problematic. The rate for NO_3-N denitrification is much higher than the rate of NO_X-N and must be considered when designing a system for glycerol use.

Another valuable significance of this research is the promising potential that an industrial waste alcohol can be a viable alternative to methanol for denitrification. MicroC 3000^{TM} is a good example of an industrial waste that has been proven to work in both lab-scale experiments as well as full-scale testing (Littleton/Englewood Wastewater Treatment Plant, CO). The issue of volatilization of an alcoholic odor is corrected after the biomass becomes adapted to using the substrate.

OVERALL REFERENCES

American Public Health Association, American Water Works Association, Water Environment Federation. (2005). *Standard Methods for the Examination of Water and Wastewater*; 21st ed.: Washington D.C.

J. C. Akuna, C. Bizeau and R. Moletta, "Nitrate and Nitrite Reductions with Anaerobic Sludge Using Various Carbon Sources: Glucose, Glycerol, Acetic Acid, Lactic Acid and Methanol," *Water Research*, **(27)** 2, April 1993, pp. 1303-1312

Al-Rekabi, W., Qiang, H., Qiang, W. (2007). Review on Sequencing Batch Reactors. *Pakistan Journal of Nutrition*. **6**(1); 11-19

Atkinson, J., Bowden, G., Esposito, K., Mahoney, K. (2007) Estimation of Air Emissions Resulting from Implementation of Supplemental Carbon to Enhance Denitrification for the NYC Stepfeed BNR WPCPs. *Proceedings of the Water Environment Federation (WEF) Technical Exhibition and Conference.* SanDiego, California, USA. 7655-7675

Barak, Y., Tal Y., Rijn T. (1998) Light-Mediated Nitrite Accumulation during Denitrification by *Pseudomonas* sp. Strain JR12. *Appl. Envrion Microbiol.*1998 March; **64**(3); 813-817

Bilyk, K., Bruton, T., Rohrbacher, J., Latimer, R., Pitt, P. (2011) Faster SDNRs with Prolonged Glycerin Addition, Carbon Storing Bugs, and the Enemy (Dissolved Oxygen), and Other Things Encountered While Piloting Supplemental Carbon at Several BNR Plants. *Proceedings of the Water Environment Federation (WEF) Nutrient Recovery and Management 2011 Conference*

Bilyk, K., Rohrbacher, J., Bruton, T., Pitt, P., Latimer, R. (2009) Evaluating the Cost and Process Performance of Carbon Alternatives to Methanol. *Proceedings of the Water Environment Federation (WEF) Nutrient Removal 2009 Specialty Conference*. 754-779. Washington D.C.

Bodík, I., Blštáková, A., Sedláček, S., Hutňan, M. (2009). Biodiesel waste as source of organic carbon for municipal WWTP defnitrification. *Bioresource Technology* 100, 2452-2456

Cappai, G.; Carucci, A.; Onnis, A. (2004) Use of Industrial Wastewaters for the Optimization and Control of Nitrogen Removal Processes. Water Sci. Technol., 50 (6), 17–24

Carrera, J., Baeza J., Vincent, T., Lafuente, J. (2003). Biological Nitrogen Removal of High-Strength Ammonium Industrial Wastewater with Two-Sludge System. *Water Res.*, **37** (17), 4211-4221

Cherchi, C., Onnis-Hayden, A., El-Shawabkeh, I., Gu, A. (2009). Implication of using different carbon sources for denitrification in wastewater treatments. *Water Environ Res. **81**:788-99*

Christensson, M,. Lie, E., Welander, T. (1994). A comparison between ethanol and methanol as carbon sources for denitrification [J]. *Water Science and Technology*, 30(6): 83–90

Czerwionka, K., Makinia, J., Kaszubowska, M., Majtacz , J., Angowski, M. (2012) Distillery wastes as external carbon sources for denitrification in municipal wastewater treatment plants. *Water Science and Technology*, Vol 65 (9), 1583-1590.

Davis, Mackenzie Leo. *Water and Wastewater Engineering: Design Principles and Practice*. New York: McGraw Hill, 2011. Print

Dold, P., Takacs, I., Mokhayeri, Y., Nichols, A., Hinojosa, J., Riffat, R., Bott, C., Bailey, W., Murthy, S. (2008). Denitrification with Carbon Addition – Kinetic Considerations. *Water Environment Research,* **80** (5), 417 427

Docket, N. (2011) Anoxic Carbon Yield Investigation Using Glycerol as the Carbon Source for Denitrification in Sequencing Batch Reactors. *M.Eng Thesis*. Howard University

EOS Communications (Personal communications, July 15, 2011)

Erdal, U., Erdal Z., Daigger,G., Randall, C. (2007) Is it PAO-GAO Competition of Metabolic Shift in EBPR System? Evidence From An Experimental Study. *Proceedings from the Water Environment Federation (WEF) Technical Exhibition and Conference.* San Diego, CA, USA

Filipe, C., Daigger, G., Grady, C. (2001) Ph as a Key Factor in the Competition between Glycogen-Accumulating Organisms and Phosphorus-Accumulating Organisms. *Water Environment Research*. **(73)** 2; 223-232

Fillos, J., Ramalingam, K., Jezek, R., Deur, A., Beckmann, K. (2007) Specific Denitrification Rates with Alternative External Sources of Carbon. *Proceedings from the 10th International Conference on Environmental Science and Technology*. Sept. 2007. Kos island, Greece

Fiss, E., Fiss, E.M., Rebodos, R., (2010) Alternative Carbon Sources for Achieving Biological Nutrient Removal at Municipal Wastewater Treatment Plants. Technical Paper for NC AWWA-WEA 90th Annual Conference, Winston-Salem, NC, USA (2010).

Gárate, H., Mar'atusalihat, E., Kleerebezem, R., van Loosdrecht, M. (2011) Microbial community engineering for biopolymer production from glycerol. *Appl. Microbiol Biotechnol, 2011* ***92****:631-639*

Ginige, M., Boywer, J., Foley, L., Keller, J., Zhiguo, Y., (2009) A comparative study of methanol as a supplementary carbon source for enhancing denitrification in primary and secondary anoxic zones. *Biodegradation* 2009, **20**: 221-234

Grabinska-Loniewska, A., Slomczynski, T., and Kanska, Z. (1985). Denitrification Studies with Glycerol as a Carbon Source. *Water Research*, **19** (12) 1471-1477

Guerrero, J., Guisasola, A., Baeza, J. (2011) The nature of the carbon source rules the competition between PAO and denitrifiers in systems for simultaneous biological nitrogen and phosphorus removal. *Water Res. 2011;* ***45****: 4793-4802*

Guerrero, J., Tayà, C., Guisasola, A., Baeza, J. (2012) Glycerol as a sole carbon source for enhanced biological phosphorus removal. *Water Res. 2012;* ***46****: 2893-91*

Guerrero, J., Tayà, C.,Guisasola, A., Baeza, J. (2012) Understanding the detrimental effect of nitrate presence on EBPR systems: effect of the plant configuration. *J Chem Technol Biotechnol, 2012,* **87;** 1508-1511

Hallin, S., Throback IN., Dicksved, J., Pell, M. (2006). Metabolic profiles and genetic diversity of denitrifying communities in activated sludge afer addition of methanol or ethanol. *Appl. Environ. Microbiol.* **7-2**:5445 5452

Hallin, S., Rothman, M., Pell, M. (1996). Adaptation of denitrifying bacteria to acetate and methanol in activated sludge. *Water Res.* **32**:13-18

Haas, T., Jaeger, B., Weber, R., Mitchell, S., King, C. (2005). New diol processes: 1,3-propanediol and 1,4 butanediol. *Applied Catalysis A: General* 280 (2005) 83–88

Henze, M., Krisetnsen, G.H., Strube, R., (1994) Rate-Capacity Characterization of Wastewater for Nutrient Removal Processes. *Water Science and Technology*, 29 (7), 101-107

Hinojosa, J., Riffat, R., Fink, S., Murthy, S., Selock, K., Bott, C., Takacs, I., Dold, P., and Wimmer, (2008). Estimating the Kinetics and Stoichiometry of Heterotrophic Denitrifying Bacteriawith Glycerol as an External Carbon Source. *Proceedings of the 81th Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC - National Conference of the Water Environment Federation),* Chicago, Illinois USA

Irwin, Richard D. "NTP Summary Report on the Metabolism, Disposition, and Toxicity of 1,4 Butanediol." *National Toxicology Program* 54th ser. (1996), United States Department of Health and Human Services

Ishikawa, Kazuhide. "1,4-Butanediol." *SIDS Initial Assessment Report for 10th SIAM*. UNEP Publications, 16 Dec. 1999

Kang, S.J., Bailey, W.F., and Jenkins, D. (1992). Biological nutrient removal at the Blue Plains Wastewater Treatment Plant in Washington, D.C. *Water Science and Technology*, **26** (9-11) 2233-2236

Katehis, D., Deur, A., Sharp R. (2011) Usage of Glycerin for Denitrification in High Rate Activated Sludge Processes: Benefits and Limitations. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

Katehis, D., Sharp, R., Deur, A., Young, P., Rubino, V., Beckman, K. (2010) Deploying Alternative Supplemental Carbon for Denitrification at Full Scale: Glycerol from Biodiesel Production. *Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation). New Orleans, Louisiana, USA.* 2422-2429

Kiss, B., Bakos, V., Liu, W., Jobbágy, A. (2011) Full-Scale use of Glycogen-Accumulating Organisms for Excess Biological Carbon Removal. *Water Environment Research* **(83)** 9; 855-864

Ledwell, Samuel (March 19, 2008). Carbon Sources for Biological Denitrification. *MWPCA Quarterly Meeting*. Bourne, MA.

Leung, G., Tam, N. (1994). Operation strategy of a sequencing batch reactor for simultaneous removal of wastewater organic matter and nutrients. *Resources, Conservation and Recycling.* **11**, 209-223

Lopez-Vasquez, C., Oehmen, A., Hooijmans, C., Brdhanovic, D., Gijzen, H., Yuan, Z., van Loosdrecht, M. (2009). Modeling the PAO-GAO competition: Effects of carbon source, pH, and temperature. *Water Research*, 43**(2)**, February 2009, 450-462

Machado, J., Batista, J. (2007) Abundance of PAO and GAO bacteria in a well performing enhanced biological phosphorus removal system. *Proceedings of the Water Environment Federation (WEF) 2007 Technical Exhibition and Conference*. San Diego, CA, USA

Martiensson, M., Schöps, R. (1999) Population Dynamics of Denitrifying Bacteria in a Model Biocommunity. *Water Res.* **(33)** 3, 639-646

Majone, M., P. Massanisso & R. Ramadori (1998) Comparison of carbon storage under aerobic and anoxic conditions. *Water Science and Technology*, 38(8-9), 77-84

McCarty, P., Beck, L., Amant P. (1969). Biological denitrification of wastewaters by addition of organic materials. 24th Ind. Waste Conf., Purdue Univ., Lafayette, Ind., pp. 1271-1285

Mokhayeri, Y., Riffat, R., Murthy, S., Bailey, W., Takacs, I., Bott, C. (2009) Balancing yield, kinetics and cost for three external carbon sources used for suspended growth post-denitrification. *Water Science and Technology*, 60 (10), 2485-2491

Mokhayeri, Y., Riffat, R., Takacs, I., Dold, P., Bott, C., Hinjosa, J., Bailey, W., Murthy, S. (2008) Characterizing

denitrification kinetics at cold temperatures using various carbon sources in lab-scale sequencing batch reactors. *Water Sci Technol*. 2008; **58** (1): 233-8

Mokhayeri, Y., Nichols, A., Murthy, S., Riffat, R., Dold, P., Takacs, I. (2006) Examining the influence of substrates and temperate on maximum specific growth rate of denitrifiers. *Water Sci. Technol.* **54**(8), 155-162

Neethling, J. (2005) WERF Project 01-CTS-3 *Biological Phosphorus Removal Survey and Investigations.*

Nyberg U., Aspegren H., Andersson, B., Jansen, J. (1992). Full-scale application of nitrogen removal with methanol as carbon source. *Wat. Sci. Technol.* **26,** 1077-1086

Oehman, A., Saunders, A., Vives, M., Yuan, Z., Keller, J. (2006) Competition between polyphosphate and glycogen accumulating organsisms in enhanced biological phosphorus removal systems with acetate and propionate as carbon sources. *Journal of Biotechnology.* 123, 22-32

Oehman, A., Vives, M., Lu, H., Yuan, Z., Keller, J. (2005) The effect of pH on the competition between polyphosphate-accumulating organisms and glycogen-accumulating organisms. *Water Research* 39, 3727 3737

Oh, J., Silverstein, J. (1999). Oxygen inhibition of activated sludge denitrification. Water Research. 1999; **33**; 1925-1937

Omari, A., Murthy, S., Takacs, I., Mokhayeri, Y., Riffat, R., Nopens, I. (2011) Modelling the use of External Carbon Substrate for Denitrification by Generalists and Specialists. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

Onnis-Hayden, A., Gu, A., (2008) Comparisons of Organic Sources for Denitrification: Biodegradability, Denitrification Rates, Kinetic Constants and Practical Implication for Their Application in WWTPs. Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation). 252-273

Peng, Y., Yong, M., Wang, S. (2007). Denitrification potential enhancement by addition of external carbon sources in a pre-denitrification process. Journal of Environmental Sciences, 19 (3), 284-289

Phillips, H., Barnard, J., deBarbadillo, C., Shaw, A., Steichen, M., Lage, C. (2009) Denitrification Rates,: Sampling, Modeling, and Design Considerations. *Proceedings from the Water Environment Federation Nutrient Removal Conference.* Washington D.C., USA

Pijuan, M., Saunders, A.,M., Guisasola, A., Baeza, J.A., Casas, C., Blackall L.L. (2004). Enhanced biological phosphorus removal in a sequencing batch reactor using propionate as the sole carbon source. *Biotechnology Bioengineering*, 85 **(1)**, 56-67

Poldrugo, F., Snead III, O., (1984). 1,4 Butanediol, γ-Hydroxybutric Acid And Ethanol: Relationships and Interactions. Neuropharmacology, Vol. 23, No. 1, 109-113

Pretorius, C.; Kilian, R.; Jannone, J. (2007) High Fructose Corn Syrup as a Substrate for Denitrification. Proceedings of the WEF-IWA Specialty Conference ‘‘Nutrient Removal 2007: State of the Art’’ [CDROM], Baltimore, Maryland, March 4–6; Water Environment Federation: Alexandria, Virginia

Ramalington, K., Filos, J., Deur, A., and Beckmann K., Specific Denitrification Rates with Alternate Sources of Organic Carbon Proceedings of 2nd External Carbon Source Water Environment Research Federation Workshop, 2007, Washington, D.C.

Randall, C., Barnard, J., Stensel, H. (1992). Design and Retrofit of Wastewater Treatment Plants for Biological Nutrient Removal. *Water Quality Management Library*. Vol. 5, Technomic Publishing Co., Lancaster, PA

Rohrbacher, J., Bilyk, K., Bruton, T., Pitt, P., Latimer, R., (2009) Evaluation of Alternative Supplemental Carbon Sources at Four BNR Facilities. *Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC – National Conference of the Water Environment Federation)* Orlando, Florida, USA. 4464-4483

Sage, M.; Daufin, G.; Gesan-Guiziou, G. (2006) Denitrification Potential and Rates of Complex Carbon Source from Dairy Effluents in Activated Sludge System. Water Res., 40, 2747–2755

Selock, K., Burton, W., Bott, C., Cutting, N., Wimmer, B., Neethling, J.B., Amad, S., Hinjosa, J., Murthy, (2008) Glycerin 101- Lessons Learned While Pilot Testing Glycerin to Enhance Denitrification. Proceedings of the Water Environment Federation Technical Exhibition and Conference (WEFTEC –National Conference of the Water Environment Federation). 6583-6602

Singh, M., Srivastava, R. (2011) Sequencing batch reactor technology for biological wastewater treatment: a review. *Asia-Pacific Journal of Chemical Engineering*. Vol. 6, 3-13

Skrinde, J., Bhagat, S. (1982). Industrial Wastes as Carbon Sources in Biological Denitrification. *Journal (Water Pollution Control Federation)*. Vol. 54, No. 4; 370-377

Sperl, GT., Hoare, DS. (1971). Denitrification with methanol: a selective enrichment for Hyphomicrobium species. *Journal of Bacteriology.* 1971, Nov. **108** (2): 733-6

Swinarski, M., Makinia J., Stensel, H., Czerwionka, K., Drewowski, J. (2011) Modeling External Carbon Addition. in Combined N-P Activated Sludge Systems with Extension of the IWA Activated Sludge Models. *Proceedings of the Water Environment Federation (WEF) 2011 Nutrient Recovery and Management Conference.* Miami, Florida, USA

Swinarski, M., Makina, J., Czerwionka, K., Chrzanowska, M., Drewnowski, J. (2009) Comparison of the Effects of Conventional and Alternative External Carbon Sources on Enhancing the Denitrification Process. *Water Environ. Res.*, **81**(9); 896-906

Tchobanoglous, George, Franklin L. Burton, and H. David. Stensel. (2003) Metcalf & Eddy. *WastewaterEngineering: Treatment and Reuse*. Boston: McGraw-Hill,. Print

Tsuchihashi, R., Bowden, G., Beckmann, K., Deur, A., and Bodniewicz, B. (2008). Evaluation of Crude Glycerin as a Supplemental Carbon Source for a High Rate Step-Feed BNR Process. *Proceedings of the 81st Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC–National Conference of the Water Environment Federation)* Chicago, Illinois, USA

Water Environment Federation (2005) Biological Nutrient Removal (BNR) Operation in Wastewater Treatment Plants, Manual of Practice No. 30; Water Environment Federation: Alexandria,Virginia

Wilderer, P., Jones, W., Dau, U. (1987) Competition in Denitrification Systems Affecting Reduction Rate and Accumulation of Nitrite. *Wat. Res.* **21**(2); 239-245

Williams, S., Wilson, A. (1994). Beckton Demonstration Biological Nutrient Removal Plant. *Water and Environment Journal*. Vol. 8 (6); 664-670

Van Dongen U, Jetten MSM, van Loosdrecht MCM. The SHARON-ANAMMOX process for treatment of ammonium rich wastewater. *Water Sci. Technol* 2001; 44:153– 60

Van Haandel, A. C.; Ekama, G. A.; Marais, G. v. R. (1981) The Activated Sludge Process 3—Single Sludge Denitrification. Water Research, 15, 1135–1152

Venkata mohan, S., N.C. Rao, K.K. Prasad, B.T.V. Madhavi, P.N. Sharma.Treatment of complex chemical wastewater in sequencing batch reactor (SBR) with an aerobic suspended growth configuration. *Process Biochem.*, **2005;** *40*(5), 1501–1508

Yuan, Q., Sparling, R., Oleszkieqicz, J. (2009) Enhanced Biological Phosphorus Removal by Glycerol Fermentation. *Proceedings of the Water Environment Federation Nutrient Removal Conference.* Washington D.C., USA

Zhang, A., Yang, S.T. (2009). Propionic acid production from glycerol by metabolically engineered Propionibacterium acidipropionici. *Process Biochemistry*, 44 **(12)**, 1346-1351

A. APPENDIX

A.1 Reactor Operation

Table A.1: Influent Characteristics

	TSS	TVSS	VSS	COD	sCOD	TKN	sTKN	TP
	mg/L	%	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
9/22/2011	135	82.00	110.70	288	114	26.20	22.2	3.36
9/29/2011	136	91.00	123.76	496	145	32.30	25.1	4.4
10/3/2011	138	96	132.48	672	102	31.60	20.30	3.82
10/5/2011	126	89	112.14	369	163	33.30	26.30	4.36
10/6/2011	118	95	112.10	330	152	34.80		4.43
10/12/2011	150	95	142.50	521	153	39.20	27.10	4.97
10/13/2011	134	88	117.92	429	144	32.60	34.00	4.97
10/17/2011	174	89	154.86	567	180	41.90	31.60	5.03
10/19/2011	154	96	147.84	581	194	42.10	34.10	4.95
10/20/2011	135	89	120.15	456	180	42.30	34.00	5.12
10/24/2011	145	88	127.60	337	163	41.7	31.3	5.33
10/26/2011	154	87	133.98	552	181	40	31.5	4.55
10/27/2011	156	88	137.28	571	173	42.6	34.6	5.25
10/31/2011	178	88	156.64	342	170	39.40	32.90	4.77
11/2/2011	154	87	133.98	612	183	39.7	32.8	5.29
11/3/2011	178	88	156.64	557	156	42.2	32.9	5.42
11/7/2011	176	86	151.36	537	161	45.6	32.3	5.85
11/9/2011	152	89	135.28	601	154	43.8	35.7	5.66
11/10/2011	206	90	185.4	862	177	49.3	36.8	5.61
11/14/2011	162	86	139.32	621	168	44.6	34.1	5.5
11/16/2011	148	88	130.24	500	196	43.5	37.3	6.15
11/17/2011	152	93	141.36	516	187	47.7	37.5	5.78
11/30/2011	126	87	109.62	614	198	48.6	38.5	6.3
12/1/2011	165	92	151.8	660	214	46.7	34.3	6.22
12/5/2011	165	88	145.2	569	198	53.1	40.6	6.79
12/7/2011	153	92	140.76	656	239	46.40	34.70	6.59
12/8/2011	158	89	140.62	689	226	49.70	38.40	7.37
12/12/2011	180	89	160.2	764	226	51.70	34.70	7.15
12/14/2011	94	96	90.24	551	204	43.10	35.30	5.13
12/15/2011	160	88	140.8	554	211	47.70	35.30	5.61

Table A.2: Methanol Effluent Characteristics

	TSS	TVSS	VSS	COD	sCOD	TKN	sTKN	TP
	mg/L	%	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
9/29/2011	4	82%	3.3	54	50	1.44	1.26	
10/3/2011	6	85	5.1	44	38	1.52	0.91	2.86
10/5/2011	4	85	3.4	48	48	1.56	0.87	0.26
10/6/2011	5	83	4.2	52	42	1.47	1.26	0.26
10/12/2011	6	86	5.2	50	45	1.59	0.96	0.20
10/13/2011	8	83	6.6	61	38	1.87	1.02	0.44
10/17/2011	5	88	4.4	50	41	1.75	1.11	0.2
10/19/2011	4	97	3.9	49	38	1.69	0.87	0.2
10/20/2011	8	92	7.4	54	40	1.89	1.32	0.34
10/24/2011	5	88	4.4	46	35	1.42	1.39	0.2
10/26/2011	4	89	3.6	51	41	1.6	0.9	0.2
10/27/2011	3	89	2.7	46	43	1.39	1.39	0.2
10/31/2011	5	89	4.5	46	36	1.78	1.52	< 0.20
11/2/2011	7	92	6.4	61	40	1.98	1.10	< 0.20
11/3/2011	6	92	5.5	55	36	1.72	1.54	< 0.20
11/7/2011	6	90	5.4	77	67	1.46	1.26	< 0.20
11/9/2011	5	87	4.4	89	74	1.58	1.09	< 0.20
11/10/2011	6	90	5.4	91	74	1.57	1.28	< 0.20
11/14/2011	5	87	4.4	37	25	1.96	1.09	< 0.20
11/16/2011	4	88	3.5	38	29	1.6	1.33	< 0.20
11/17/2011	3	97	2.9	31	27	1.45	1.35	< 0.20
11/30/2011	5	92	4.6	63	53	1.63	1.29	0.27
12/1/2011	5	98	4.9	59	54	1.86	0.87	0.25
12/5/2011	5	90	4.5	46	45	1.30	0.91	0.21
12/7/2011	6	87	5.2	51	45	1.53	1.23	0.20
12/8/2011	2	86	1.72	41	41	1.7	1.09	< 0.20
12/12/2011	3	97	2.91	45	37	1.24	1.04	0.21
12/14/2011	2	95	1.9	44	41	1.43	0.93	< 0.20
12/15/2011	3	94		46	42	1.29	1.20	< 0.20

Table A.3: Glycerol Effluent Characteristics

	TSS	TVSS	VSS	COD	Scod	TKN	Stkn	TP
	mg/L	%		mg/L	mg/L	mg/L	mg/L	mg/L
9/29/2011	5	83%	4	53	48	1.26	1.54	0.29
10/3/2011	6	84	5	34	26	1.34	1.05	2.14
10/5/2011	5	86	4	32	33	1.32	0.87	0.28
10/6/2011	5	86	4	35	30	1.18	1.31	0.21
10/12/2011	5	87	4	70	30	1.50	0.82	0.21
10/13/2011	6	85	5	48	29	1.64	0.96	0.28
10/17/2011	15	83	12	46	28	1.63	1.04	0.42
10/19/2011	17	85	14	46	28	1.68	0.88	0.39
10/20/2011	9	92	8	49	32	1.99	1.46	0.37
10/24/2011	10	88	9	40	25	2.12	1.24	0.3
10/26/2011	8	82	7	98	33	1.51	1.01	0.2
10/27/2011	5	89	4	55	31	1.51	1.3	0.2
10/31/2011	7	90	6	41	25	1.71	1.39	0.21
11/2/2011	6	87	5	33	28	1.73	0.99	< 0.20
11/3/2011	5	89	4	38	31	1.54	1.15	< 0.20
11/7/2011	11	87	10	41	28	1.78	1.12	0.31
11/9/2011	15	86	13	33	25	1.94	1.03	0.27
11/10/2011	5	91	5	39	30	1.23	1.13	< 0.20
11/14/2011	9	84	8	44	26	1.8	1.03	0.22
11/16/2011	4	88	4	35	27	1.67	1.25	< 0.20
11/17/2011	12	88	11	36	29	1.61	1.14	< 0.20
11/30/2011	4	95	4	82	66	1.17	1.15	0.22
12/1/2011	7	90	6	82	44	1.68	0.83	0.39
12/5/2011	5	90	5	75	32	1.80	0.99	0.60
12/7/2011	3	92	3	32	30	1.56	1.22	< 0.20
12/8/2011	5	85	4	144	37	1.62	1.13	< 0.20
12/12/2011	3	96	3	36	32	1.41	1.05	0.22
12/14/2011	2	96	2	32	33	1.22	0.89	< 0.20
12/15/2011	11	86	9	42	43	1.44	1.04	0.22

Table A.4: BGW Effluent Characteristics

	TSS	TVSS	VSS	COD	sCOD	TKN	Stkn	TP
	mg/L	%		mg/L	mg/L	mg/L	mg/L	mg/L
9/29/2011	4	83	3.32	50	45	1.23	1.39	0.22
10/3/2011	9	81	7.29	49	27	1.51	1.02	1.93
10/5/2011	5	85	4.25	35	32	1.20	1.17	0.27
10/6/2011	5	84	4.2	38	26	1.20	1.05	0.32
10/12/2011	5	91	4.55	59	44	1.30	1.03	0.31
10/13/2011	7	84	5.88	37	35	1.64	1.19	0.29
10/17/2011	7	84	5.88	45	29	1.52	1.12	0.24
10/19/2011	13	72	9.36	77	26	2.06	0.93	0.44
10/20/2011	10	81	8.1	55	28	1.85	1.03	0.39
10/24/2011	5	91	4.55	39	29	1.34	1.07	0.2
10/26/2011	5	84	4.2	44	31	1.51	1.14	0.2
10/27/2011	6	86	5.16	39	28	1.5	1.03	0.2
10/31/2011	6	88	5.28	35	28	1.7	1.23	< 0.20
11/2/2011	6	86	5.16	43	30	1.59	1.04	< 0.20
11/3/2011	5	90	4.5	40	28	1.45	1.05	< 0.20
11/7/2011	8	92	7.36	46	30	1.46	1.04	0.47
11/9/2011	16	84	13.44	42	< 25	2.8	1.01	0.55
11/10/2011	6	92	5.52	42	27	1.51	1.01	0.21
11/14/2011	4	89	3.56	44	< 25	1.38	1.23	< 0.20
11/16/2011	11	87	9.57	37	29	2.08	1.12	0.27
11/17/2011	3	97	2.91	34	32	1.31	1.01	< 0.20
11/30/2011	13	88	11.44	45	38	1.38	1.22	0.24
12/1/2011	5	88	4.4	40	34	1.79	0.91	0.23
12/5/2011	4	90	3.6	31	28	1.48	0.89	0.22
12/7/2011	14	82	11.48	33	32	1.90	1.32	0.41
12/8/2011	8	87	6.96	54	38	1.52	1.13	< 0.20
12/12/2011	40	86	34.4	57	32	2.17	1.02	0.34
12/14/2011	7	86	6.02	36	33	1.17	0.78	< 0.20
12/15/2011	14	86	12.04	36	36	1.30	0.95	< 0.20

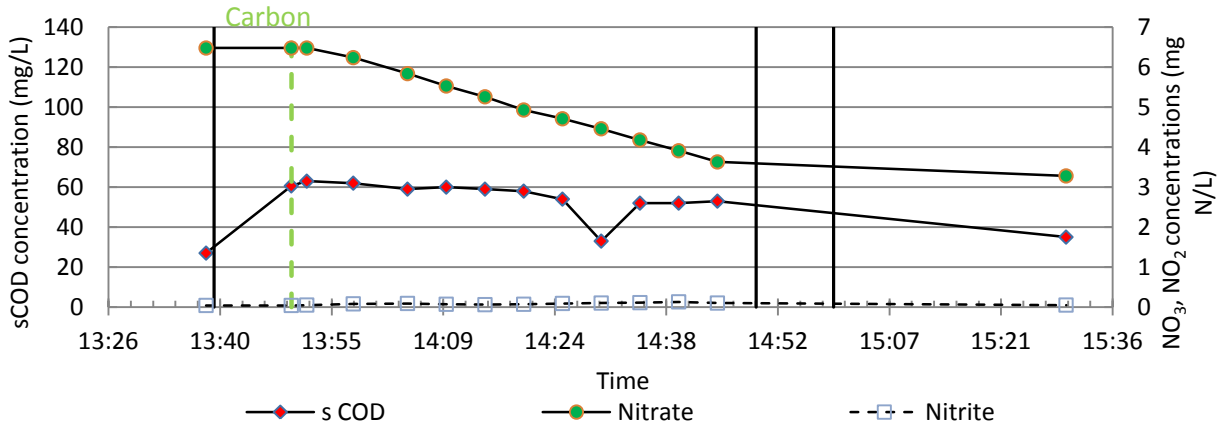
Table A.5: MicroC 3000 Effluent Characteristics

	TSS	TVSS	VSS	COD	sCOD	TKN	sTKN	TP
	mg/L	%		mg/L	mg/L	mg/L	mg/L	mg/L
9/29/2011	4	86%	3.44	50	47	1.25	1.16	0.21
10/3/2011	9	81	7.29	49	30	1.61	1.16	3.57
10/5/2011	6	84	5.04	47	41	1.60	1.27	0.80
10/6/2011	6	80	4.80	47	38	1.55	1.16	0.80
10/12/2011	5	85	4.25	44	42	1.44	1.06	1.35
10/13/2011	6	82	4.92	47	30	1.50	1.07	0.64
10/17/2011	4	93	3.72	41	51	1.35	1.29	0.78
10/19/2011	5	96	4.80	50	25	1.46	1.05	0.2
10/20/2011	6	85	5.10	47	33	1.65	1.08	0.21
10/24/2011	5	89	4.45	35	29	1.6	1.14	0.24
10/26/2011	5	88	4.40	42	35	1.54	1.21	0.2
10/27/2011	5	85	4.25	42	31	1.57	1.21	0.2
10/31/2011	4	90	3.60	71	36	1.34	1.27	< 0.20
11/2/2011	8	90	7.20	43	27	1.51	1.1	< 0.20
11/3/2011	8	90	7.20	46	26	1.76	1.35	0.2
11/7/2011	6	89	5.34	76	69	1.48	1.12	< 0.20
11/9/2011	5	83	4.15	80	71	1.2	0.99	< 0.20
11/10/2011	5	96	4.80	81	67	1.46	1.1	< 0.20
11/14/2011	7	81	5.67	33	25	1.56	0.94	0.24
11/16/2011	4	90	3.60	34	29	1.51	1.04	< 0.20
11/17/2011	3	88	2.64	40	29	1.09	0.85	< 0.20
11/30/2011	17	85	14.45	53	48	1.82	1.02	0.44
12/1/2011	5	96	4.80	47	44	1.69	1.10	0.22
12/5/2011	4	85	3.40	40	32	1.90	1.08	< 0.20
12/7/2011	3	93	2.79	38	40	1.42	1.05	0.21
12/8/2011	4	85	3.4	46	42	1.55	1.16	< 0.20
12/12/2011	3	97	2.91	42	38	1.40	1.02	< 0.20
12/14/2011	4	93	3.72	67	36	0.97	0.81	< 0.20
12/15/2011	5	85	4.25	44	39	1.89	0.88	0.50

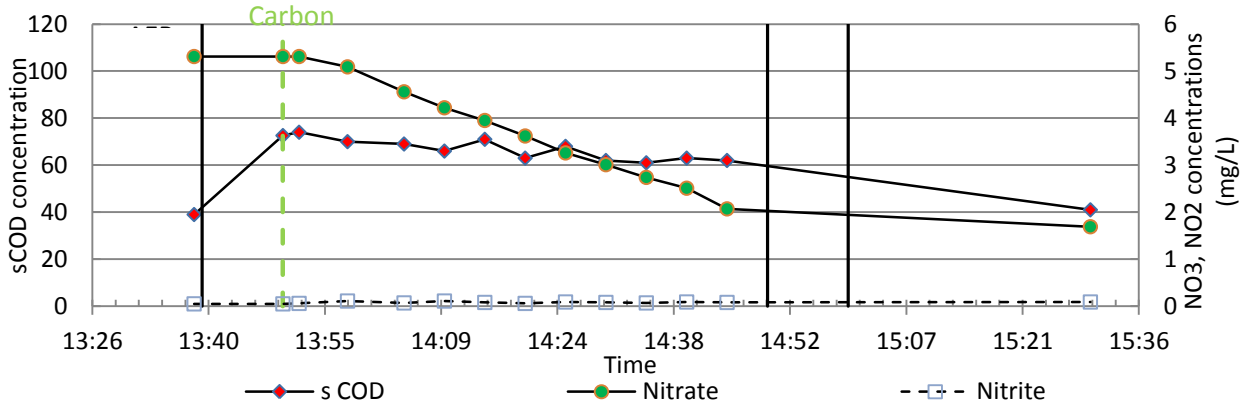
Table A.6: Reactor MLSS/MLVSS

	Methanol		Glycerol		BGW		MicroC 3000	
Date	MLSS	MLVSS	MLSS	MLVSS	MLSS	MLVSS	MLSS	MLVSS
9/29/2011	3620	2787	4420	3447.6	3880	2987.6	3920	3018.4
10/3/2011	4040	3192	3380	2636.4	3720	2901.6	3500	2730
10/5/2011	3420	2633	3940	3073.2	3560	2848	3300	2541
10/6/2011	3160	2528	3880	3065.2	3440	2752	3240	2559.6
10/12/2011	2580	2038	3280	2656.8	2960	2427.2	2540	2032
10/13/2011	2680	2171	3000	2430	3560	2883.6	2680	2090.4
10/17/2011	2640	2112	3500	2765	2920	2482	2500	2025
10/19/2011	2340	1989	3140	2574.8	2820	2397	2280	1869.6
10/20/2011	2380	2023	2960	2427.2	2960	2456.8	2300	1909
10/24/2011	2140	1819	2880	2649.6	2160	2008.8	1780	1637.6
10/26/2011	2100	1743	2900	2407	2260	1898.4	1940	1571.4
10/27/2011	2360	1912	2960	2486.4	2740	2356.4	2160	1706.4
10/31/2011	2040	1714	2620	2200.8	2880	2390.4	2040	1693.2
11/2/2011	2360	1959	3180	2607.6	2880	2419.2	2340	1942.2
11/3/2011	2500	2050	3060	2539.8	2900	2436	2380	1951.6
11/7/2011	2300	1932	3200	2784	3000	2580	2400	2016
11/9/2011	2460	2042	3320	2855.2	3360	2788.8	2220	1887
11/10/2011	2540	2388	3140	2763.2	3140	2731.8	2420	2105.4
11/14/2011	2280	1915	3060	2601	3240	2689.2	2150	1806
11/16/2011	2740	2274	3340	2872.4	3420	2941.2	2680	2251.2
11/17/2011	2680	2385	3300	2871	3240	2818.8	2460	2091
11/30/2011	4080	3386.4	4100	3362	3780	3137.4	4120	3378.4
12/1/2011	3960	3207.6	4240	3519.2	3820	3132.4	4000	3200
12/5/2011	3820	3132.4	4260	3578.4	4200	3486	3900	3198
12/7/2011	3680	3091.2	3960	3326.4	3760	3158.4	3460	2871.8
12/8/2011	3720	3087.6	3940	3388.4	4060	3410.4	3560	2990.4
12/12/2011	3540	2973.6	3840	3264	3660	3111	3000	2490
12/14/2011	3260	2771	3720	3124.8	3720	3124.8	3100	2635
12/15/2011	3460	2906.4	3960	3366	3760	3233.6	3400	2890

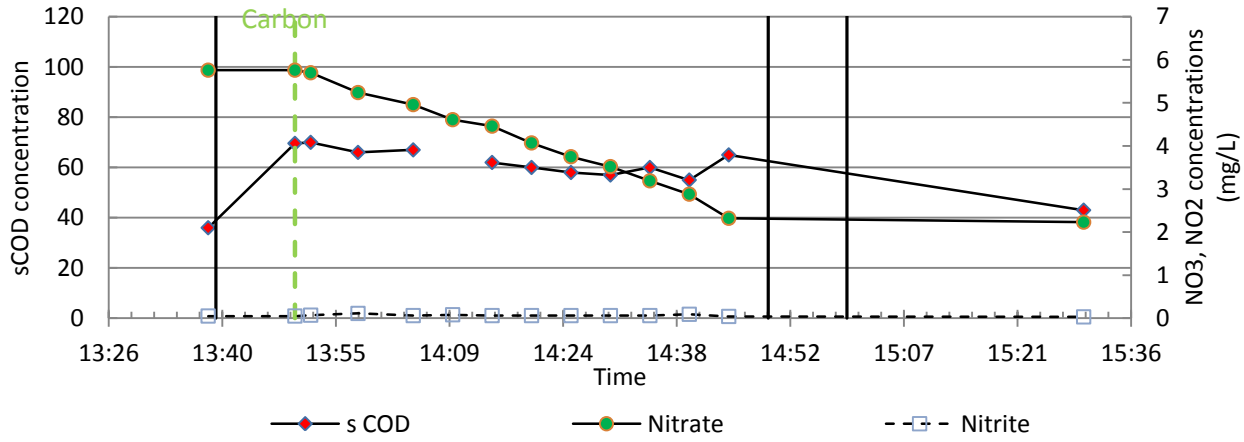
A.2 Methanol



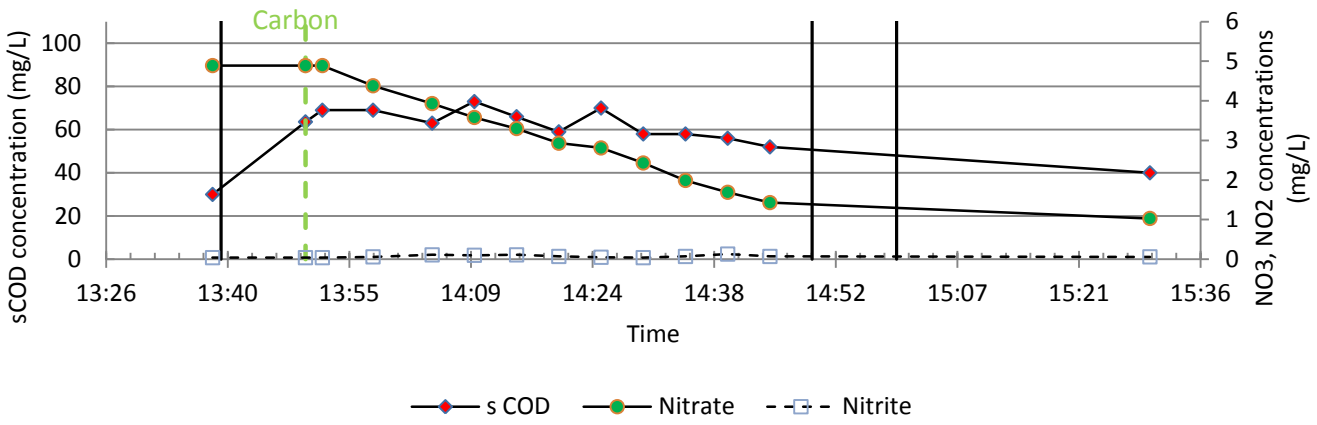
A.1: 10/24/11 Second Anoxic Zone Profile



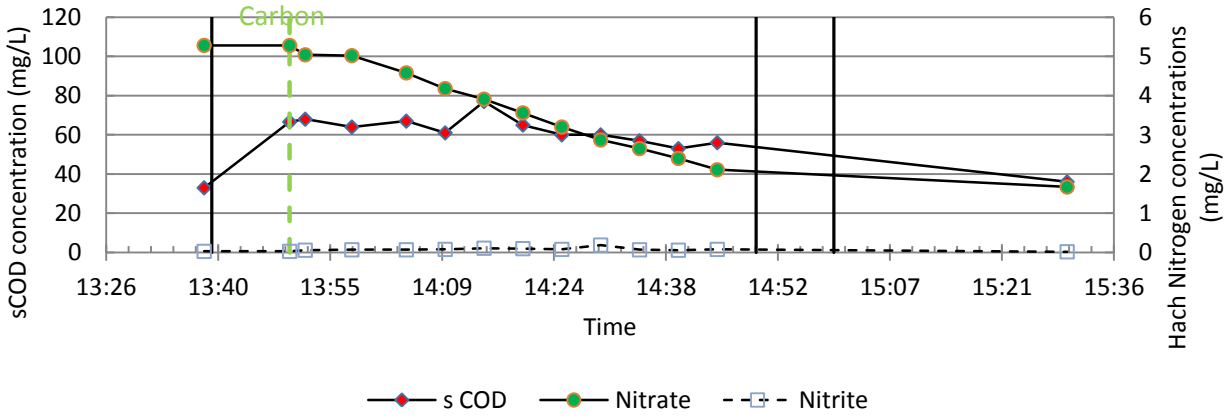
A.2: 10/26/11 Second Anoxic Zone Profile



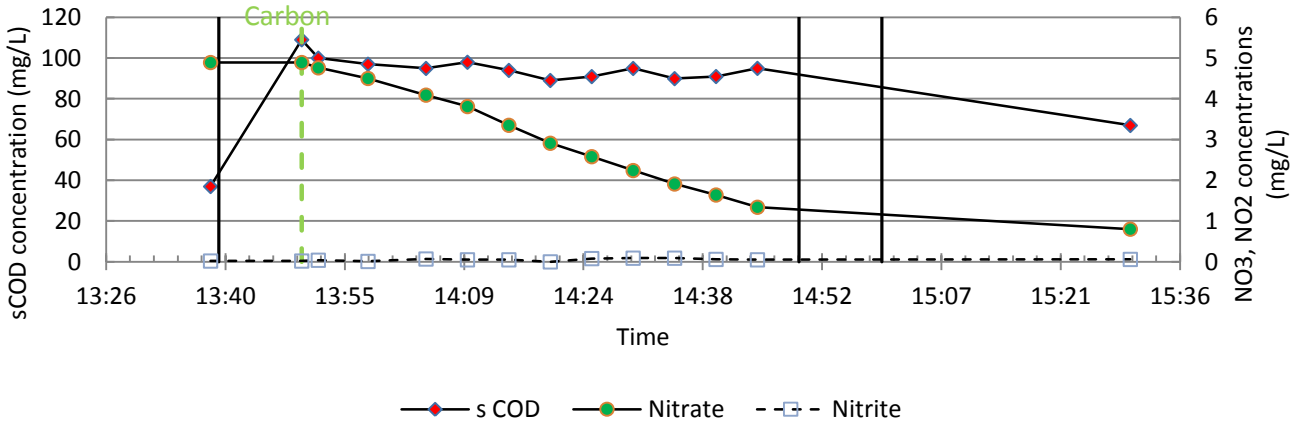
A.3: 10/27/11 Second Anoxic Zone Profile



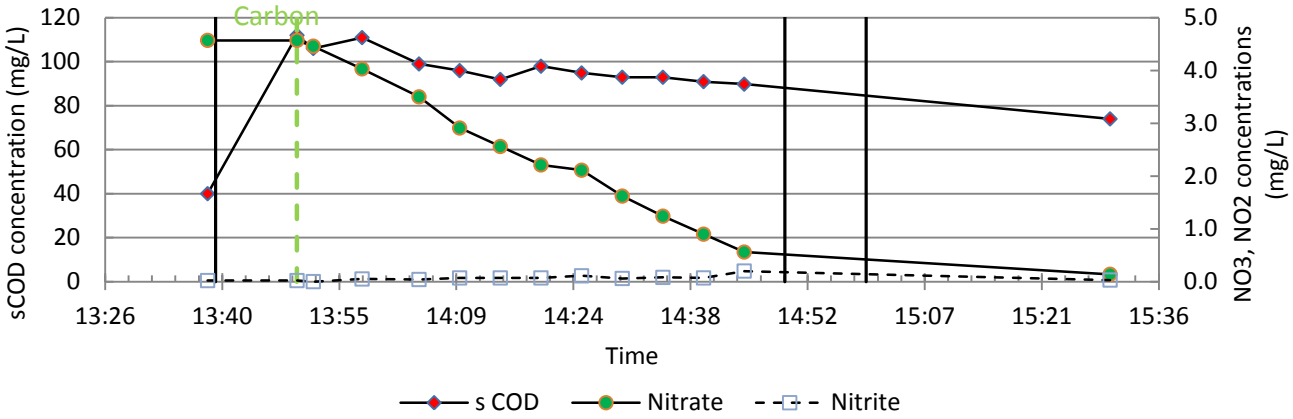
A.4: 11/2/11 Second Anoxic Zone Profile



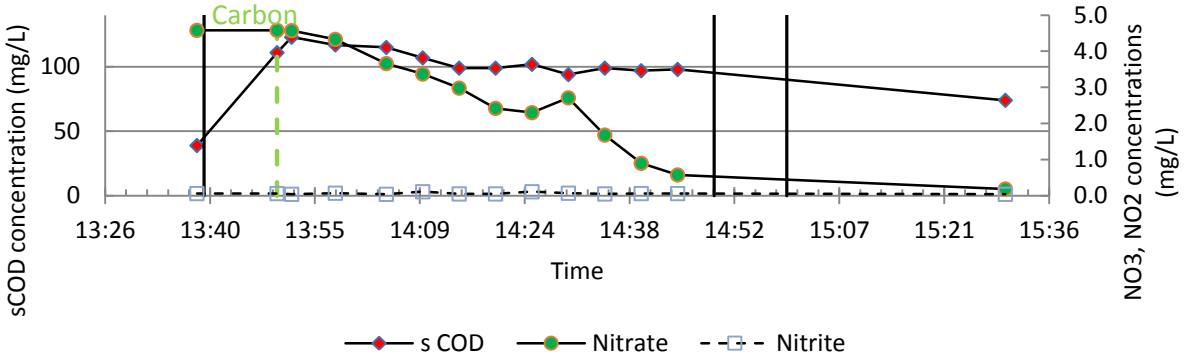
A.5: 11/3/11 Second Anoxic Zone Profile



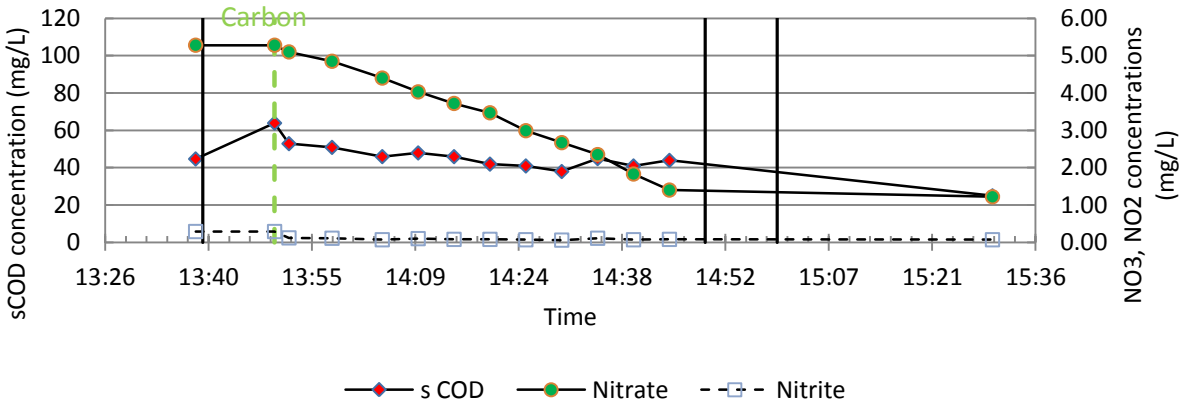
A.6: 11/7/11 Second Anoxic Zone Profile



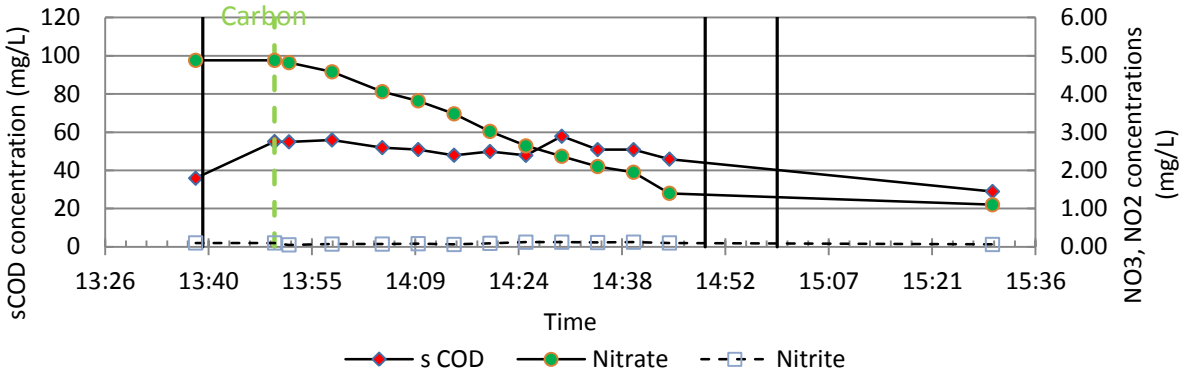
A.7: 11/9/11 Second Anoxic Zone Profile



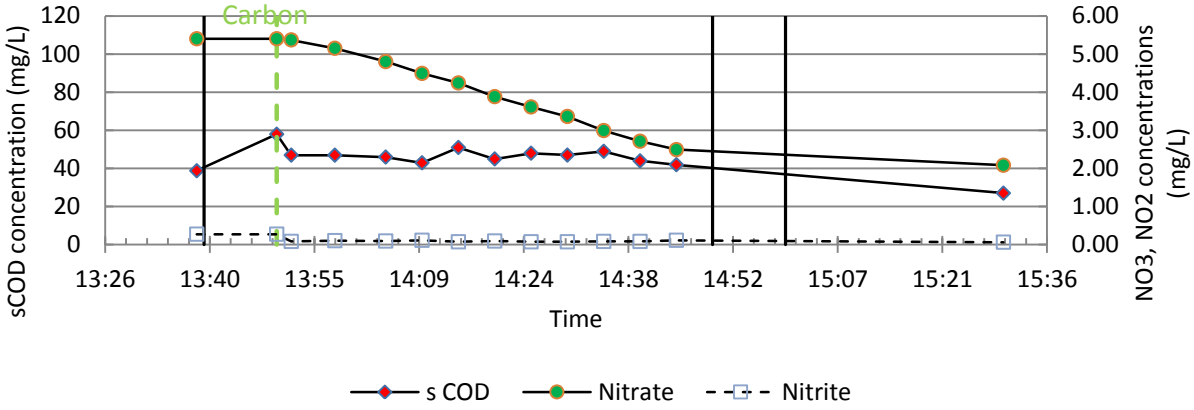
A.8: 11/10/11 Second Anoxic Zone Profile



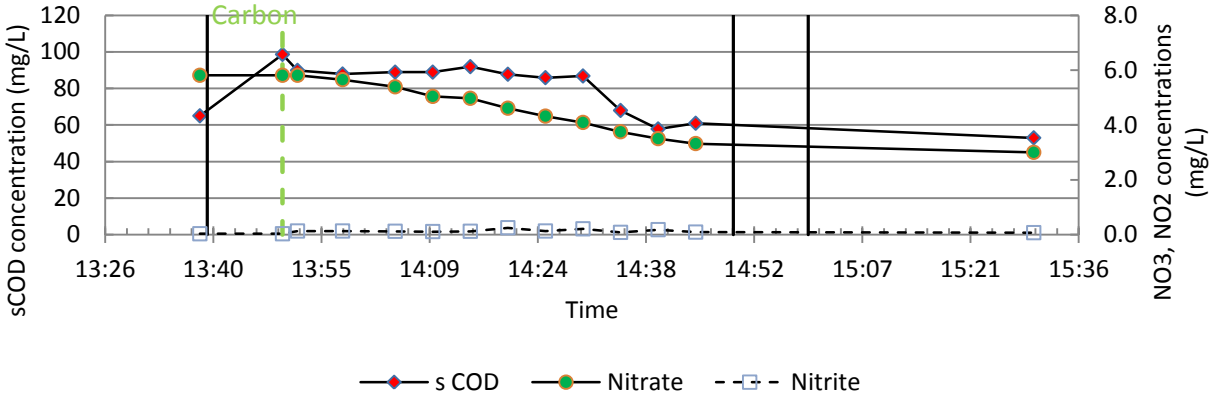
A.9: 11/14/11 Second Anoxic Zone Profile



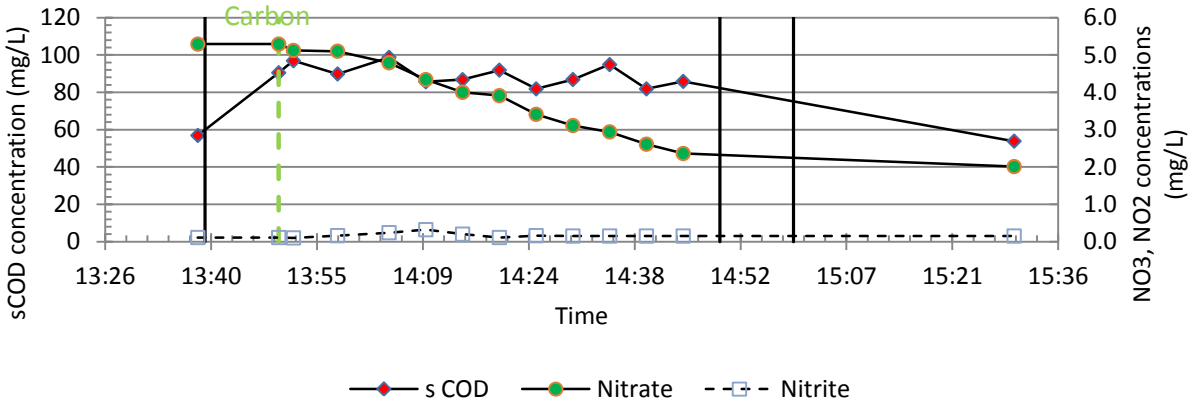
A.10: 11/16/11 Second Anoxic Zone Profile



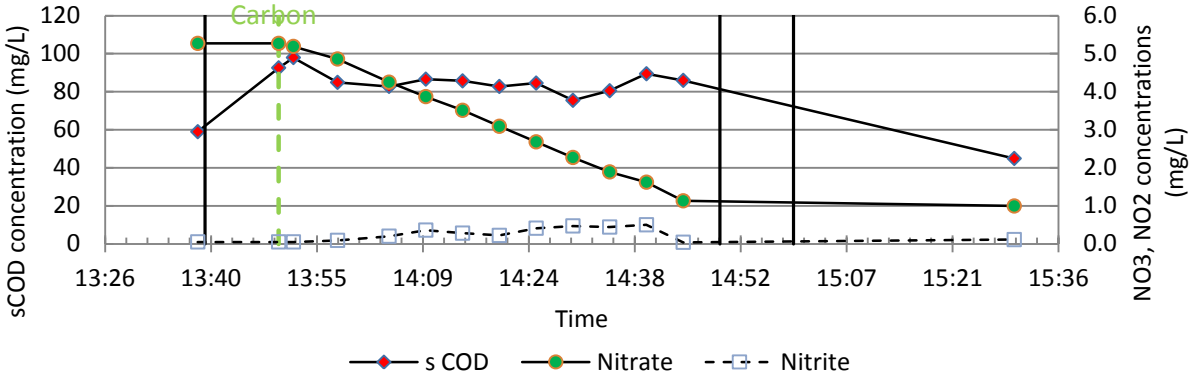
A.11: 11/17/11 Second Anoxic Zone Profile



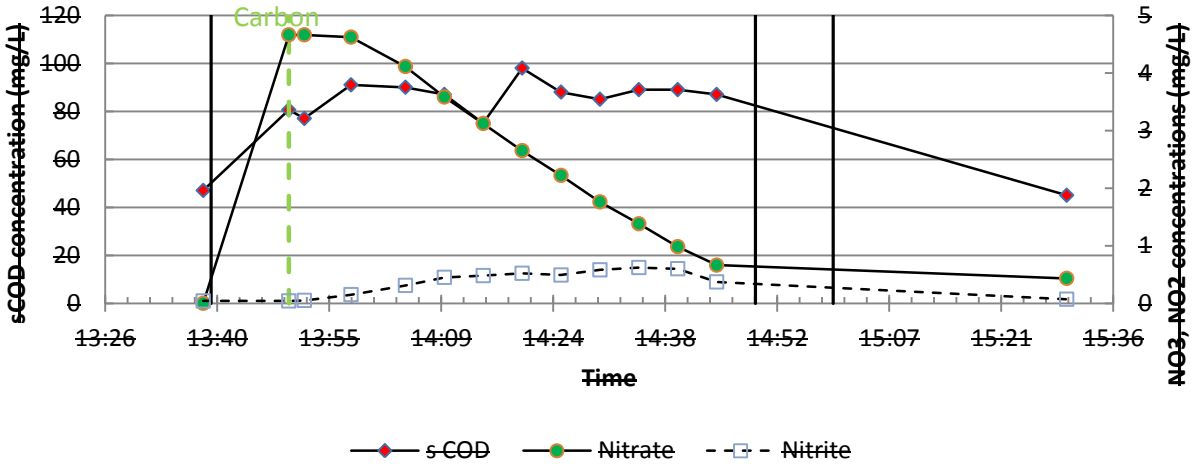
A.12: 11/30/11 Second Anoxic Zone Profile



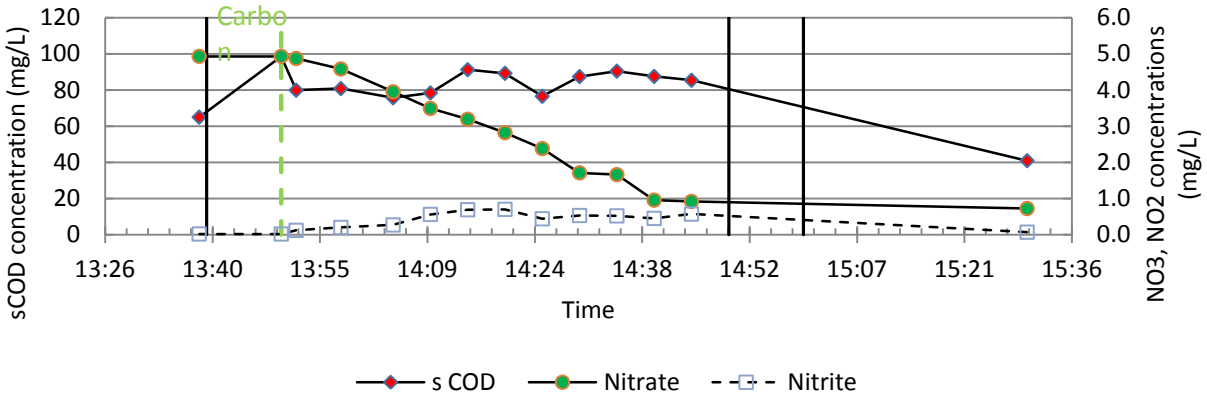
A.13: 12/1/11 Second Anoxic Zone Profile



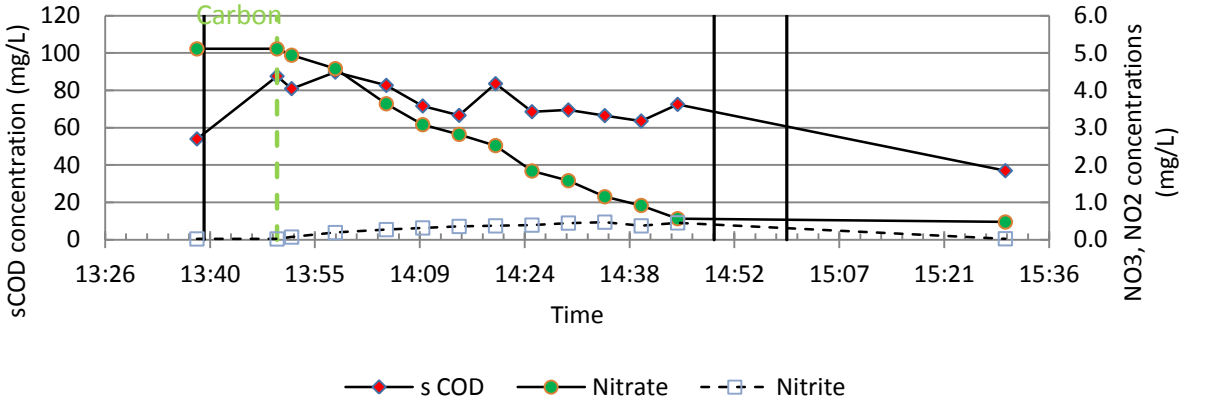
A.14: 12/5/11 Second Anoxic Zone Profile



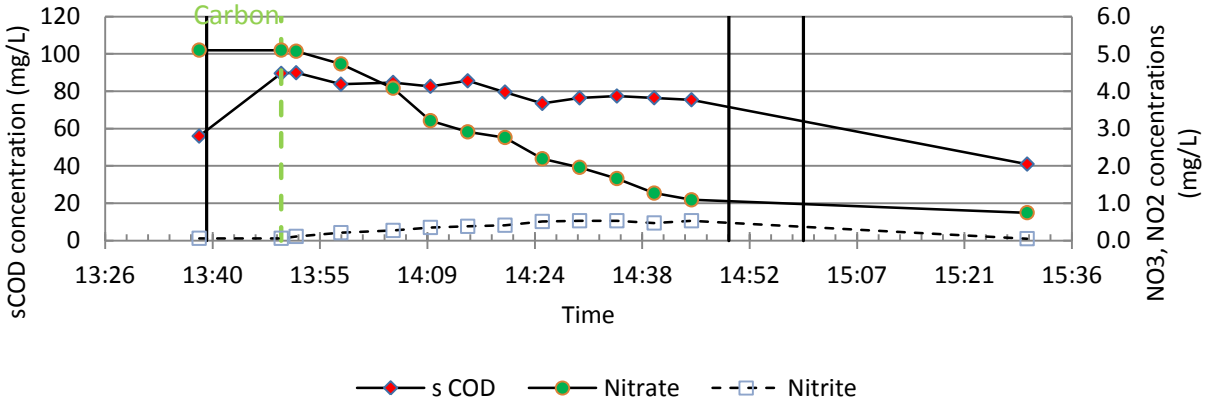
A.15: 12/7/11 Second Anoxic Zone Profile



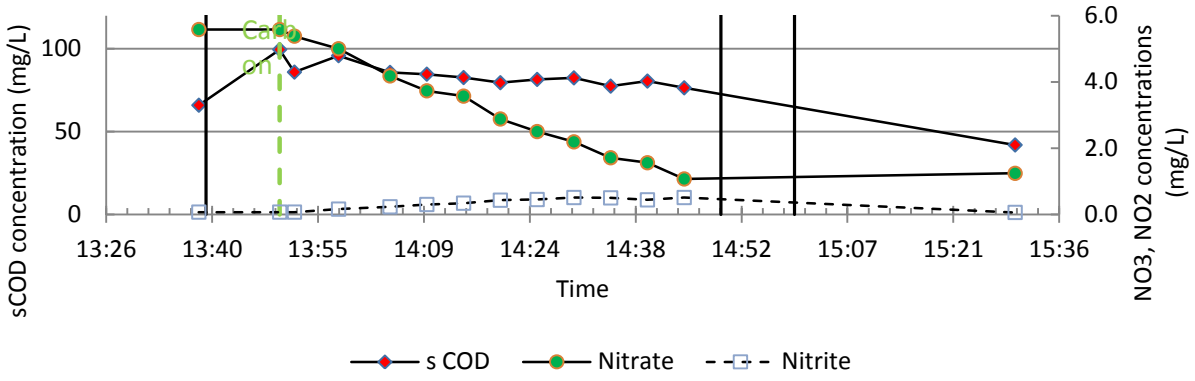
A.16: 12/8/11 Second Anoxic Zone Profile



A.17: 12/12/11 Second Anoxic Zone Profile

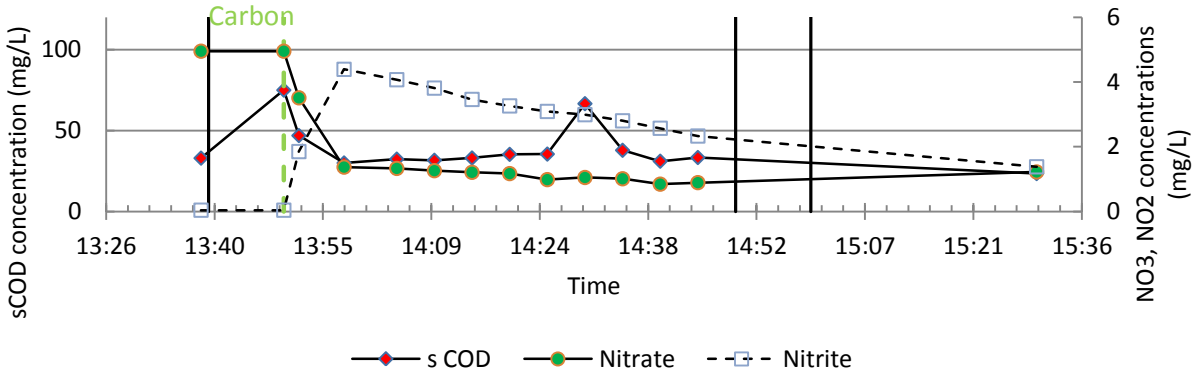


A.18: 12/14/11 Second Anoxic Zone Profile

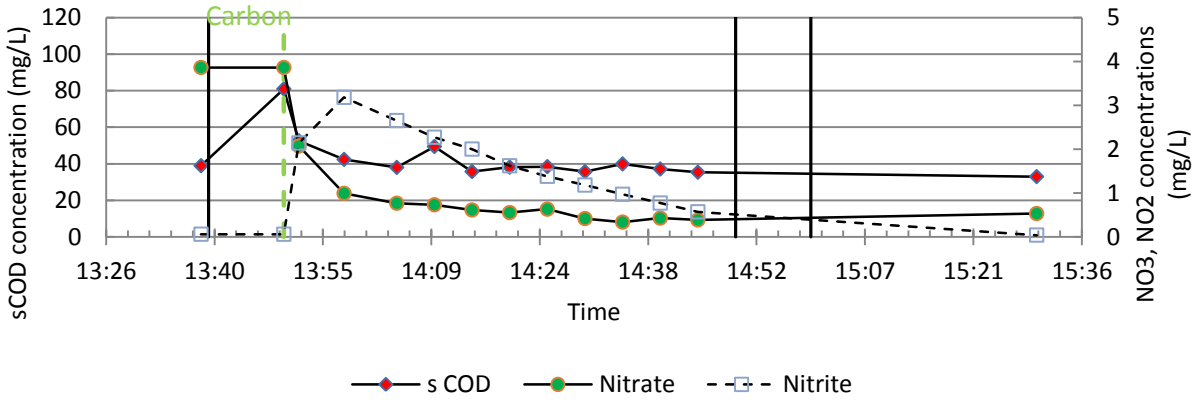


A.19: 12/15/11 Second Anoxic Zone Profile

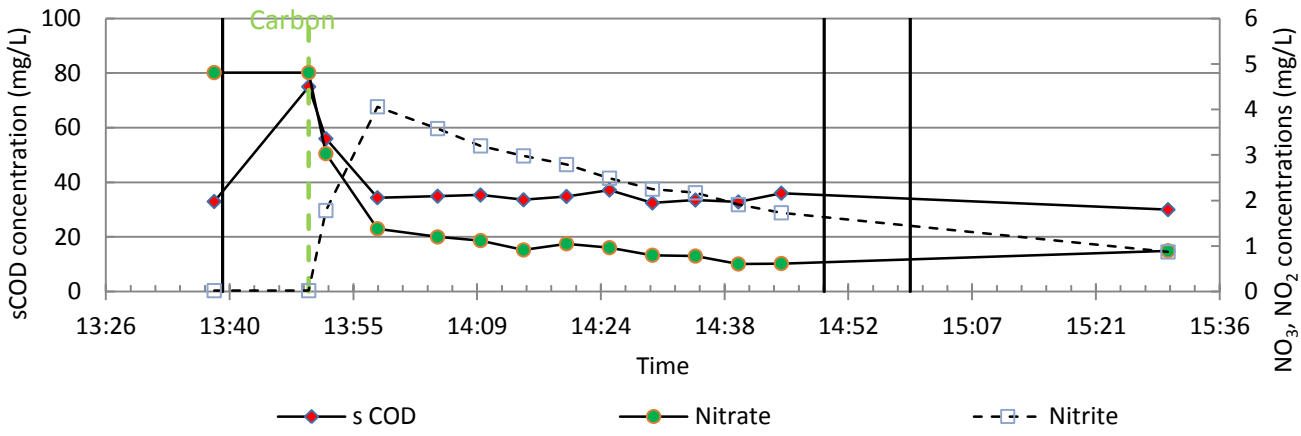
A.3 GLYCEROL



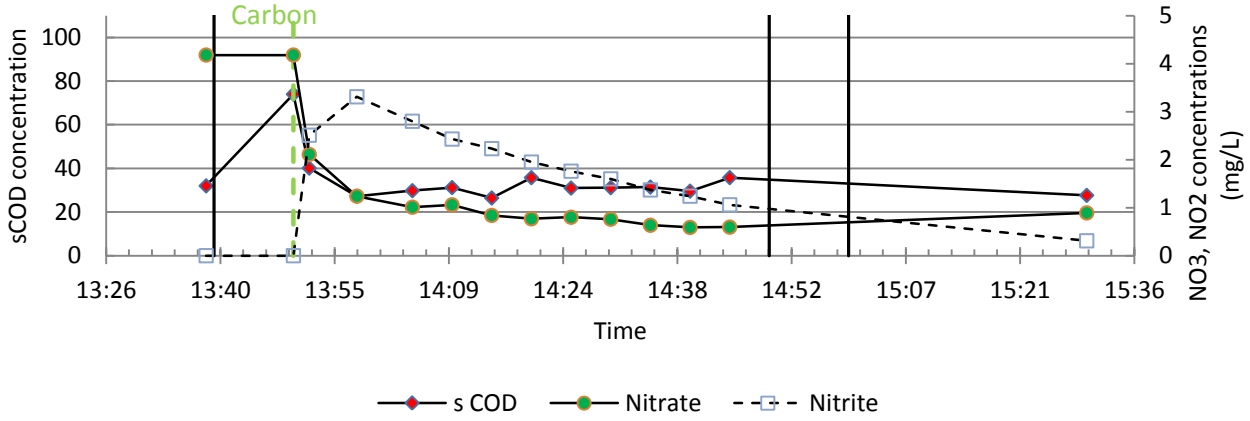
A.20: 10/24/11 Second Anoxic Zone Profile



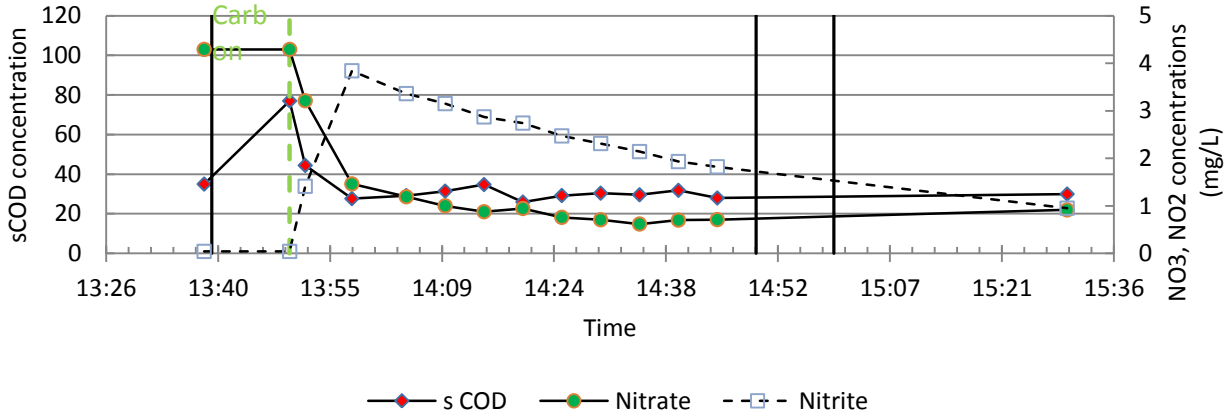
A.21: 10/26/11 Second Anoxic Zone Profile



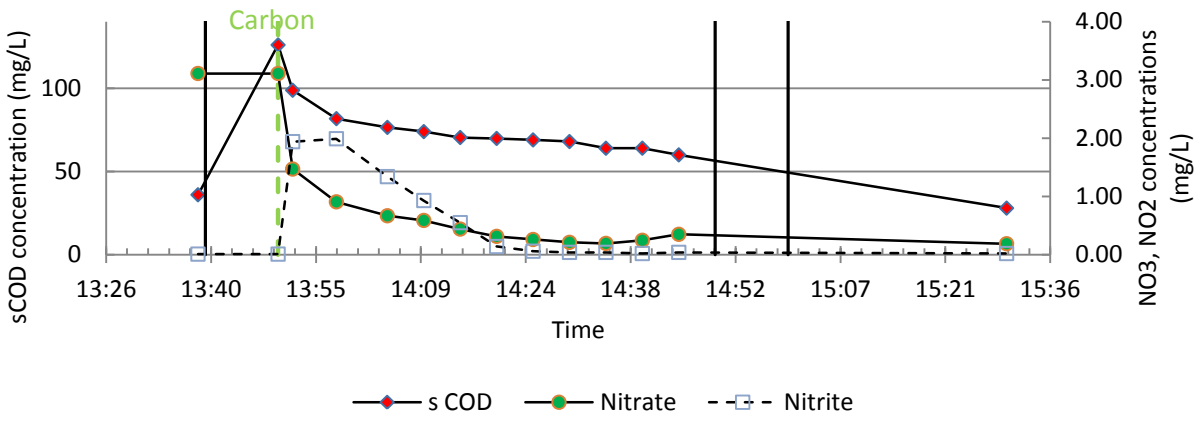
A.22: 10/27/11 Second Anoxic Zone Profile



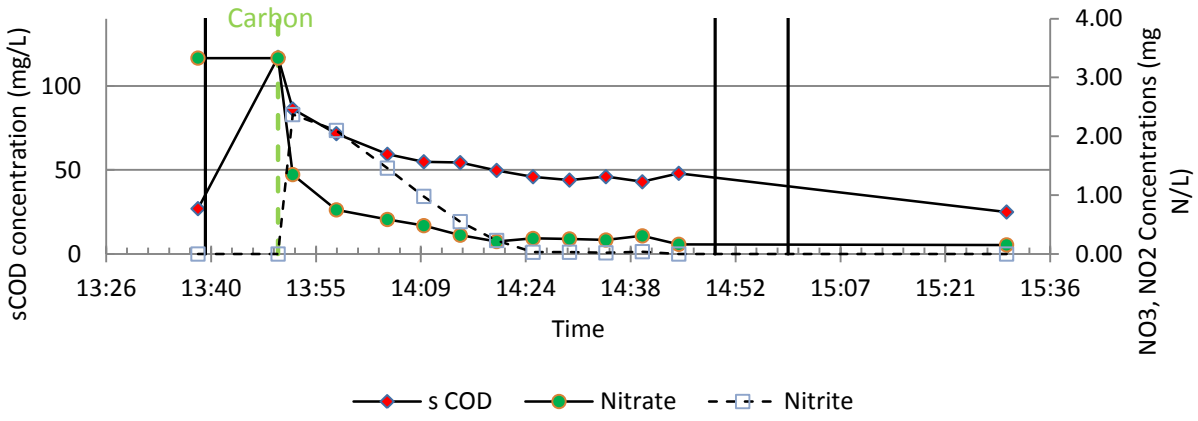
A.23: 11/2/11 Second Anoxic Zone Profile



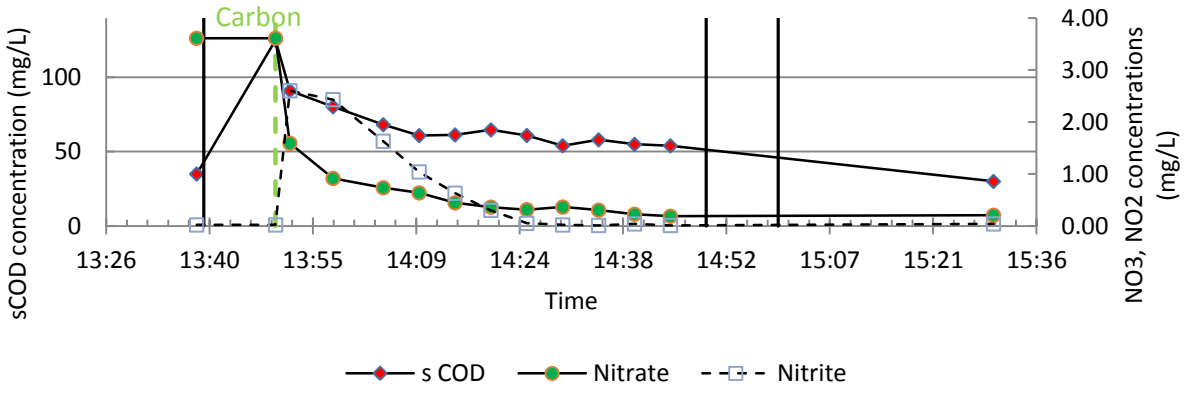
A.24: 11/3/11 Second Anoxic Zone Profile



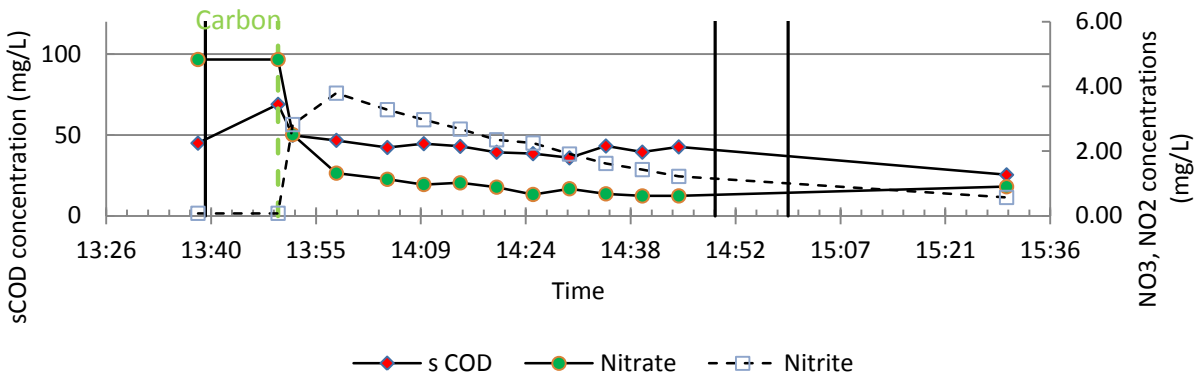
A.25: 11/7/11 Second Anoxic Zone Profile



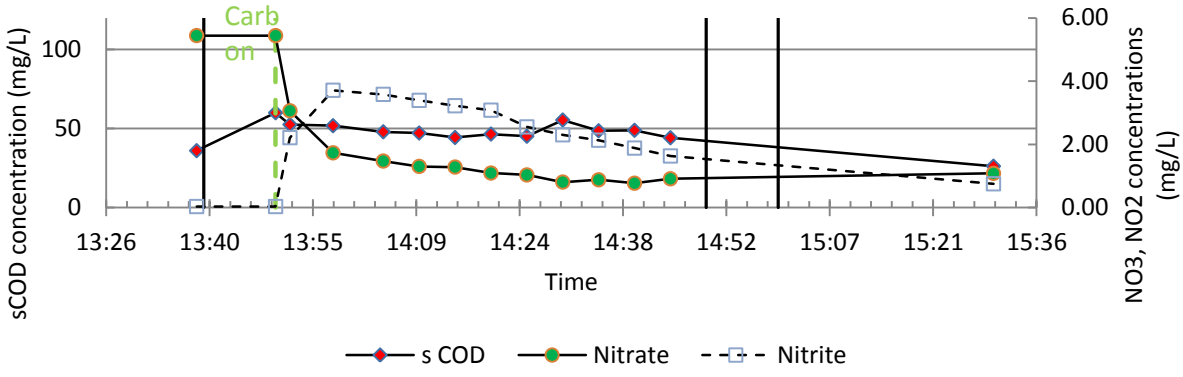
A.26: 11/9/11 Second Anoxic Zone Profile



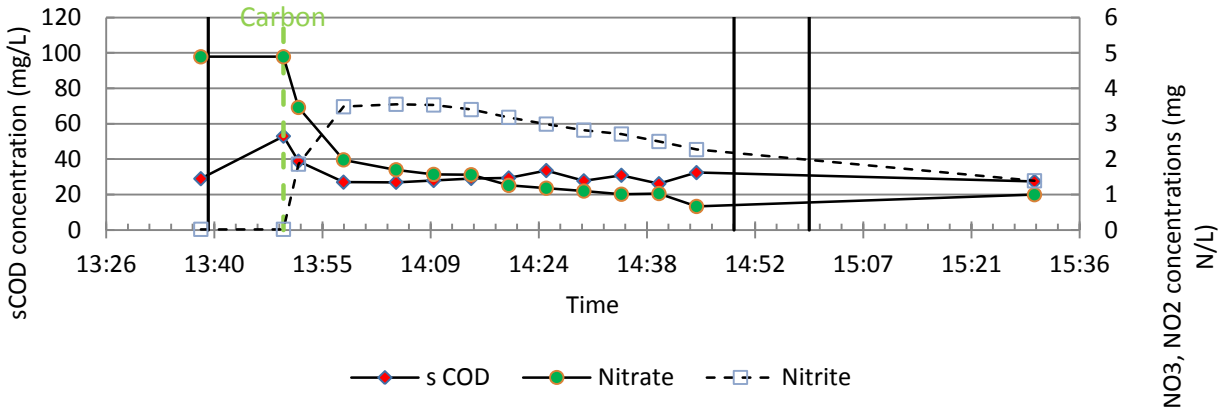
A.27: 11/10/11 Second Anoxic Zone Profile



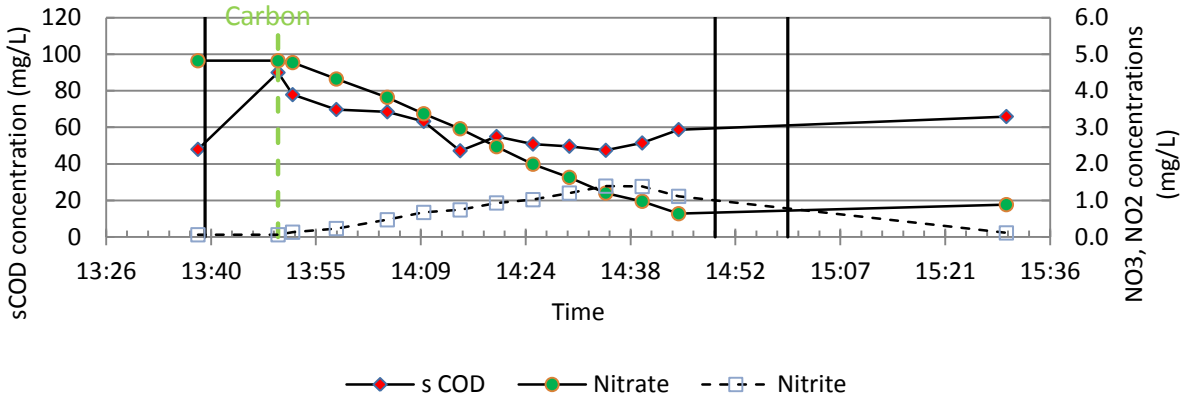
A.28: 11/14/11 Second Anoxic Zone Profile



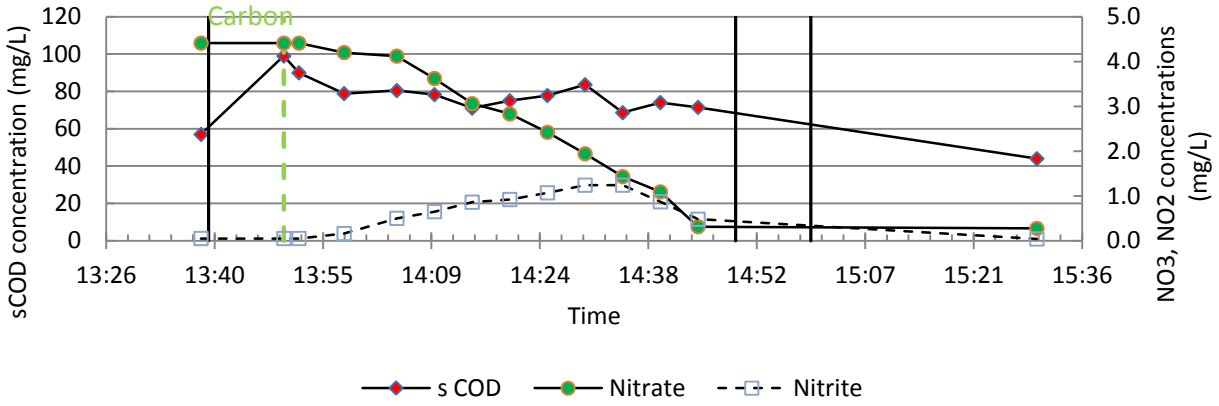
A.29: 11/16/11 Second Anoxic Zone Profile



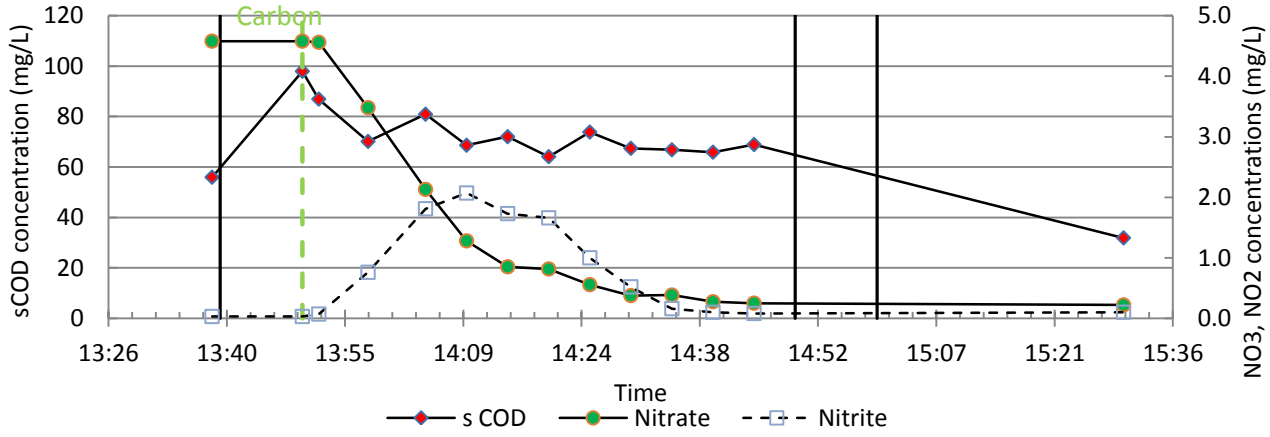
A.30: 11/17/11 Second Anoxic Zone Profile



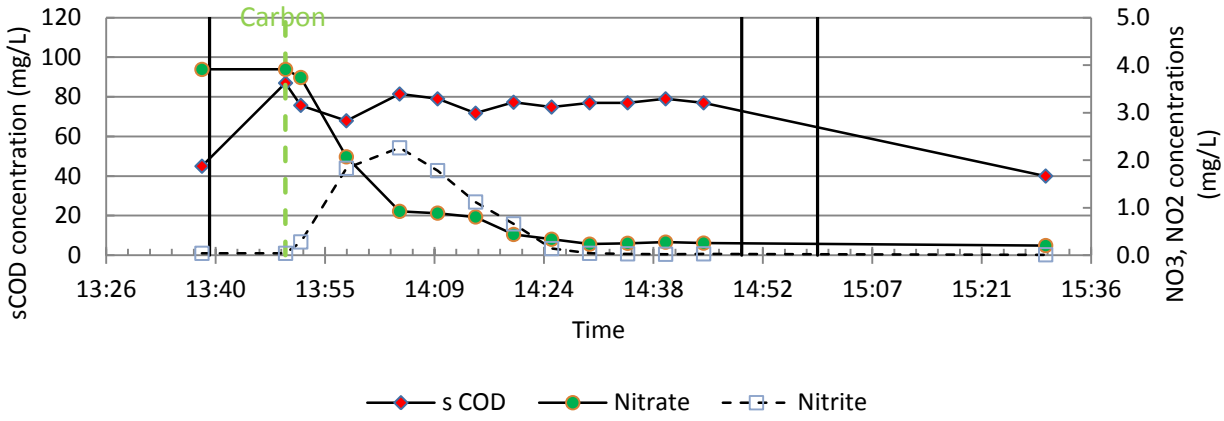
A.31: 11/30/11 Second Anoxic Zone Profile



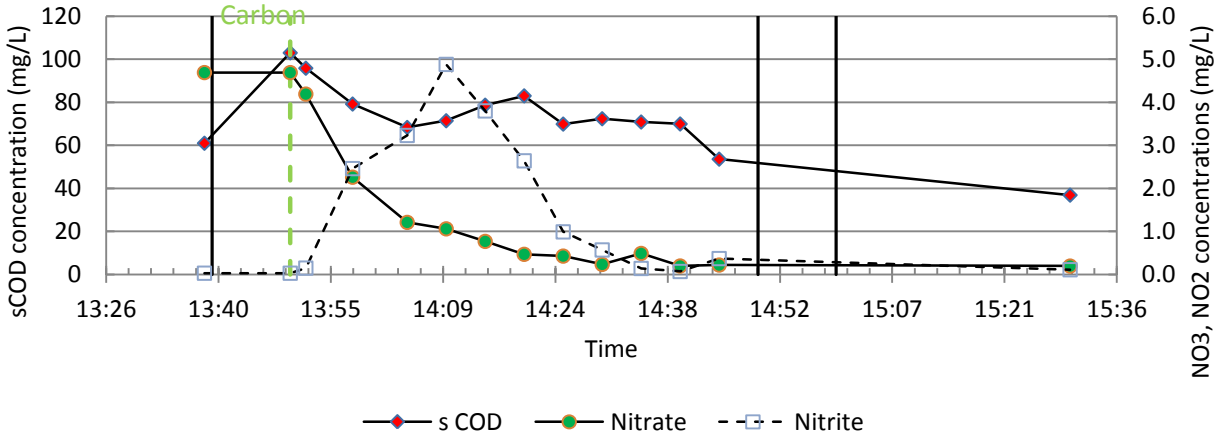
A.32: 12/1/11 Second Anoxic Zone Profile



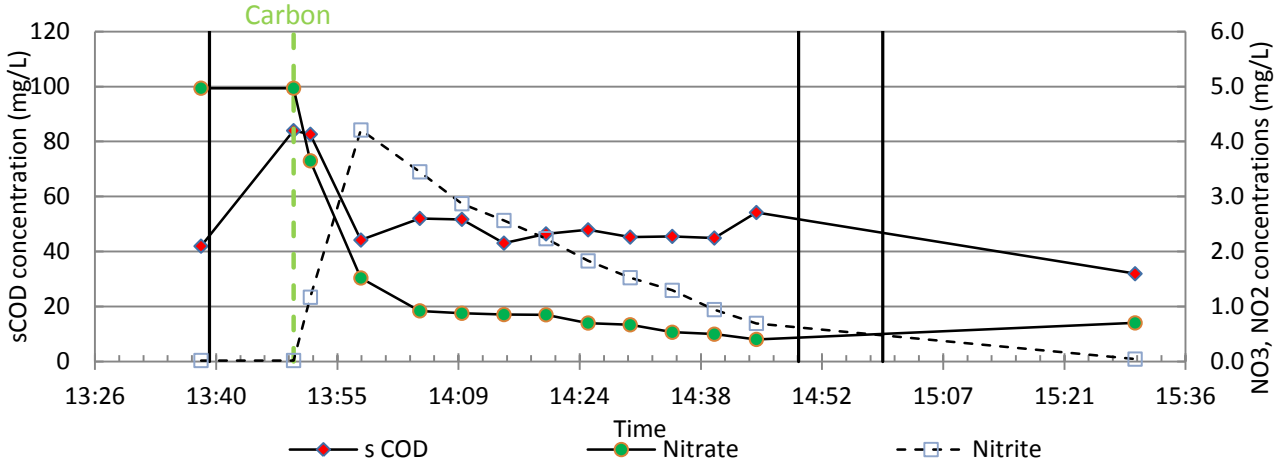
A.33: 12/5/11 Second Anoxic Zone Profile



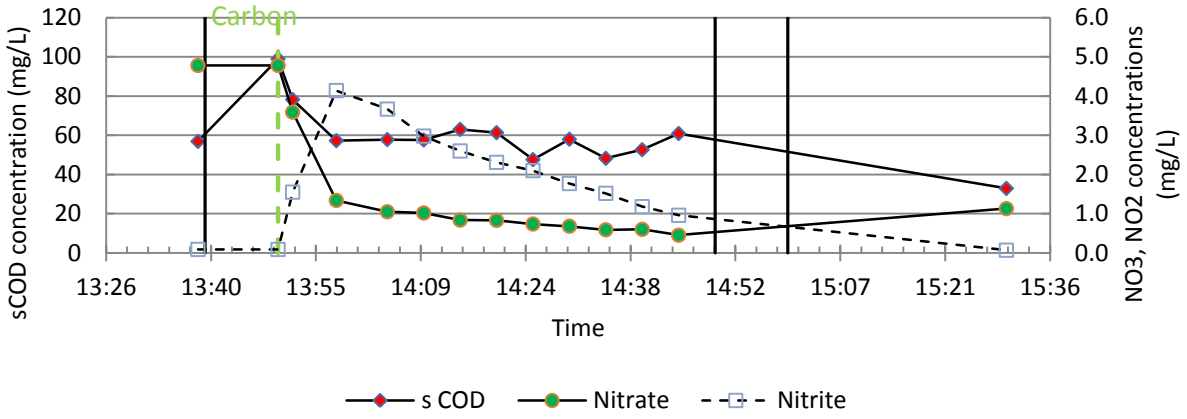
A.34: 12/7/11 Second Anoxic Zone Profile



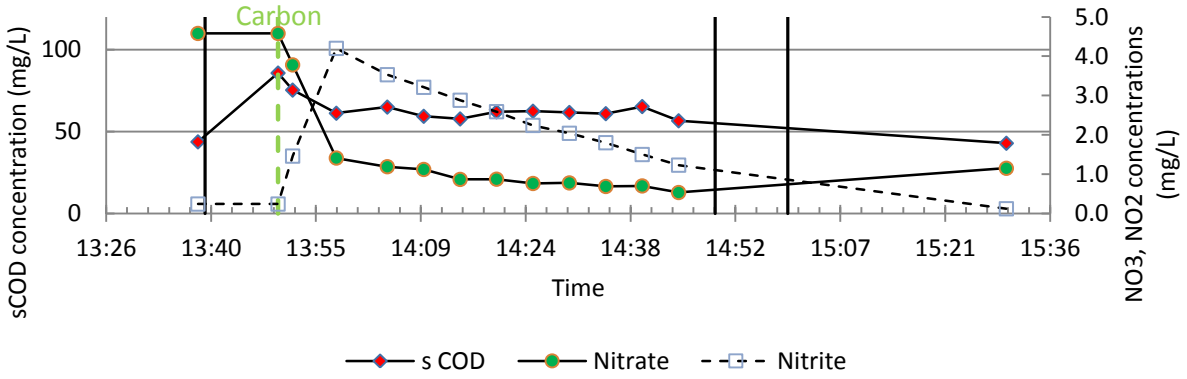
A.35: 12/8/11 Second Anoxic Zone Profile



A.36: 12/12/11 Second Anoxic Zone Profile

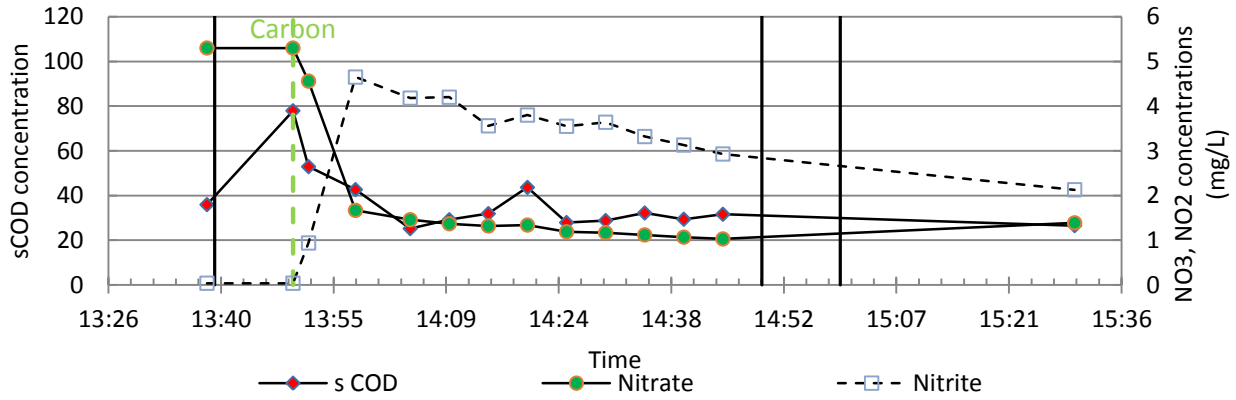


A.37: 12/14/11 Second Anoxic Zone Profile

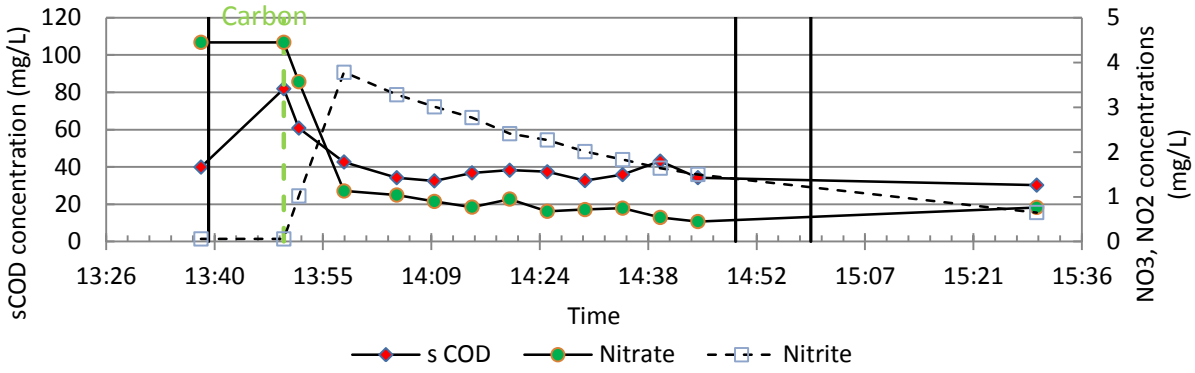


A.38: 12/15/11 Second Anoxic Zone Profile

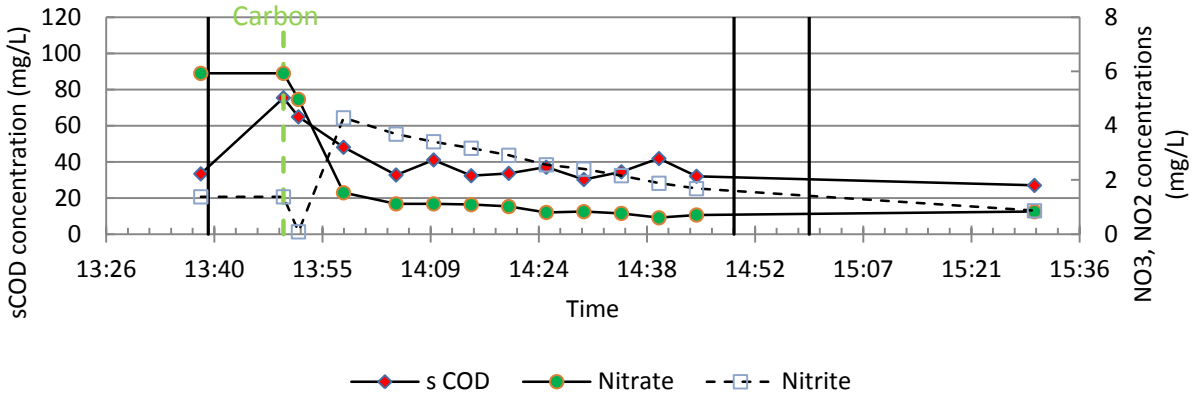
A.4 BGW



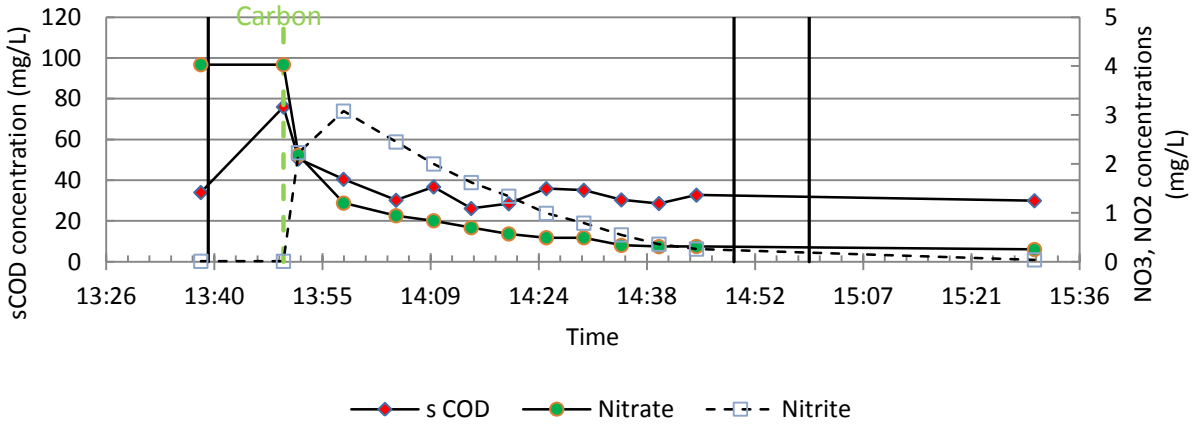
A.39: 10/24/11 Second Anoxic Zone Profile



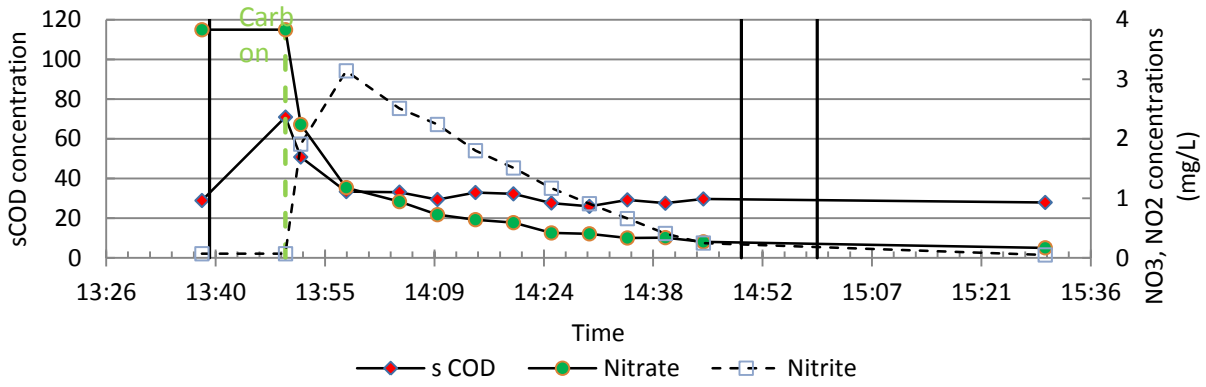
A.40: 10/26/11 Second Anoxic Zone Profile



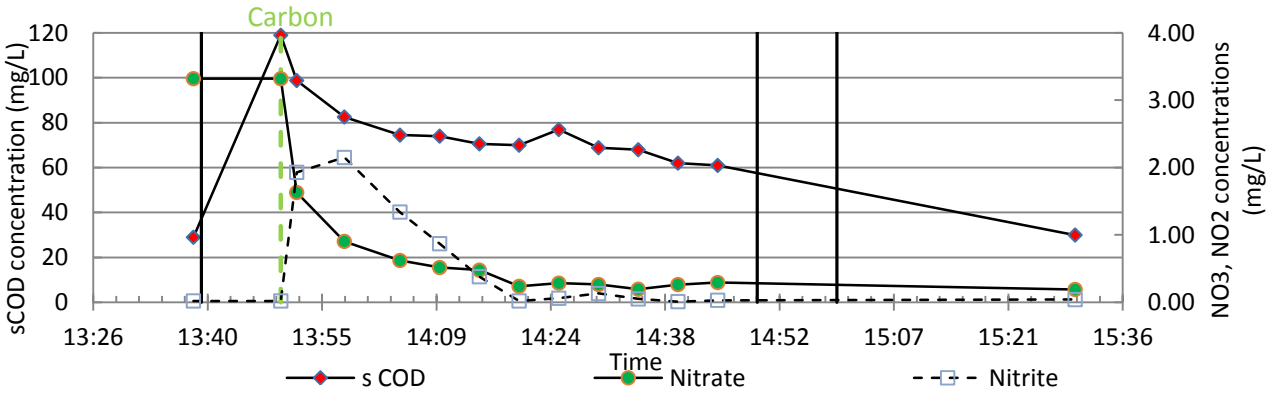
A.41: 10/27/11 Second Anoxic Zone Profile



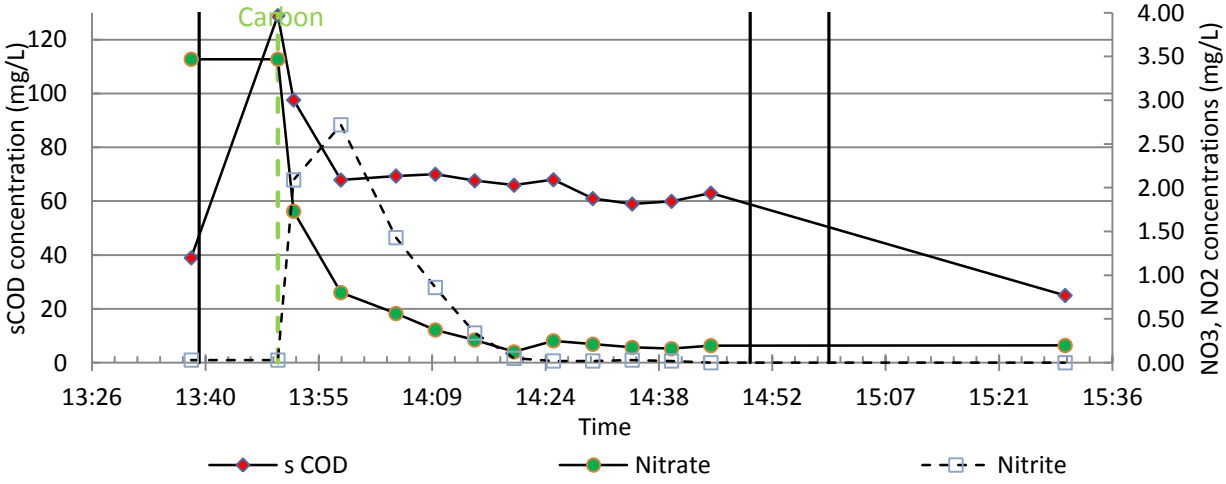
A.42: 11/2/11 Second Anoxic Zone Profile



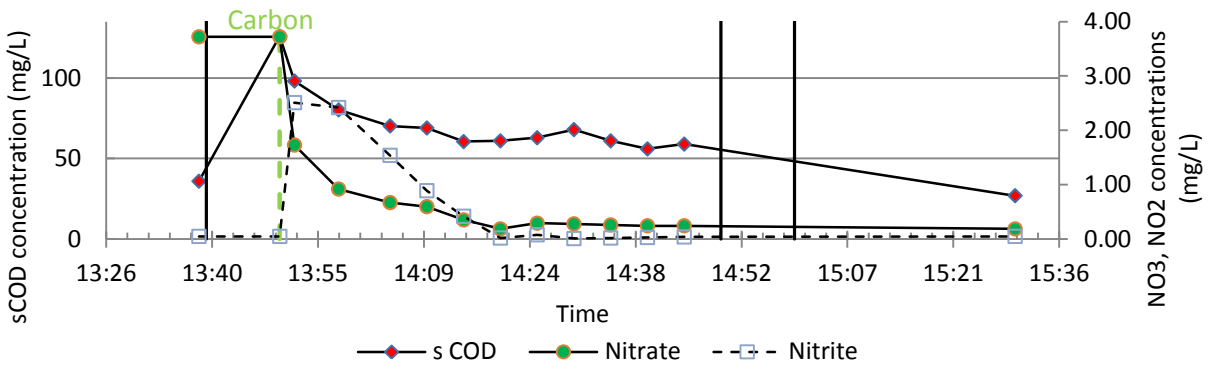
A.43: 11/3/11 Second Anoxic Zone Profile



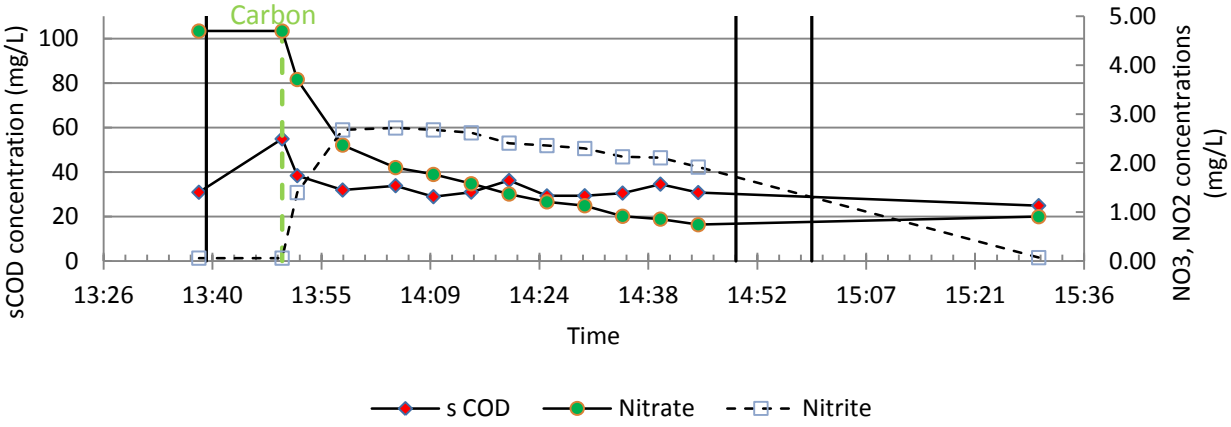
A.44: 11/7/11 Second Anoxic Zone Profile



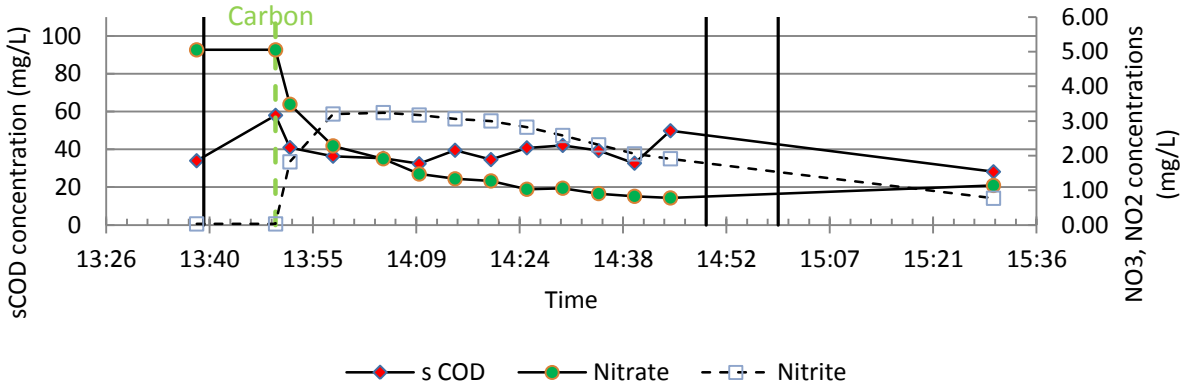
A.45: 11/9/11 Second Anoxic Zone Profile



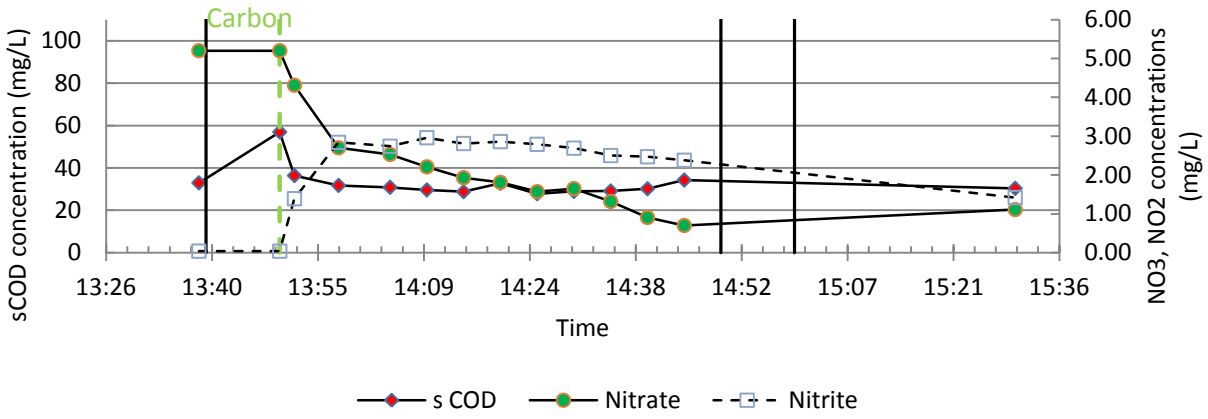
A.46: 11/10/11 Second Anoxic Zone Profile



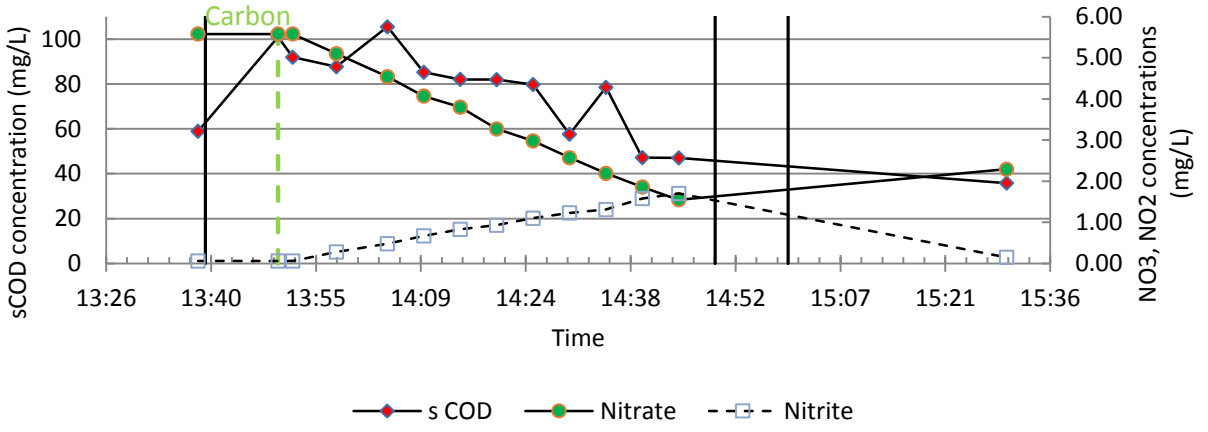
A.47: 11/14/11 Second Anoxic Zone Profile



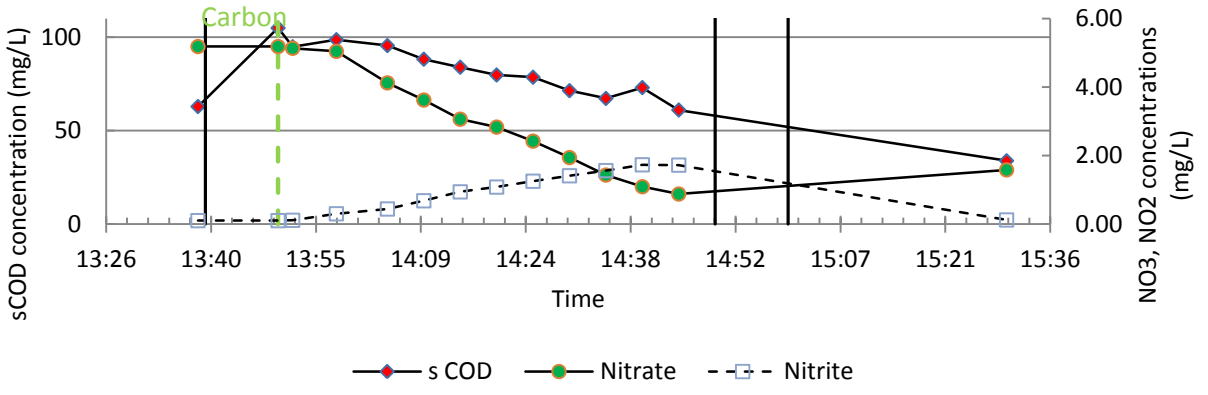
A.48: 11/16/11 Second Anoxic Zone Profile



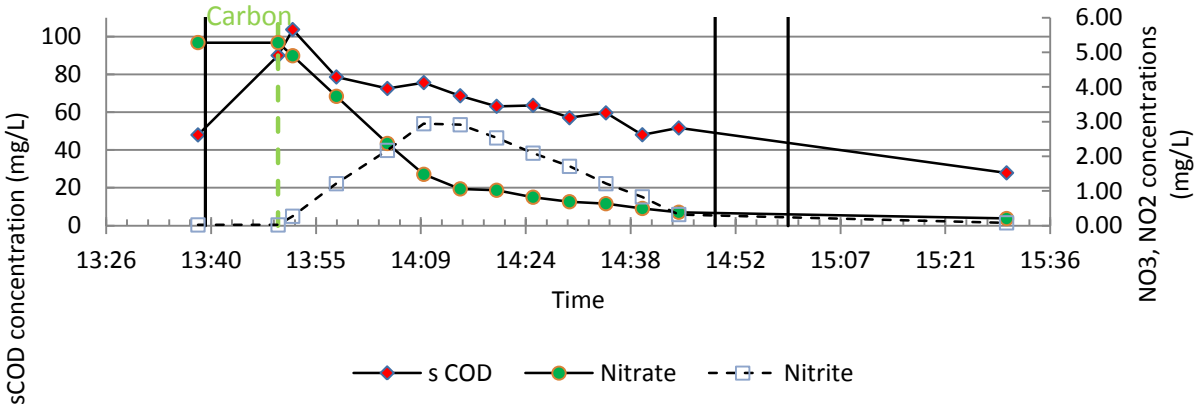
A.49: 11/17/11 Second Anoxic Zone Profile



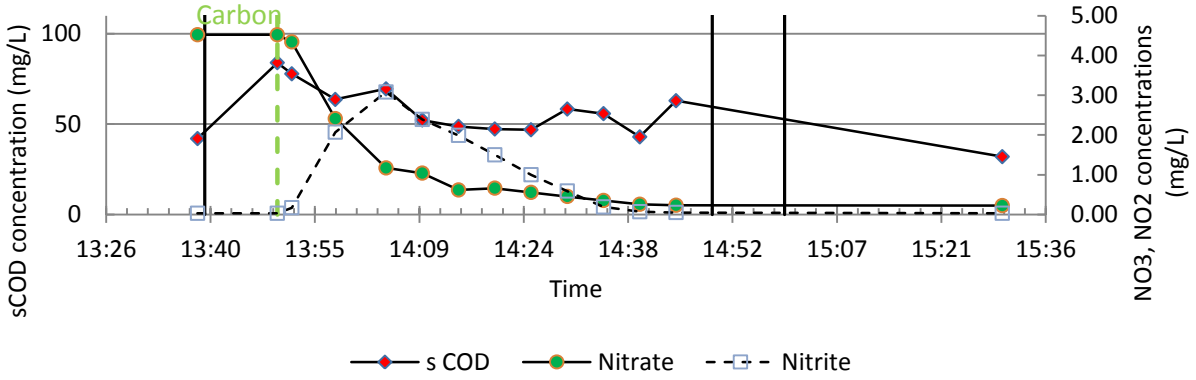
A.50: 11/30/11 Second Anoxic Zone Profile



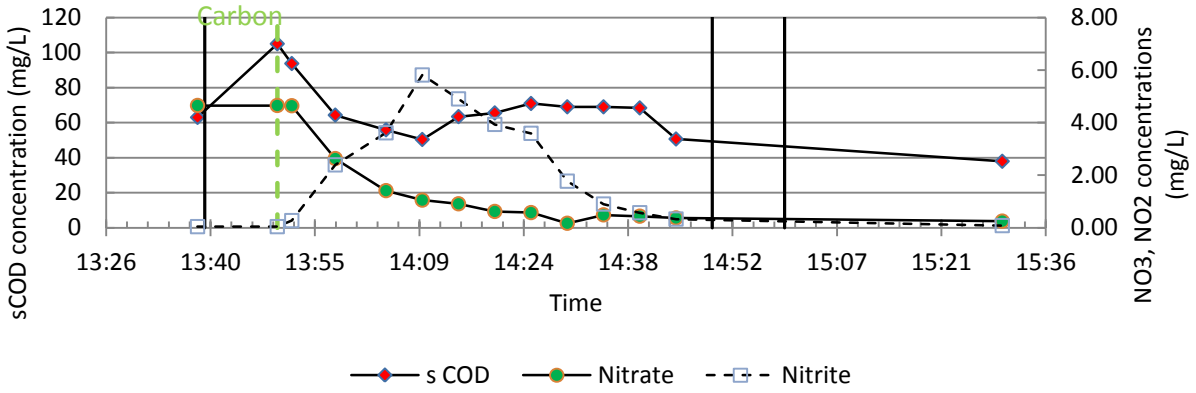
A.51: 12/1/11 Second Anoxic Zone Profile



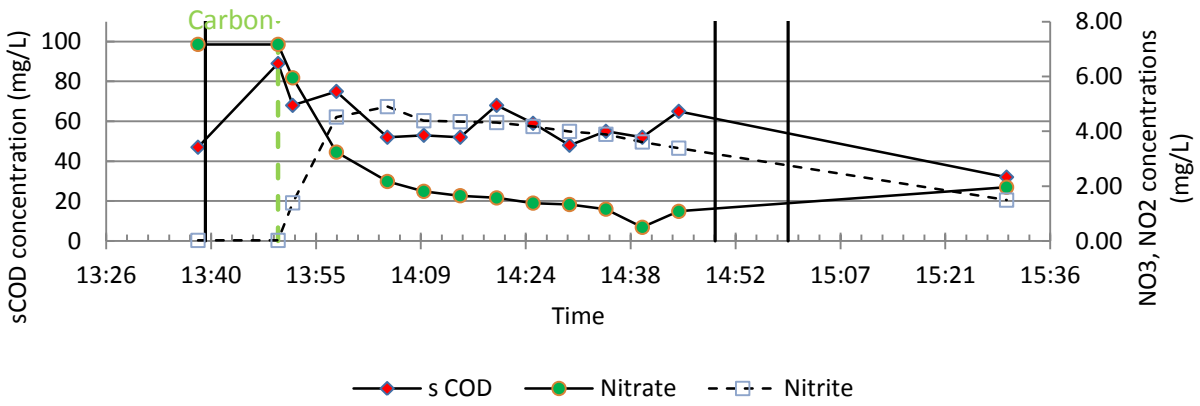
A.52: 12/5/11 Second Anoxic Zone Profile



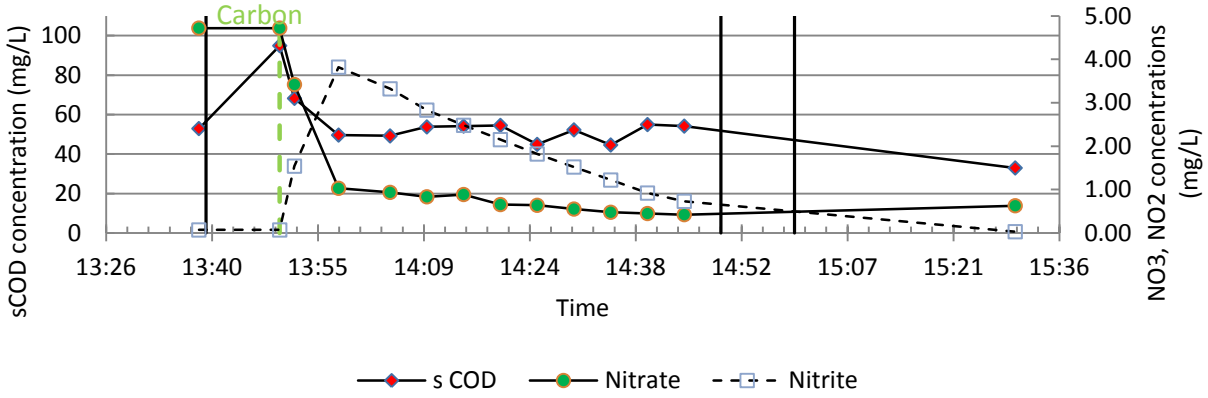
A.53: 12/7/11 Second Anoxic Zone Profile



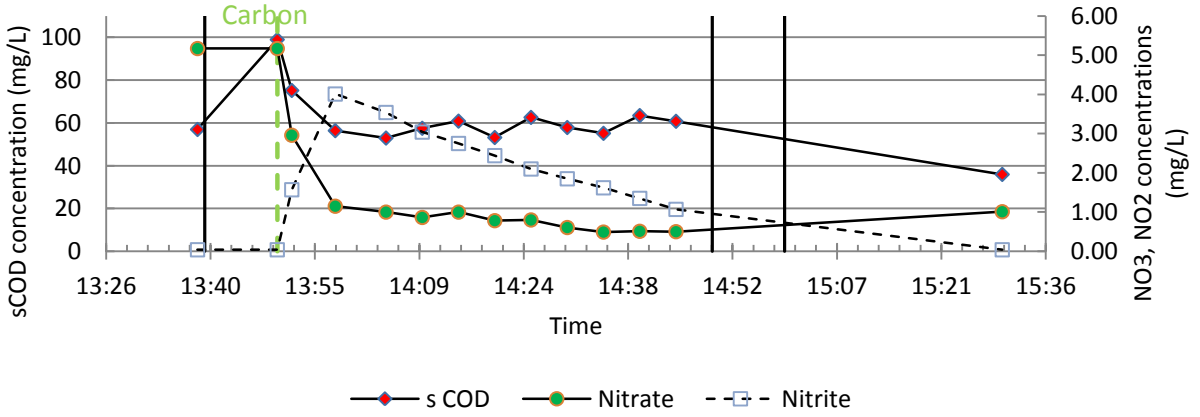
A.54: 12/8/11 Second Anoxic Zone Profile



A.55: 12/12/11 Second Anoxic Zone Profile

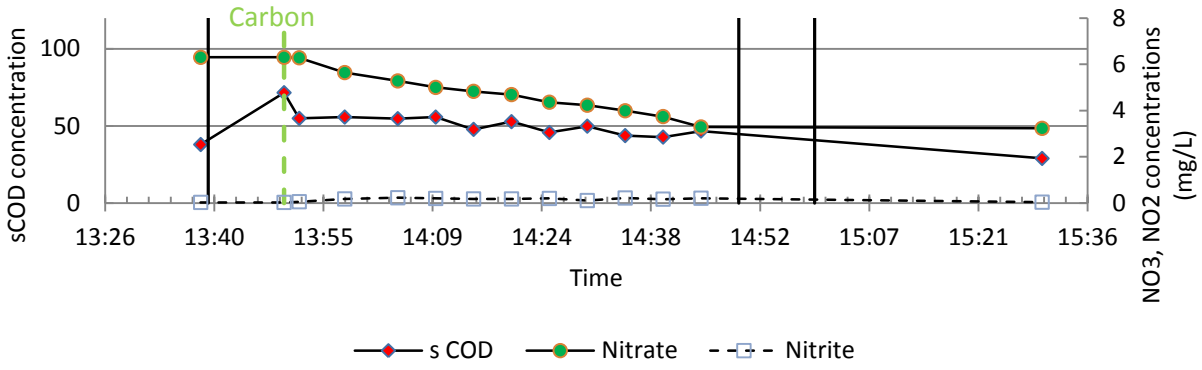


A.56: 12/14/11 Second Anoxic Zone Profile

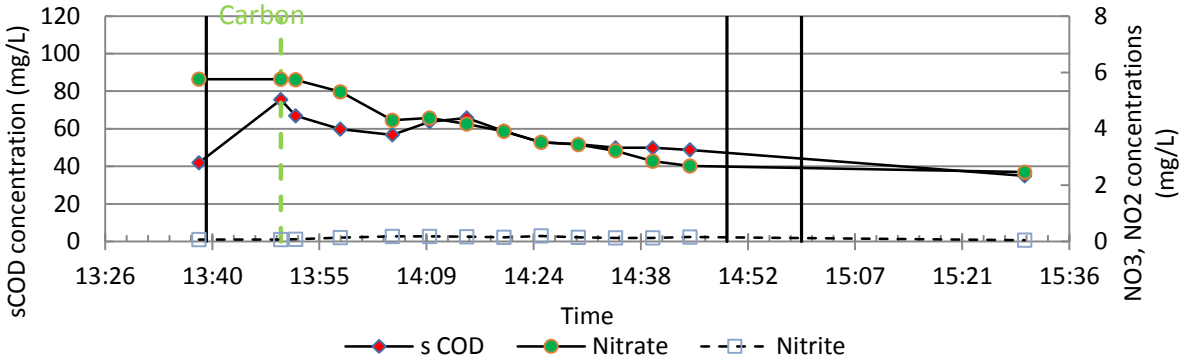


A.57: 12/15/11 Second Anoxic Zone Profile

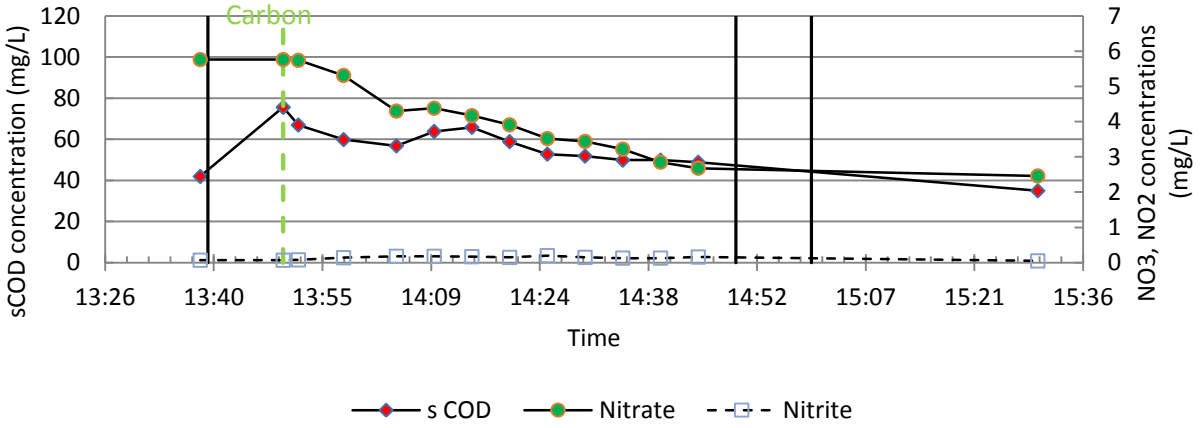
A.5 Waste Alcohol



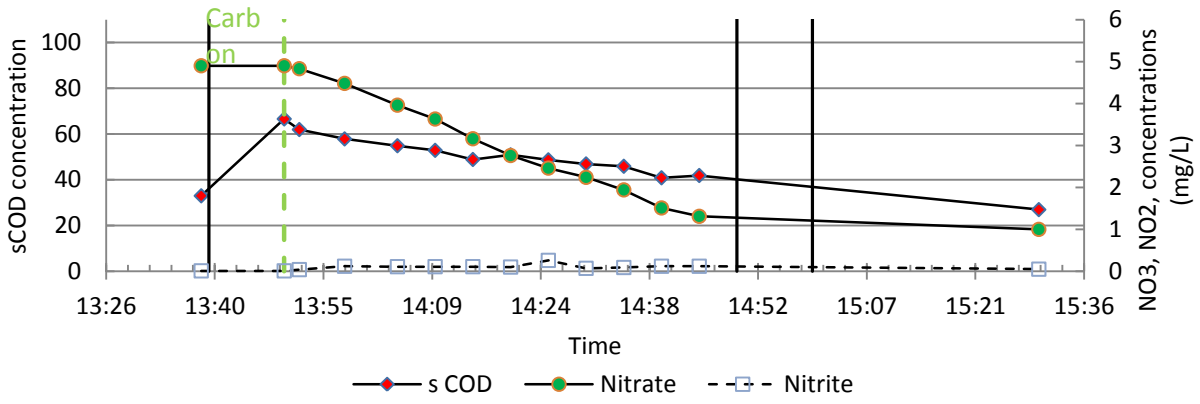
A.58: 10/24/11 Second Anoxic Zone Profile



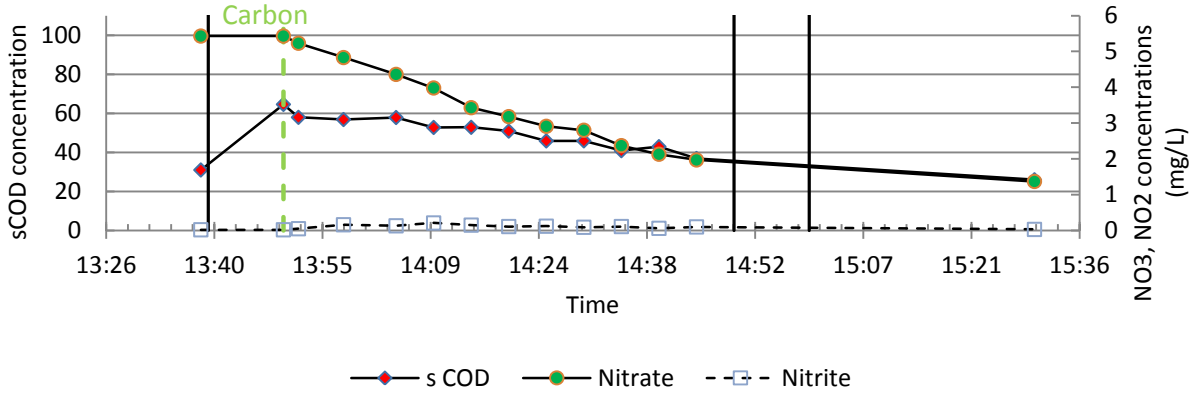
A.59: 10/26/11 Second Anoxic Zone Profile



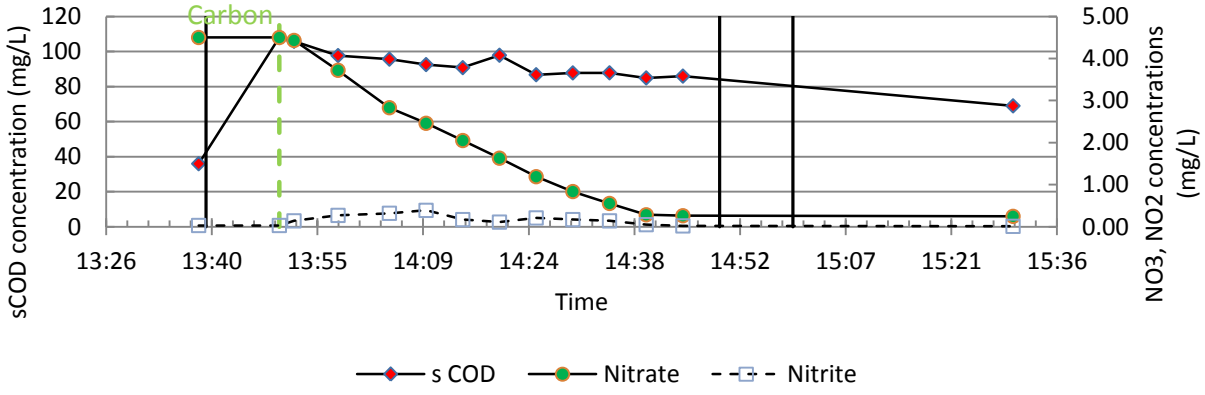
A.60: 10/27/11 Second Anoxic Zone Profile



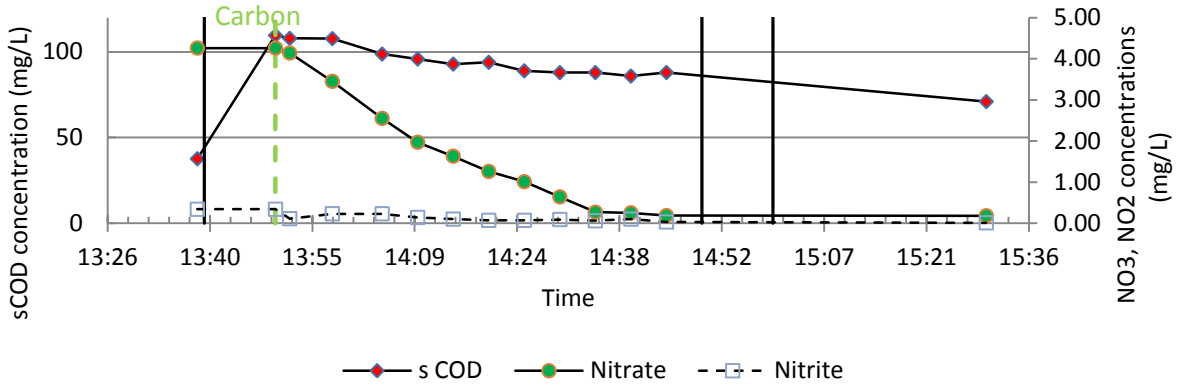
A.61: 11/2/11 Second Anoxic Zone Profile



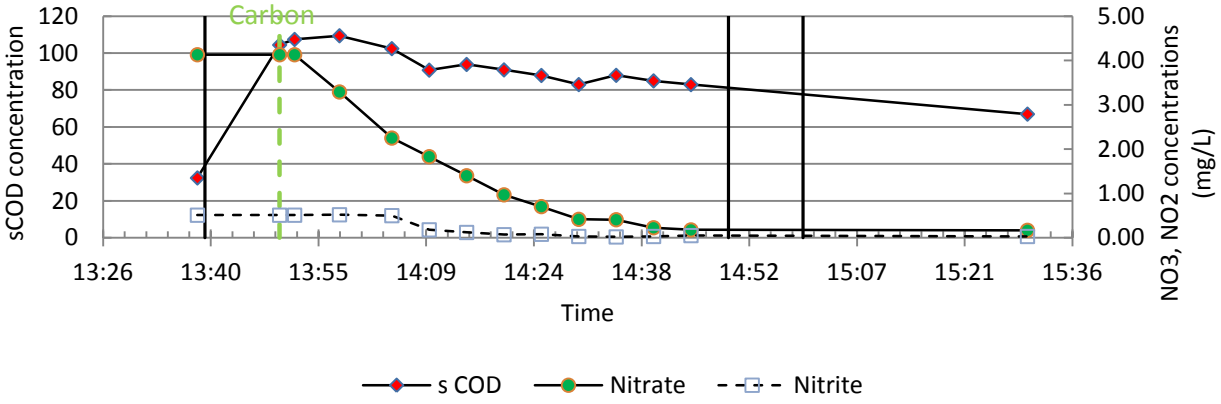
A.62: 11/3/11 Second Anoxic Zone Profile



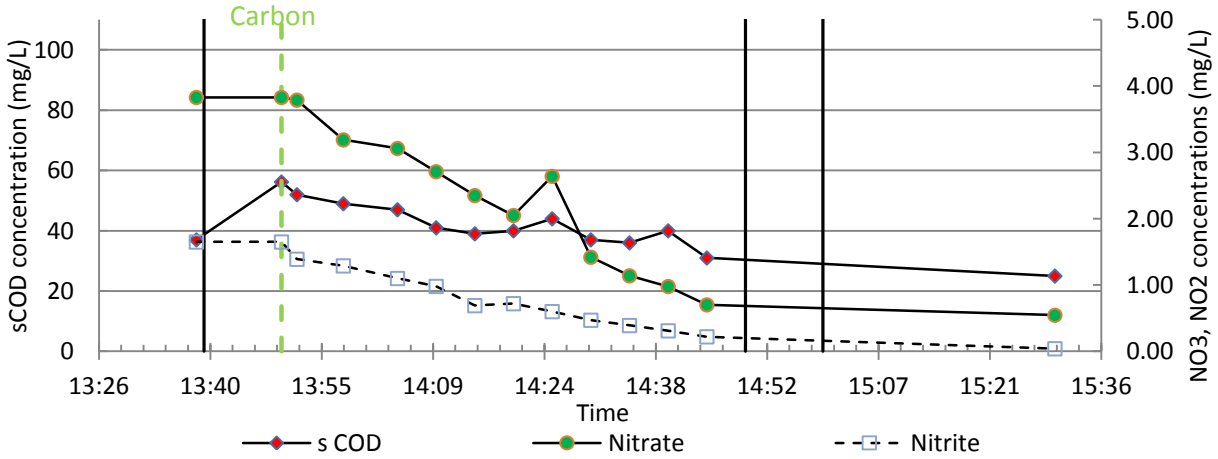
A.63: 11/7/11 Second Anoxic Zone Profile



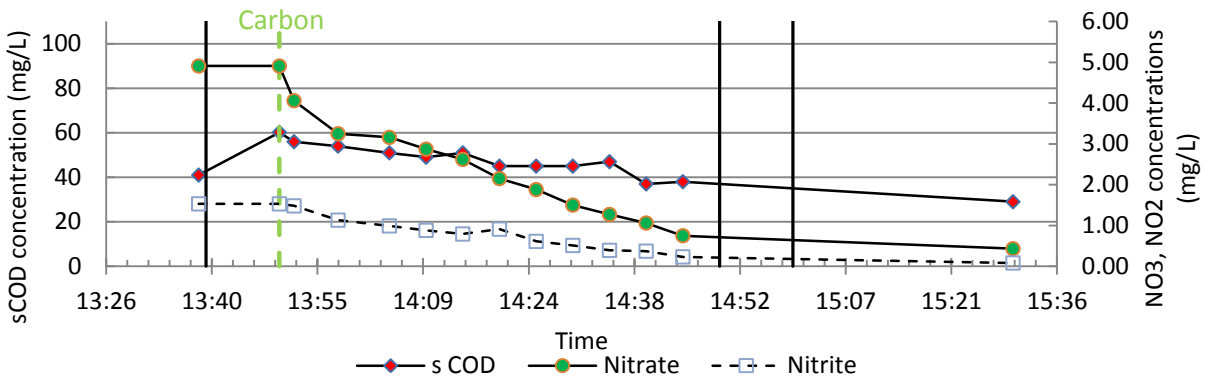
A.64: 11/9/11 Second Anoxic Zone Profile



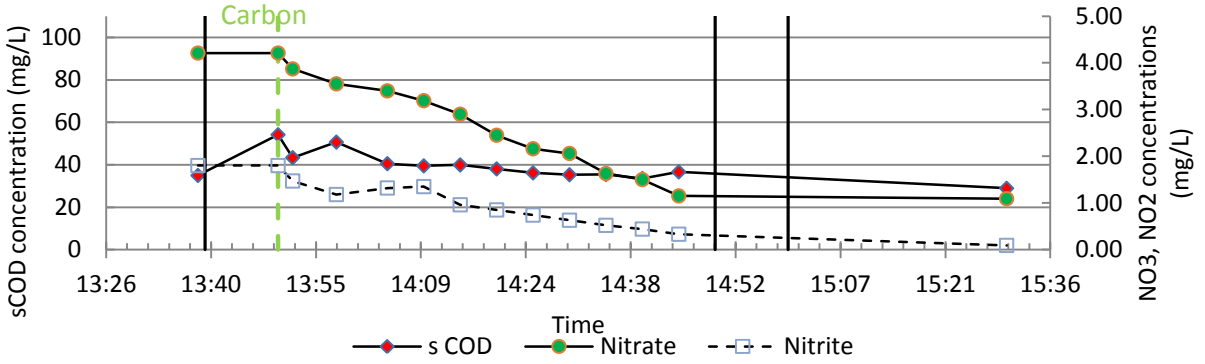
A.65: 11/10/11 Second Anoxic Zone Profile



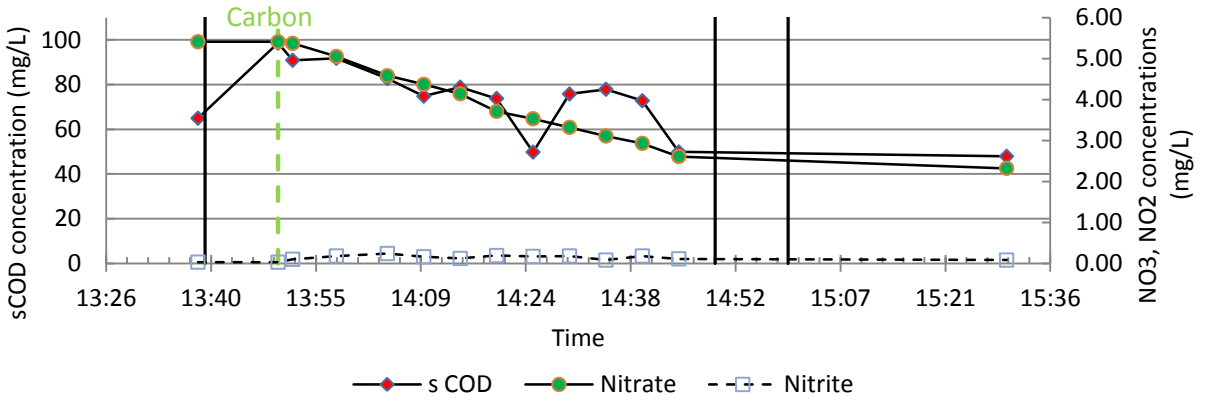
A.66: 11/14/11 Second Anoxic Zone Profile



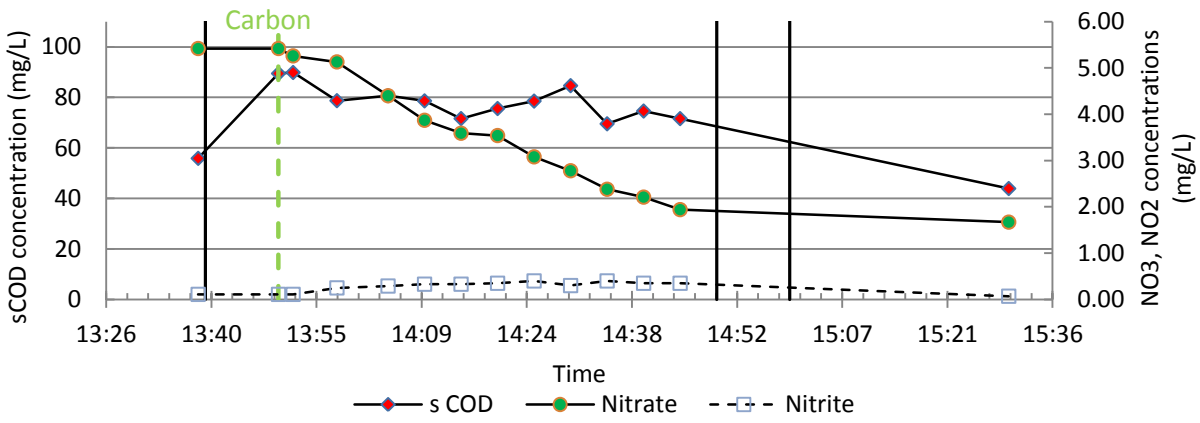
A.67: 11/16/11 Second Anoxic Zone Profile



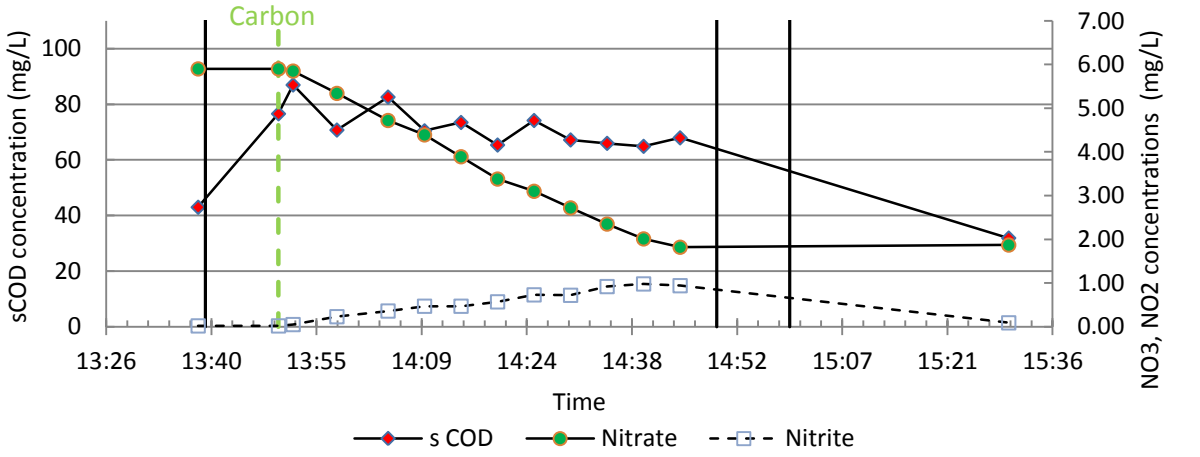
A.68: 11/17/11 Second Anoxic Zone Profile



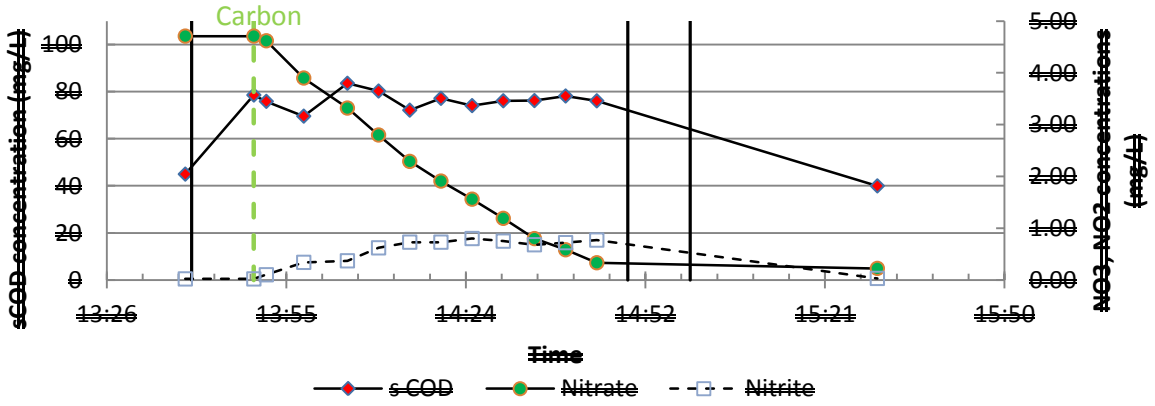
A.69: 11/30/11 Second Anoxic Zone Profile



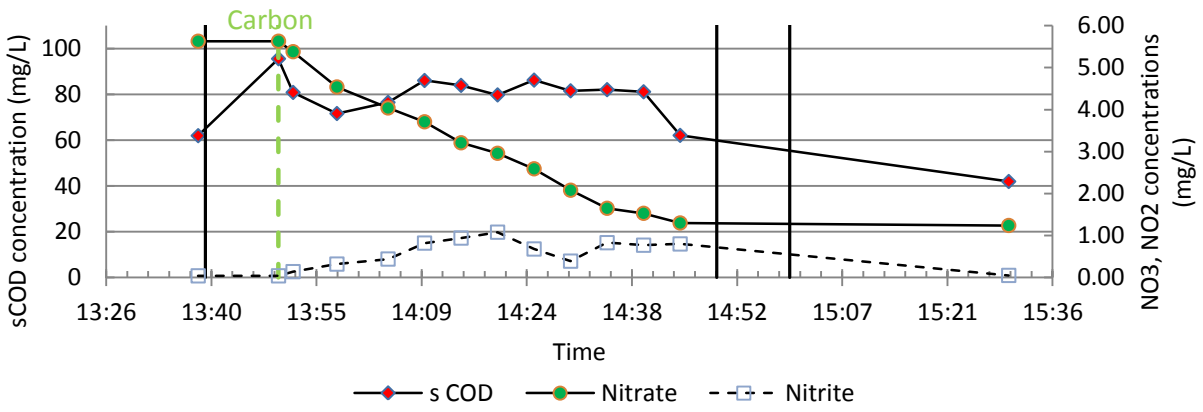
A.70: 12/1/11 Second Anoxic Zone Profile



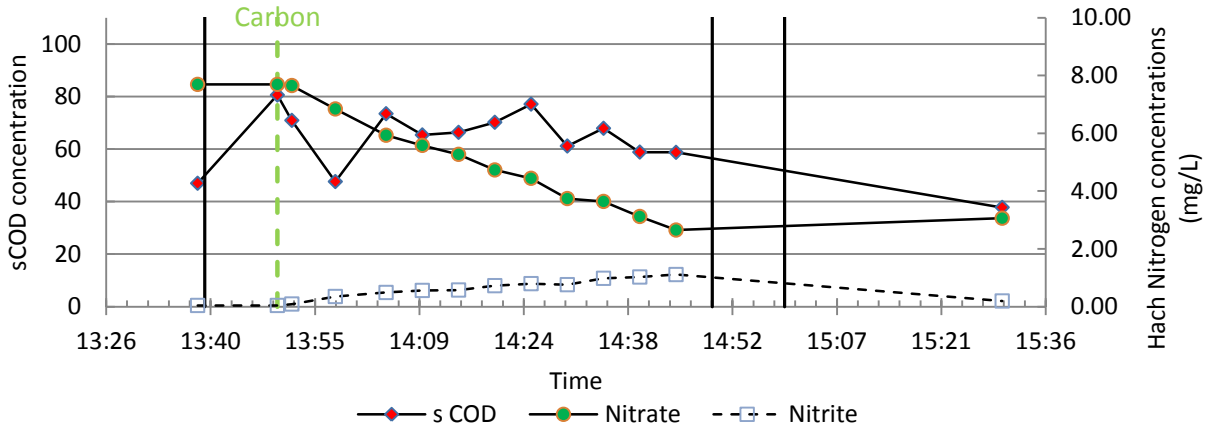
A.71: 12/5/11 Second Anoxic Zone Profile



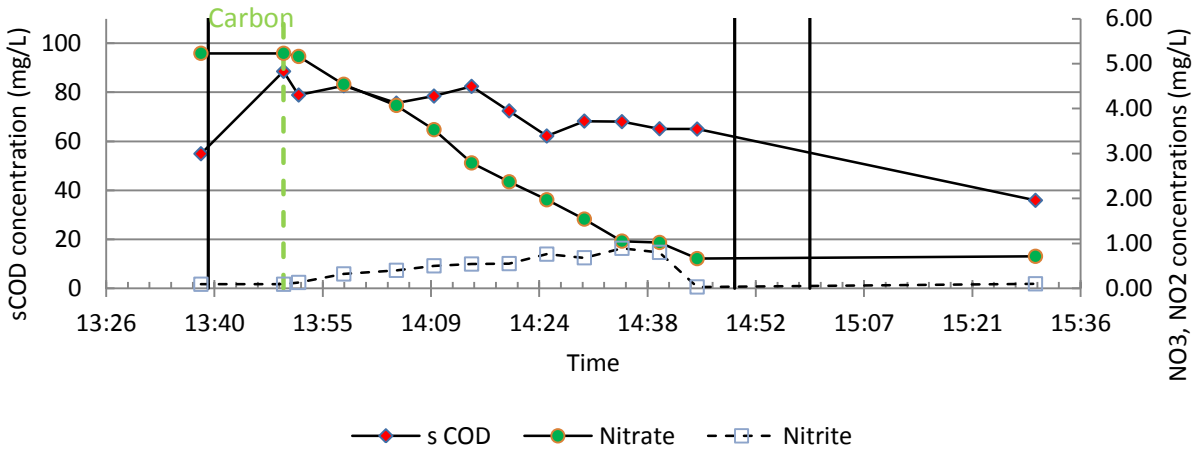
A.72: 12/7/11 Second Anoxic Zone Profile



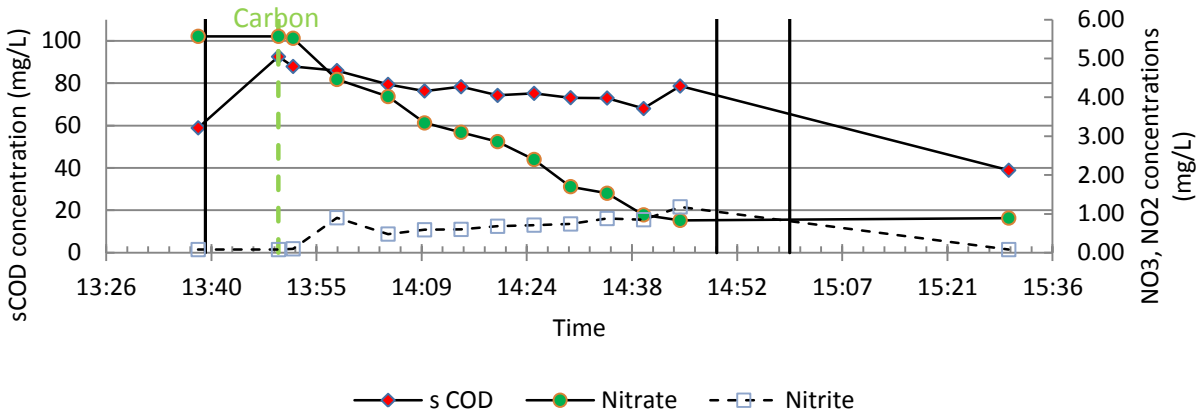
A.73: 12/8/11 Second Anoxic Zone Profile



A.74: 12/12/11 Second Anoxic Zone Profile

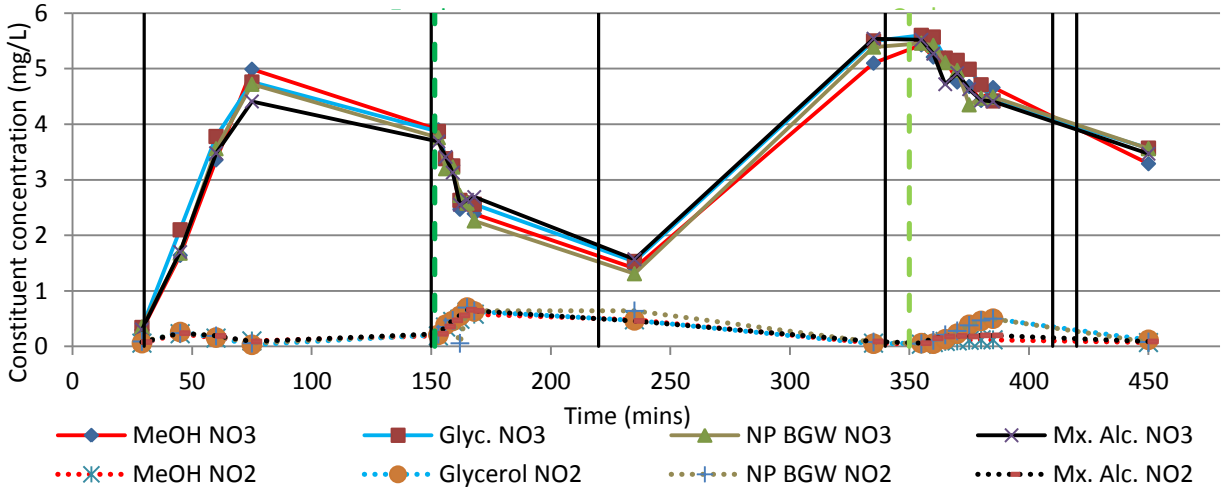


A.75: 12/14/11 Second Anoxic Zone Profile

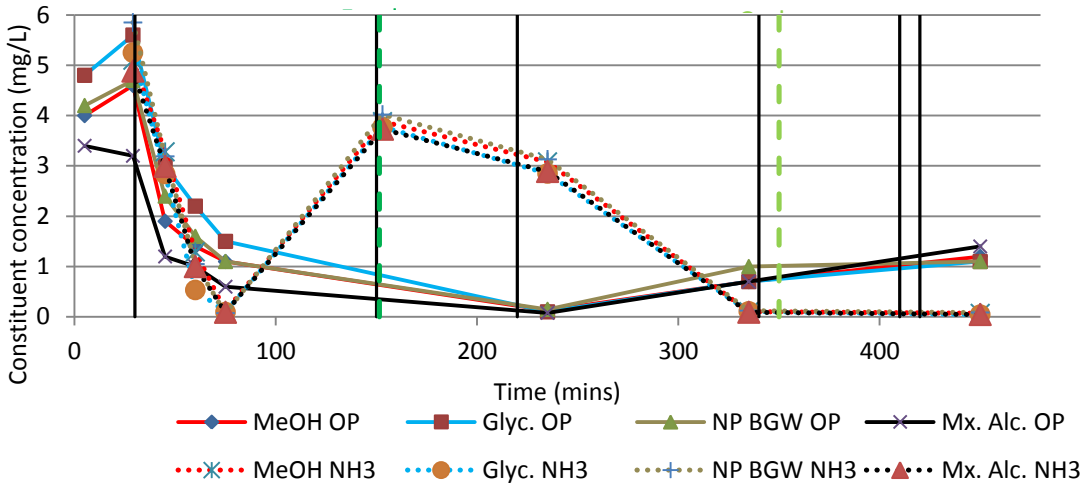


A.76: 12/15/11 Second Anoxic Zone Profile

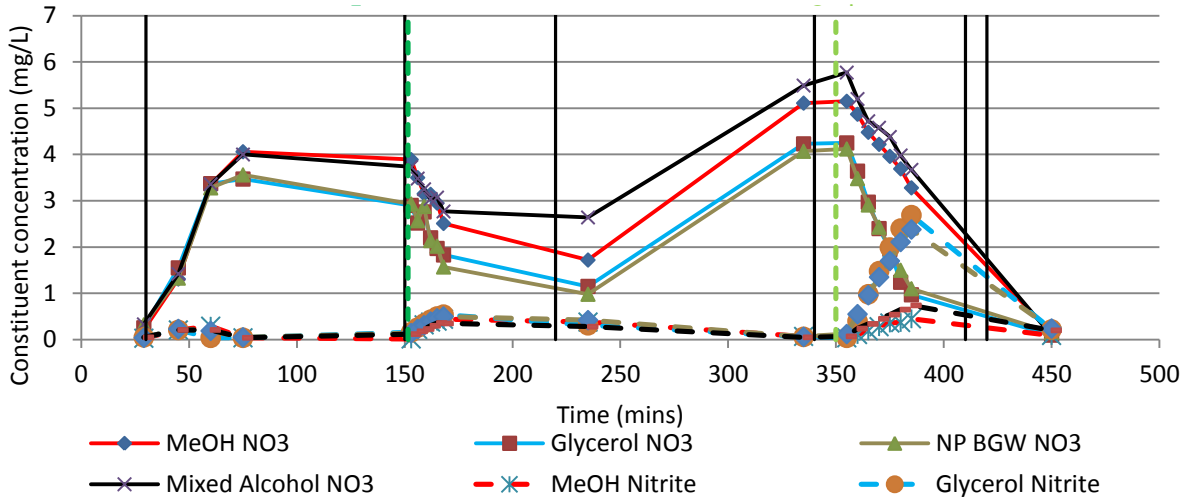
A.6 ALL REACTOR DAILY SAMPLING PROFILES



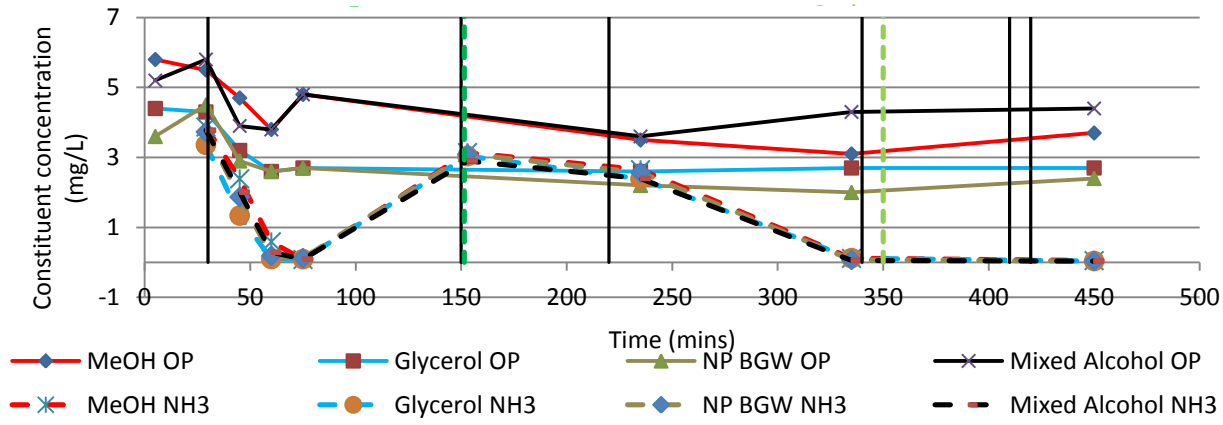
A.77: 9/29/11 Nitrate and Nitrite Profiles



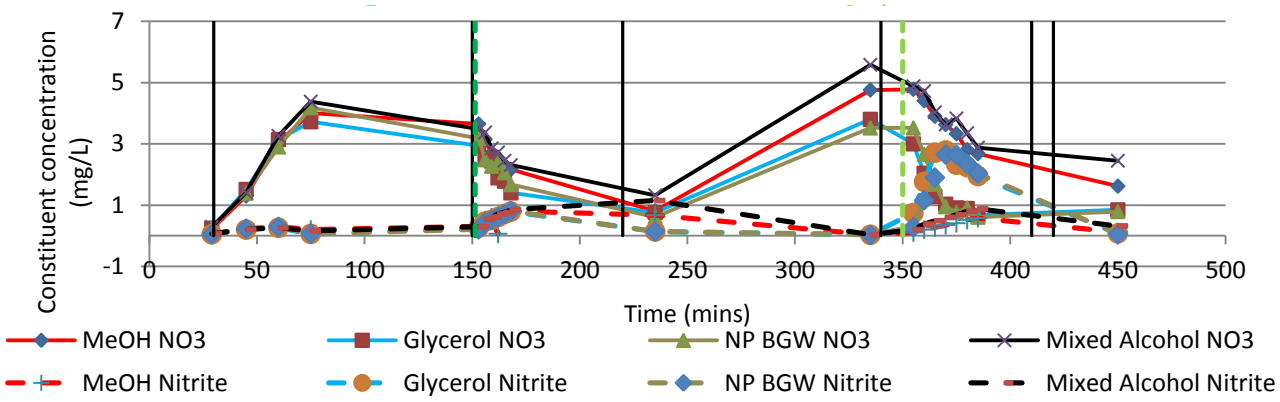
A.78: 9/29/11 Ammonia and Orthophosphate Profiles



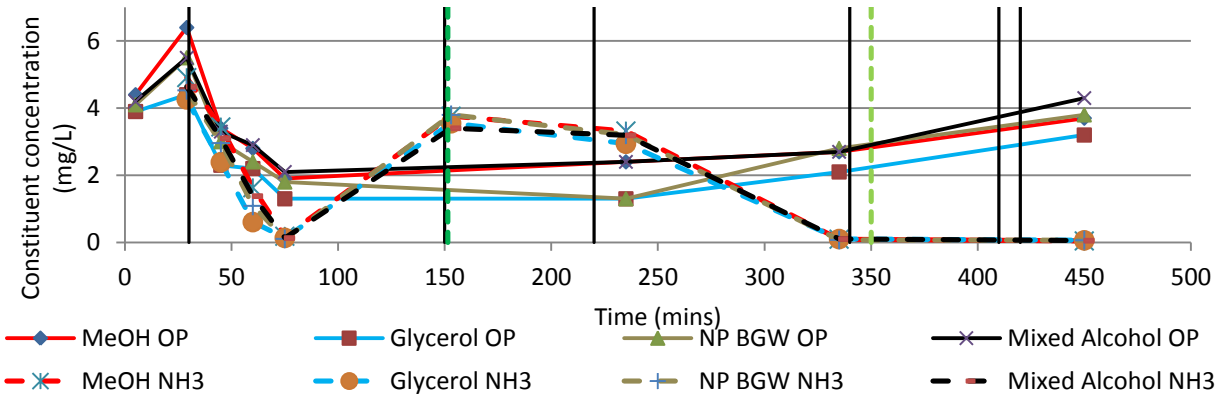
A.79: 10/3/11 Nitrate and Nitrite Profiles



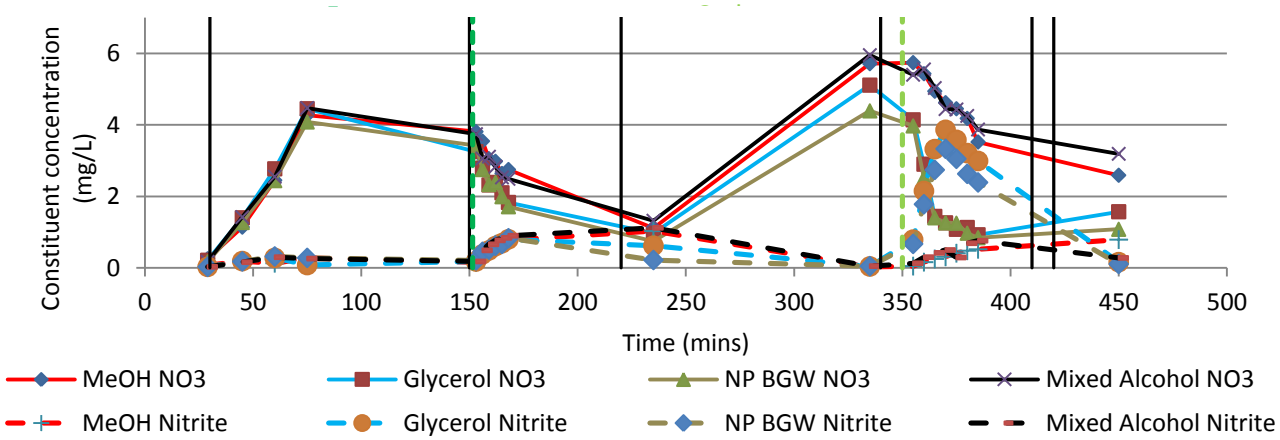
A.80: 10/3/11 Ammonia and Orthophosphate Profiles



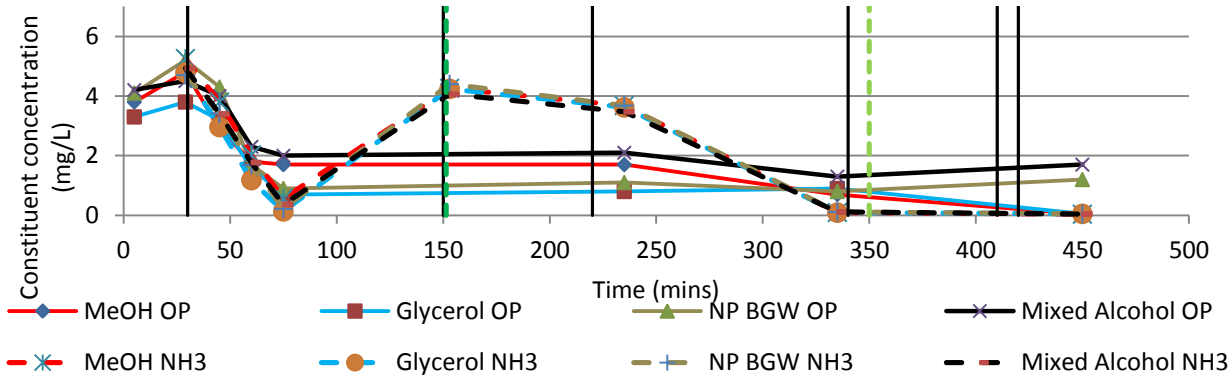
A.81: 10/5/11 Nitrate and Nitrite Profiles



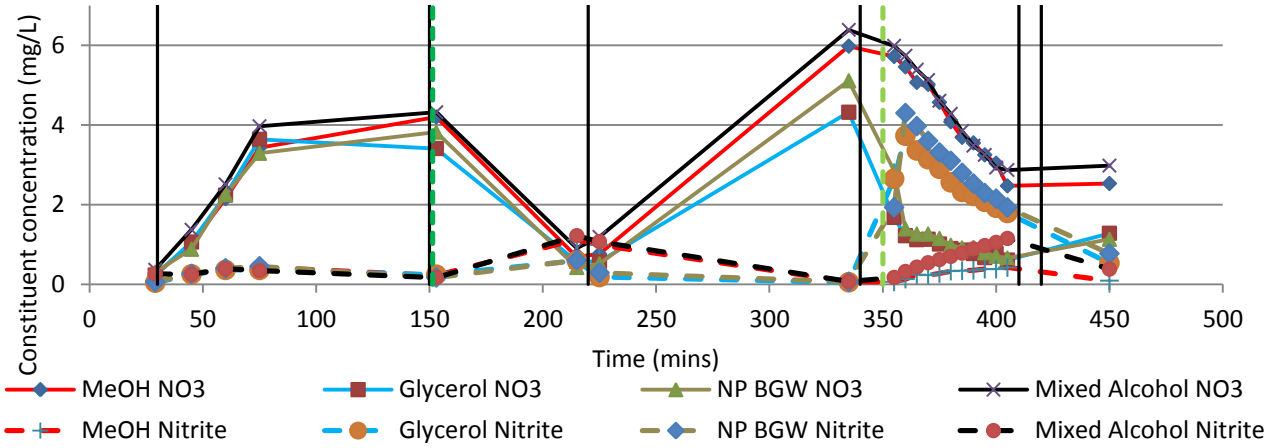
A.82: 10/5/11 Ammonia and Orthophosphate Profiles



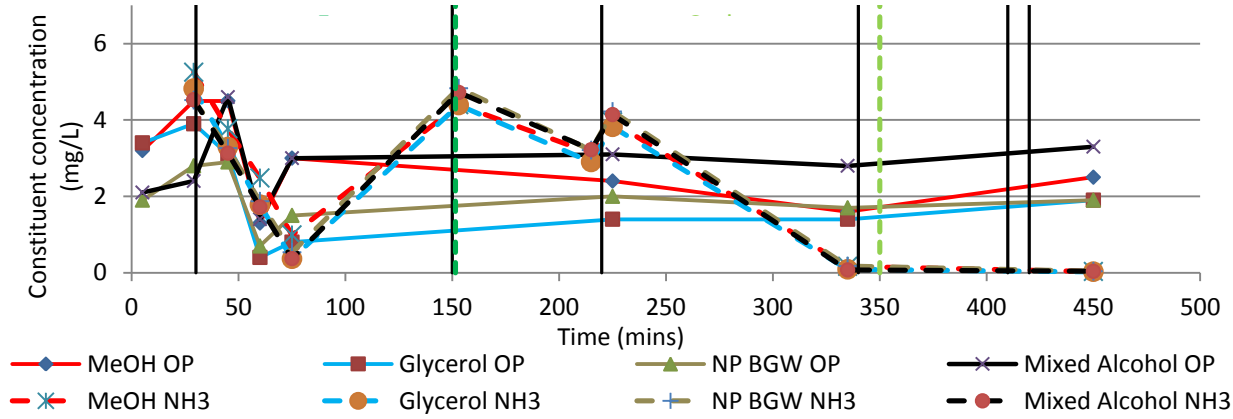
A.83: 10/6/11 Nitrate and Nitrite Profiles



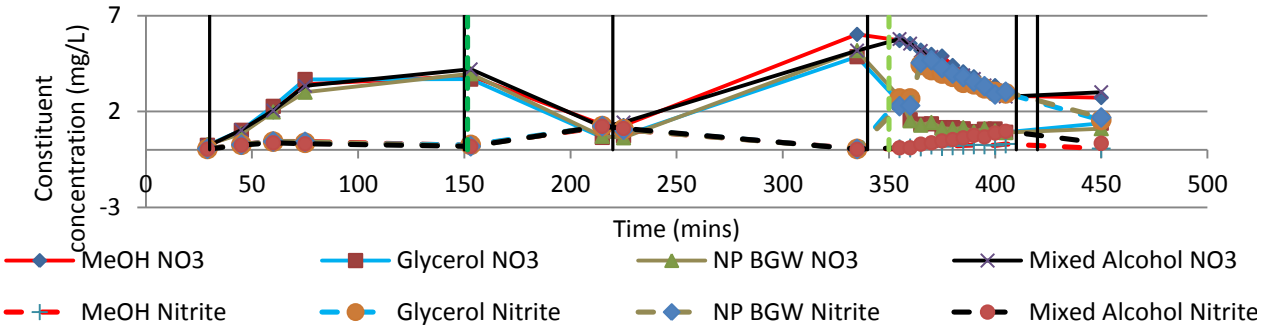
A.84: 10/6/11 Ammonia and Orthophosphate Profiles



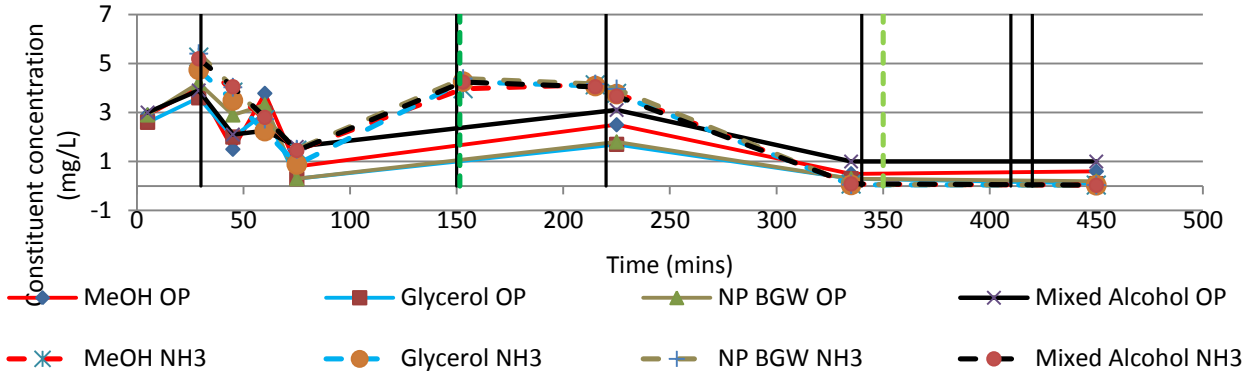
A.85: 10/12/11 Nitrate and Nitrite Profiles



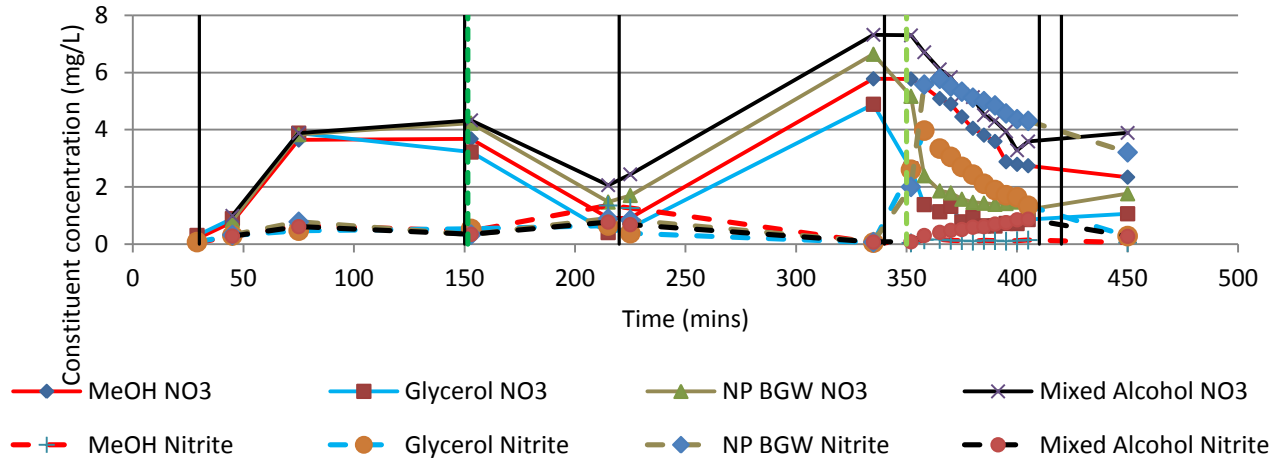
A.86: 10/12/11 Ammonia and Orthophosphate Profiles



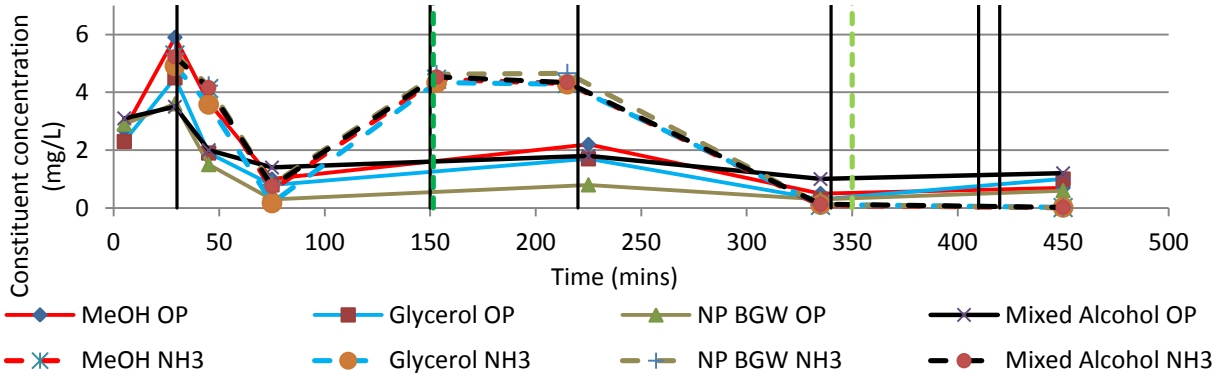
A.87: 10/13/11 Nitrate and Nitrite Profiles



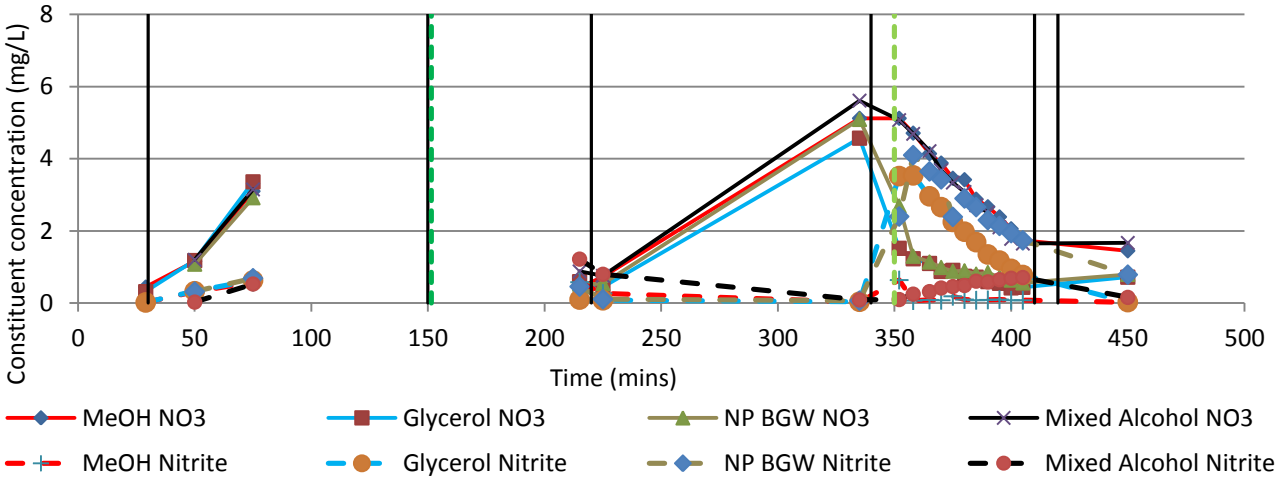
A.88: 10/13/11 Ammonia and Orthophosphate Profiles



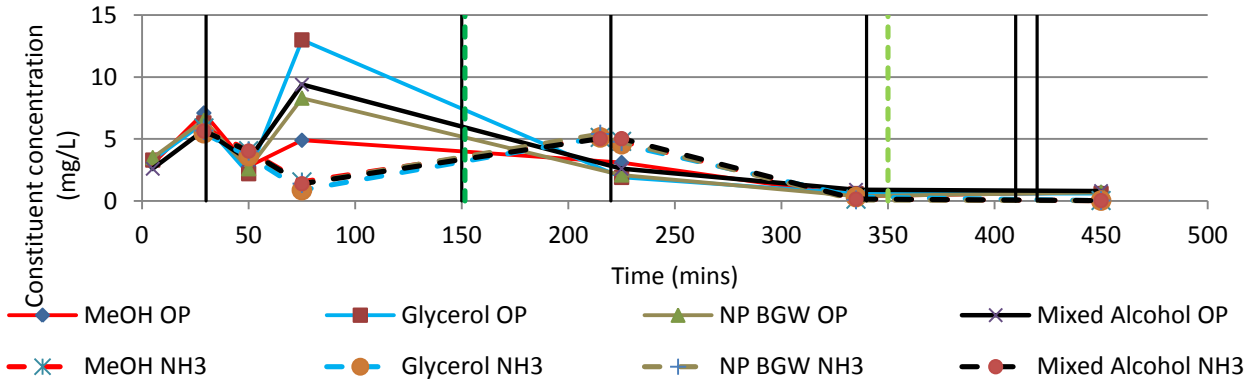
A.89: 10/17/11 Nitrate and Nitrite Profiles



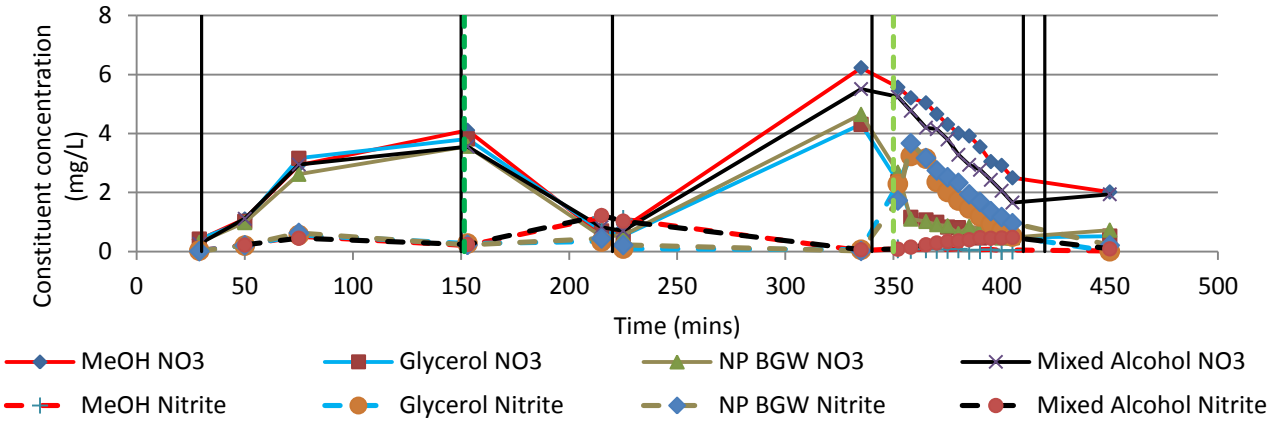
A.90: 10/17/11 Ammonia and Orthophosphate Profiles



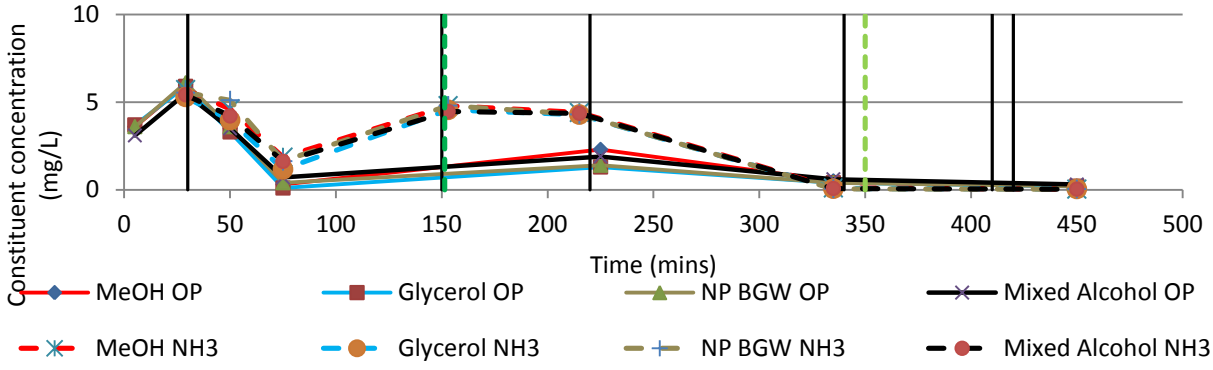
A.91: 10/19/11 Nitrate and Nitrite Profiles



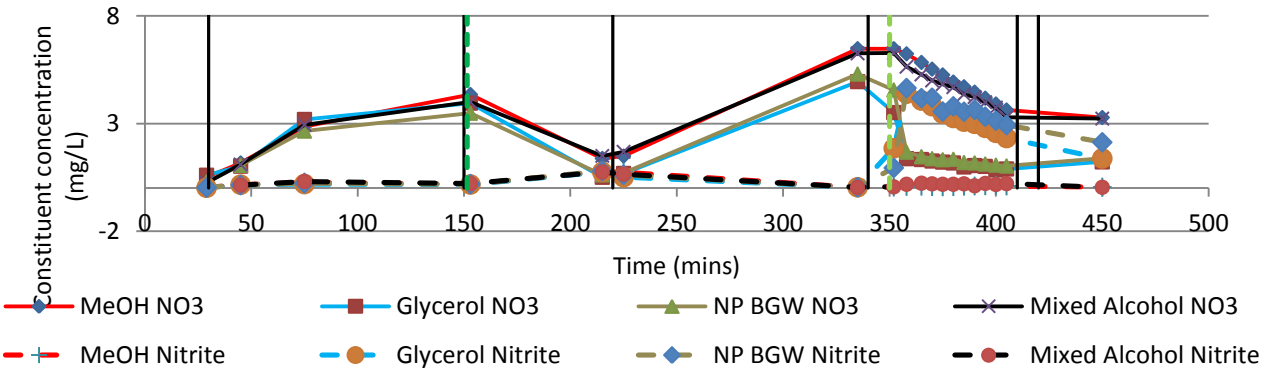
A.92: 10/19/11 Ammonia and Orthophosphate Profiles



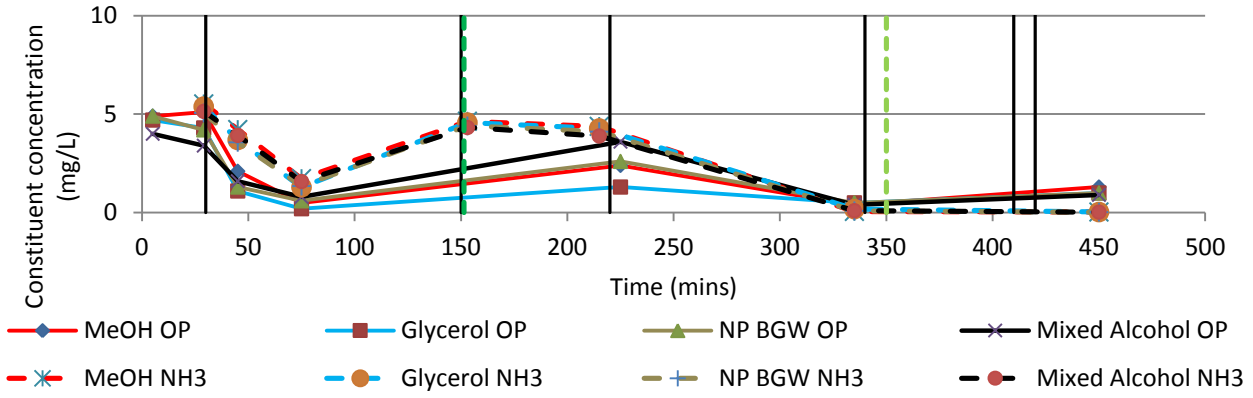
A.93: 10/20/11 Nitrate and Nitrite Profiles



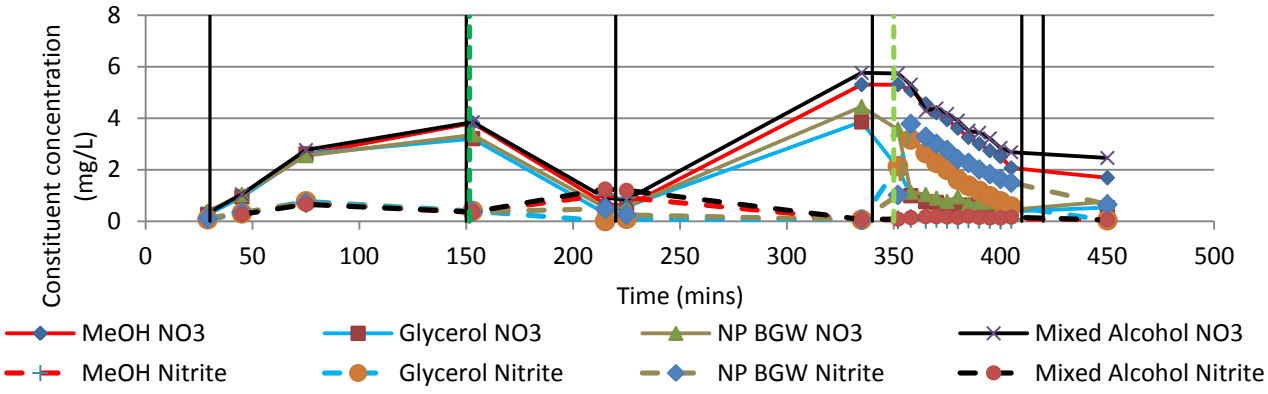
A.94: 10/20/11 Ammonia and Orthophosphate Profiles



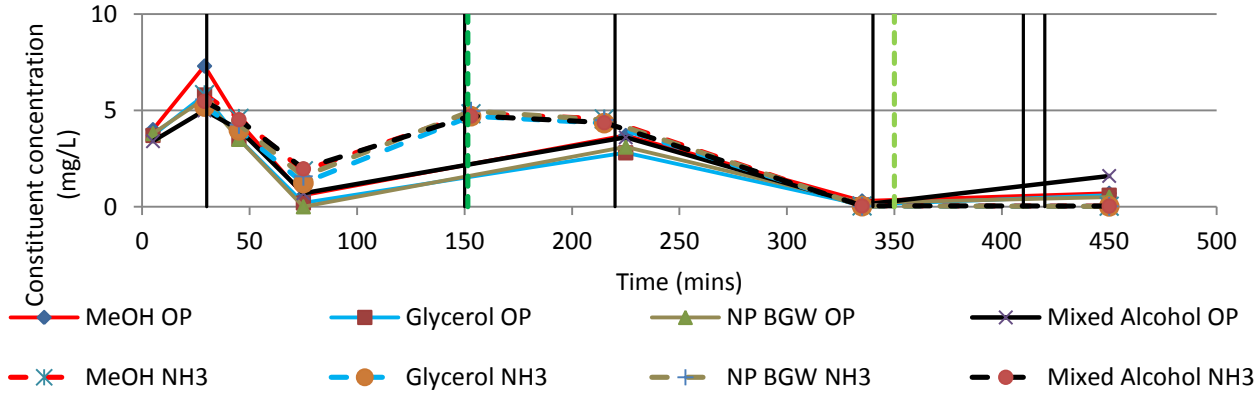
A.95: 10/24/11 Nitrate and Nitrite Profiles



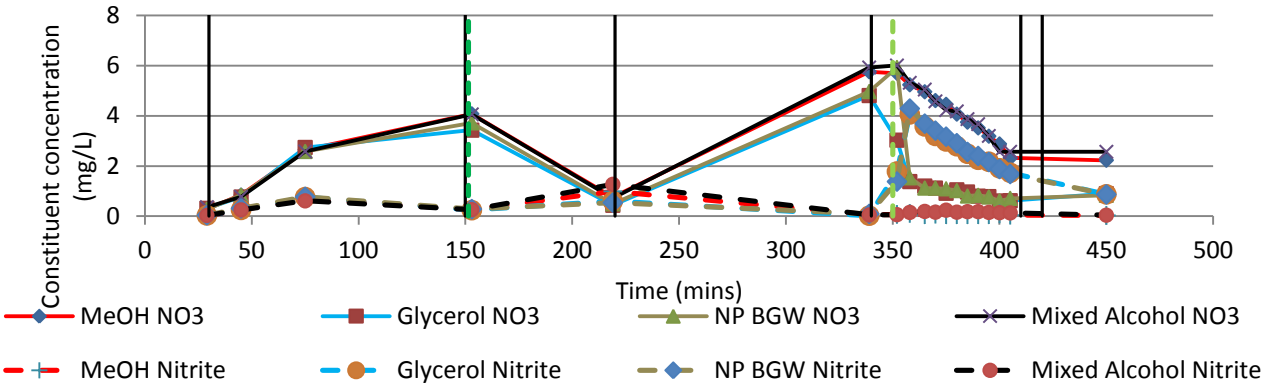
A.96: 10/24/11 Ammonia and Orthophosphate Profiles



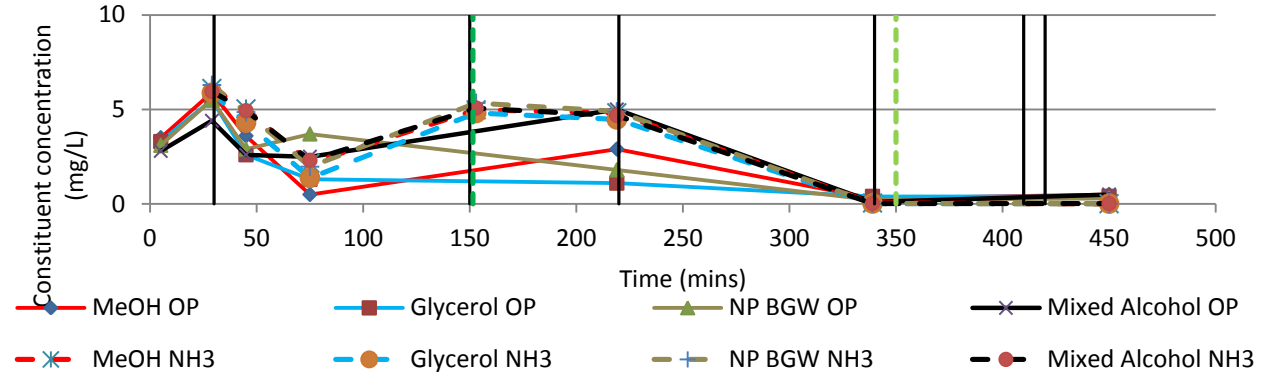
A.97: 10/26/11 Nitrate and Nitrite Profiles



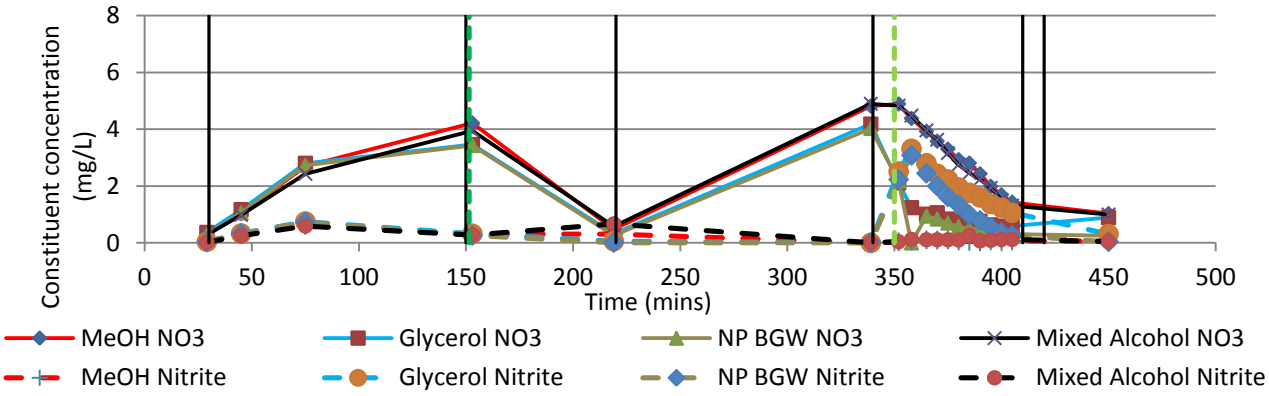
A.98: 10/26/11 Ammonia and Orthophosphate Profiles



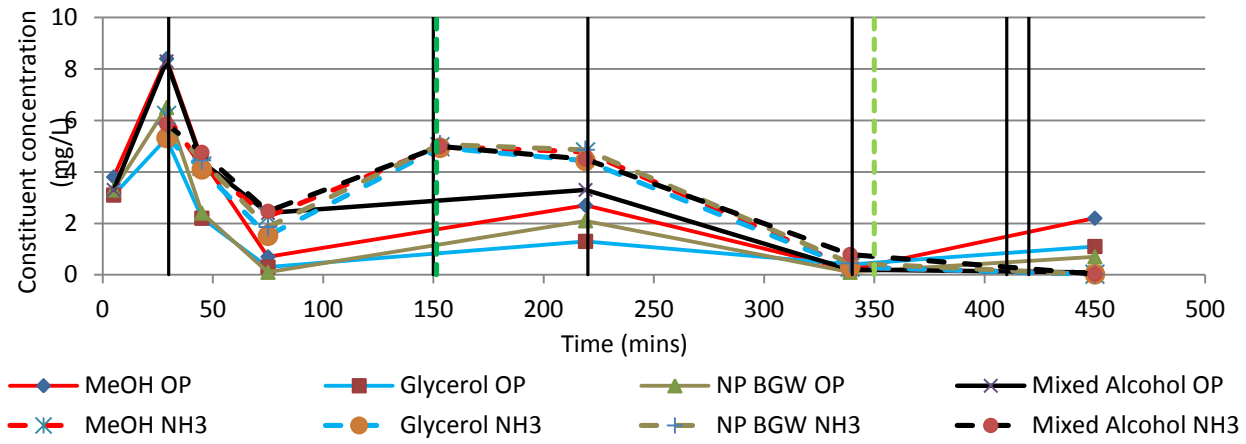
A.99: 10/27/11 Nitrate and Nitrite Profiles



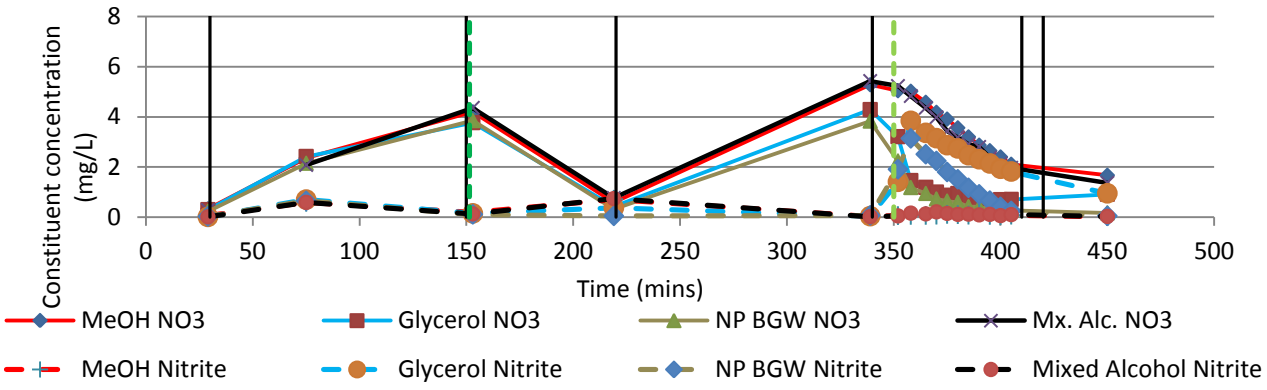
A.100: 10/27/11 Ammonia and Orthophosphate Profiles



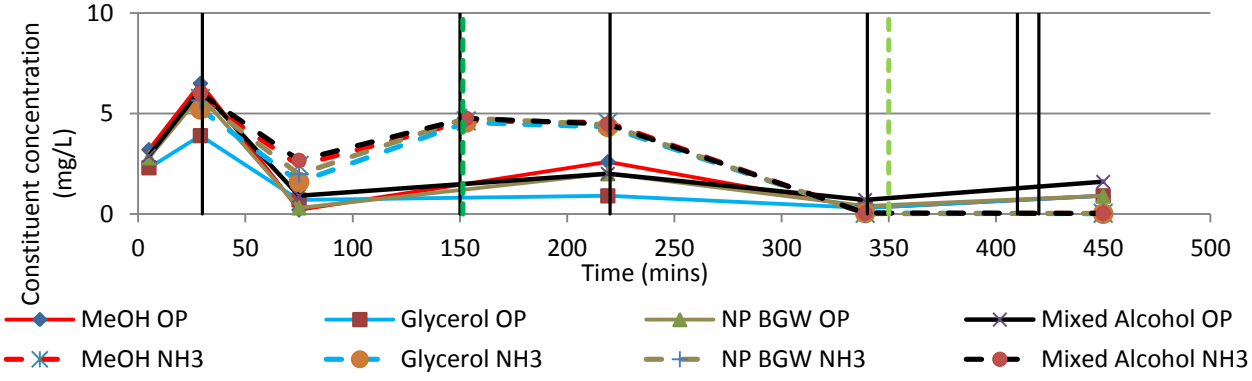
A.101: 11/2/11 Nitrate and Nitrite Profiles



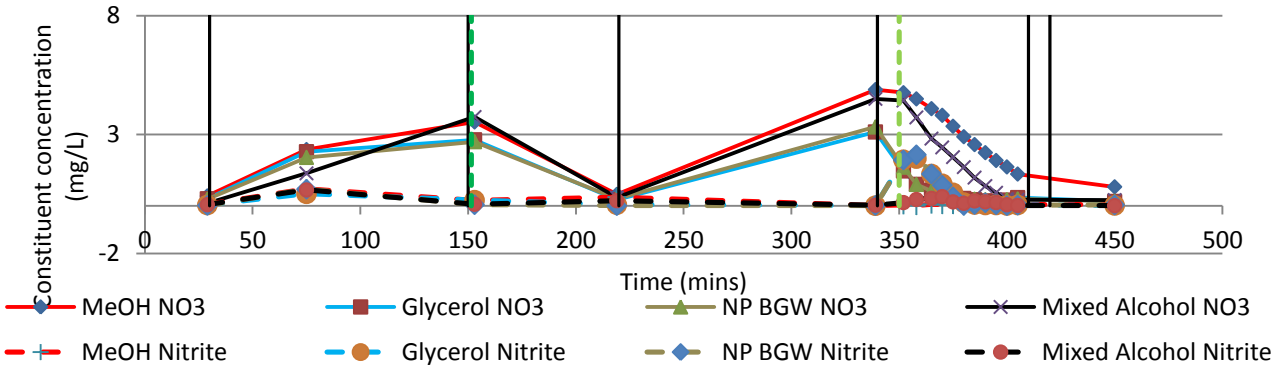
A.102: 11/2/11 Ammonia and Orthophosphate Profiles



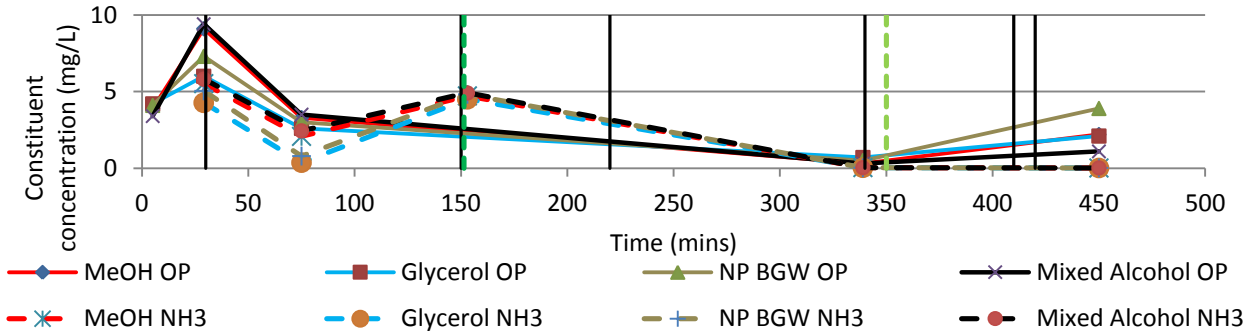
A.103: 11/3/11 Nitrate and Nitrite Profiles



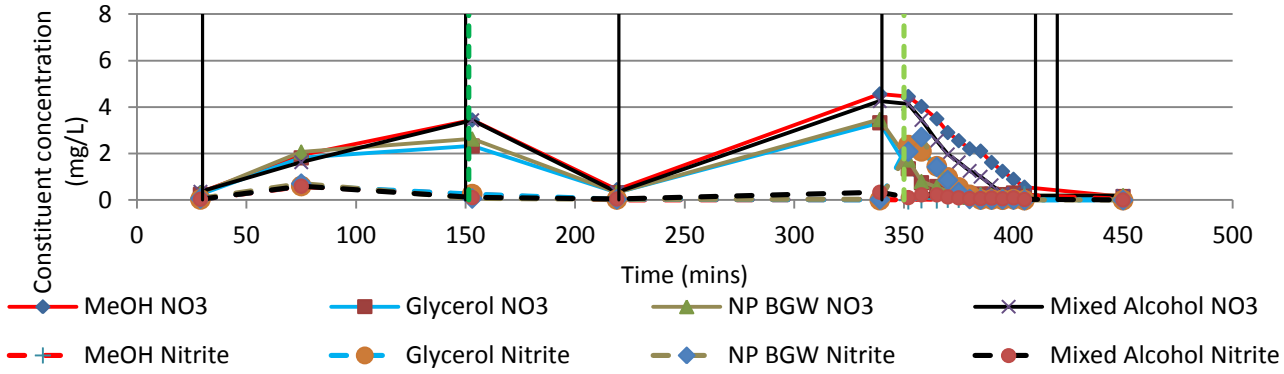
A.104: 11/3/11 Ammonia and Orthophosphate Profiles



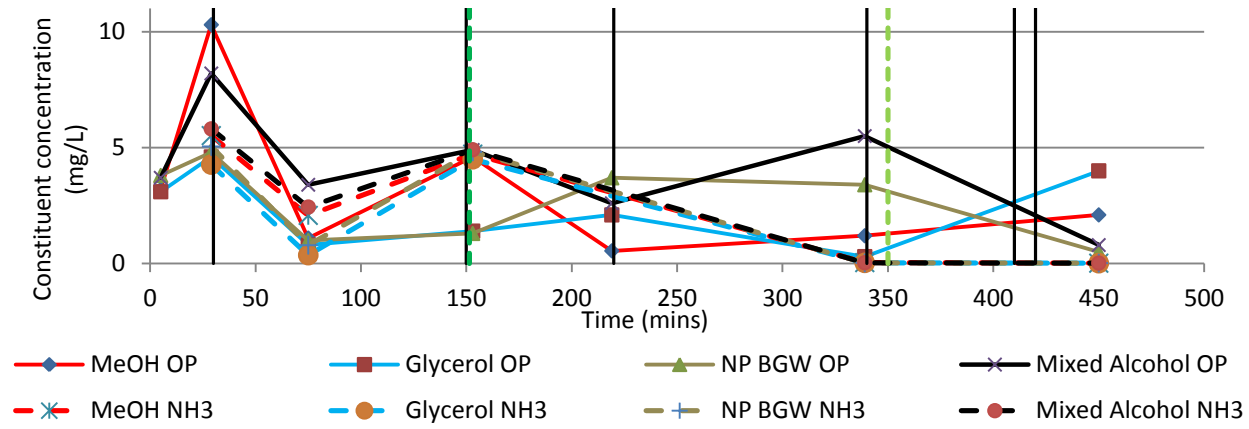
A.105: 11/7/11 Nitrate and Nitrite Profiles



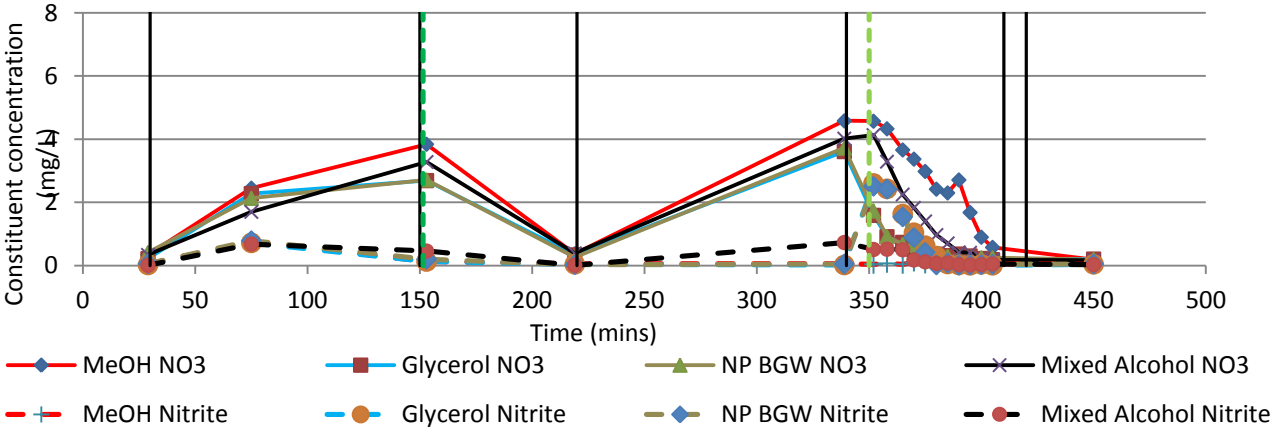
A.106: 11/7/11 Ammonia and Orthophosphate Profiles



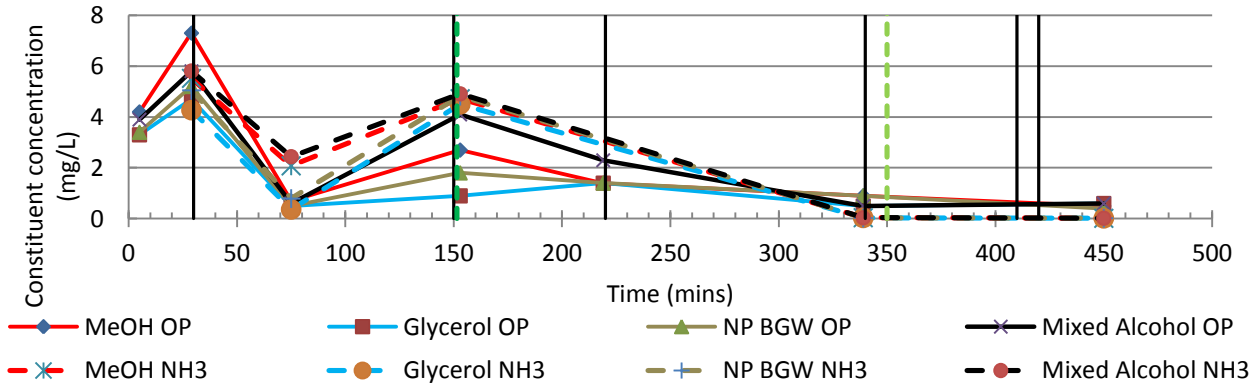
A.107: 11/9/11 Nitrate and Nitrite Profiles



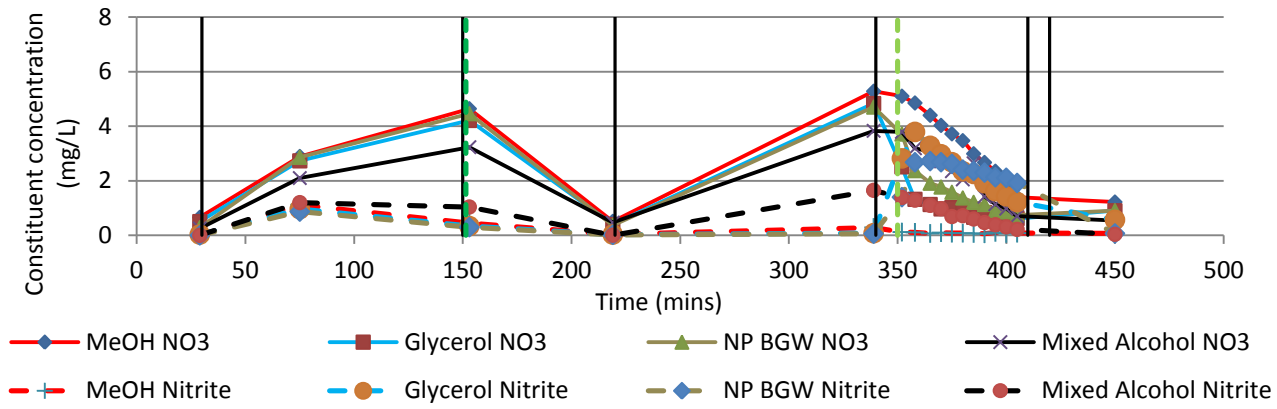
A.108: 11/9/11 Ammonia and Orthophosphate Profiles



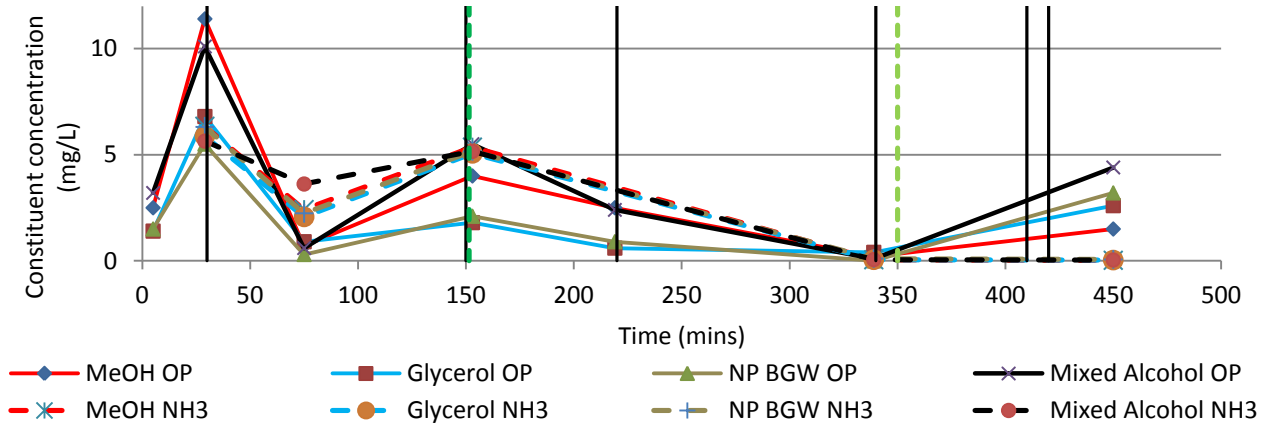
A.109: 11/10/11 Nitrate and Nitrite Profiles



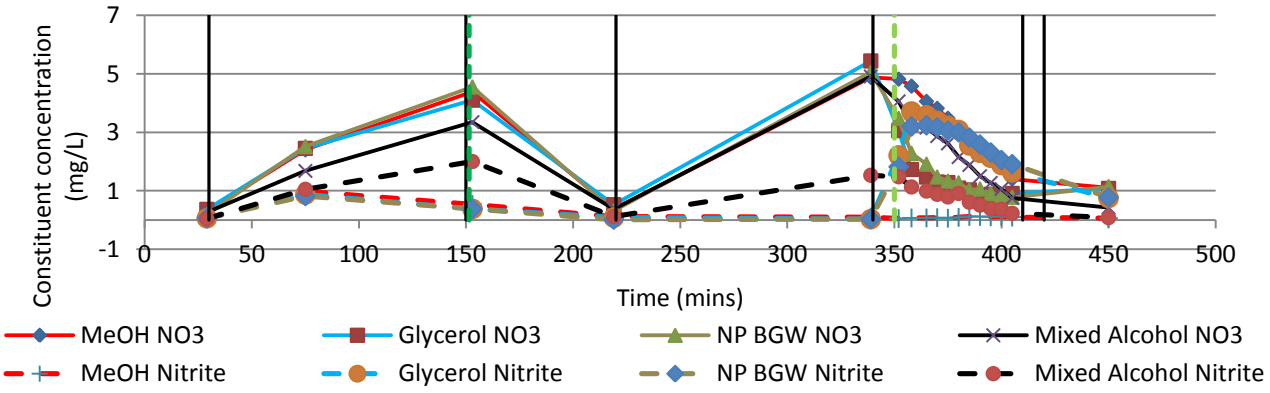
A.110: 11/10/11 Ammonia and Orthophosphate Profiles



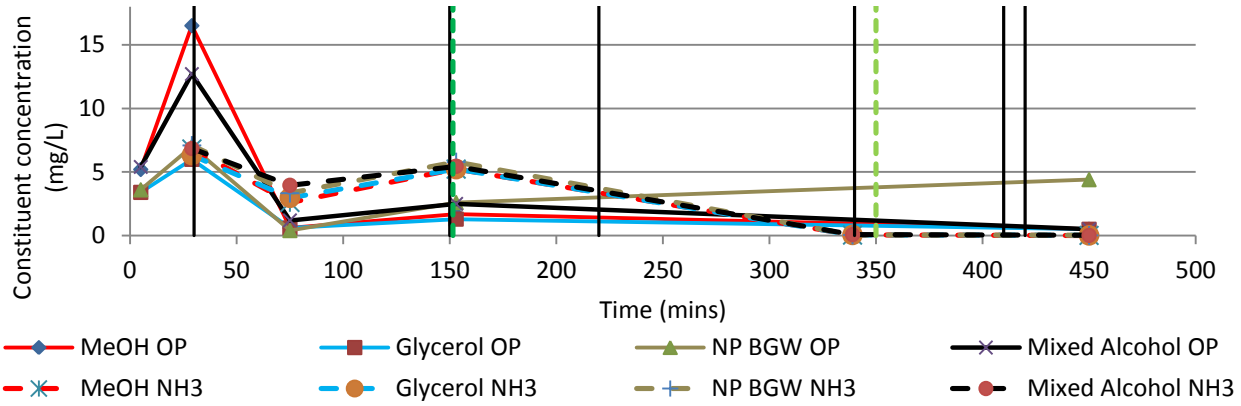
A.111: 11/14/11 Nitrate and Nitrite Profiles



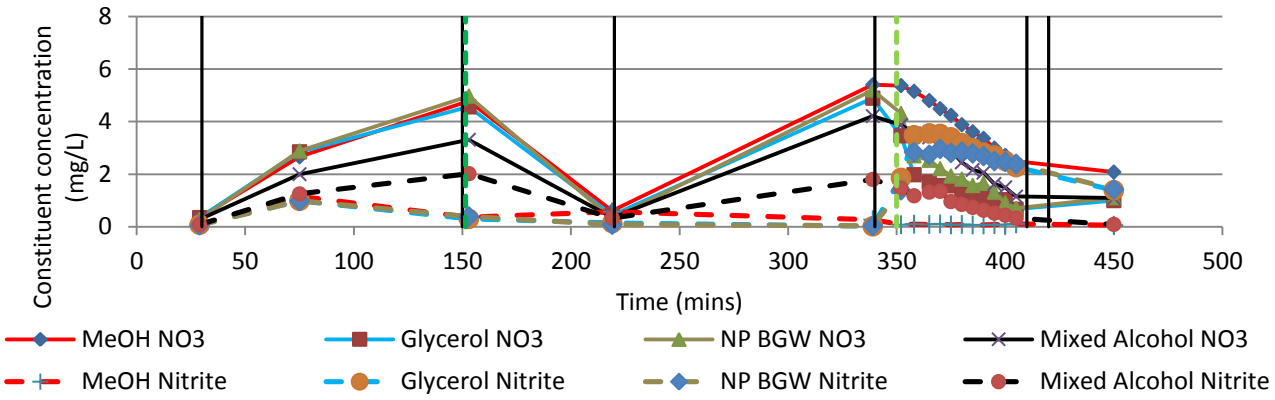
A.112: 11/14/11 Ammonia and Orthophosphate Profiles



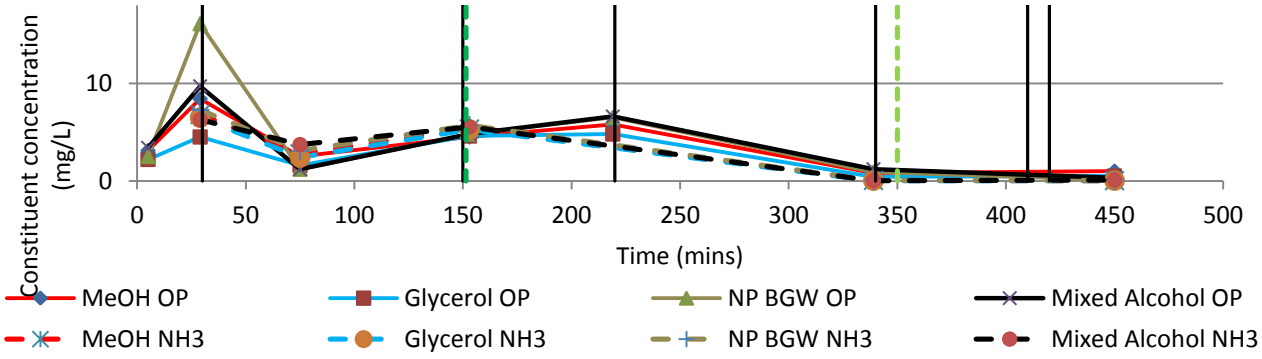
A.113: 11/16/11 Nitrate and Nitrite Profiles



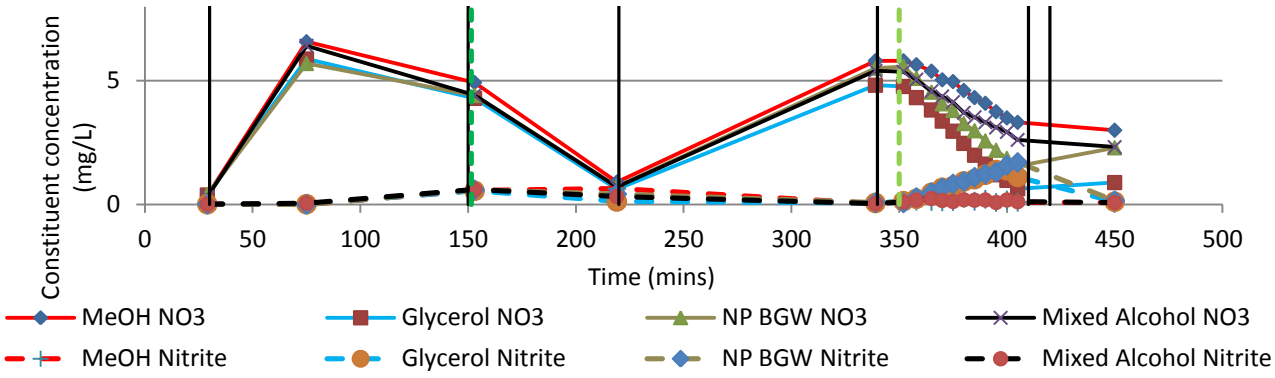
A.114: 11/16/11 Ammonia and Orthophosphate Profiles



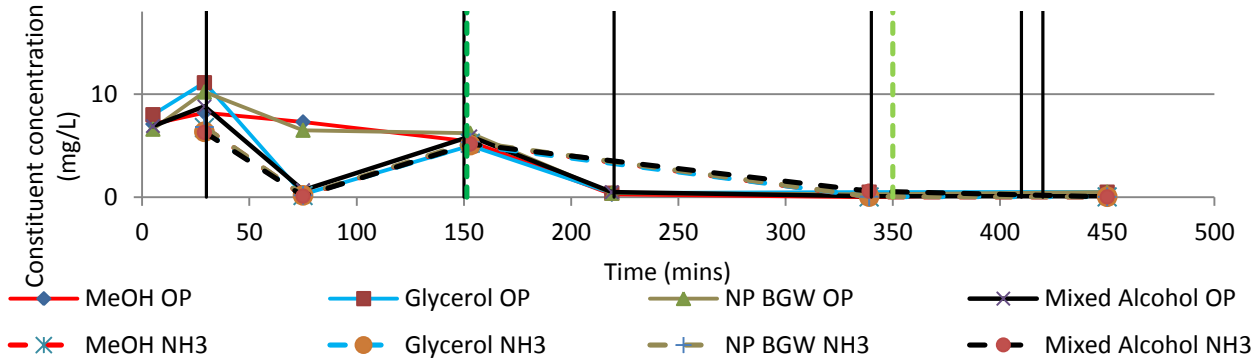
A.115: 11/17/11 Nitrate and Nitrite Profiles



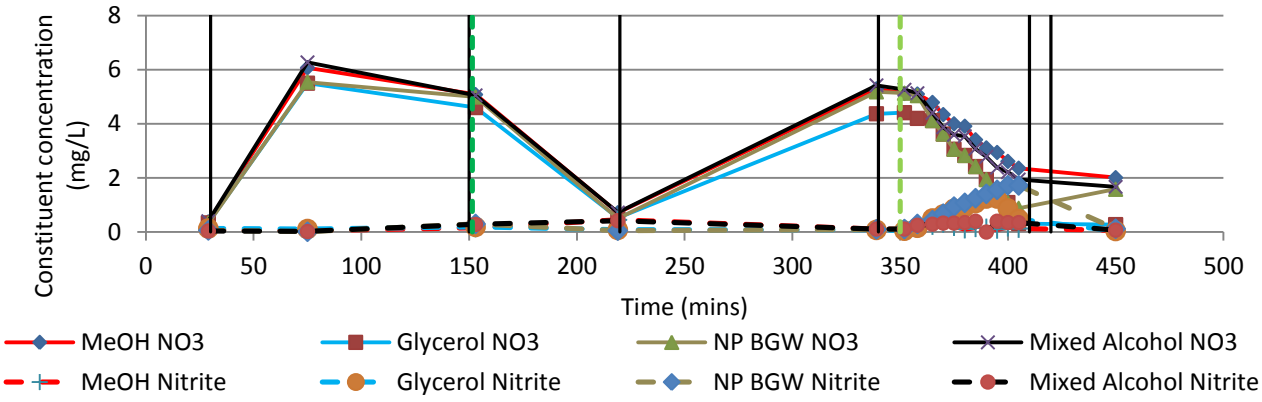
A.116: 11/17/11 Ammonia and Orthophosphate Profiles



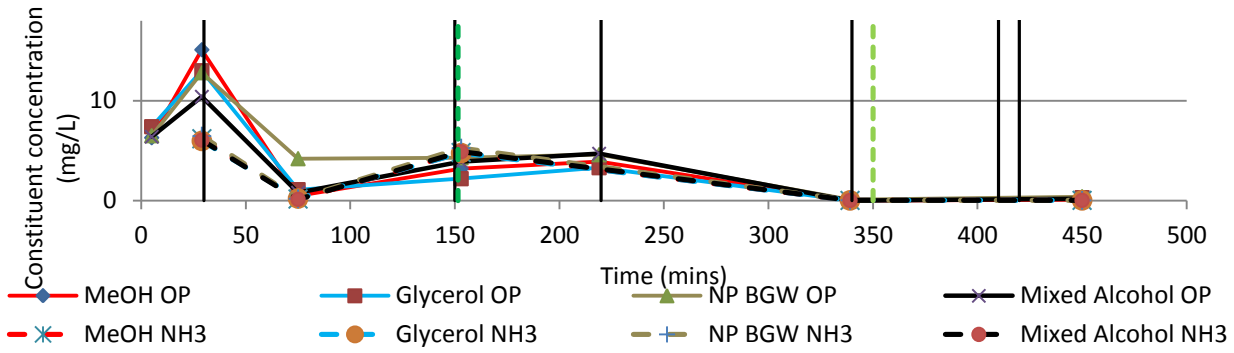
A.117: 11/30/11 Nitrate and Nitrite Profiles



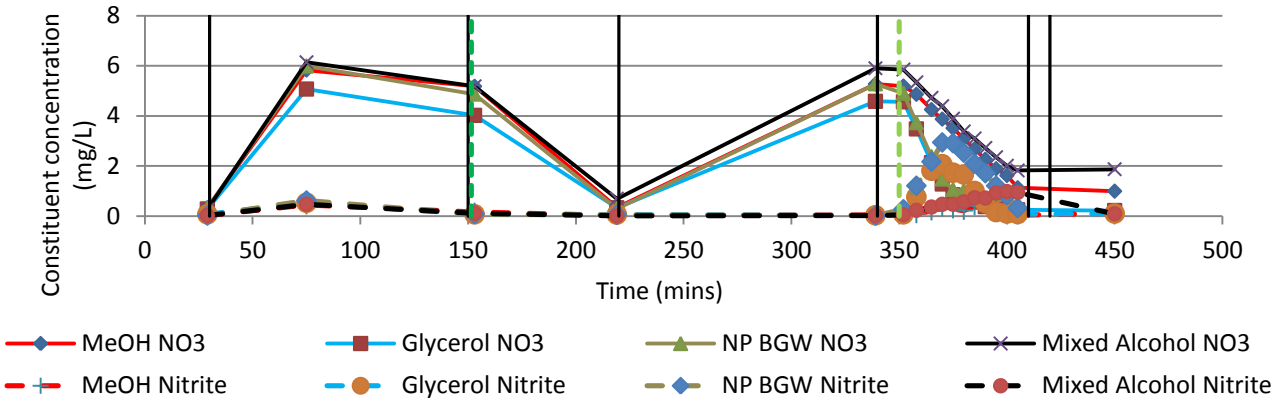
A.118: 11/30/11 Ammonia and Orthophosphate Profiles



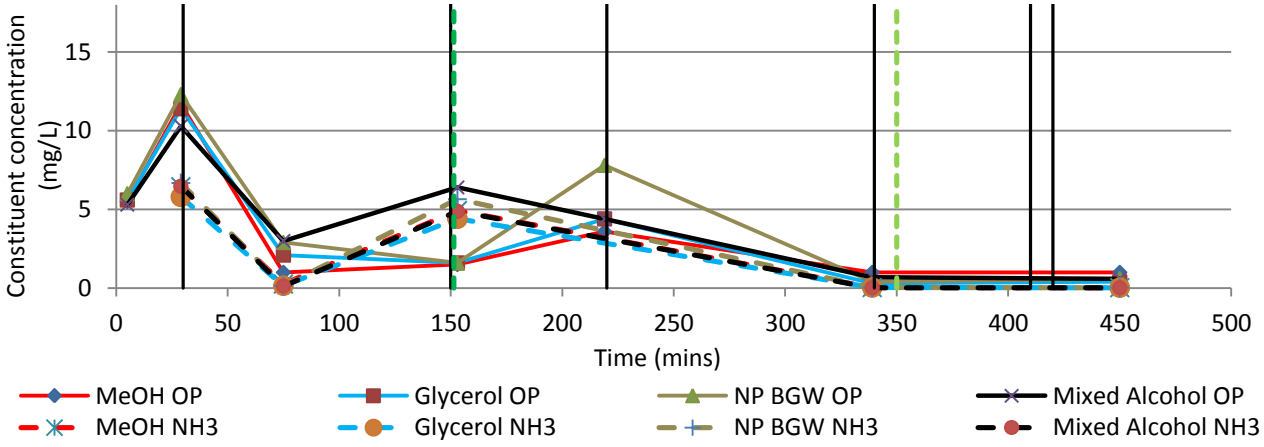
A.119: 12/1/11 Nitrate and Nitrite Profiles



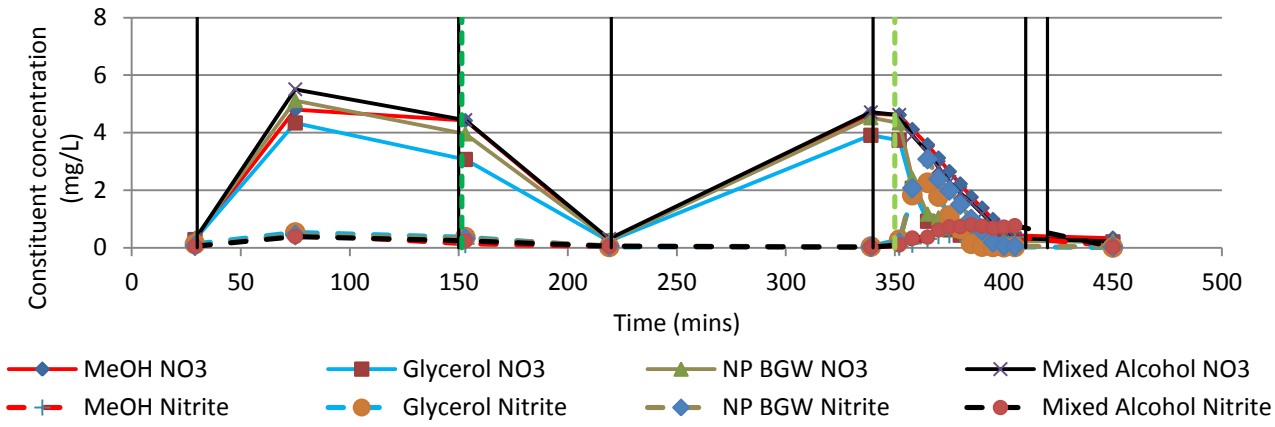
A.120: 12/1/11 Ammonia and Orthophosphate Profiles



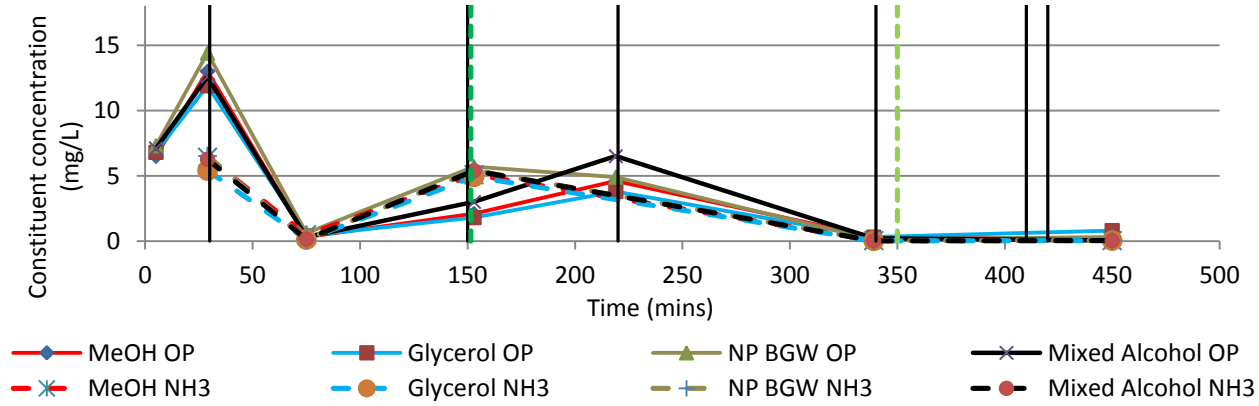
A.121: 12/5/11 Nitrate and Nitrite Profiles



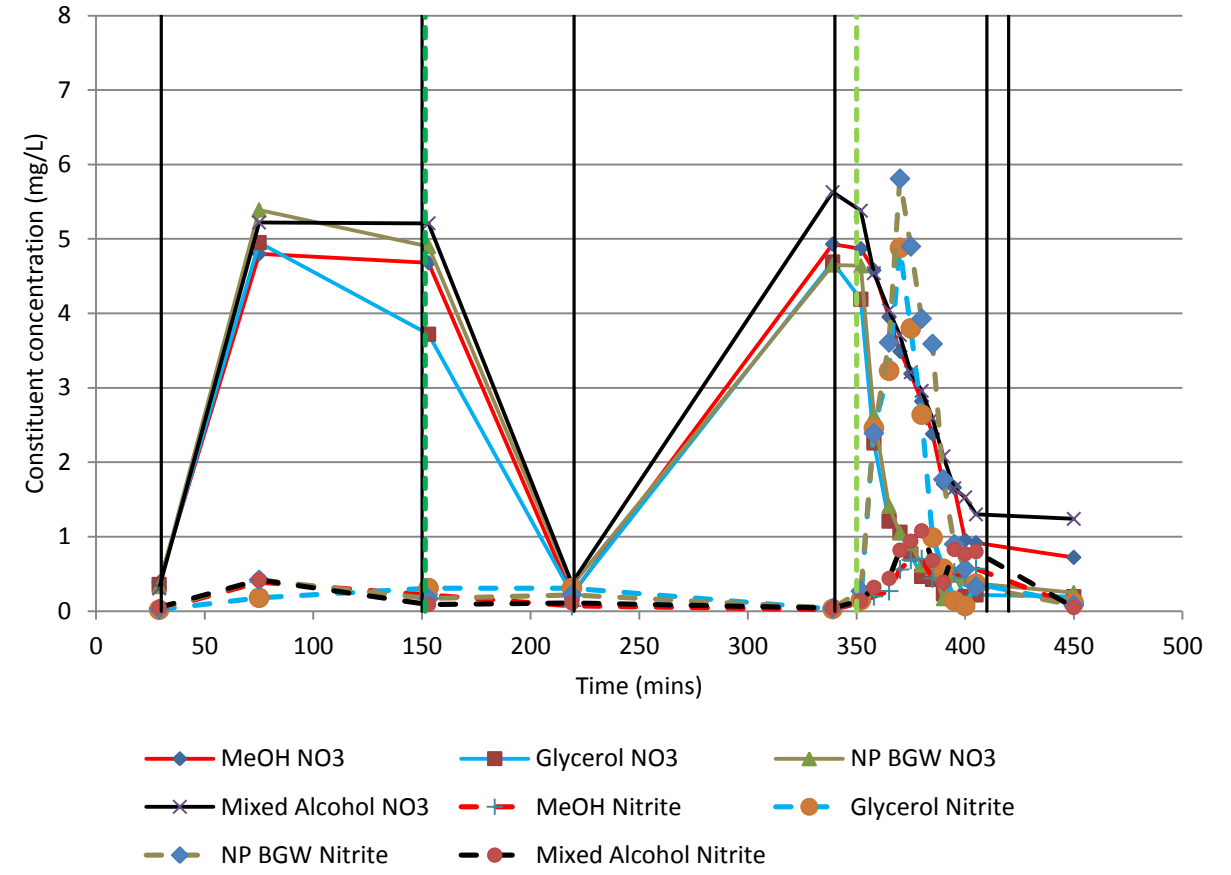
A.122: 12/5/11 Ammonia and Orthophosphate Profiles



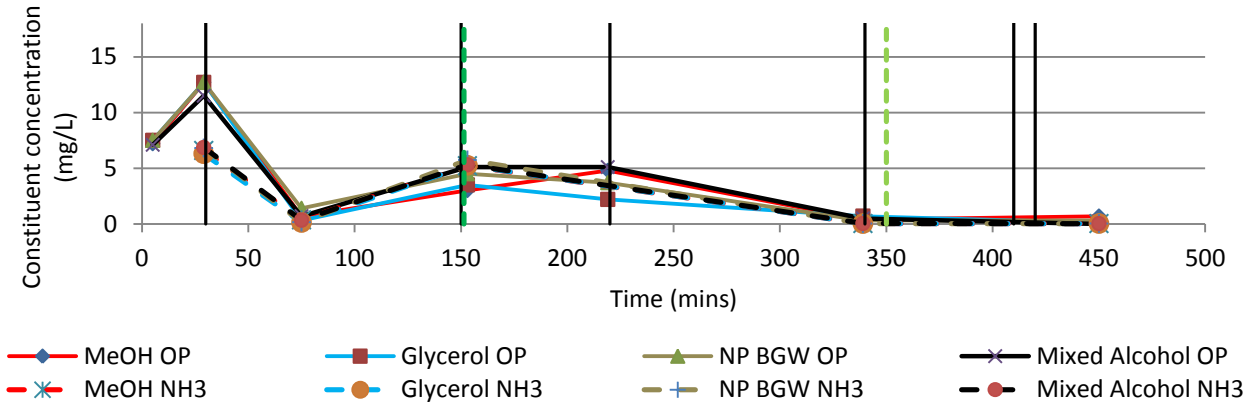
A.123: 12/7/11 Nitrate and Nitrite Profiles



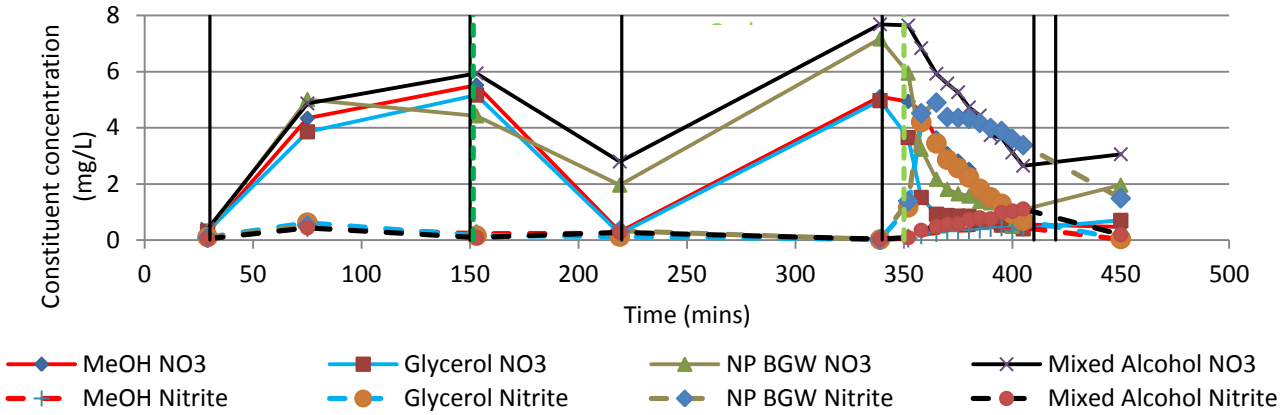
A.124: 12/7/11 Ammonia and Orthophosphate Profiles



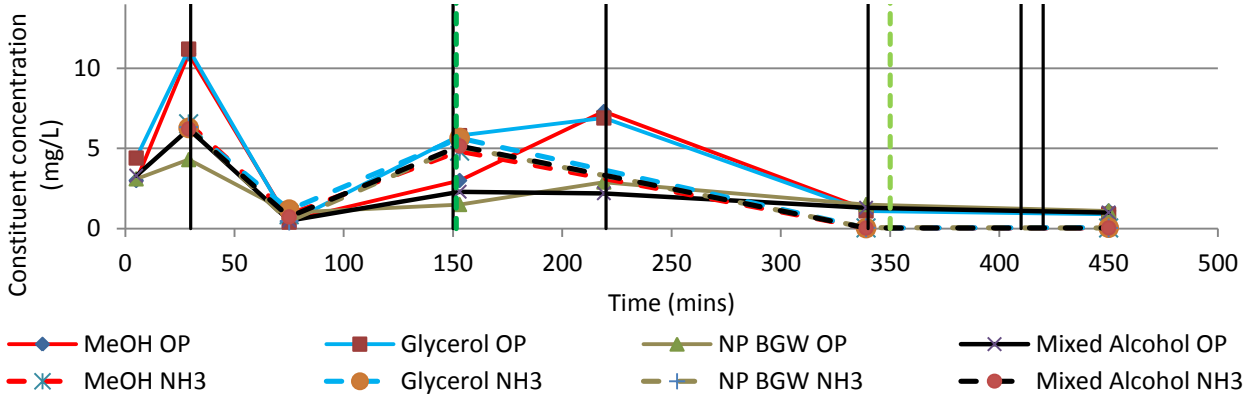
A.125: 12/8/11 Nitrate and Nitrite Profiles



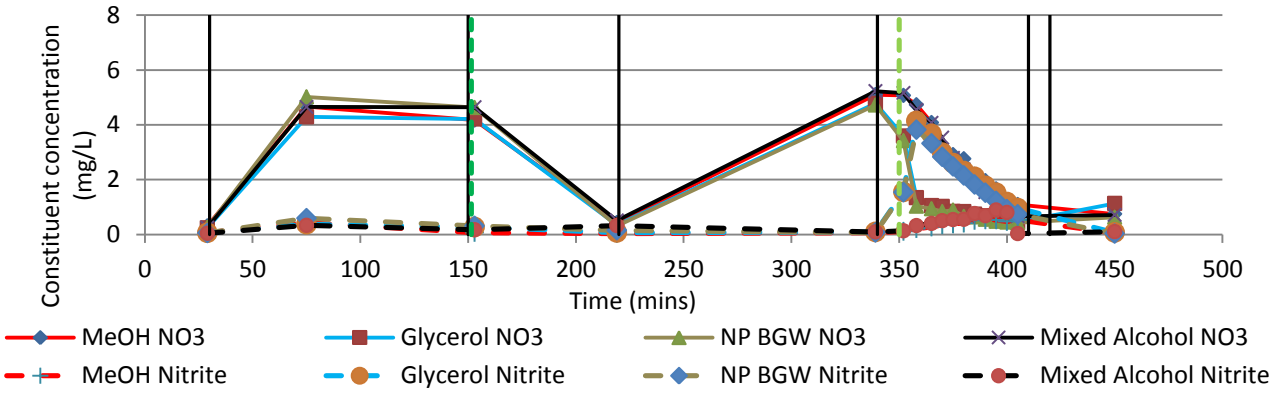
A.126: 12/8/11 Ammonia and Orthophosphate Profiles



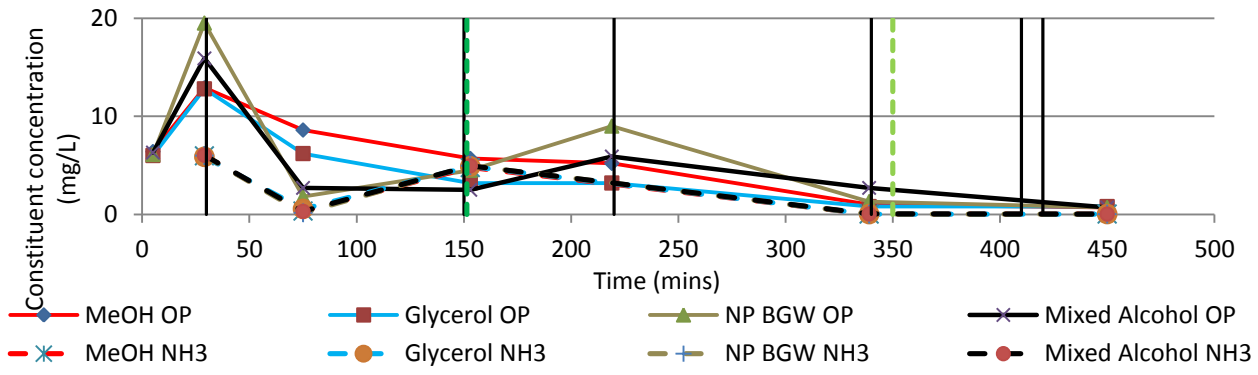
A.127: 12/12/11 Nitrate and Nitrite Profiles



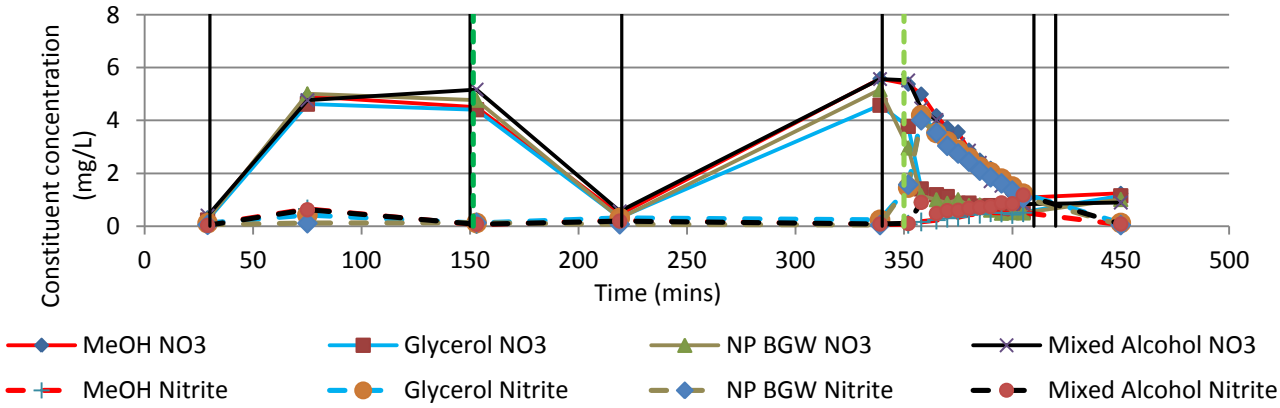
A.128: 12/12/11 Ammonia and Orthophosphate Profiles



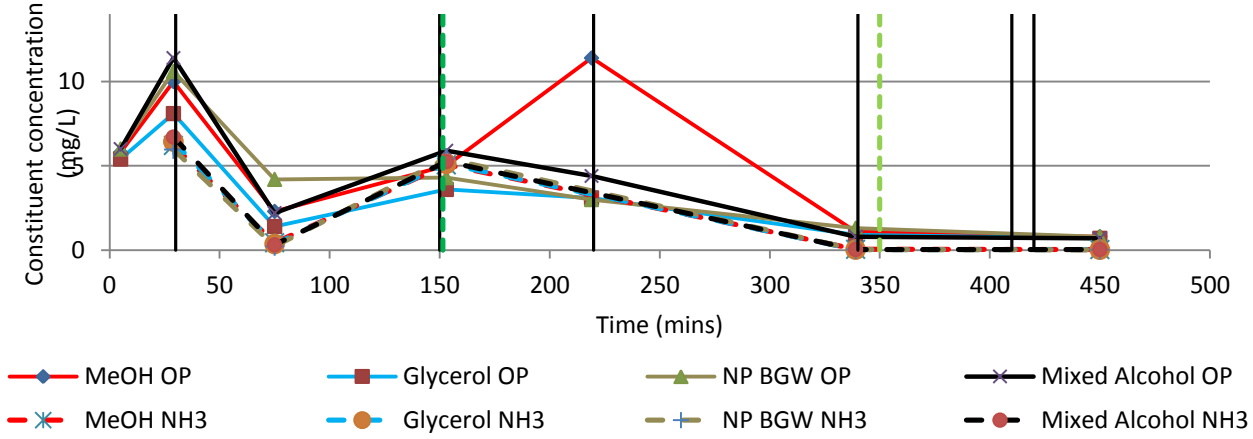
A.129: 12/14/11 Nitrate and Nitrite Profiles



A.130: 12/14/11 Ammonia and Orthophosphate Profiles

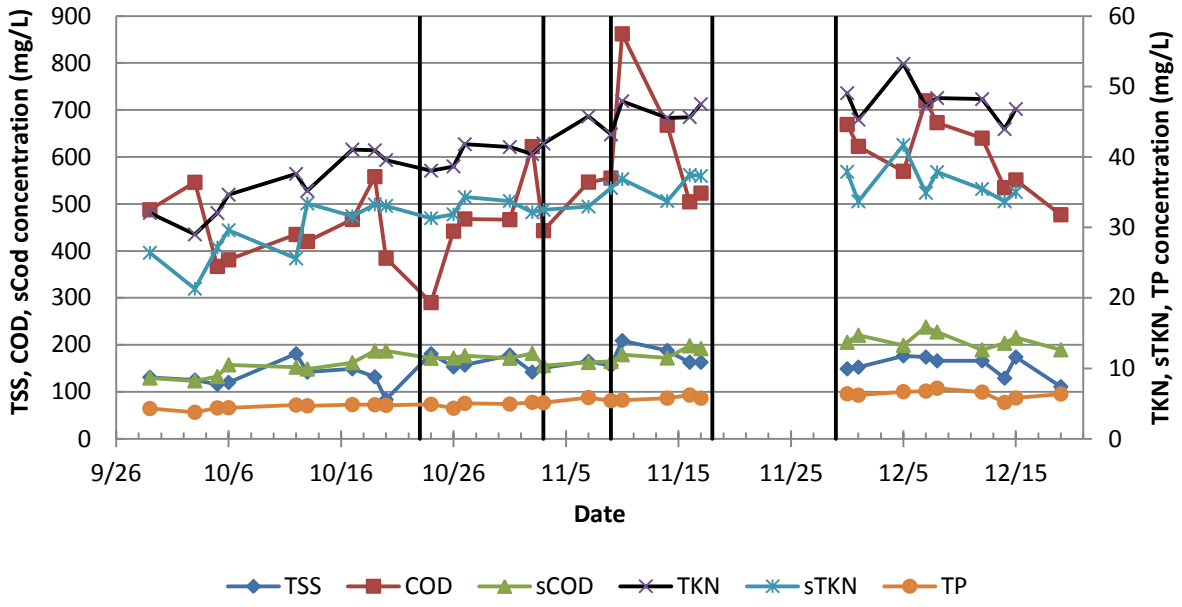


A.131: 12/15/11 Nitrate and Nitrite Profiles

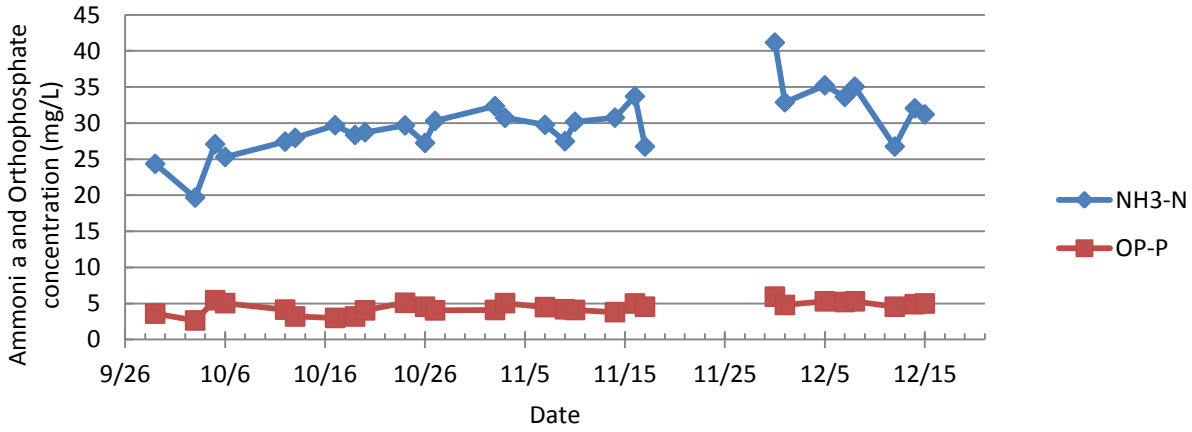


A.132: 12/14/11 Ammonia and Orthophosphate Profiles

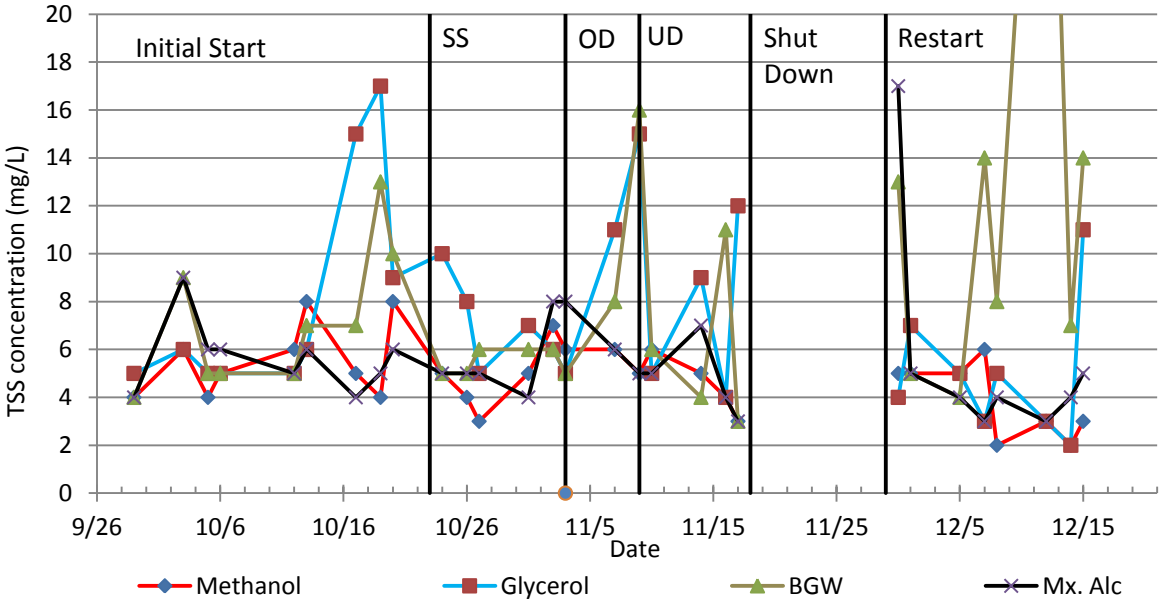
A.7 Influent and Effluent Characteristics



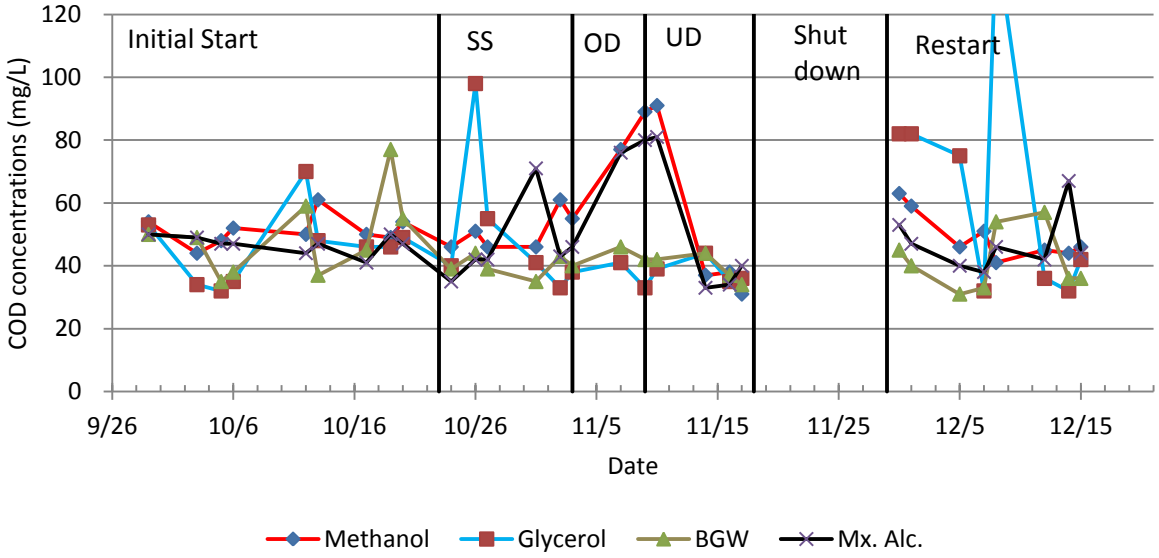
A.133: Chesapeake Elizabeth RWI Characteristics



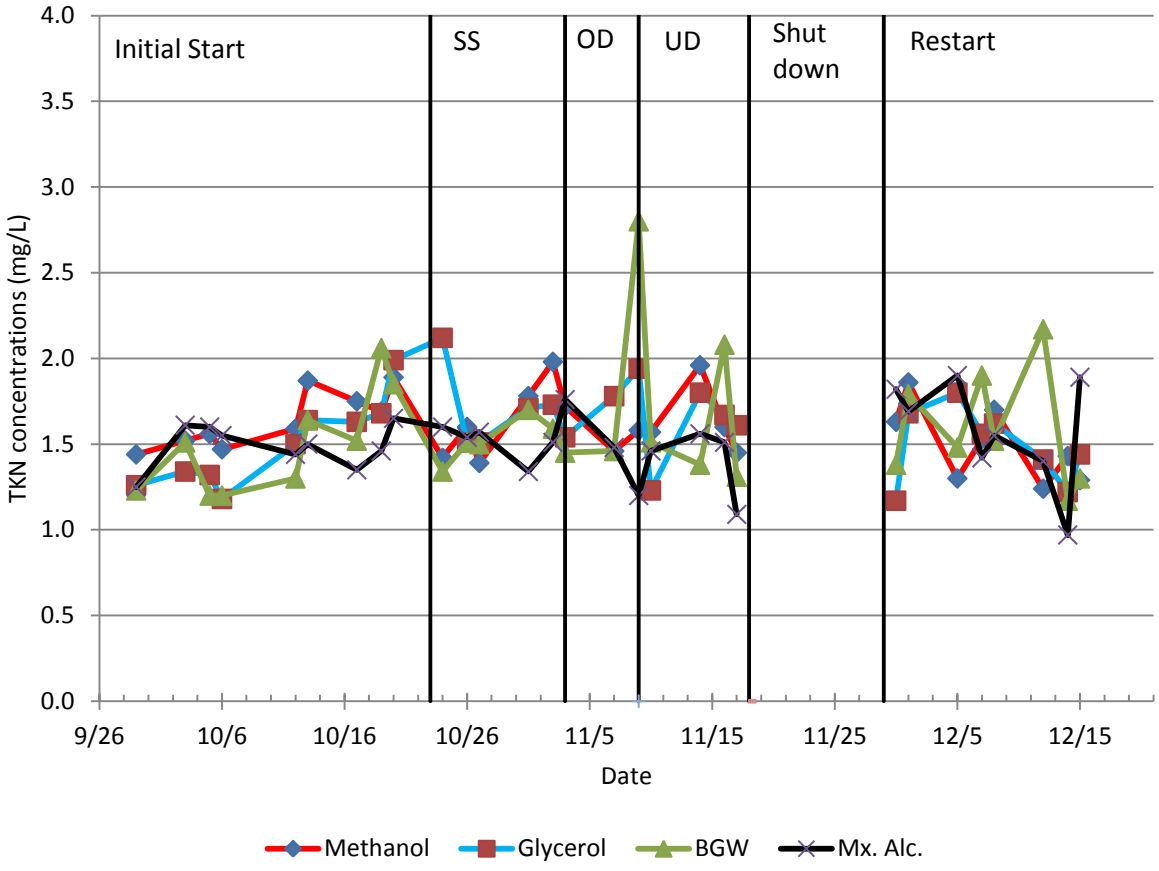
A.134: Chesapeake Elizabeth Influent Ammonia and Orthophosphate Concentrations



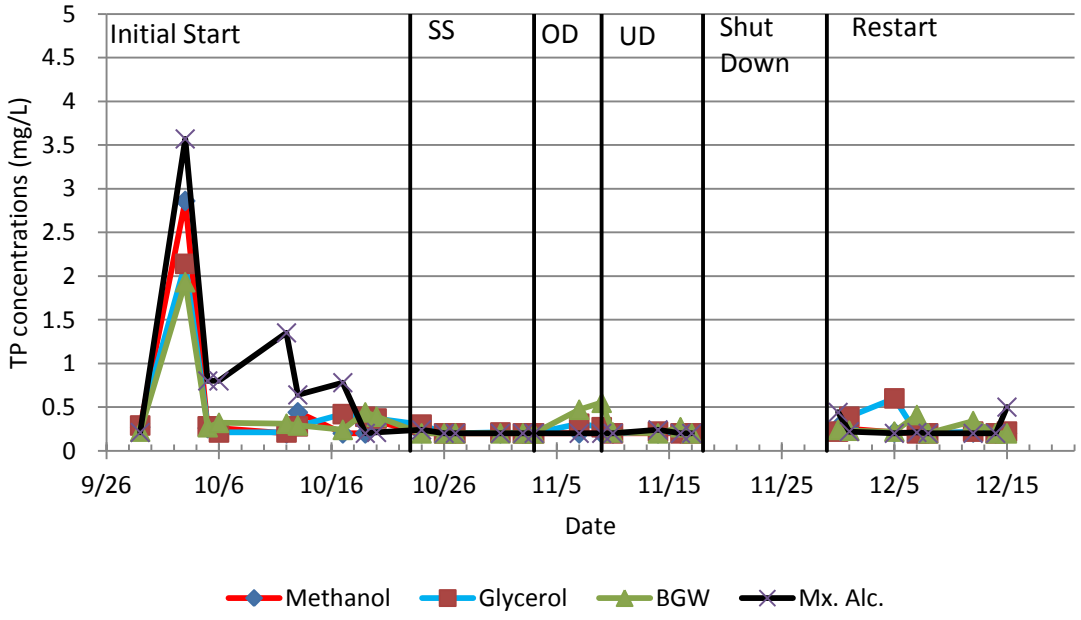
A.135: Effluent Total Suspended Solids



A.136: Effluent COD



A.137: Effluent TKN



A.138: Effluent TP