Evaluation of an Industrial By-product Glycol Mixture as a Carbon Source for Denitrification

Wei Liang

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Gregory D. Boardman
Charles B. Bott
John T. Novak

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ABSTRACT

In order to meet increasingly stringent total nitrogen limits, supplemental carbon must be added to improve the performance of the biological nutrient removal process. An industrial by-product that contained ethylene glycol and propylene glycol was used as a substitute carbon source for methanol in this study. The objectives of this study were to investigate the efficiency of using the glycol mixture as carbon source, including the calculation of denitrification rate and yield at two different initial concentrations of glycols. Possible inhibition effect on nitrification was also investigated. Three SBR reactors were operated by adding methanol, a low dosage of glycol, and a high dosage of glycol into the reactors. The low dosage glycol reactor exhibited the best performance, with the highest denitrification rate of 11.55 mg NO_x-N/g MLVSS•h and the lowest yield of 0.21 mg VSS/mg COD. Small nitrite accumulation was observed in the low dosage glycol reactor (COD=185±15 mg/L), but effluent quality was not influenced. Excess glycol in the reactor caused deteriorated performance. The high dosage glycol reactor (COD=345±20 mg/L) performed with the lowest denitrfication rate of 8.56 mg NO_x-N/g MLVSS•h and the highest yield of 0.55 mg VSS/ mg COD. The reactor with the high dosage of glycol also inhibited the lowest nitrification rate of 1.15 mg NH_3-N oxidized/g MLVSS•h, which indicated that excess glycol may cause nitrification inhibition.

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1 INTRODUCTION AND OBJECTIVES

1.1 Introduction

Nitrogen is one of the most abundant elements in the air we breathe and it is also an essential nutrient for plant and animal growth. But excess nitrogen in the environment can cause serious air and water pollution. Discharging nitrogen into receiving water can cause a lot of serious environmental problems, such as eutrophication, hypoxia, and toxicity to aquatic organisms. Furthermore, high nitrite/nitrate concentrations will cause the "blue baby syndrome". Thus it is very important and necessary to achieve low a nitrogen level in wastewater treatment plant effluent. With the increasingly stringent wastewater effluent standards on nitrogen, more and more wastewater treatment plants are using biological nitrogen removal process. In this process, nitrification followed by a denitrification process is commonly used. In the biological nitrogen removal process, nitrification converts ammonia into nitrate, and the denitrification process turns nitrate into nitrogen gas. To fulfill complete denitrification, a minimum COD/N ratio of 3.5-4.8 (Stern and Marais, 1974; Marsden and Marais, 1976; Henze and Harremoes, 1990, 1992; Nyberg *et al.*, 1992) is required due to the heterotrophic characteristics of denitrifying bacteria. Thus a supplemental carbon source is needed to meet the minimum COD/N ratio for denitrification. Methanol has been chosen as the most prevalent carbon source based on its relatively low cost and availability on the market. It still has disadvantages, such as having fluctuating nitrogen effluent (Christensson *et al*., 1994), a long adaptation time for generation of methanol utilized organisms-methylotrophs before achieving satisfactory denitrification rates (Nyberg et al., 1992), and a potential safety issue with its toxicity and flammability. All these shorts comings have led researchers to explore suitable carbon substitutes for methanol. The choice of a carbon source is mainly based on its denitrification efficiency and cost. Ethanol, acetate, methane, and glycerol have long been proven to be capable of serving as a denitrification carbon source (Christensson *et al*., 1994, Isaacs and Henze, 1995, Thalasso *et al*., 1997, Grabinska-Loniewska *et al*., 1985). Glycol as a colorless, odorless organic group of low volatility

and high hygroscopy (Cox, 1987) has been widely used in industrial applications. Ethylene glycol and propylene glycol are widely used in antifreeze, polyester fiber and polyester resins (Staples *et al*., 2001), and their high solubility decides that they can be readily degraded. Based on the great quantity available on the market and their physical and chemical properties, the thought of using them as supplemental carbon source was developed.

1.2 Objectives

In this research, we were focused on using an ethylene glycol (EG)/ propylene glycol (PG) Coproduct stream as a carbon source for a denitrification. This EG/PG mixture is a by-product from the glycerol to propylene glycol hydrogenolysis process.

The major hypotheses of this study included:

1. Glycols can be readily used as a supplemental carbon source. Glycol can effect satisfactory denitrification rate and low level of effluent nitrogen.
2. Using glycols as carbon source has higher sludge yield compare to methanol.
3. The EG/PG Coproduct stream does not inhibit on nitrification or denitrification.

The objectives of this research were to:

1. Compare nitrification and denitrification rates when using methanol and the EG/PG mixture as carbon sources.
2. Compare the C/N ratio of using methanol and glycols as denitrification carbon sources.
3. Study the possible inhibition of nitrification and denitrification when using the EG/PG Coproduct.

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2 LITERATURE REVIEW

2.1 Biological Nitrogen Removal

Nitrogen is one of the most abundant elements in the air we breathe and it is also an essential nutrient for plant and animal growth. However excess nitrogen in the environment, especially when it comes from human activities, can cause serious air and water pollution. When too much ammonia or nitrate is discharged into lakes and rivers, a lot of problems will arise. Ammonia oxidation to nitrate and nitrite can lead to oxygen depletion in the receiving water and can kill fish. Ammonia itself is also toxic to fish and other aquatic organisms. Besides that, excess nitrate in receive water can stimulate the growth of algae, which contributes to the eutrophication of lakes and rivers. In addition to these environmental problems, high nitrogen effluent level can also threaten human health. High nitrate and nitrite levels in drinking water can cause methemoglobinemia in infants, and also contribute to the formation of cancinogenic nitrosamines (Wiesmann, 1994). The major nitrogen sources are runoff of fertilizers containing nitrate and wastewater with high nitrogen levels and deposition of nitrogen-containing compounds from the atmosphere. Among these, the most controllable source is wastewater effluent. Therefore, increasingly stringent regulations on the effluent nitrogen level of wastewater treatment plants have been developed.

To meet the more and more strict regulations, wastewater treatment plants normally use a process known as biological nutrient removal. Biological nutrient removal processes are those active sludge processes that incorporate anaerobic or anoxic sections to provide nitrogen and phosphorus removal. In a biological nutrient removal process, different zones provide different functions. A biological nitrogen removal process normally contains an aerobic zone in which the nitrification process happens and an anoxic zone, where denitrification happens.

2.1.1 Nitrification

Nitrification is a process in which ammonia is converted to nitrite and then nitrate. This process happens naturally in the environment and occurs through specialized bacteria. Ammonia is toxic to aquatic life and naturally occurring nitrification can cause oxygen depletion in receiving water, which can kill fish and other aquatic life. To treat wastewater with high ammonia concentration, a nitrification process under human control is desirable. By controlling aeration and other conditions, one hopes to transform most of the ammonia to nitrite or nitrate before the wastewater is discharged.

A nitrification process consists of two processes: nitritation and nitratation. Two different types of bacteria, ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), carry out these two processes respectively (Tchobanoglous *et al.*, 2003). Both of these bacteria groups are chemo-litho-autotrophic, which means they utilize chemicals as their energy source, inorganic compounds as electron-donor, and carbon dioxide as their carbon source. These bacteria are known as "nitrifiers"; they must have free dissolved oxygen to perform their work.

The first step of nitrification is nitritation, in which ammonia is oxidized into nitrite. The reaction is as follows:

$$NH_3 + 1.5O_2 \longrightarrow NO_2^- + H^+ + H_2O \qquad 2\text{-}1$$

The second step is nitratation, in which nitrite is oxidized into nitrate. The reaction is as follows:

$$NO_2 + 0.5O_2 \longrightarrow NO_3^- \qquad 2\text{-}2$$

The total nitrification reaction is as follows:

$$NH_3 + 2O_2 \longrightarrow NO_3^- + H^+ + H_2O \qquad 2\text{-}3$$

The two processes, nitritation and nitratation, can happen at different velocities. If nitritation is faster than nitratation, nitrite will build-up; if nitratation is faster, then little nitrite will be detectable. Nitrite build-up can be inhibitory to both AOB and NOB.

The nitrification rate is highly dependent on a few environmental factors. One of the most important is oxygen concentration. It is reported that the minimum dissolved oxygen (DO) concentration for efficient nitrification is 2 mg/L. When the DO concentration is less than 0.5 mg/L, nitrification is inhibited (Grady *et al*., 1999). So it is

very important and normally costly, for wastewater treatment plants to provide enough oxygen in the nitrification zone. Temperature is also a crucial factor. Nitrifying bacteria are temperature sensitive. The best nitrification performance is acquiring at temperatures between 20 ℃ and 40 ℃. There is a critical temperature below which the rate of nitratation is less than the rate of nitritation, because it is reported that the nitrate formers, such as *Nitrobacter*, are more sensitive to decreased temperatures than nitrite formers, such as *Nitrosomonas*. A build-up of nitrite would happen if temperature drops below that critical temperature (Randall *et al*., 1984). The maximum specific growth rate of nitrifiers monotonically increases as a function of temperature in the range of 15-25 ℃ (Antoniou *et.al*., 1990).

During nitrification, some hydrogen ions are produced. This acid formation lowers the pH in the aeration tank. The optimum pH for nitrifiers is reported in a wide range. Meyerhof (1917) suggested that the optimum pH for *Nitrosomonas* is 8.3 to 8.8 and for *Nitrobacter* is 8.3 to 9.3. While Hofman *et al*. (1953) found the optimum pH for *Nitrosomonas* is 8.3 and for *Nitrobacter* is 7.7. In general, the nitrification rate decreases as the pH decreases. Painter reported that nitrification ceased under pH of 5 to 5.5. To maintain the pH in an optimum range, an alkalinity of between 50 and 150 mg/L as $CaCO_3$ is normally required. Other substances, such as heavy metals and free ammonia, can also inhibit nitrification.

2.1.2 Denitrification

As mentioned before, it is very important to convert ammonia to nitrate in wastewater, but this process does not actually remove any nitrogen from wastewater. Excess nitrate in receiving water can also cause serious environmental problems, such as eutrophication. And high concentration of nitrate in drinking water can threaten the health of babies. Denitrification is a process that facilitates nitrate reduction and ultimately produces nitrogen gas.

Generally, denitrification is a respiratory process that happens in environment with depleted oxygen. This process is a reduction-oxidation reaction that needs an electron donor to provide electrons that are transferred to an electron acceptor. Organic matter

serves as the electron donor and nitrate serves as the electron acceptor instead of oxygen. Denitrification is a stepwise reduction process as follows:

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2$$

The denitrification process is performed primarily by heterotrophic bacteria and several species are involved, such as *Achromobacter* species, *Alcaligenes facalis*, *Bacillus*, *Chromobacterium Violaceum*, *Paracoccus* denitrificans, and *Pseudomonas* (Knowles, 1982). Several enzymes are involved in this process at different steps. The reduction of nitric oxide to nitrous oxide is mediated by NO reductases. The reaction is as follows:

$$2NO+2H^++2e^- \longrightarrow N_2O+H_2O \qquad 2\text{-}4$$

The reduction of nitrous oxide to dinitrogen is performed by N_2O reductases, as follows:

$$N_2O+2H^++2e^- \longrightarrow N_2+H_2O \qquad 2\text{-}5$$

The complete denitrification process can be expressed as the following equation:

$$2NO_3^-+10e^-+12H^+ \longrightarrow N_2+6H_2O \qquad 2\text{-}6$$

The completion of denitrification is very important because the intermediates such as nitric oxide and nitrous oxide are greenhouse gases. They can react with sunlight and ozone to produce nitric acid, a component of acid rain. There are several factors that influence the denitrification rate and the completion of denitrification. First of all, an anoxic environment with very low levels of dissolved oxygen is crucial to the denitrification process. This is because oxygen is a more energetically favorable electron acceptor than nitrate. Only under oxygen depleted conditions, can nitrate be utilized as a substitute electron acceptor. Besides that, the presence of oxygen blocks nitrate reduction enzymes and increases N_2O production (Focht, 1974). The other crucial factor related to denitrification rate is the carbon source. As mentioned before, to complete the process, organic matter must be present to act as an electron donor. In general, easily degraded materials such as methanol and ethanol give the highest denitrification rates. A minimum of COD/N ratio of 3.5-4.8 (Stern and Marais, 1974; Marsden and Marais, 1976; Henze and Harremoes, 1990, 1992; Nyberg *et al.*, 1992) is required to complete denitrification. The organic matter in raw wastewater is usually not easily degraded and does not fulfill the minimum COD/N ratio, especially when the wastewater is treated by post denitrificaion process where nearly all organic matter has already been oxidized in the aerobic tank. It is important for wastewater plant personnel to choose appropriate type

and quantities of an external carbon source that can be used in the denitrification process. Denitrification is also sensitive to pH. The optimum pH range is 7.0 to 8.0 (Delwiche, 1974; Muller, 1980; Nommik, 1956; Van Cleemput, 1974, Wijler 1954). At low pH values, the N_2O reductases are progressively inhibited, decreasing the overall denitrification rate. Furthermore, a decreasing pH tends to increase the proportion of N_2O in the products (Focht, 1974). The rate of denitrification increases with temperature in a similar manner to nitrification rate.

2.2 Supplemental Carbon Source

To complete nitrate reduction, easily degradable organic matter must be provided. In general, the denitrification potential is primarily a function of available organic carbon, which is usually expressed as COD/N (Kujawa, 1999). The required COD/N ratio to satisfy completion of denitrification is reported in a wide range of 4-15 gCOD/gN. As the IAWQ-model No. 1 and 2 indicated, there are two fractions of total COD in wastewater (Henze *et al*., 1987,1996): soluble, readily biodegradable COD (Ss) and particulate, slowly biodegradable COD (Xs). During denitrification with wastewater as an electron donor, there are different linear phases of nitrate reduction related to different fractions of COD (Stern and Marais, 1974; Van Haandel *et al*., 1981). The Ss allows for the highest denitrification rate, while a lower denitrification rate is with Xs. However, the organic load in raw wastewater is always low, especially the soluble, readily biodegradable COD. This contributes to the incompletion of denitrification. Therefore, an external carbon source should be added to increase Ss and thus to enhance the denitrification rate.

Several kinds of carbon sources have been proven to be applicable, such as methanol, ethanol, acetate, glycerol, methane and some waste products (Christensson *et al*., 1994, Isaacs and Henze, 1995, F. Thalasso *et al*., 1997, Grabinska-Loniewska *et al*., 1985). To choose the most suitable carbon source, certain criteria should be considered. The efficiency of the carbon source to enhance denitrification is the most important. This characteristic can be evaluated by specific denitrification rate (SDNR) and yield. When carbon source is introduced as electron donor, it is used for two different purposes. One part of it is oxidized to produce energy by denitrification of nitrate to nitrogen gas, whereas the other part goes towards new cell synthesis. Low yield means more energy

was used for denitrification enhancement and less sludge production, more importantly, it means that less carbon source is need for denitrificaiton. Yield can be measured by the COD/N ratio.

The other important factor of choosing a carbon source is the cost. Carbon source is consumed in great quantities at wastewater treatment plants. A lower cost carbon source can significantly reduce the investment of wastewater treatment plants. Availability on the market and the safety of transportation and storage are also important factors related to carbon source selection. In general, it is popular to choose a low cost and low yield carbon source that can perform stable, efficient denitrification. To meet the increasingly stringent effluent regulations, it is important to explore the use of new carbon sources that can greatly enhance denitrification efficiency.

2.2.1 Methanol

Among all of the available carbon sources, methanol has served as the most prevalent electron donor in wastewater treatment plants worldwide. The advantages of choosing methanol as a supplemental carbon source include the higher denitrification efficiency it can bring, the relatively lower yield, lower cost and broad availability in the market. Methanol is divided into two parts. One part of it goes towards bacterial energy for denitrification. The overall energy reaction can be expressed as:

$$6NO_3^- + 5CH_3OH \longrightarrow 3N_2 + 5CO_2 + 7H_2O + OH^- \quad (2\text{-}7)$$

The other part of methanol goes to cell synthesis. The cell formula suggested by Hoover and Porgess (1952) as $C_5H_7NO_2$ is used. The equation is as follows:

$$14CH_3OH + 3NO_3^- + 4H_2CO_3 \longrightarrow 3C_5H_7NO_2 + 20H_2O + 3HCO_3^- \quad (2\text{-}8)$$

The overall nitrate removal reaction is as follows (Metcalf and Eddy, 2010):

$$NO_3^- + 1.08CH_3OH + H^+ \longrightarrow 0.065C_5H_7NO_2 + 0.467N_2 + 0.76CO_2 + 2.44H_2O \quad (2\text{-}9)$$

It is reported that about 25 to 30% of methanol is used for bacterial cell synthesis (Mateju *et al.*, 1992). Based on experimental results, a formula for determining the requirement of methanol for denitrification has been developed. The formula is as follows (McCarty *et al.*, 1969):

$$c_{met} = 2.47NO_3^- - N + 1.53NO_2^- N \quad (2\text{-}10)$$

The common working value of 3.2 kg of methanol per kg NO_3^--N removed is higher than the stoichiometric amount of 2.47 kg. This might because of the deoxygenation consumption at the beginning of anoxic period.

Over the past decades, more and more wastewater treatment plants are exploring the use of new carbon sources to replace methanol. This is because several disadvantages of using methanol have arisen. It has been reported that a long adaptation time is needed before satisfactory denitrification rates are achieved (Nyberg *et al*., 1992). To use methanol as a carbon source, a specific methanol-using denitrifying bacteria, called a methylotroph has to build up (Christesson *et al*., 1994). The length of the lag period depends on the solids retention time of the reactors. It normally takes 4 to 8 weeks before achieving significant methanol uptake and satisfactory denitrification (Selock *et al*., 2008). It is also observed that methanol cannot achieve as stable denitrification as other carbon sources can, such as ethanol (Christesson *et al*., 1994). Additionally, the growth rate of specific methanol-using denitrifying bacteria decreases at lower temperatures and thus could cause washout of denitrifying bacteria from the system and deteriorated denitrification. Results from the study at Blue Plains AWTP showed that the maximum growth rate of methanol utilizers at 13℃ decreases by half at 19℃ (Mokhayeri *et al*. 2006). The same problem happens in those wastewater treatment plants that encounter low temperatures in winter. The only way to enhance the denitrification performance in winter is to utilize a substitute carbon source in place of methanol. Besides that, methanol is a flammable and toxic compound; the safety issue associated with its transportation and storage is costly. It is reported that compared to a non-flammable, non-hazardous product, an additional 25 to 31% of the capital construction cost is required to meet the safety standards. Additional costs may be required for methanol storage, pumping, and delivery (CDM, 2007), which weakens its advantages over other carbon sources.

2.2.2 Glycols

Glycols are a class of organic compounds belonging to the alcohol family that is composed of one to four units of ethylene oxide, which are connected to each other by ether linkages (Cox, 1987). The glycols have physical properties such as low volatility, high hygroscopicity, and they are colorless and odorless (Cox, 1987). Glycols are widely

used in industrial applications due to their features mentioned above. They are used as heat-transfer fluids, lubricants, brake fluids, dye solvents, humectants polyester resin softening agents, and selective solvents (Cox, 1987). Ingestion of ethylene glycol could hurt a human's central nervous system and even cause death. No health effects were reported when people were chronically exposed to ethylene glycol. Ethylene glycol also has low toxicity to aquatic organisms. The toxic thresholds for microorganisms are above 1000 mg/L (World Health Organization, 2000). From their vast production and utilization, a considerable amount of glycols are introduced into the environment and cause potential environmental problems. Thus, a lot of studies of their fate of degradation and environment risks have been done. Among all of the glycols, the two simplest compounds in the class, ethylene glycol and propylene glycol, are most widely used and studied.

Ethylene glycol is also called 1,2-ethanediol and has the molecular formula $HOCH_2CH_2OH$. It is a viscous hygroscopic liquid with a sweet taste, but no color or odor (Staples *et al*., 2001). On a worldwide base, about two-thirds of ethylene glycol is used in the manufacture of polyesters, in which it performs as a chemical intermediate. Over one-quarter of ethylene glycol is used as antifreeze in engine coolants (World Health Organization, 2000). The major release of ethylene is use as antifreeze, which then is introduced into hydrosphere. Ethylene glycol has a low vapor pressure of 7.0 Pa at 20℃ (Eisenreich *et al*., 1981) and low Henry's law constant of $6.08\times10^{-3}Pa\bullet m^3/mol$, which indicates that ethylene glycol will not easily volatilize from water bodies or soil surfaces (Howard, 1991). Ethylene glycol is not expected to be hydrolyzed in the environment (Lyman *et al*., 1982). Low soil partition coefficients (log K_{oc}) of 0-0.62 were suggested by Lokke (1984), which indicated that ethylene glycol has a high mobility in soil and could leach into groundwater eventually. Ethylene glycol also has low octanol/water partition coefficient (log K_{ow}) of -1.93 to -1.35. Through this parameter, it can be determined that ethylene glycol has a low potential for bioaccumulation. According to the standard biodegradation tests made by the Organization for Economic Co-operation and Development (OECD), such as US environmental Protection Agency (USEPA), ethylene glycol is readily biodegradable (World Health Organization, 2000). The biodegradation

of ethylene glycol in both aerobic and anaerobic conditions have been studied, and in both conditions it has been proven to be biodegradable.

The biodegradation rates of ethylene glycol were reported over a wide range. Whether it has a lag period and the length of the lag period is in controversy. However, most of studies were focused on the aerobic degradation of glycol. Under aerobic conditions, Means and Anderson (1981) reported that 71% biodegradation of ethylene glycol to 10% or less of the original concentration took 21 days. In the study of Boatman *et al*. (1986) the ethylene glycol was degraded by day 21, acclimated sewage sludge was chosen as inoculum. Removal of ethylene glycol within 210 h was reported by Pitter (1976) to be 96.8% and 63% degradation after 5 days was reported by Wolff *et al*. (1979) when using previously adapted sewage sludge as inoculum. McGohey and Bowwer (1992) reported the biodegradation rate of ethylene glycol in groundwater, to be 0.76/day at 25℃. Complete biodegradation of ethylene glycol in fresh water at day 20 was reported by Price *et al*. (1974). According to Boatman *et al*. (1986), significant ethylene glycol degradation did not occur until day 14, and a lag period of 8-10 days was estimated. A lag period of 3 days was suggested by McGahey and Bouwer (1992). No lag period was observed in the study of Zahn and Wellens (1980). Under anaerobic conditions, Kameya *et al*. (1995) found 89% degradation of ethylene glycol within 7 days. Based on the studies mentioned above, it can be concluded that ethylene glycol is an easily biodegradable compound and using it as the denitrification carbon source would not cause carbon accumulation in the reactor.

Several kinds of bacteria have been proven to be capable of degrading ethylene glycol. Soil bacteria *Pseudomonas aeruginosa* and *Acinetobacter* strains, can degrade ethylene glycol using oxygen (Haines *et al*., 1975; Watson *et al*., 1977). Another bacterium, *Flavobacterium*, was not able to degrade ethylene glycol, but under strongly aerobic conditions, it can convert ethylene glycol to glycolate and eventually carbon dioxide (Willetts, 1981). *Methanobacterium sp*. and *Desulfavibrio sp*. are capable of degrading ethylene glycol under methanogenic conditions (Dwyer *et al*., 1993). Anaerobic bacteria, *Clostridium glycolicum* and *Acetobacter*, were isolated and proved to be able to degrade ethylene glycol (Gaston *et al*., 1963; Kaushal *et al*., 1951; Hrotmatka

et al., 1962). Few studies reported the bacteria related to the denitrification by using of ethylene glycol.

Temperature is a key factor that influences the biodegradation rate of ethylene glycol. The biodegradation rate decreases as the temperature goes down (Evans *et al*., 1974; McGahey *et al*., 1992). However, complete biodegradation can be achieved in both high and low temperatures. Another factor found to be related to the biodegradation rate was the glycol concentration. McGahey *et al*. (1992) reported that a high concentration of ethylene glycol could cause a diminished biodegradation rate.

Propylene glycol is also called 1,2-propanediol and has a molecular formula $HOCH_2CHOHCH_3$. It has similar physical properties as ethylene glycol, but the two do not have a similar degree of toxicity in mammals. Propylene glycol is much less toxic than ethylene glycol. It is listed as a "Generally Recognized as Safe Additive" in foods (FDA, 1982) and it is not on the Toxic Release Inventory in US or Canada, so little data are available on the releases of propylene glycol into the environment. The main uses of propylene glycol are for polyester resins and for consumables. It is used as a humectant, solvent and preservative in food and tobacco products. It is also the major component in aircraft deicers. Runoff of the deicers is the main source of propylene glycol release into environment. Propylene glycol has low vapor pressure of 0.07 mmHg at 20℃, low Henry's law constant values of 1.2×10^{-8} to 1.7×10^{-8} atm-m^3/mol and low soil partition coefficients (Simmons *et al*., 1976; Swarm *et al*., 1983). The physical properties of propylene glycol indicate that it has high mobility in soil and will eventually leach into groundwater. Thus, the majority of released propylene glycol in the environment is expected to be transported in aqueous media (EPA, 1979). Propylene glycol also has a low octanol/water partition coefficient K_{ow}, as does ethylene glycol, which indicates that propylene glycol is readily biodegradable and bioconcentration/biomagnification are not likely to happen.

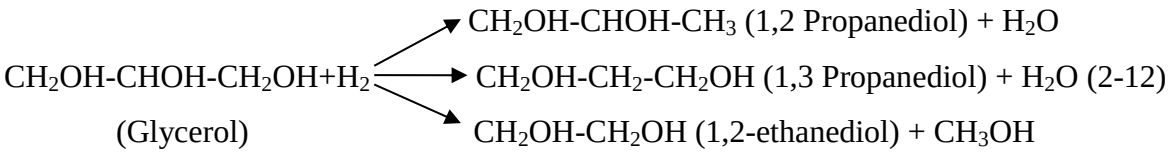
Propylene glycol can be degraded by a variety of acclimated and unacclimated microorganisms under both aerobic and anaerobic conditions. The general half-lives of propylene glycol biodegradation range from 1 to 4 days under aerobic conditions, and 3 to 5 days under anaerobic conditions (EPA, 1987). Kaplan *et al*. (1982) reported propylene glycol was completely degraded after 4 days under aerobic conditions and 9

days under anaerobic condition, when it was used as sole carbon source. Raja *et al*. (1991) suggested a biotreatment process using *Pseudomonas* and *Aerobacter* bacteria to degrade propylene glycol. *Pseudomonas* was able to convert propylene glycol to a volatile acid, and then *Aerobacter* degraded the volatile acid to carbon dioxide and water. The soil microbe, *C. glycolicum*, isolated by Gaston *et al*. (1963) was able to degrade propylene glycol to acid and alcohol end products under anaerobic conditions. A lag period of 2-4 days was observed by Gooden (1998). Generally, the biodegradation rate of propylene glycol was higher with low glycol concentrations and high temperatures. There was also a report that the degradation rates of propylene glycol were limited by the availability of nitrogen and phosphorus in the water (Gooden, 1998). However, the bacteria of degrading propylene glycol under denitrifying conditions were not reported.

Both ethylene glycol and propylene glycol are major components of aircraft deicer because of their unique physical properties. There are many different formulations of the deicer according to the different usages, such as aircraft deicing, aircraft anti-icing, pavement deicing and so on. In most of the deicers, ethylene glycol and propylene glycol are used together (Switzenbaum, 2001). To deice and prevent ice formation on aircraft is very important for ensuring wintertime flight safety. Large quantities of ethylene glycol and propylene glycol based mixtures are used. According to USEPA (1995), deicing a large commercial aircraft takes 2-4 m^3 of aircraft deicing fluid; over 1000 m^3 of fluid would be used by a medium-sized airport during the entire winter season. (Betts, 1999) Deicing fluid runoff becomes one of the main sources of ethylene glycol and propylene glycol release in water. It is estimated that about 21 million gallons of aircraft deicing fluids are discharged into surface water every year in the US, and 2 million gallons of fluids are discharged into public wastewater treatment plants (EPA, 2000). Deicing fluids releasing into the environment leads to considerable organic loads in receiving water and cause some environmental problems. Toxic signs such as ethylene poisoning, fish kills and reduced biodiversity have been reported in the vicinity of airports (World Health Organization, 2000). The biochemical oxygen demand of wastewater during decomposition occurring over a 5-day period (BOD_5) of an airport runoff can be as high as 245,000 mg/L according to Veltman *et al*. (1998). Thus, collecting the fluids and utilizing them as a denitrification carbon source have come into people's mind.

A lot of studies have found that deicing fluids are capable of being used as a carbon source. Similar specific denitrification rates (SDNR) have been reported. B. Rusten *et al.* (1997) suggested that deicing fluids have a SDNR of 10.6 $mgNO_x$-N/gVSS•h, which was higher than the denitrification rates when using methanol and ethanol as carbon sources. Denitrification rates of 8 $mgNO_3$-N/gVSS•h and 6.5-8.4 $mgNO_x$-N/gVSS•h were reported by J. Chen *et al*. (2013) and J. Trela (1998) respectively. The C/N ratio of using deicing fluids as a carbon source was reported to be between 4.7 g COD/g NO_3-N to 10 g COD/g NO_3-N (Chen *et al*., 2013; Trela, 1998; Plaza *et al*., 1990). Generally, deicing fluids required less time for acclimation by denitrifying bacteria than methanol did. It took only 20 h to achieve 80% nitrogen removal after the deicing fluids were introduced (Trela, 1998). A lag time of 3 days for a response of denitrifying bacteria to deicing fluids was reported by Chen (2013). Besides that, deicing fluids can be switched to ethanol and switched back without a lag time (Rusten, 1997). Based on all of these studies, it can be concluded that ethylene/propylene glycol mixtures can perform well as an external carbon source and even have some advantages over methanol.

Over the past decade, a form of diesel fuel manufactured from vegetable oils and animal fats has arisen, and is called "biodiesel". It is a safe and biodegradable fuel with less air pollution problems than petroleum-based diesel. Glycerol, as the main by-product of the biodiesel fuel process, is produced in large quantities. It is reported that about 1 kg of crude glycerol by-product is formed when 9 kg of biodiesel is produced (Dasari, *et al*., 2005). To deal with the by-product and increase profitability, biodiesel production plants can convert glycerol to propylene glycol through hydrogenolysis (Dasari, *et al*., 2005). The overall reaction of converting glycerol to propylene glycol (1,2 Propanediol, 1,3 Propanediol) and ethylene glycol (1,2-ethanediol) is as follows:



As the formula above shows, a by-product of propylene/ethylene glycol is produced during this process, which has similar components as deicers. So, instead of disposing of

the mixture, perhaps it should be used as a supplemental denitrification carbon source. In this study, this kind of proplylene/ethylene glycol mixture was used as the carbon source and certain parameters related to its denitrification efficiency were tested.

2.3 Sequencing Batch Reactor (SBR)

Sequencing batch reactors are a variation of activated-sludge processes, combining all of the treatment steps into a single tank, where all metabolic reactions and solid-liquid separation takes place. It has been used worldwide since the 1920s (Al-Rekabi *et al*., 2007). There are several advantages of choosing SBR. First of all, SBRs are very suitable in treatment plants with limited space, since all of the treatment steps happen in a single tank instead of multiple tanks. A low total suspended solids value can be achieved by effective decanters in SBRs, so a separate clarifier can be eliminated. The other advantage of SBRs is that the treatment cycle can be adjusted to achieve different biological nutrient removal goals. If the react periods are aerobic throughout, then only carbon oxidation and nitrification would happen. If aeration is eliminated, but mixing is maintained, denitrification would occur. If SBR is operated with short SRT, so no nitrate is produced and then mixed without aeration, which allows the SBR to behave like a A/O continuous system, then phosphorus removal will be achieved. Besides that, the SBR is easily retrofitted from older wastewater treatment facilities by using the existing basins. SBRs are cost-effective to achieve lower effluent limits with increasingly stringent discharge permits (NEIWPCC, 2005). A SBR could save 60% of the costs of conventional activated sludge processes required to achieve a high quality effluent. It is reported that SBRs were able to get more than 90% biochemical oxygen demand (BOD) removal (Metcalf and Eddy, 2010).

The SBR operation is based on the fill-and draw principle, which contains five steps:

1. Fill: During the fill period, influent wastewater is introduced into basins. The influent provides essential nutrients for microbial growth and creates an environment for biochemical reactions. There are three scenarios of a fill period: static fill, mixed fill and aerated fill. Under static fill, no mixing or aeration happens. This is often used during the initial start-up phase of a facility to save energy. During mixed fill, the mechanical mixers are active but there is no aeration, so denitrification is promoted.

For the aerated fill, both mixers and aerators are active, which allows for organic oxidation and nitrification.

2. React: No wastewater enters or discharges from the basin and mechanical mixers and aerators are in action. Most of the carbon oxidation and nutrient removal happens in this period. The treatment process is controlled by turning the air on and off to create aerobic, anaerobic and anoxic conditions (Singh *et al*., 2011).
3. Settle: During this phase, activated sludge is allowed to settle; both mixers and aerators are stopped. The entire basin acts as a clarifier during this time. This period is a critical part of the whole process because if the sludge does not settle rapidly or the settling period is too short, some sludge can be drawn off and thereby influences effluent quality.
4. Decant: A decanter is used to remove the upper liquid during this phase. It is important to avoid collecting the surface foam or scum. The decanted volume should be about the same as influent volume.
5. Idle: This step occurs between fill and decant. The length of this step depends on the influent flow rate and operating strategy (NEIWPCC, 2005).

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3 Evaluation of an Industrial By-product Glycol Mixture as a Carbon Source for Denitrification

Abstract

In order to meet increasingly stringent total nitrogen limits, supplemental carbon must be added to improve the performance of the biological nutrient removal process. An industrial by-product that contained ethylene glycol and propylene glycol was used as a substitute carbon source for methanol in this study. The objectives of this study were to investigate the efficiency of using the glycol mixture as carbon source, including the calculation of denitrification rate and yield at two different initial concentrations of glycols. Possible inhibition effect on nitrification was also investigated. Three SBR reactors were operated by adding methanol, a low dosage of glycol, and a high dosage of glycol into the reactors. The low dosage glycol reactor exhibited the best performance, with the highest denitrification rate of 11.55 mg NO_x-N/g MLVSS•h and the lowest yield of 0.21 mg VSS/mg COD. Small nitrite accumulation was observed in the low dosage glycol reactor (COD=185±15 mg/L), but effluent quality was not influenced. Excess glycol in the reactor caused deteriorated performance. The high dosage glycol reactor (COD=345±20 mg/L) performed with the lowest denitrfication rate of 8.56 mg NO_x-N/g MLVSS•h and the highest yield of 0.55 mg VSS/ mg COD. The reactor with the high dosage of glycol also inhibited the lowest nitrification rate of 1.15 mg NH_3-N oxidized/g MLVSS•h, which indicated that excess glycol may cause nitrification inhibition.

KEYWORDS: supplemental carbon, methanol, glycol, nitrite, denitrification, nitrification, yield, sequencing batch reactor

3.1 Introduction

Nitrogen is one of the most abundant elements in the air and is also an essential nutrient for plant and animal growth. However, excess nitrogen in the environment can

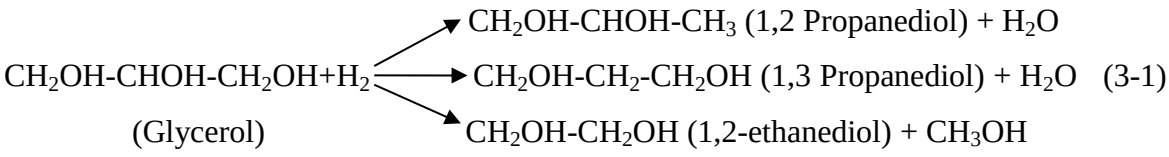
cause serious air and water pollution. When too much ammonia or nitrate is discharged into lakes and rivers, a lot of problems will arise. Ammonia oxidation to nitrite and nitrate can lead to oxygen depletion in receiving water and can kill aquatics. Ammonia itself is toxic to fish and other aquatic organisms. Besides that, excess nitrate in receiving water can stimulate the growth of algae, which contributes to the eutrophication of lakes and rivers. In addition to these environmental problems, high nitrate and nitrite levels in drinking water can cause methemoglobinemia in infants, and contribute to the formation of carcinogenic nitrosamines (Wiesmann, 1994). With the increasingly stringent wastewater effluent standards for nitrogen, the biological nitrogen removal process is being used more commonly. In this process, nitrification is typically followed by a denitrification process. Nitrification is a process in which ammonia is converted to nitrite and then nitrate and denitrification is a process that facilitates nitrate reduction through the production of nitrogen gas. To fulfill complete denitrification, a minimum COD/N ratio of 3.5-4.8 (Stern and Marais, 1974; Marsden and Marais, 1976; Henze and Harremoes, 1990, 1992; Nyberg *et al.*, 1992) is required due to the heterotrophic characteristics of denitrifying bacteria. Supplemental carbon is therefore needed to meet the minimum COD/N ratio for denitrification.

Several types of carbon sources have proven to be applicable, such as methanol, ethanol, acetate, glycerol, methane and some waste products (Christensson *et al*., 1994, Isaacs and Henze, 1995, F. Thalasso *et al*., 1997, Grabinska-Loniewska *et al*., 1985). Methanol has been chosen as the most prevalent carbon source by the wastewater treatment industry based on its relatively low cost and availability. However, it has disadvantages such as having fluctuating nitrogen effluent (Christensson *et al*., 1994), a long adaptation time for generation of methanol utilized organisms-methylotrophs before achieving satisfactory denitrification rates (Nyberg et al., 1992), and potential safety issues due to its toxicity and flammability. All these shortcomings have led to countless studies to identify a suitable substitute for methanol. The choice of a carbon source is mainly based on denitrification efficiency and cost. In general, the goal is to choose some with a low cost and low yield that can accomplish stable, efficient denitrification. To meet increasingly stringent effluent regulations, it is important to explore the efficiency of additive carbon sources.

Glycols are a class of organic compounds belonging to the alcohol family that are composed of one to four units of ethylene oxide. The units connected to each other by ether linkages (Cox, 1987). Among all of the glycols, the two simplest compounds in the class, ethylene glycol and propylene glycol, are most widely used and studied. Propylene glycol and ethylene glycol are readily degradable under both aerobic and anaerobic conditions and they are the major components of aircraft deicers because of their unique physical and chemical properties. Runoff of the deicer is one of the main sources of propylene glycol and ethylene glycol releases into the hydrosphere. The BOD_5 of an airport deicing fluid runoff can be as high as 245,000 mg/L, according to Veltman *et al*. (1998). Deicing fluids released into the environment lead to considerable organic loads in receiving waters and cause environmental problems. Toxic signs such as ethylene poisoning, fish kills, and reduced biodiversity have been reported in the vicinity of airports (World Health Organization, 2000).Thus, collecting the fluids and utilizing them for denitrification might be beneficial for treatment and protect the environment. Many studies have shown that deicing fluids are capable of being used as a supplemental carbon source. The specific denitrification rates (SDNR) of deicing fluids were reported to be between between 6.5 and 10.6 mgNO_x-N/gVSS •h, and were generally higher than the SDNR of methanol (Rusten *et al*., 1997; Chen *et al*., 2013; Trela, 1998). Besides that, deicing fluids required less time for acclimation by denitrifying bacteria than methanol. It took only 20 h to achieve 80% nitrogen removal after the deicing fluids were introduced (Trela, 1998). A lag time of 3 days for the response of denitrifying bacteria to deicing fluids was reported by Chen (2013). However, in an average year, the amount of spent deicing fluids will not be sufficient to cover the year-round carbon source requirement at municipal wastewater treatment plants (Rusten *et al*., 1997). Thus, the use of other carbon sources should be explored. Using the propylene/ethylene glycol by-product in this study was inspired by the success of using deicing fluids as a supplemental denitrification carbon source.

Over the past decade, a form of diesel fuel manufactured from vegetable oils and animal fat has arisen, and is called “biodiesel”. It is a safe and biodegradable fuel with less air pollution problems than petroleum-based diesel. Glycerol, as the main by-product of the biodiesel production process, is produced in large quantities. It is reported that

about 1 kg of crude glycerol by-product is formed when 9 kg of biodiesel produced (Dasari, *et al*., 2005). To deal with the by-product and increase profitability, biodiesel production plants can convert glycerol to propylene glycol through hydrogenolysis (Dasari, *et al*., 2005). The overall reaction of converting glycerol to propylene glycol (1,2 Propanediol, 1,3 Propanediol) and ethylene glycol (1,2-ethanediol) is as follows:



(3-1)

As the formula above shows, a by-product mixture of propylene/ethylene glycol is produced which have similar components to aircraft deicing fluids. It therefore appear to be a good candidate as a supplemental denitrification carbon source. The Archer Daniels Midland Company (ADM) produced an ethylene/propylene (EG/PG) waste material used in this study.

The major hypotheses of this study included:

4. Glycols can be readily used as a supplemental carbon source. Glycol can effect satisfactory denitrification rate and low level of effluent nitrogen.
5. Using glycols as carbon source has higher sludge yield compare to methanol.
6. The EG/PG Coproduct stream does not inhibit on nitrification or denitrification.

The objectives of this research were to:

4. Compare nitrification and denitrification rates when using methanol and the EG/PG mixture as carbon sources.
5. Compare the C/N ratio of using methanol and glycols as denitrification carbon sources.
6. Study the possible inhibition of nitrification and denitrification when using the EG/PG Coproduct.

3.2 Materials and Methods

3.2.1 Experimental Setup

Three 4-L glass beakers were used as sequencing batch reactors (SBR). The three reactors were operated at the same solids retention time (SRT) and hydraulic retention time (HRT), which were 12 days (d) and 12 hours (hr) respectively. The reactors were operated in a 6-hr cycle. The maximum and minimum volumes of liquid in the reactors were 3 L and 1.5 L, respectively. At the beginning of one cycle, synthetic wastewater was pumped into the reactors to the 3 L level. After settling period, 1.5 L of upper liquid was discharged into a drain; thereby maintaining a 12-hr HRT. The SRT was maintained by wasting the proper amount of mixed liquor. Effluent total suspended solids (TSS) and volume of the samples taken for analysis were taken into consideration when calculating the SRT.

The reactors were all connected to four peristaltic pumps (Col-Parmer, Vernon Hills, IL), including one pump for carbon source addition, one for synthetic wastewater addition, one for mixed liquor wasting, and one for decanting. Three 400 mL glass bottles were used as carbon source containers that were prepared every week. Synthetic wastewater was contained in a 64 L bucket and was prepared every three days. Magnetic stirrers were inserted in those containers to ensure homogeneity of the solutions. Three 1-L cylinders were set to collect wasted activated sludge, and proper amounts of sludge were returned to the reactors to compensate the sludge taken out for testing and maintain the SRT as 12 d. The air in reactors was supplied by a fish pump (Aquatic Ecosystems, Inc., Apopka, FL) through two diffusor stones in each reactor. Three stainless steel paddles rotated by Dayton Gearmotors (Grainger, Roanoke, VA) were used to maintain homogeneity in the reactors. Floating styrofoam was used in each reactor to minimize oxygen transfer through the surface during the anoxic period, thus ensuring the dissolved oxygen (DO) level during the anoxic period were below 0.2 mg/L. The system was controlled by two programmable timers (ChronTrol Corporation, San Diego, CA). All three reactors were operated at room temperature of 20℃. Fig. 3-1 shows the schematic layout of the system.

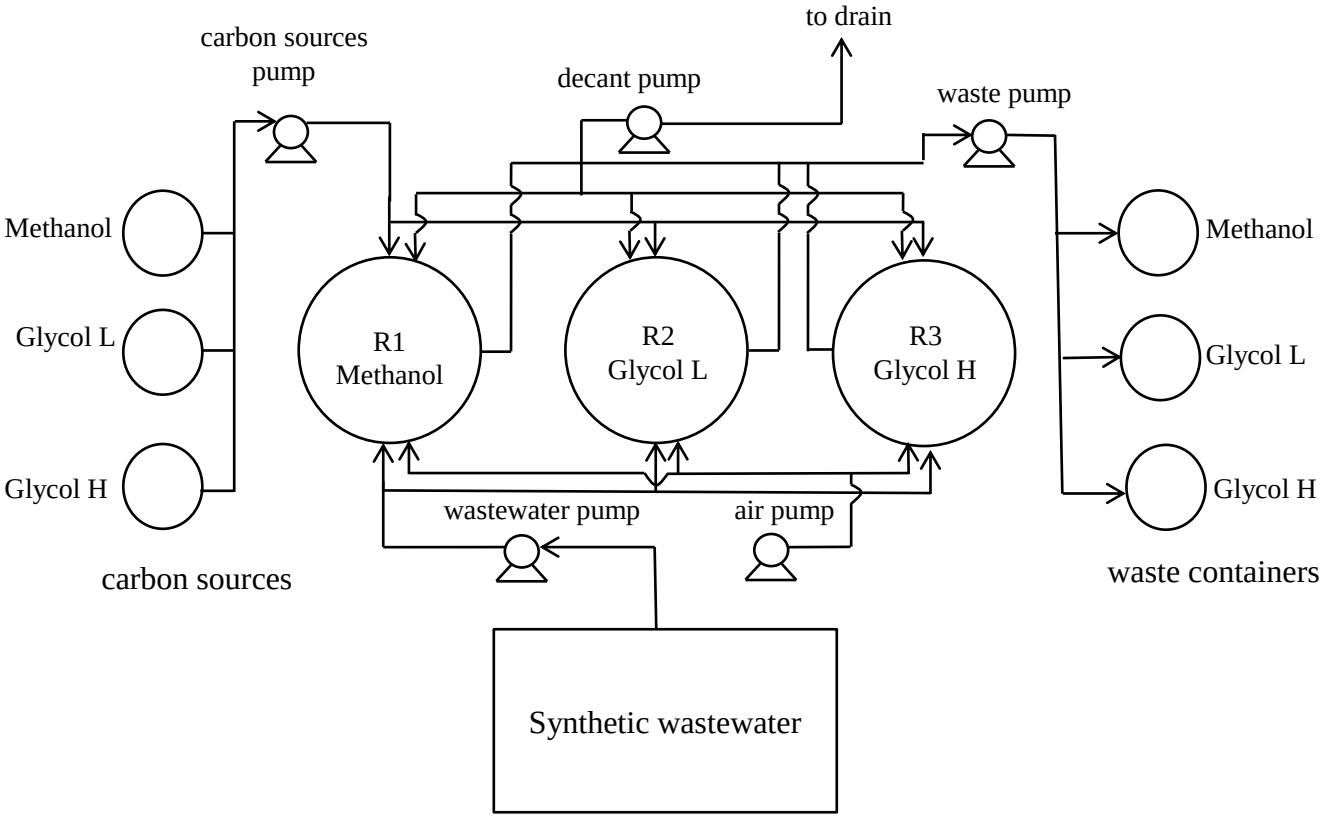


Fig. 3-1 Schematic of SBR Set-up

3.2.2 Sequence Schedule

The system was operated in 6-hr cycles, four cycles a day. The reactors stimulated the post-denitrification process which has aerobic-anoxic-aerobic periods (Fig. 3-2). In the first 15 minutes, synthetic wastewater was pumped into the reactors with the air turned on and mixing. The nitrification process occurred in the first aerobic period which lasted for 160 minutes. After that, the air was shut off. Mixing for 15 min without aeration was set for deoxygenation, which was very important to ensure DO in the reactors was below 0.2 mg/L. Then, carbon sources were added into the reactors within 20 seconds and the air was off for denitrification in 100 minutes. After the anoxic period, another 15-min aerobic period was set for stripping nitrogen gas produced during denitrification process, in order to enhance the settling performance of the reactors. In the last 2 minutes of this period, excess activated sludge was wasted. The mixer were then stopped, activated sludge settled for 45 minutes, and the upper liquid were discharged

into the drain over 10 minutes. Fig. 3-2 provides a schematic diagram of the system schedule.

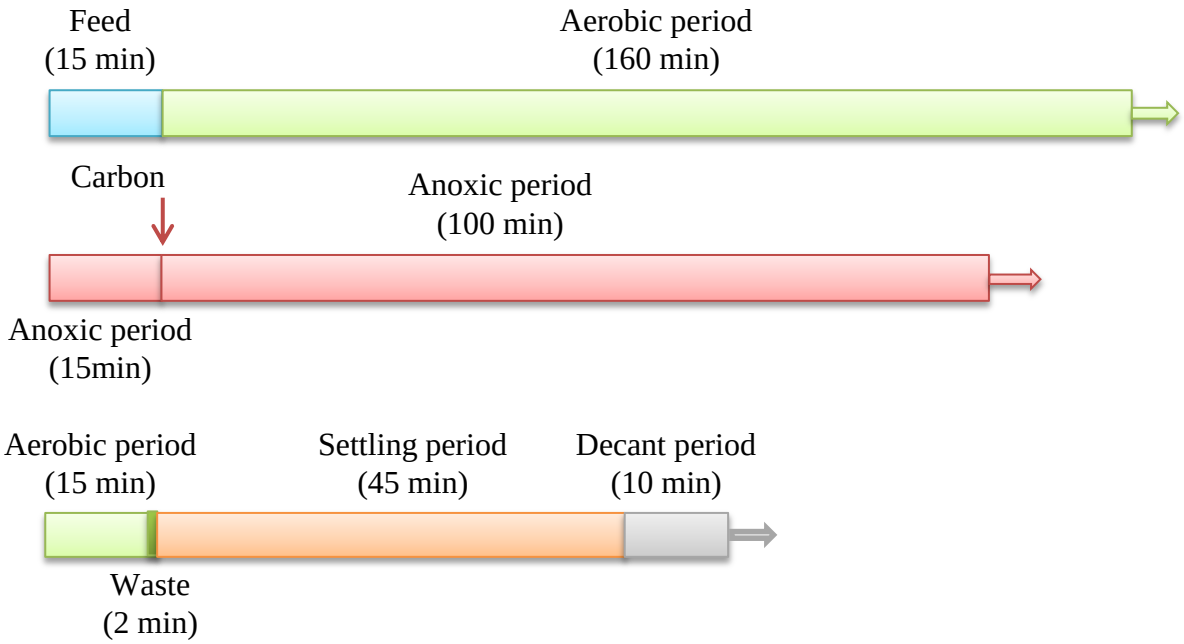


Fig. 3-2 Schematic of the SBR Schedule

3.2.3 Reactor Feed

At the beginning of experiment, the reactors were inoculated with 1.5 L of return activated sludge (RAS) from the Blacksburg-VPI Municipal Wastewater Treatment Plant. One and a half liters of synthetic wastewater was fed into the reactors in the first 15 minutes of every cycle. The synthetic wastewater was comprised of bactopeptone (Spectrum Chemical Mfg. Corp, New Brunswick, NJ), an enzymatic digested animal protein, which provided about 5% of COD in the reactors, and salts needed for bacterial growth. The cation concentrations used in the synthetic wastewater were taken from Maharajh (2010). The monovalent to divalent ratio was kept at around 3:2, which was within the optimum range of < 2 for floc formation and high quality settlement (Higgins and Novak, 1997). The effluent alkalinity was kept at approximately 100 mg/L as $CaCO_3$ to maintain the pH in optimum range for nitrification and denitrification. The cation concentrations in the synthetic wastewater are provided in Table 3-1:

Table 3-1 Influent Cation Concentration

Cation	Concentration (meq/L or mg/L)	Compound used	Desired influent concentration (mg/L)
Ca^{2+}	1.8 meq/L	$CaCl_2 \cdot 2H_2O$	132
	0.7 meq/L	CaO	20
Mg^{2+}	2 meq/L	$MgSO_4 \cdot 7H_2O$	246
K^+	0.75 meq/L	KH_2PO_4	102
	0.75 meq/L	K_2HPO_4	130
Na^+	5 meq/L	$NaHCO_3$	650
	0.5 meq/L	Na_2CO_3	134
Fe^{3+}	6 mg/L	$FeCl_3$	17
Al^{3+}	3 mg/L	$Al_2(SO_4)_3 \cdot 18H_2O$	37

Inorganic nitrogen in the form of ammonia was added to the synthetic wastewater at a concentration of 60 mg/L as N. No nitrate or nitrite was added to the synthetic wastewater. All chemicals were mixed with tap water. The wastewater was prepared every three days.

There were two kinds of carbon sources added into the reactors, laboratory grade methanol (Fisher Scientific, Pittsburg, PA) and the EG/PG Coproduct stream (ADM, Decatur, IL). Two batches of glycol mixture were used during the experiment. They had slightly different component. Table 3-2 shows the components of the two glycol batches.

Table 3-2 Components of Glycol Mixture

Batch 1			Batch 2		
Component	Result	Units	Component	Result	Units
Water	0.07	%	Water	0.02	%
Ethylene Glycol	70.04	%	Ethylene Glycol	83.85	%
Propylene Glycol	22.32	%	Propylene Glycol	9.79	%
Dipropylene Glycol	0.64	%	Total Diols	2.94	%
Butanediols	3.97	%	Total Dipropylene Glycol	0.74	%
Pentanediols	0.01	%	Iron	7.07	ppm
Iron	4.73	ppm			

The amount of COD added was based on the assumed yields and 30 mg/L NO_3-N in reactors. Two concentrations of glycols were added to reactors, high dosage and low dosage. The methanol (MeOH) and high dosage glycol (GLYH) were designed to be

overdosed with COD, while the low dosage glycol (GLYL) was designed to be fully nitrified but without COD overdosed. All carbon sources were diluted and stored in 400 mL beakers. Magnetic stirrers were inserted in each beaker and stirred at slow speed to ensure the homogeneity of the solutions and minimum the volatilization. Carbon sources were prepared every week.

3.2.4 Sample Collection and Analysis

To calculate the nitrification rates, denitrification rates, and C/N ratio, intensive sample collections were performed over one cycle. These intensive collections were conducted three times a week after the system was at steady state. There were three weeks of intensive sampling in total, and between every two weeks of profiling, there were 15 days of gap time to ensure the independence between every set of data. During the time of intensive sampling, samples were taken at different times throughout a 6-hour cycle. Overing the nitrification period, due to the relatively slower rate, samples were taken every 10 minutes. During denitrification, samples were taken every 4 minutes for the first 40 minutes. After that, the sampling intervals were longer because nearly all nitrate was removed during the first 40 minutes. Samples were collected with a 60 mL plastic syringe and immediately vacuum-filtered using 47 mm membrane (Watman). The filtered samples were stored at 4℃ until analysis.

In order to determine the performance of different carbon sources, ammonia, nitrite, nitrate, chemical oxygen demand (COD), mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were tested. The parameters such as ammonia, nitrite and COD were measured by means of a HACH DR2800 spectrophotometer (Hach Company, Loveland, CO).

Ammonia was measured using two methods because of different concentration ranges. Nitrogen-Ammonia Reagent set, TNT, AmVer, High Range test vials were used to test the ammonia concentrations of samples collected from the first 80 minutes in a cycle. The rest of the samples were tested by means of Hach TNT plus 831. Both tests are the salicylate method, which is based on the reaction of ammonium ions with hypochlorite ions and salicylate ions in the presence of sodium nitroprusside to act as a

catalyst to form a blue-colored compound (indophenol), the depth of color is proportional to the amount of ammonia-nitrogen present in the sample.

Nitrite was measured with NitriVer 3 Nitrite Reagent Powder Pillows (Hach Method 8507). This method is based on the reaction of nitrite with sulfanilic acid to form an intermediate diazomium salt. This reaction couples with chromotropic acid to produce a pink-colored compound, which is directly proportional to the amount of nitrite in samples. All samples were diluted 1:20 before testing.

COD was also determined using two methods with different test ranges, COD Digestion Vials High Range (20-1500 mg/L) and COD Digestion Vials Low Range (3-150 mg/L). Both methods are referred to a Hach Method 8000, which is approved by US EPA for wastewater analysis. The sample was mixed with sulfuric acid and potassium dichromate, a strong oxidizing agent, and heated for two hours. Oxidizable organic compounds reduced dichromate ion ($Cr_2O_7^{2-}$) to green chromic ion (Cr^{3+}) with silver as a catalyst.

Nitrate concentrations were analyzed with a DIONEX DX-120ion chromatograph (IC) and AS-9HC column (DIONEX Corp., Sunnyvale, CA). Details of the method are given in the study of Kazasi (2011).

For monitoring purposes, the effluent total suspended solids (TSS), volatile suspended solids (VSS), MLSS, MLVSS, sludge volume index (SVI), pH, DO, and temperature were measured. They were all measured every two days before intensive sample collections. DO and pH were tested at the beginning and end of both the nitrification and denitrification periods. The effluent TSS, effluent VSS, MLSS were determined according to Method 2540D, and the VSS, MLVSS were determined according to Method 2540E (APHA, 2005). SVI was determined according to Method 2710C and 2710D (APHA, 2005). Measurements of pH were made with an Oakton pH 110 Series meter and Acumet electrode. DO and temperature in reactors were measured with a Model 85, YSI meter and probe.

3.2.5 Data Analysis

Software packages R 2.14.1 and JMP 10.0.1 were employed to statistically analyze the data. To determine the denitrification rates for the three carbon sources, linear regression

was employed. The first point was chosen as the point right after carbon sources were added. Then, the first point after NO_3 or NO_x level decreased below 1 mg/L was chosen as the end point of the line. By applying linear regression to the points between the first and end points, the denitrification rate were calculated from the slope of the regressed-line. Linear regression also was used to define the relationships between COD and NO_3 for the different carbon sources. The C/N ratio was decided by the same points as denitrification based on NO_3 reduction because that the COD in all of the three reactors was not limiting throughout the whole process. After finding the denitrification rates and C/N ratios, several statistic tests were used to test the differences between each carbon source. Firstly, the Shapiro-Wilk test was employed to test the normality of each set of data, The Bartlett test was employed to test the homogeneity of variance between the three populations. When both normality and equal variance were found to be true, then an ANOVA was used to compare the means between the three populations. To further identify which population was different from others, Tukey's Honestly Significant Difference (HSD) test was used. For all the tests mentioned above, the significance level α was set as 0.05.

3.3 Results and Discussion

To monitor the system's performance, DO and pH in the three reactors were measured at the beginning and the end of both aerobic period and anoxic period as shown in Table 3-3. Where the Aer_1 refers to ten minutes after the system was fed; Aer_2 refers to ten minutes before the end of aerobic period; Anx_1 refers to ten minutes after the carbon sources were added into reactors; Anx_2 refers to ten minutes before the end of anoxic period. Table 3-3 shows that during the entire operation time of the system, the pH in three reactors was kept in optimum range for nitrification and denitrification. The DO during the aerobic period was kept above 2 mg/L for nitrification and the DO during anoxic period was kept below 0.2 mg/L for denitrification. Table 3-4 shows the average MLVSS and effluent TSS in the three reactors during the entire operation time. The VSS/TSS ratios in three reactors were above 80%, which indicated that none of the carbon sources inhibited sludge growth. The effluent TSS was measured to calculate the SRT in each reactor and adjust the volume of returned sludge.

887 Table 3-3 DO and pH Values in Three Reactors

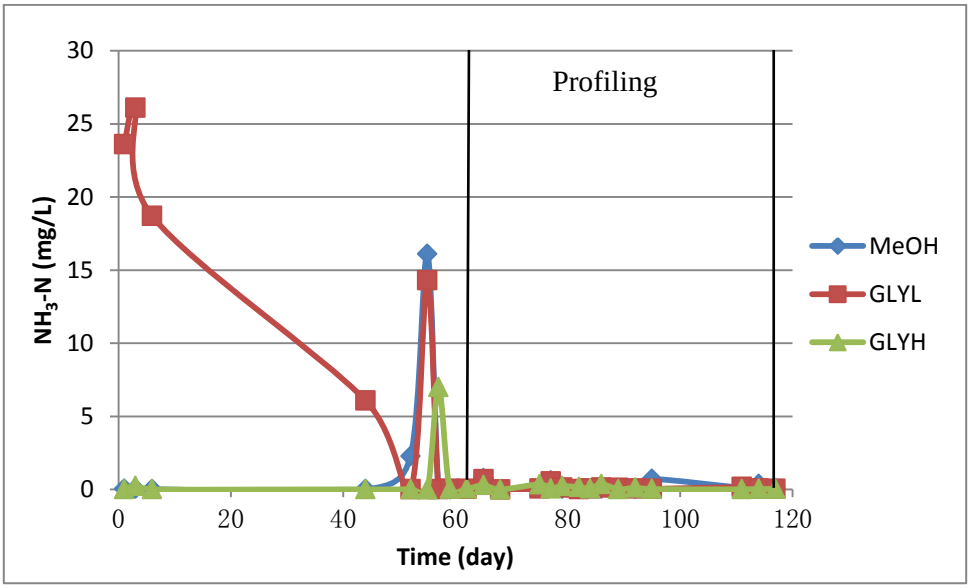
	MeOH		GLYL		GLYH	
	pH	**DO (mg/L)**	**pH**	**DO (mg/L)**	**pH**	**DO (mg/L)**
Aer_1	7.93±0.15	2.09±0.2	7.85±0.19	3.04±1.39	7.87±0.13	2.9±0.9
Aer_2	6.84±0.61	6.96±0.58	6.88±0.84	6.57±1.51	7.14±0.63	6.63±0.61
Anx_1	6.98±0.55	0.11±0.10	6.73±0.72	0.11±0.07	7.12±0.50	0.09±0.03
Anx_2	7.55±0.42	0.11±0.02	7.22±0.59	0.09±0.01	7.33±0.25	0.08±0.01

888

889 Table 3-4 Solids Values in Three Reactors

	MeOH	**GLYL**	**GLYH**
MLVSS (mg/L)	2295±473	2115±476	2574±260
VSS/TSS (%)	86.56±7.35	80.68±1.58	84.77±2.07
Effluent TSS (mg/L)	23.08±14.06	9.64±5.48	10.36±6.71

890 After the reactors were seeded, the effluent ammonia levels were monitored to
891 determine the steady sate. Fig. 3-3 shows the effluent ammonia concentrations of the
892 reactors. It shows that at the first 60 days, the effluent ammonia concentrations fluctuated
893 significantly. After that, the system came into the steady state where the effluent
894 ammonia was consistently low. By that time, the profiling of the system's performance
895 was made.



896 Fig. 3-3 Effluent Ammonia Concentrations of Three Reactors

3.3.1 Denitrification

Table 3-5 shows the effluent nitrogen concentrations of the three reactors. The effluent nitrogen levels of all three reactors were very low, which shows that by using both methanol and the EG/PG mixture, at low and high dosage, nitrification and denitrification processes were nearly complete.

Table 3-5: Effluent Nitrogen Concentrations at Steady State (mg/L)

	NH_3-N	**NO_3-N**	**NO_2-N**
MEOH	0.18±0.24	0.02±0.03	0.08±0.09
GLYL	0.06±0.05	0.01±0.02	0.04±0.02
GLYH	0.04±0.04	0.02±0.02	0.12±0.24

The typical NO_3-N concentration changes during a 6-hr cycle for each reactor are depicted in Fig. 3-4. It shows that in all of the reactors, the denitrification process happened rapidly soon after the carbon sources were introduced, and nearly all of the nitrate was removed. The effluent nitrate level was very low and this suggests that the carbon sources in these three reactors could be left at the end of the cycle and carried into the start of the next cycle. From the figure, it is clearly shown that the denitrification rates in different reactors were different. To further investigate the differences of denitrification rates in different reactors, the linear regression model was used. The point where carbon sources were added was selected as the first point, and the first data point where nitrate was below 1 mg/L was selected as the last point. All of the data between the two were used in fitting a linear regression (Fig. 3-5). According to the slopes of regressed lines, denitrification rates (DNR) were defined. The specific denitrification rates (SDNR) were calculated by dividing the respective DNRs by the MLVSS of each reactor (Table 3-6). Because that the sludge growth rates in the three reactors were not the same and the sludge in MeOH reactor grew in the lowest rate, so the MLVSS values in the MeOH reactor was keeping decreasing during the profiling.

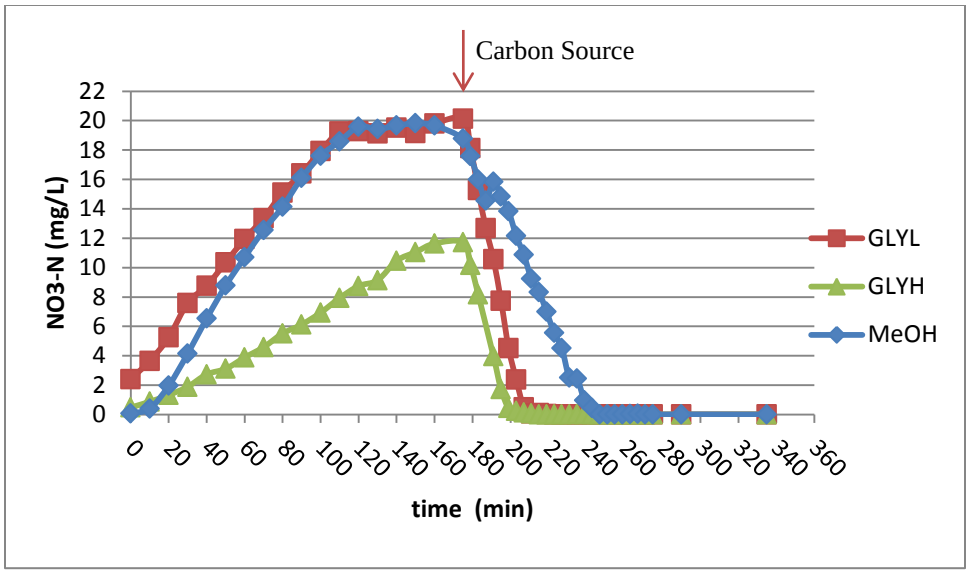
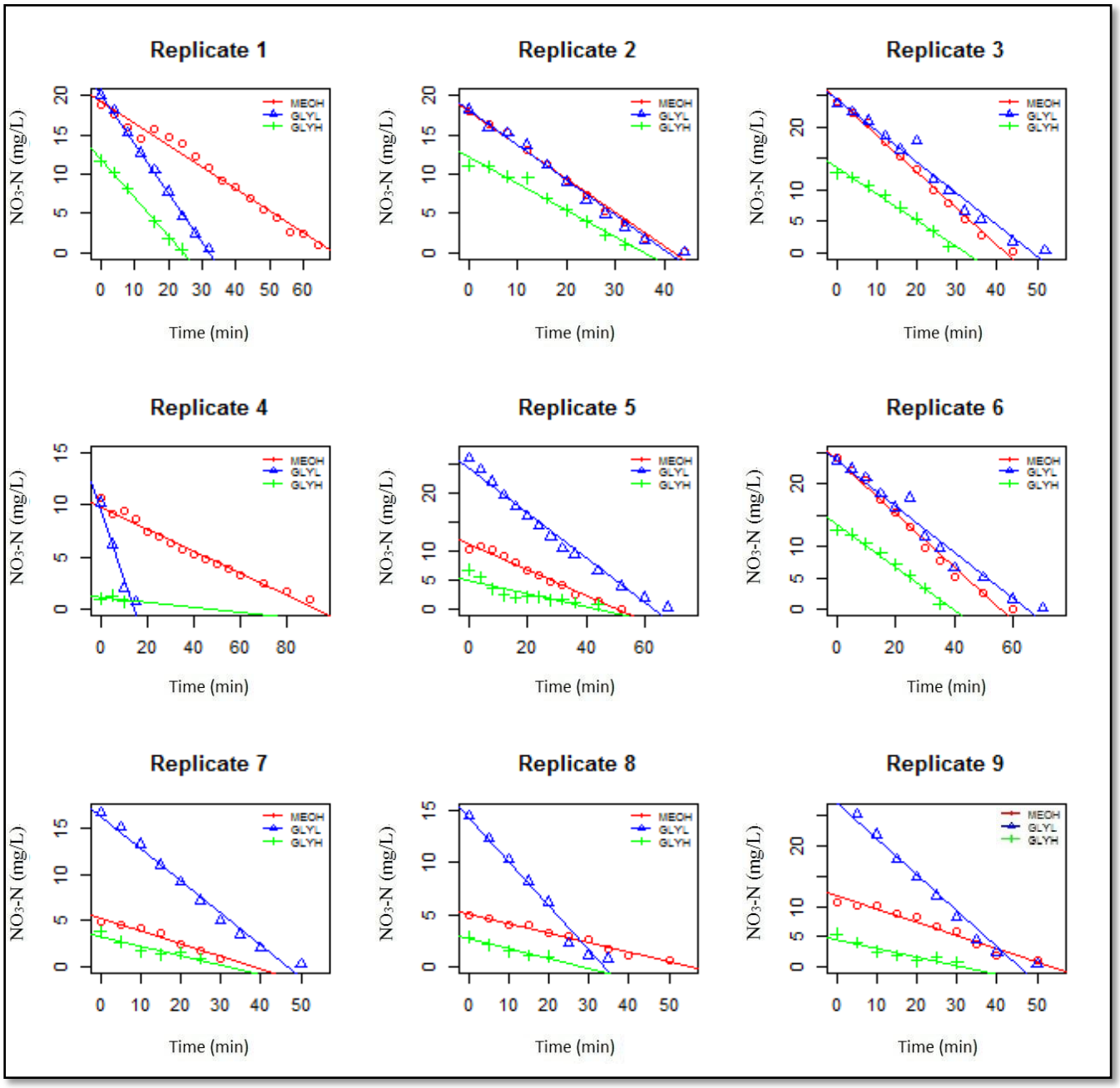


Fig. 3-4 Typical NO_3-N Profile in Three Reactors

Table 3-6: Denitrification Rates Based on NO_x-N

	MeOH			GLYL			GLYH		
Replicate	MLVSS (mg/L)	DNR,mg NO_x-N/L•h	SDNR, mgNO_x-N/gMLVSS•h	MLVSS (mg/L)	DNR,mg NO_x-N/L•h	SDNR, mgNO_x-N/gMLVSS•h	MLVSS (mg/L)	DNR,mg NO_x-N/L•h	SDNR, mgNO_x-N/gMLVSS•h
1	2978	16.84	5.66	2905	31.68	13.31	2980	38.22	15.41
2	2978	25.96	8.72	2380	19.04	8.00	2480	21.31	8.59
3	2781	35.08	12.61	1271	23.67	18.63	2442	21.31	8.73
4	2410	6.49	2.69	2615	26.33	10.07	2940	3.61	1.23
5	2400	20.32	8.47	2410	18.52	7.68	2803	17.15	6.12
6	2085	31.56	15.14	2085	17.07	8.19	2620	33.17	12.66
7	1842	26.64	14.46	2383	21.13	8.87	2624	17.29	6.59
8	1542	21.42	13.89	2114	24.84	11.75	2246	21.54	9.41
9	1418	24.41	17.21	2133	37.16	17.42	2465	20.40	8.28
Average	2270±589	23±8	11±5	2255±453	24±7	12±4	2622±245	22±10	9±4



919 Fig. 3-5 NO_3-N (mg/L) vs. Time (min) Regression Models for the Three Intensive Profiling Trials

920

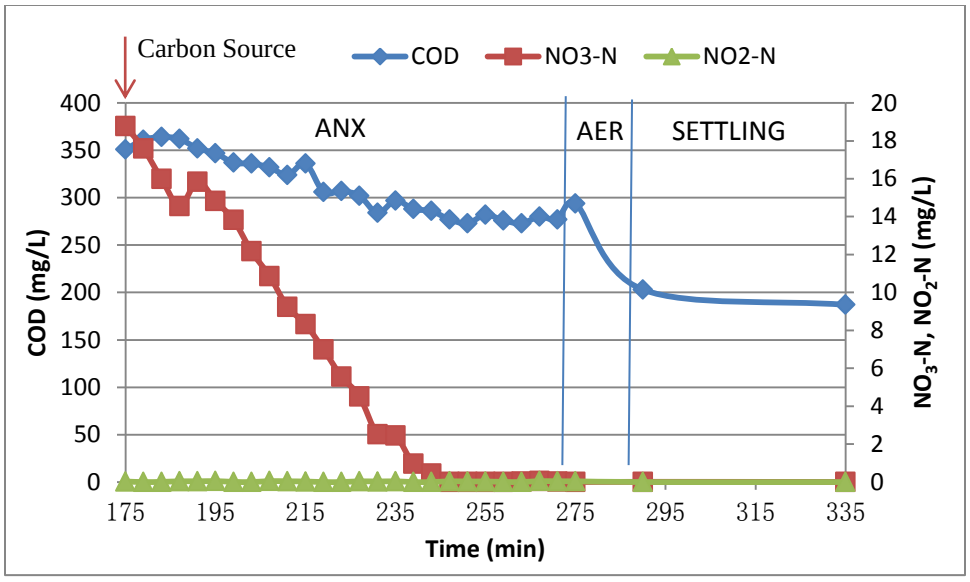
921 As what Fig. 3-5 shows, the starting NO_3-N levels of the MeOH reactor and the

922 GLYH reactor were always lower than the GLYL reactor, which indicates that only

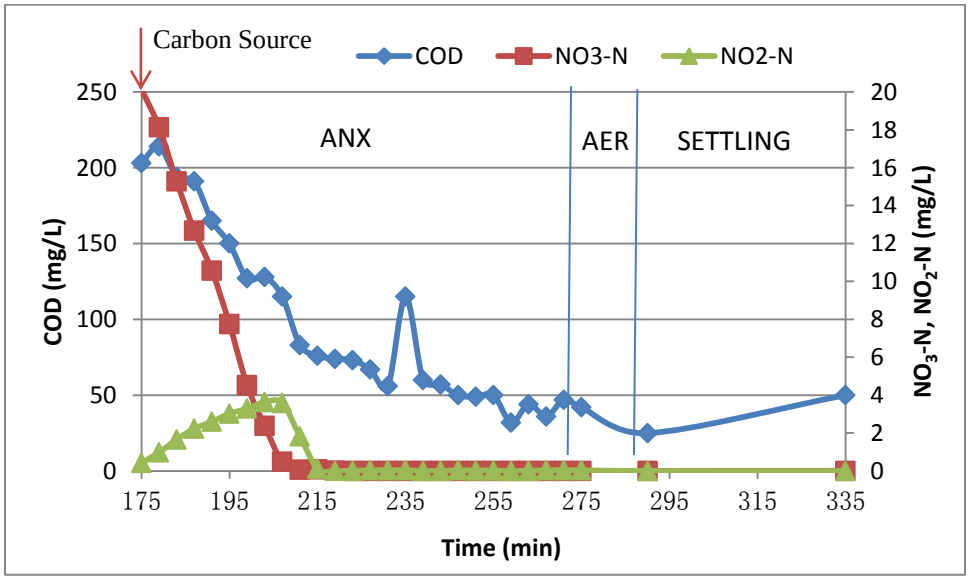
partial ammonia in these two reactors were oxidized into nitrate and nitrite accumulation may happened in these two reactors at the end of aerobic period.

Based on the Shapiro-Wilk and Barlett tests, the three populations of SDNRs were proven to be normally distributed (P_{MeOH}=0.28, P_{GLYL}=0.07, P_{GLYH}=0.42) and had equal variance ($P_{MeOH-GLYL}$=0.52, $P_{GLYL-GLYH}$=0.68, $P_{MeOH-GLYH}$=0.82). The Tukey HSD test was performed to determine the differences between the three populations. The results indicated that the denitrification rate in reactors fed with the low dosage EG/PG glycol mixture was statistically different from the other two reactors ($P_{GLYL-GLYH}$=0.0019, $P_{GLYL-MeOH}$=0.0106); whereas, the denitrification rate in the high dosage glycol reactor was not statistically different from the methanol reactor ($P_{GLYH-MeOH}$=0.7579). Among the three reactors, the GLYL reactor had the highest SDNR, an average of13.39 mgNO_3-N/gMLVSS•h. The average SDNRs of GLYH reactor and MeOH reactor were 5.74 mgNO_3-N/gMLVSS•h and 7.14 mgNO_3-N/gMLVSS•h, respectively.

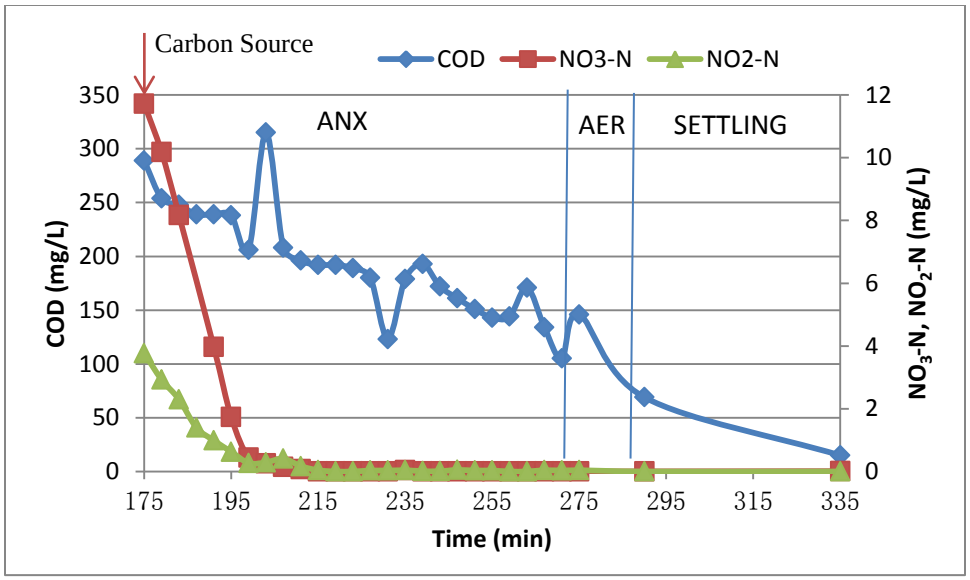
Fig. 3-6, 3-7 and 3-8 depict typical NO_3-N, NO_2-N and COD profiles determined for the reactors under anoxic conditions. The figures show that COD was simultaneously removed with nitrate reduction in the three reactors. In all reactors, the effluent nitrate and nitrite levels were very low, which suggests that in none of them was COD limiting. In fact, in the MeOH and GLYH reactors, the COD was overdosed. As a consequence, no nitrite accumulated in the two reactors. In the MeOH reactor the nitrite level was remained at a low level while nitrate were reduced. In the GLYH reactor, nitrification was incomplete at the end of aerobic period, so nitrite was left over for the denitrification period. When carbon was introduced, both nitrite and nitrate were rapidly reduced. However, in the GLYL reactor, temporary nitrite accumulation was detected. Nitrate reduction was more rapid than nitrite reduction, and coincided with more rapid COD consumption during nitrate reduction than COD consumption during nitrite reduction. Comparison of Fig. 3-7 and 3-8 suggests that excess COD can induce more rapid nitrite reduction. Nevertheless, both Fig. 3-7 and 3-8 show that the EG/PG glycol Coproduct can be readily used as a carbon source.



952 Fig. 3-6 Representative MeOH Reactor NO_x and COD Profiles during the Anoxic Period

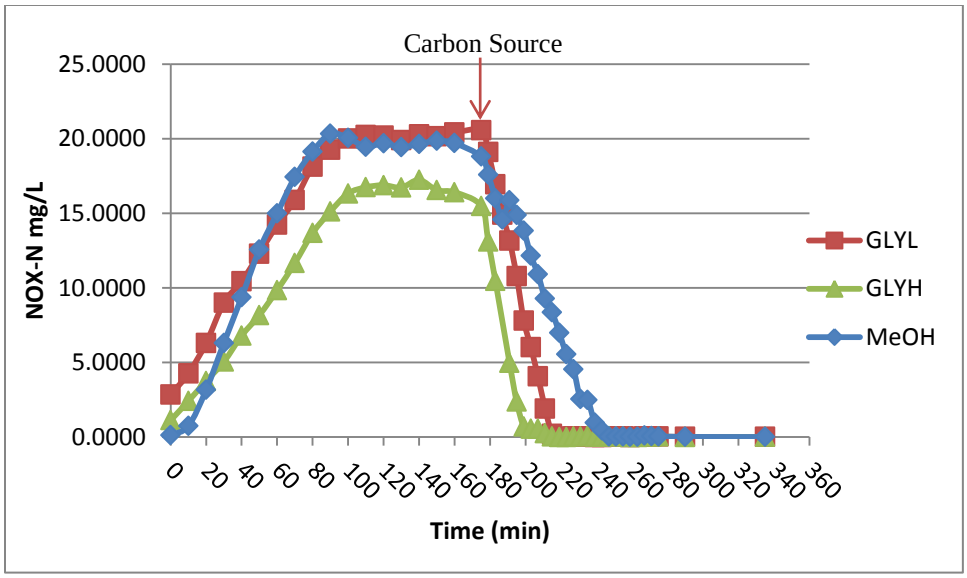


953 Fig. 3-7 Representative GLYL Reactor NO_x and COD Profiles during the Anoxic Period



954 Fig. 3-8 Representative GLYH Reactor NO_x and COD Profiles during the Anoxic Period

955 In order to more accurately represent the denitrification capacity of the carbon
956 sources, NO_2-N should be considered when calculating the denitrification rates. Thus, Fig.
957 3-9 depicts the NO_x-N changes in one 6-hr cycle. As Fig. 3-9 shows, NO_x-N in all
958 reactors rapidly decreased after carbon was added and the effluent NO_x level was low. A
959 linear regression model was fitted in the same way as before based on NO_3-N, by which
960 the denitrification rates based on NO_x-N were achieved. The between relationships are
961 shown in Fig. 3-10. The SDNR based on NO_x-N for the three reactors were calculated in
962 the same way as SDNR based on NO_3-N. The same statistical tests were performed on
963 these SDNRs, but different results were achieved. The Shapiro-Wilk test proved that all
964 of the three populations were normally distributed (P_{MeOH}=0.6, P_{GLYL}=0.09, P_{GLYH}=0.76)
965 and the Barlett test showed that those three population had an equal variance (P_{MeOH-}
966 $_{GLYL}$=0.79, $P_{GLYL-GLYH}$=0.94, $P_{MeOH-GLYH}$=0.6). Tukey HSD indicated that the SDNRs of
967 three reactors were not statistically different ($P_{MeOH-GLYL}$=0.96, $P_{GLYL-GLYH}$=0.33.P_{MeOH-}
968 $_{GLYH}$=0.47). The mean SDNRs of the MeOH reactor, GLYL reactor and GLYH reactor
969 were 10.98 mgNO_x-N/gMLVSS•h, 11.55 mgNO_x-N/gMLVSS•h and 8.56 mgNO_x-
970 N/gMLVSS•h, respectively.



971 Fig. 3-9 Typical NO_X-N Profiles for the Three Reactors

Table 3-7: Denitrification Rates Based on NO_x-N

	MeOH			GLYL			GLYH		
Replicate	MLVSS (mg/L)	DNR,mg NO_x-N/L•h	SDNR, mgNO_x-N/gMLVSS•h	MLVSS (mg/L)	DNR,mg NO_x-N/L•h	SDNR, mgNO_x-N/gMLVSS•h	MLVSS (mg/L)	DNR,mg NO_x-N/L•h	SDNR, mgNO_x-N/gMLVSS•h
1	2978	16.84	5.66	2905	31.68	13.31	2980	38.22	15.41
2	2978	25.96	8.72	2380	19.04	8.00	2480	21.31	8.59
3	2781	35.08	12.61	1271	23.67	18.63	2442	21.31	8.73
4	2410	6.49	2.69	2615	26.33	10.07	2940	3.61	1.23
5	2400	20.32	8.47	2410	18.52	7.68	2803	17.15	6.12
6	2085	31.56	15.14	2085	17.07	8.19	2620	33.17	12.66
7	1842	26.64	14.46	2383	21.13	8.87	2624	17.29	6.59
8	1542	21.42	13.89	2114	24.84	11.75	2246	21.54	9.41
9	1418	24.41	17.21	2133	37.16	17.42	2465	20.40	8.28
Average	2270 ±589	23 ±8	11 ±5	2255 ±453	24 ±7	12 ±4	2622 ±245	22 ±10	9 ±4

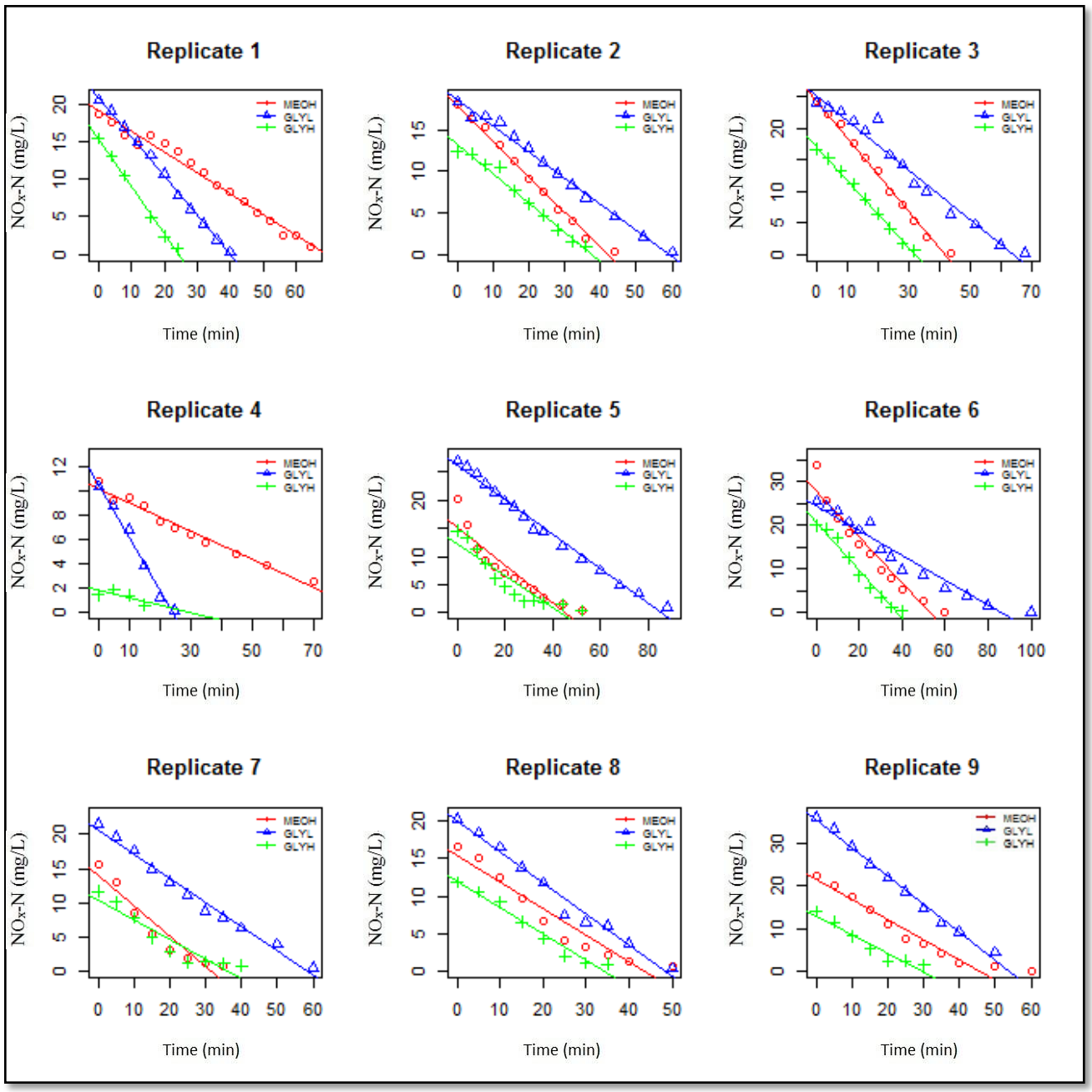


Fig. 3-10 NO_x-N (mg/L) vs. Time (min) Regression Models for the Three Intensive Profiling Trials

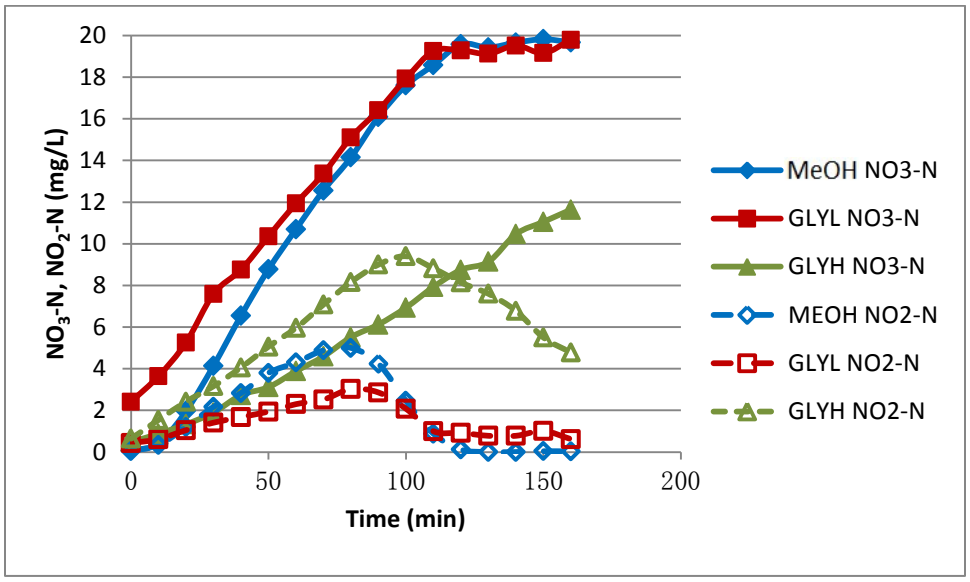
Fig. 3.10 clearly shows that the initial NO_x-N concentrations in the MeOH and GLYH reactors were still consistently lower than the GLYL reactor. Since both MeOH and GLYH reactors were overdosed with COD and great quantities of COD was left over at the beginning of the aerobic period from last cycle, ammonia assimilation and simultaneous nitrification/denitrification can explain the loss of nitrogen in the reactors.

3.3.2 Nitrification

Linear regression models were also fitted to data during the aerobic period to calculate the nitrification rates and specific nitrification rates (SNR) in the three reactors, as shown in Table 3-8. Since the populations did not have an equal variance, the data were log transformed to determine possible differences. The results indicated that the SNR of the GLYH reactor was statistically different from the other two reactors ($P_{GLYH-GLYL}$=0.0001, $P_{GLYH-MeOH}$=0.0003), while the SNR of MeOH reactor and GLYL reactor were not statistically different ($P_{MeOH-GLYL}$=0.32). Among the three reactors, the GLYH reactor had the lowest SNR, with an average of 1.15 mgNH_3-N oxidized/gMLVSS•h. The MeOH reactor's SNR was slightly lower than the GLYL reactor. The average SNR for the MeOH reactor and GLYL glycol reactors were 2.94 mgNH3-N oxidized/gMLVSS•h and 3.86 mgNH3-N oxidized/gMLVSS•h, respectively. Fig. 3-11 indicates that there was nitrite accumulation in GLYH reactor during the aerobic period. Ammonia was rapidly oxidized to nitrite, but nitrite was oxidized to nitrate at a slow rate. At the end of the aerobic period, there was still a high concentration of nitrite left in the reactor. Since in the GLYH reactor COD was overdosed, excess glycol was left over in aerobic period from previous cycle. Fig. 3-11 suggests that glycols may inhibit the nitrification process and cause partial nitrification.

Table 3-8: Nitrification Rates

	MeOH			GLYL			GLYH		
Replicate	MLVSS (mg/L)	NR,mg NH_3-N/L•h	SNR, mgNH_3-N/gMLVSS•h	MLVSS (mg/L)	NR,mg NH_3-N/L•h	SNR, mgNH_3-N/gMLVSS•h	MLVSS (mg/L)	NR,mg NH_3-N/L•h	SNR, mgNH_3-N/gMLVSS•h
1	2978	11.06	3.71	2905	9.33	3.21	2980	4.36	1.46
2	2978	10.55	3.54	2380	8.46	3.55	2480	4.29	1.73
3	2781	10.93	3.93	1271	9.34	7.35	2442	4.56	1.87
4	2410	4.86	2.02	2615	9.58	3.66	2940	2.37	0.81
5	2400	3.98	1.66	2410	8.64	3.59	2803	2.31	0.82
6	2085	10.93	5.24	2085	9.04	4.34	2620	4.56	1.74
7	1842	4.10	2.23	2383	6.39	2.68	2624	1.90	0.72
8	1542	2.01	1.30	2114	4.61	2.18	2246	0.98	0.44
9	1418	4.03	2.84	2133	9.00	4.22	2465	1.77	0.72
Aeverage	2270±589	7±3.8	3±1.3	2255±453	8±1.7	4±1.5	2622±245	3±1.4	1±0.5



990 Fig. 3-11 Typical NO_3-N, NO_2-N Profiles During Aerobic Period

991 **3.3.3 Carbon to Nitrogen Ratios**

992 To investigate the yields of the different reactors, carbon-use-to-nitrate-consumption
993 ratios were calculated. Linear regression models were used to quantify the relationship
994 between COD and NO_3-N (Figure 3.12). The data of which neither COD nor NO_3-N were
995 limiting were chosen to be fitted by linear regression. The slopes of fitted lines were the
996 amount of COD consumed per NO_3-N denitrified.

997

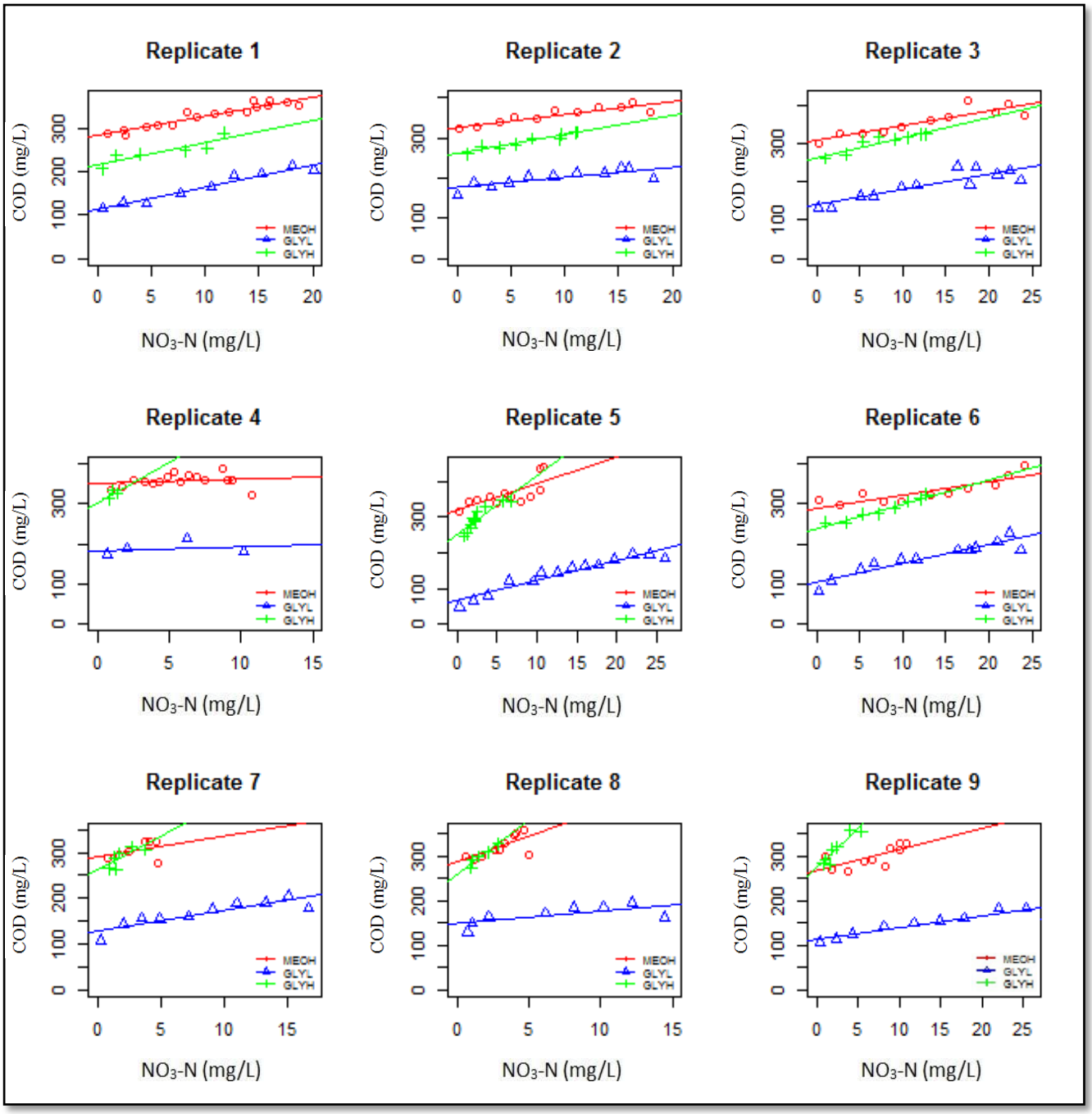


Fig. 3-12 COD (mg/L) vs. NO_3-N (mg/L)

The same statistical tests as before were performed on C/N ratios for the three reactors. The C/N ratios were log transformed because they did not have an equal variance and they then were compared to each other by the Tukey HSD test. Results indicated that the GLYH reactor was statistically different from the other two reactors ($P_{GLYH\text{-}GLYL}$=0.0046, $P_{GLYH\text{-}MeOH}$=0.013), having the highest C/N ratio of 12.73 g COD/g NO_3-N. The C/N ratios in the MeOH reactor and GLYL reactor were not statistically different and were smaller than C/N ratio for the GLYH reactor. The C/N ratios were 4.87 g COD/g NO_3-N and 4.07 g COD/g NO_3-N in the MeOH and GLYL reactors, respectively. The observed growth yields for denitrifier (mg VSS/ mg COD) in the three reactors were estimated based on equation 3-2:

$$Y_{HD} = (1 - \frac{2.86}{COD/N})/1.42 \qquad 3\text{-}2$$

Where Y_{HD} refers to the heterotrophic denitrifitier yield; 2.86 refers to the oxygen equivalent of using nitrate as electron acceptor; 1.42 refers to the equivalent COD of MLVSS by assuming of the organism formula of $C_5H_7O_2N$ (Metcalf and Eddy, 2010).

The calculated observed growth yields of denitrification were 0.29 mg VSS/mg COD in the MeOH reactor, 0.21 mg VSS/mg COD in the GLYL reactor and, 0.55 mg VSS/ mg COD in the GLYH reactor. However, the overall sludge growth yields in MeOH, GLYL and GLYH reactors were 0.31 mg VSS/mg COD, 0.22 mg VSS/mg COD and 0.26 mg VSS/mg COD, respectively. Among those values, the calculated growth yield in the GLYH reactor was about twice higher than overall growth yield, which indicates that the C/N ratio in the GLYH reactor was unrealistically high. The C/N ratio not only depends on theoretical yield, but also other factors, such as possible activity of phosphorus-accumulating bacteria (PAOs) (Naidoo et al., 2000), and the death and decay of microbes in reactors. In this study, phosphorus was not tested, so further research should be done to explain the phenomena of unrealisticthe high C/N ratio.

3.3.4 Results Comparison with Other Studies

Table 3.8 shows how the data from this study compare with values from the literatures. The slight differences may be due to the different dosages of COD in reactors, different treatment processes, or different ways of analyzing the data. The COD/N ratio

of methanol in this study was close to values in the literature and whereas the COD/N ratios for glycol were not. However, the COD/N values in this study, and in other studies for glycol vary greatly. In this study, it appeared that the dosage of glycol significantly influenced the COD/N ratio. This phenomenon may have also been a factor

Table 3-9: Results Comparison

	SDNR, mg NO_3-N/gMLVSS•h			SDNR,mg NO_x-N/gMLVSS•h			g COD/g NO_3-N		
	This study	Literature	Reference	This study	Literature	Reference	This study	Literature	Reference
Methanol	7.14	10	Chen *et al.*(2013)	10.98	6.9	Rusten *et al.*(1997)	4.87	4.9	Chen *et al.*(2013)
		3.2	Peng *et al.*(2007)					3.8-4.5	Narkis *et al.*(1979)
		9.2	Mokhayeri et al.(2008)					4.16	Mycielski *et al.*(1983)
Glycol	13.39 (GLYH) 5.74 (GLYL)	8	Chen *et al.*(2013)	11.55 (GLYH) 8.56 (GLYL)	6.5-8.4	Trela (1998)	4.07 (GLYH) 12.74 (GLYL)	4.7	Chen *et al.*(2013)
					10.6	Rusten *et al.*(1997)		6.3-7.1	Trela (1998)
								4.1-6.3	Rusten *et al.*(1997)

3.4 Conclusions

The results of this study showed that the EG/PG glycol co-product can be used in denitrification to effect efficient nitrogen removal. The concentration of glycols added in reactor significantly influenced the reactor performance. At high glycol concentrations, both denitrification and nitrification rates decreased. The glycol appeared to inhibit nitrification. Nitrite accumulation occurred at high dosages of glycols. Among the three reactors, the GLYL reactor exhibited the best performance, providing the highest

denitrification rate and lowest sludge yield. Although a slight nitrite accumulation was seen at the beginning of denitrification, the nitrite and nitrate levels in effluent were low.

The EG/PG glycol co-product proved to be a suitable supplemental carbon source to replace methanol. As a by-product of an industrial process, the EG/PG co-product should also have price advantage over methanol. However, before it is used, further study need to be done with the investigation of the optimum COD dosages of glycols to achieve the most efficient nutrient removal performance. The research about the storage phenomenon and PAOs' activities under over-dose of COD conditions should also be applied. The required dosage should be carefully determined, since over-dosage could cause significant deterioration of the nitrification and denitrification.

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4 CONCLUSIONS AND ENGINEERING SIGNIFICANCES

This study showed that the EG/PG glycol co-product can be used in denitrification to effect efficient nitrogen removal. The concentration of glycols added in reactor significantly influenced the reactor performance. At high glycol concentrations, both denitrification and nitrification rates decreased. The glycol appeared to inhibit nitrification. Nitrite accumulation occurred at high dosages of glycols. Among the three reactors, the GLYL reactor exhibited the best performance, providing the highest denitrification rate and lowest sludge yield. Although a slight nitrite accumulation was seen at the beginning of denitrification, the nitrite and nitrate levels in effluent were low.

The EG/PG glycol co-product proved to be a suitable supplemental carbon source to replace methanol. As a by-product of an industrial process, the EG/PG co-product should also have price advantage over methanol. However, before it is used, further study need to be done with the investigation of the optimum COD dosages of glycols to achieve the most efficient nutrient removal performance. The research about the storage phenomenon and PAOs' activities under over-dose of COD conditions should also be applied. The required dosage should be carefully determined, since over-dosage could cause significant deterioration of the nitrification and denitrification.

The significance of study is to provide another possible carbon source for wastewater treatment plants, which has high nitrogen removal efficiency and low sludge yields at low concentrations. Proper denitrification carbon source could help the wastewater treatment plants meet the increasingly decreased nitrogen limits and reduce the costs of operation at the same time. This study also provides a good way to deal with the by-product from the hydrogenolysis process of converting glycerol to propylene glycol.

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6 APPENDICES

6.1 Start-up Data

Table 6-1 pH, DO, and T Values in Aerobic Period

	10 min									end								
	pH			DO, mg/l			T, ℃			pH			DO, mg/l			T, ℃		
Day	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H
1	7.87	8.07	7.8	0.5	4.16	3.26	21.1	21.2	21.3	7.04	7.87	7.36	6.95	5.39	7.11	20.4	20.6	21.1
3	7.8	8.05	7.78	0.56	3.5	2.95	20.6	20.4	20.4	7	7.9	7.25	6.9	5.09	6.54	20.4	20.4	20.4
6	7.78	7.93	7.69	0.45	2.66	2.46	21.3	21.3	21.4	6.49	7.67	6.9	6.79	5.12	7	20.9	21	21
9	7.83	8.23	7.74	0.32	6.51	3.08	22.1	20.8	21	6.23	7.58	6.85	7.49	5.31	7.05	20.8	21	21
44	7.91	7.73	7.66	6.28	5.34	2.91	19.9	19.5	19.9	6.75	6.07	6.4	6.55	7.56	7.25	19.8	19.5	20.1
52	7.73	7.68	7.78	4.26	4.61	4.64	21.5	21.2	21.2	6.34	5.89	6.34	6.21	6.68	6.19	21	20.8	20.9
55	7.65	7.51	7.83	5.11	2.81	2.53	21	21	21.1	6.29	5.82	7.06	7.09	7.5	6.78	20.6	20.6	20.6
57	7.92	7.73	7.77	0.82	2.6	4.6	20.8	20.5	20.3	6.62	6.56	6.38	5.76	7.48	6.7	21.3	20.8	20.9
59	8.01	7.79	7.92	0.31	1.73	1.42	21.4	21.1	21.1	7.15	6.97	7.38	7.51	7.08	5.34	20.9	20.9	21
62	8.08	7.87	7.97	1.34	1.46	1.62	21.5	21.3	21.2	7.32	7.28	7.68	7.65	7.18	6.16	20.8	20.6	20.9
68	7.95	7.78	7.87	0.47	1.7	1.84	21.1	21.1	21.1	6.24	6.01	6.88	7.09	6.5	5.87	20.2	20.4	20.5
75	8.1	7.69	8.05	4.24	3.03	2.03	21	20.8	20.8	7.64	6.36	8	8.11	8.63	8.06	20.7	20.6	20.5
77	7.83	7.67	7.82	1.11	2.25	2.52	21.1	20.9	20.8	6.4	5.94	6.86	6.5	6.84	6.15	21.1	20.7	20.7
79	7.82	8.13	7.78	2.09	1.18	2.86	21	20.8	20.7	6.22	7.2	6.59	6.54	2.03	6.22	20.7	20.9	20.6
82	8.04	7.83	7.92	0.63	2.78	4.07	21	20.9	20.7	6.5	6.72	6.54	7.41	7.24	6.96	20.3	20.7	20.8
84	8.07	7.92	8	5.2	2.57	2.9	20.7	20.1	20.1	7.21	7.34	7.71	7.17	7.34	6.42	19.9	19.9	20.2
86	8.12	7.72	8.05	0.8	3.58	3.37	20.3	20.1	20.2	7.21	6.11	7.67	6.28	7.55	6.65	20	20	20.2
89	8.21	8.06	8.14	3.07	2.26	3.08	21.4	21.3	21.2	8.55	8.59	8.64	7.25	7.68	6.8	20.4	19.8	20.1

1279 Table 6-2 pH, DO, and T Values in Anoxic Period

		10 min								end								
	pH			DO, mg/l			T, ℃			pH			DO, mg/l			T, ℃		
Day	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H
1	7.38	7.76	7.39	0.08	0.075	0.09	20.6	20.7	20.7	7.94	7.79	7.37	0.09	0.09	0.09	20.6	20.7	20.8
3	7.26	7.78	7.32	0.09	0.063	0.08	19.9	20.4	20.6	7.79	7.73	7.29	0.09	0.1	0.09	20.6	20.6	20.8
6	6.48	7.52	6.96	0.09	0.099	0.08	20.7	21	21	7.13	7.83	7.2	0.14	0.08	0.08	20.6	20.8	21
9	6.4	7.47	6.94	0.5	0.089	0.1	20.7	20.8	21	7.63	7.85	7.13	0.09	0.1	0.06	20.8	20.9	21.1
44	7.68	6.42	7.45	0.09			20.4	20.6	20.6	7.13	7.01	6.96	0.1	0.08	0.09	20.3	20.2	20.5
52	6.32	5.94	6.5	0.08	0.09	0.07	21.2	20.9	21	6.98	6.97	7.03	0.08	0.08	0.07	21.2	21.1	21.1
55	6.14	5.67	7.09	0.06	0.29	0.08	20.7	20.6	20.6	7.08	5.88	7.25	0.1	0.09	0.07	20.8	20.6	20.7
57	6.62	6.46	6.24	0.08	0.08	0.2	21	20.9	20.9	7.34	7.33	7.3	0.09	0.08	0.08	21.2	21	21
59	7.11	6.96	7.52	0.06	0.07	0.07	21.1	20.9	21	7.66	7.42	7.47	0.13	0.1	0.09	21.2	21.1	21
75	7.67	6.14	7.94	0.09	0.26	0.1	20.7	20.6	20.6	8.22	6.14	7.69	0.1	0.12	0.09	20.8	20.6	20.7
77	6.74	5.87	6.97	0.1	0.1	0.09	20.9	20.9	20.8	7.33	6.94	7.23	0.1	0.12	0.08	21	21	20.9
79	6.56	7.02	6.61	0.09	0.08	0.08	20.9	21	20.7	7.2	7.35	7.04	0.11	0.09	0.08	21	21.1	20.8
82	7.08	6.76	6.52	0.09	0.09	0.1	20.5	20.8	20.8	7.55	7.34	7.58	0.12	0.1	0.09	20.6	20.8	20.9
84	7.79	7.27	7.7	0.09	0.08	0.07	20.1	20.1	20.3	8.2	7.66	7.7	0.12	0.09	0.09	20.4	20.3	20.4
86	7.48	5.98	7.66	0.1	0.09	0.08	20.1	20.1	20.3	8.02	7.02	7.69	0.12	0.09	0.09	20.3	20.4	20.5

Table 6-3 SRT of Three Reactors

	Effluent Volume			Smple Volume			Effluent TSS			MLSS			SRT		
Day	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H	MeOH	Gly L	Gly H
1	1330	1130	1000	130	126	220	43.0952	74.1052	33.256	2747.586	2789.63	3422.308	13.45897	12.8554	14.16061
3	1250	1140	930	194	304	270	50.0106	36.1227	64.0873	2568.228	2947.917	3302.083	13.15105	13.7808	13.43546
5	1200	1120	920	182	110	218	41.2718	59.127	29.2932	2827.606	2691.787	3186.17	13.62573	13.21482	14.25431
45	820	850	880	364	386	400	50.5374	46.1171	52	2380	2270	3474.809	13.32497	13.32679	13.68894
48	770	1000	800	390	330	368	31.0263	45.4228	49.1876	2600	2412.857	3471.905	14.02902	13.3311	13.85378
50	870	940	900	338	352	372	37.3168	62.045	51.753	2461.905	2420	3352.381	13.7419	12.86864	13.65892
52	920	860	1060	376	376	416	43.8625	43.3748	52.6422	2497.381	2371.364	3243.182	13.46728	13.47662	13.39537
54	900	950	960	354	416	420	44.6524	52.6442	57.5855	2509.091	2435.178	3719.048	13.49428	13.07016	13.5521
56	840	900	830	384	392	418	62.5	29.0937	57.9758	2750.718	2428.571	4204.091	13.16882	13.92254	13.8115
58	710	830	800	352	334	348	18.5207	17.738	20.4762	2356.315	2134.565	3152.381	14.39903	14.30801	14.46084
61	840	780	980	344	342	390	27.638	19.9111	22.3768	2530	1868.182	2950	14.08886	14.15373	14.2591
63	870	950	960	372	396	388	12.8771	14.8148	14.9394	2355.952	1655.901	2695	14.50758	14.14813	14.45975
68	770	870	860	378	388	416	33.0207	26.621	20.8611	2630.208	1752.105	2358.125	13.99172	13.69152	14.19862
70	620	830	790	426	406	416	31.9907	16.3319	13.1697	2438.776	1839.951	2781.667	14.037	14.21996	14.58365
72	910	850	900	420	440	432	22.75	13.625	11.7954	2295	1999.608	2160	14.07234	14.36851	14.47361
76	720	900	890	422	424	446	64.0058	21.4486	27.1276	2755	2010	1975	13.24318	14.01029	13.73937
79	990	980	940	440	444	442	16.0801	17.2918	13.7981	2345	1905	1930	14.29894	14.08942	14.29386
82	760	910	750	402	410	390	41.9234	28.5282	34.0241	2496.25	2311.633	2091.837	13.66648	13.87025	13.72732
84	800	900	1120	398	390	440	26.6827	20.0267	15.4545	2244.792	1895	1926.277	14.00299	14.04278	14.11659
86	540	830	650	416	420	420	29.5156	17.7424	14.5	2121.429	1534.898	2015.918	14.06464	13.98933	14.44417

6.2 Profiling Data

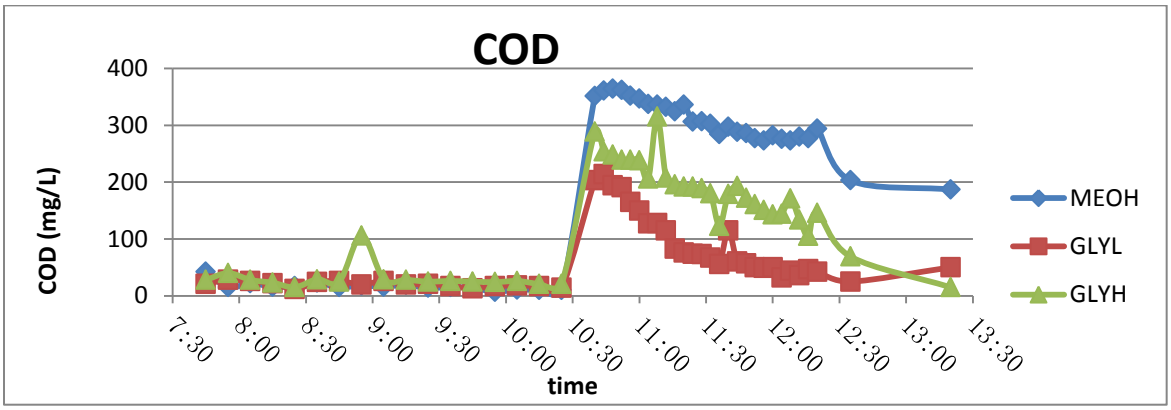


Fig. 6-1 2/11 COD (mg/L) Profiles

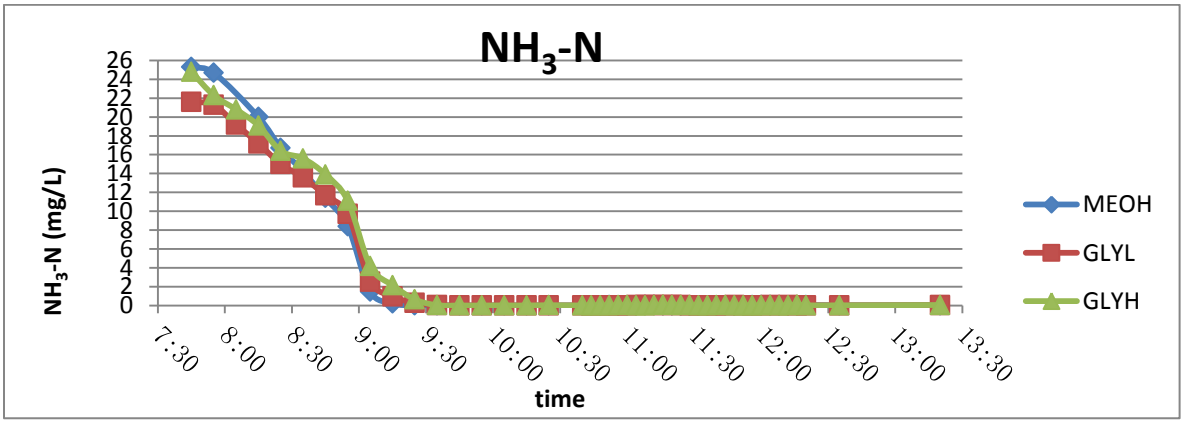


Fig. 6-2 2/11 NH3 (mg/L) Profiles

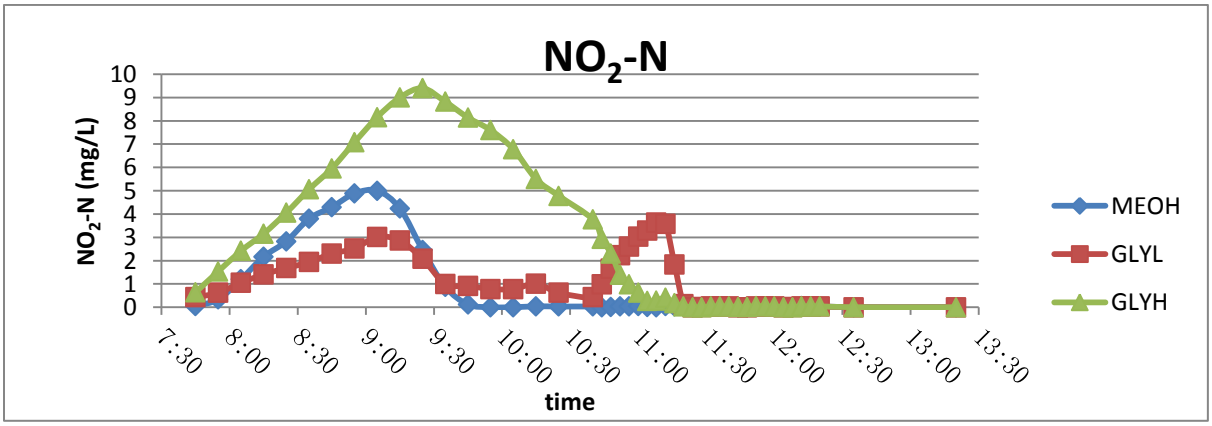


Fig. 6-3 2/11 NO_2-N (mg/L) Profiles

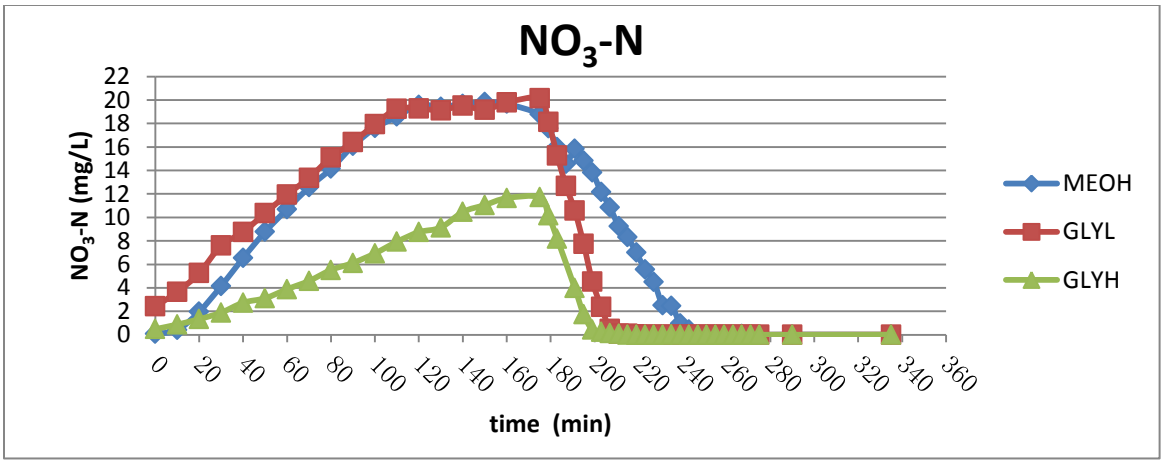


Fig. 6-4 2/11 NO3-N (mg/L) Profiles

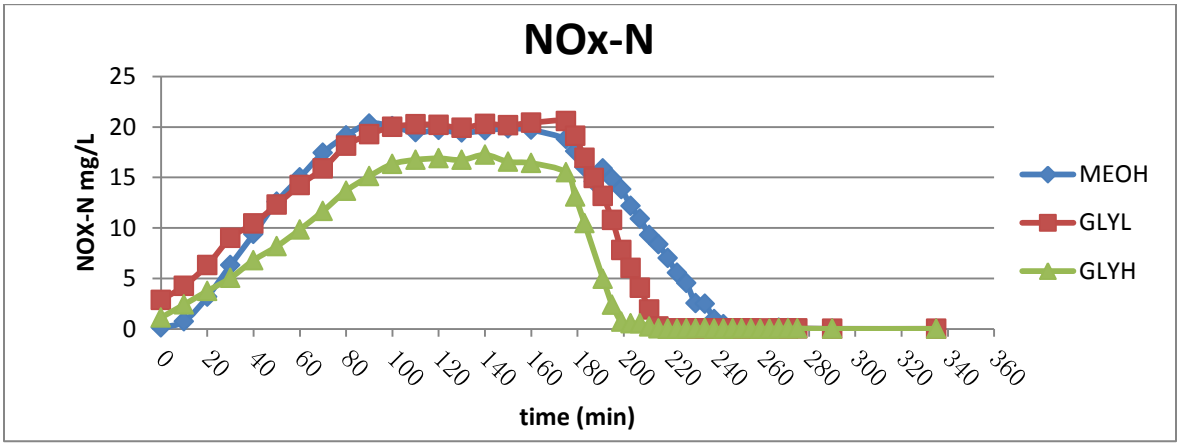


Fig. 6-5 NOx-N (mg/L) Profiles

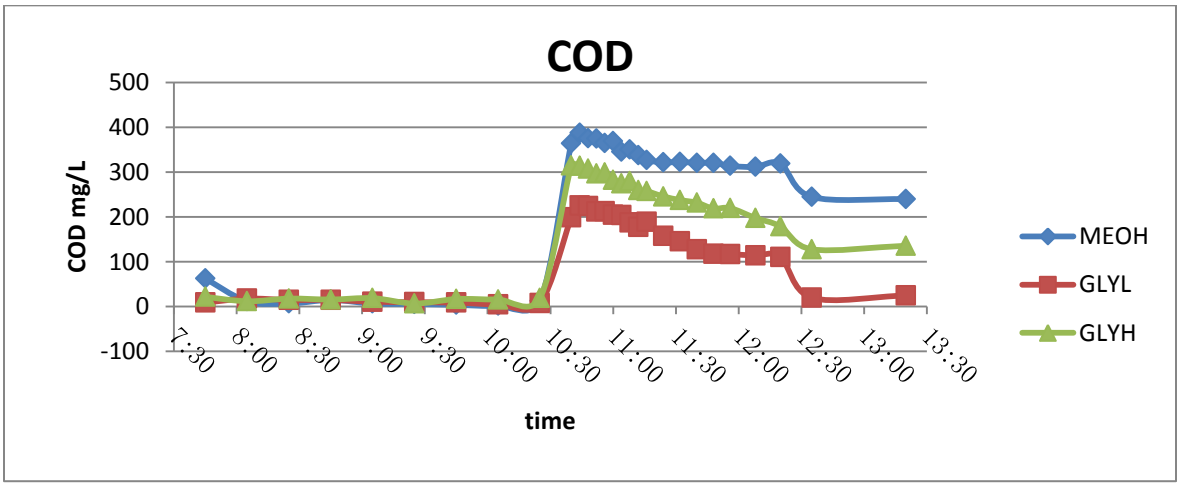


Fig. 6-6 2/14 COD (mg/L) Profiles

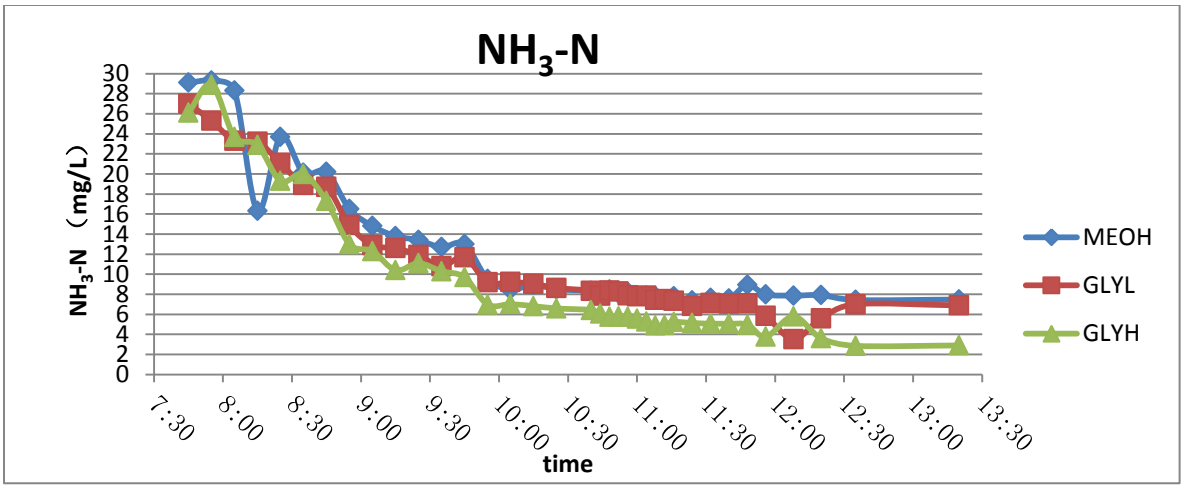


Fig. 6-7 2/14 NH_3-N (mg/L) Profiles

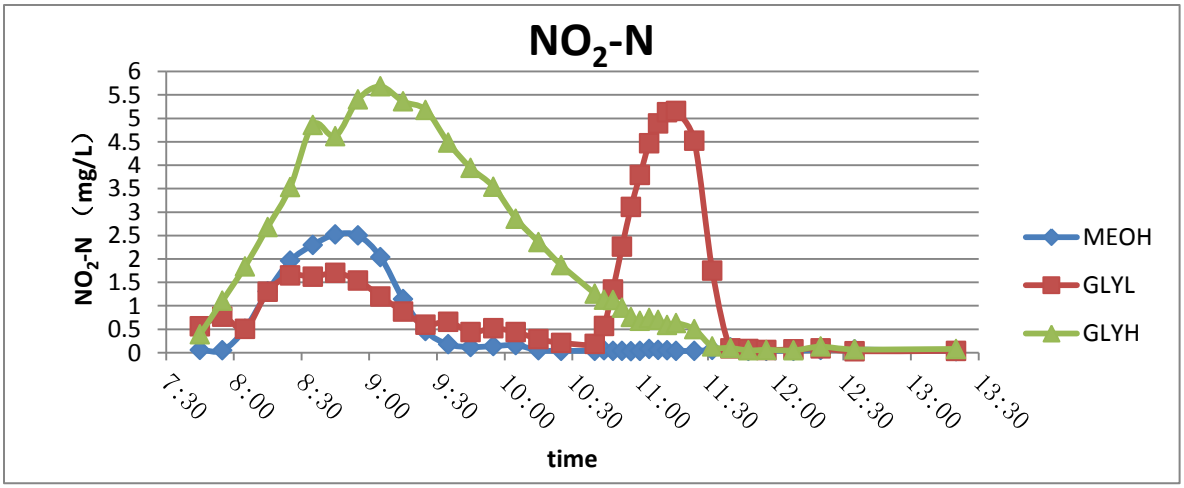


Fig. 6-8 2/14 NO_2-N (mg/L) Profiles

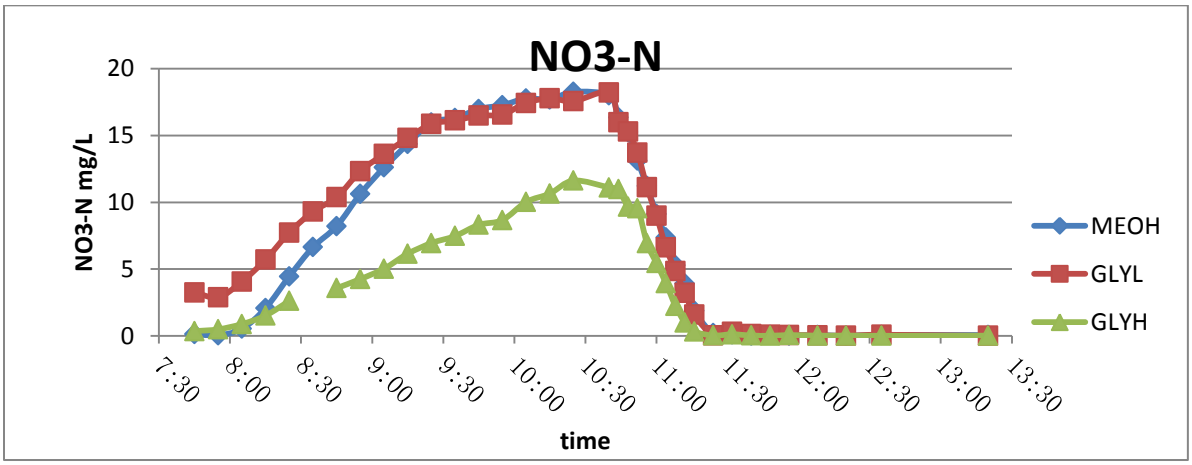


Fig. 6-9 2/14 NO3-N (mg/L) Profiles

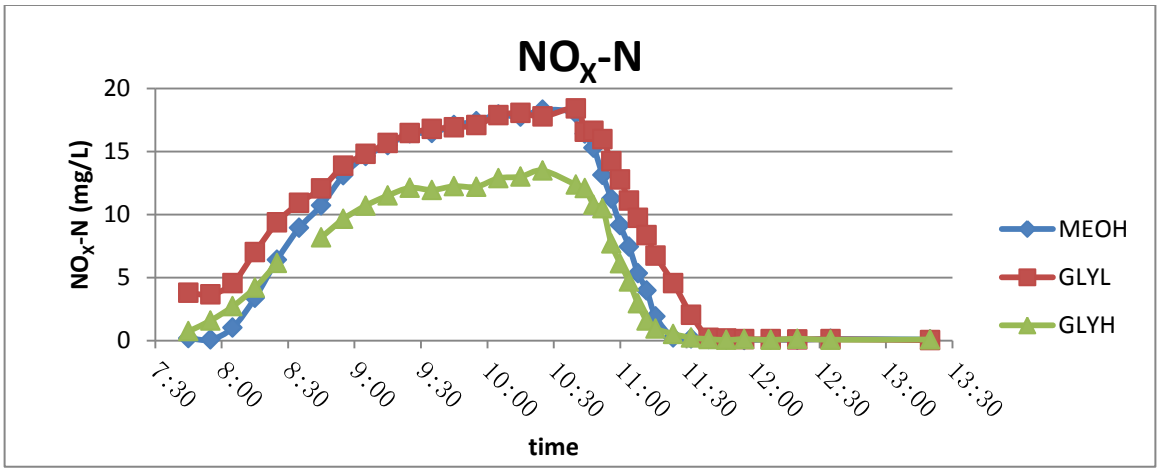


Fig. 6-10 2/14 NO_x-N (mg/L) Profiles

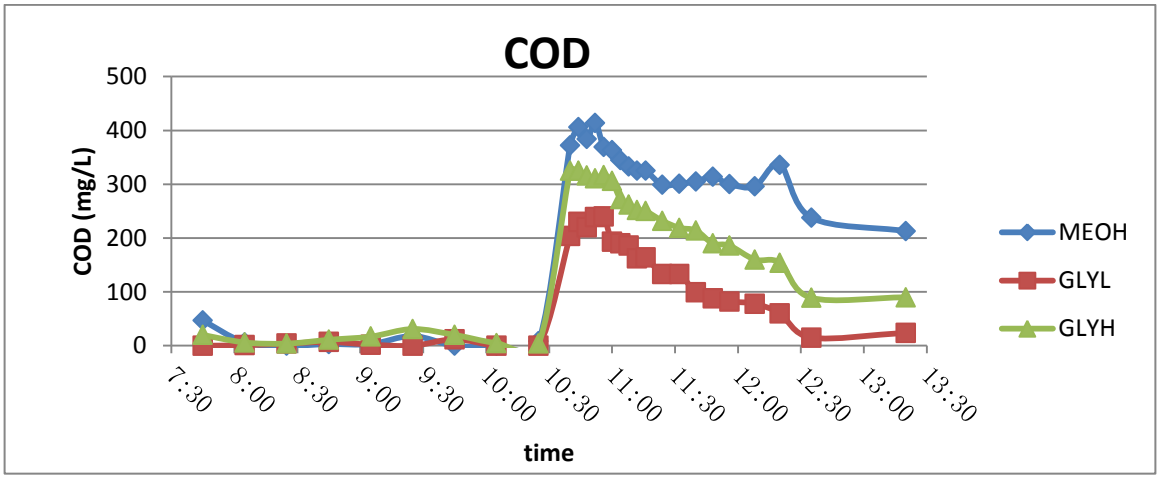


Fig. 6-11 2/17 COD (mg/L) Profiles

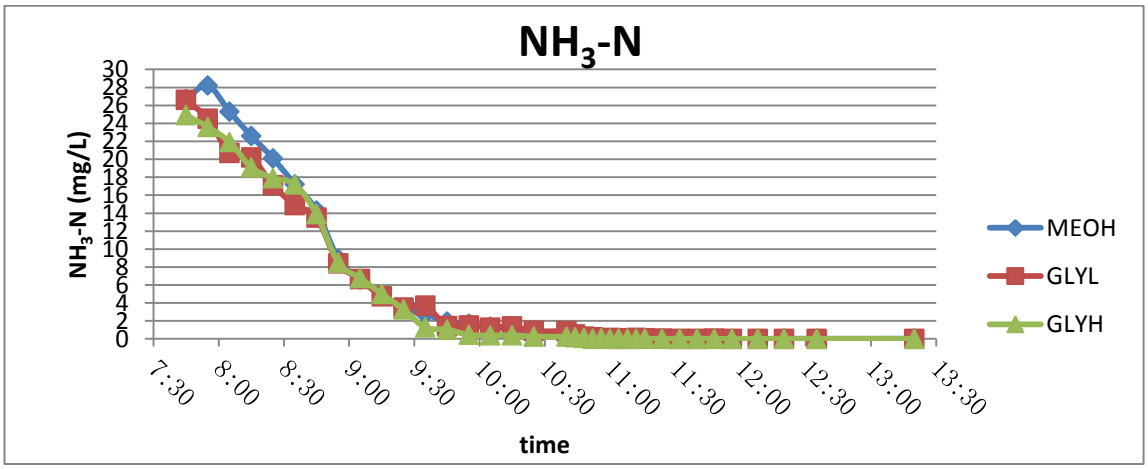


Fig. 6-12 2/17 NH_3-N (mg/L) Profiles

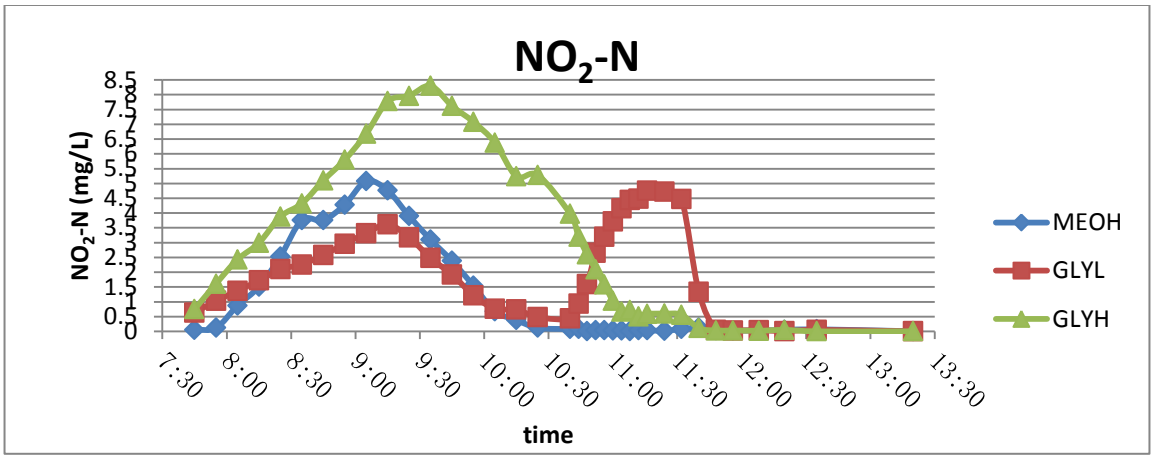


Fig. 6-13 2/17 NO_2-N (mg/L) Profiles

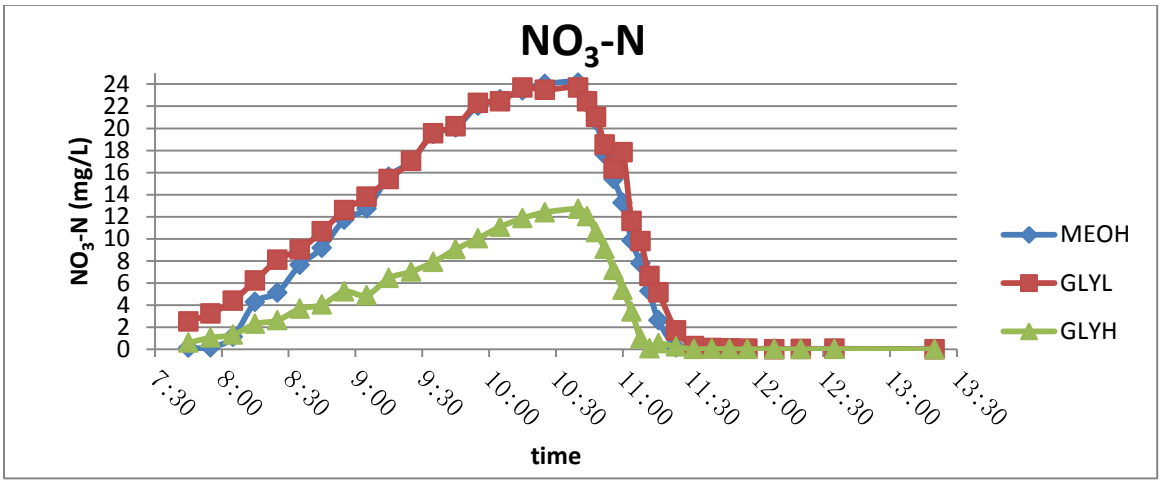


Fig. 6-14 2/17 NO_3-N (mg/L) Profiles

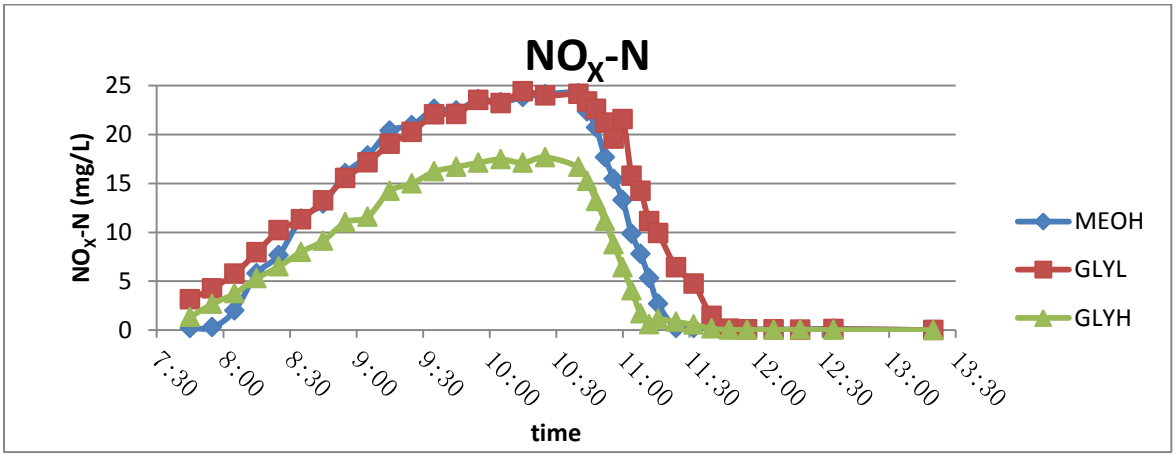


Fig. 6-15 2/17 NO_x-N (mg/L) Profiles

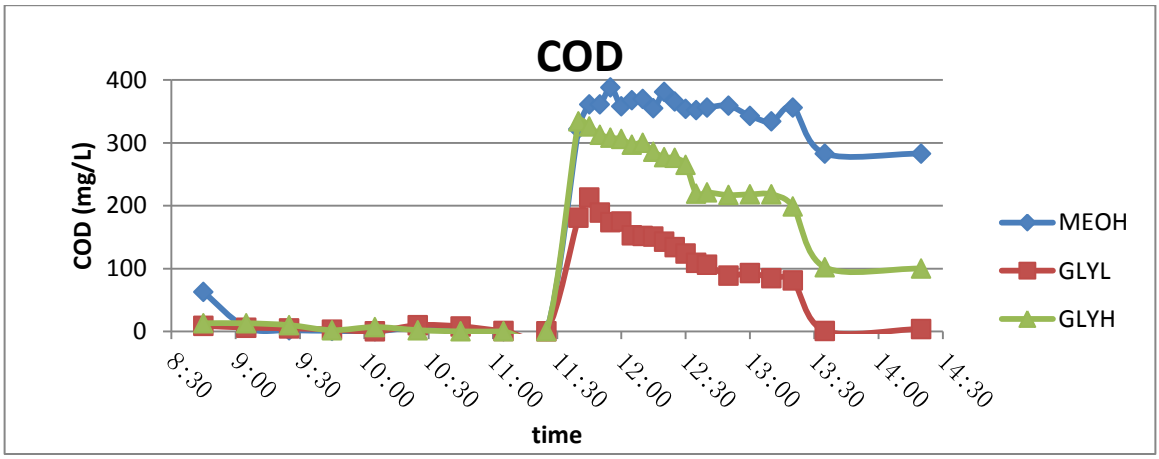


Fig. 6-16 3/11 COD (mg/L) Profiles

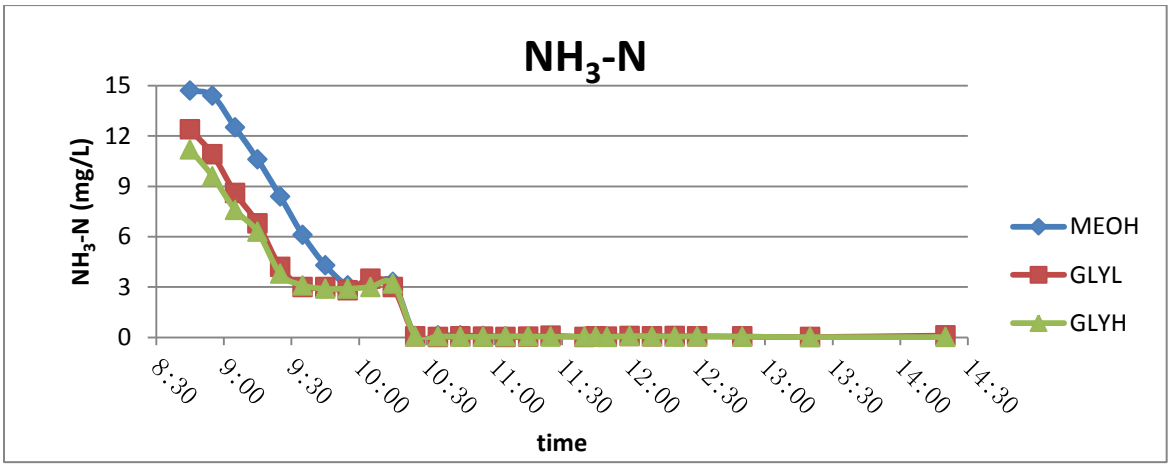
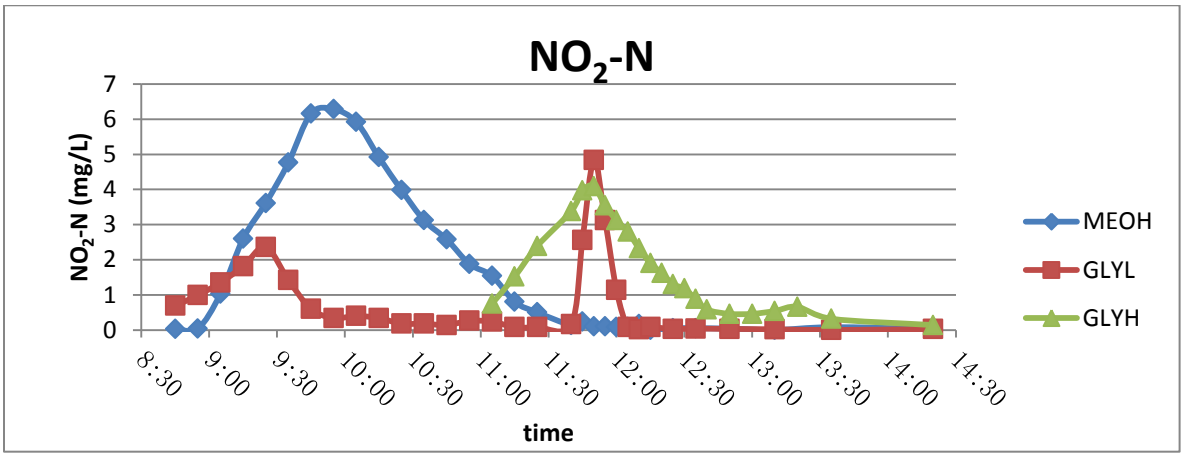


Fig. 6-17 3/11 NH_3-N (mg/L) Profiles



1282 Fig. 6-18 3/11 NO_2-N (mg/L) Profiles

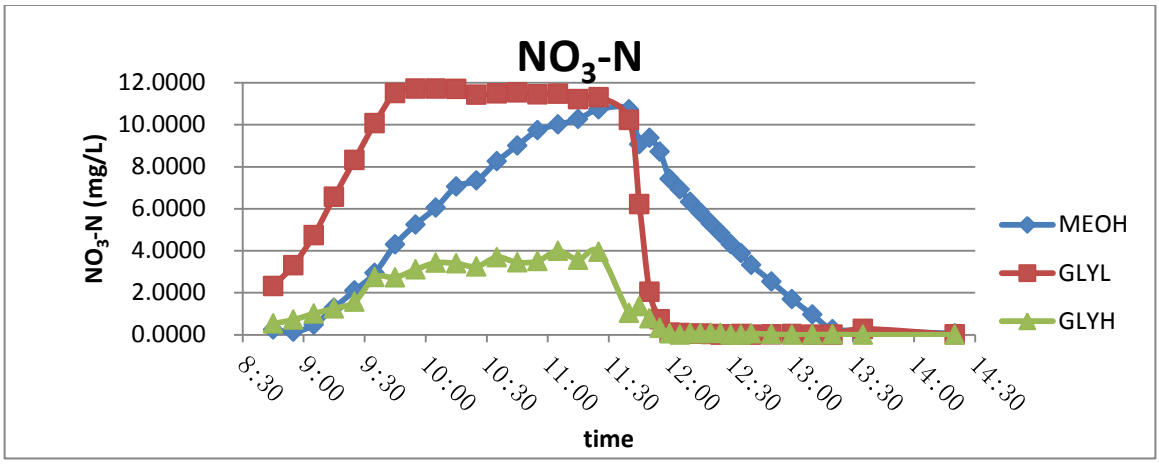


Fig. 6-19 3/11 NO_3-N (mg/L) profiles

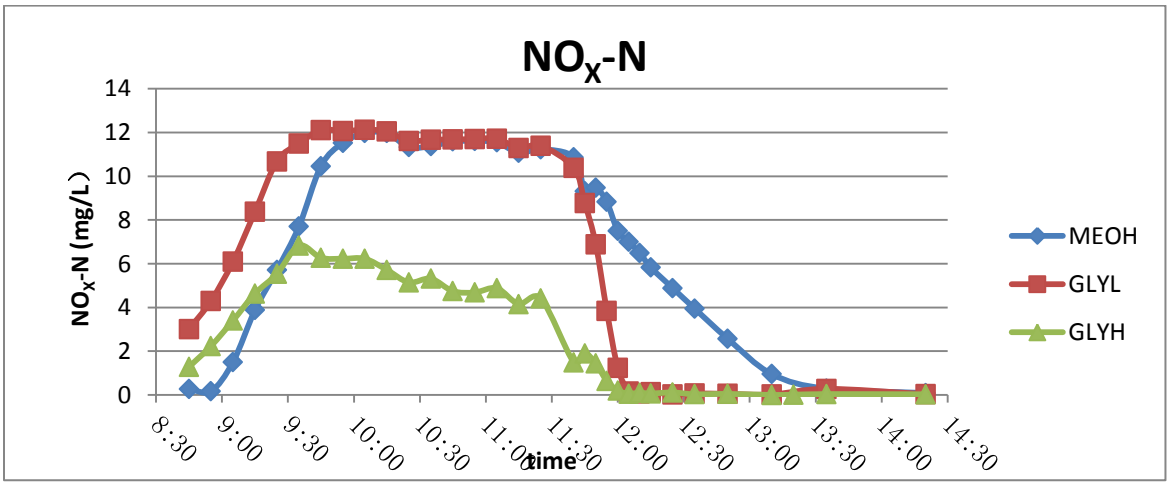


Fig. 6-20 3/11 NO_x-N (mg/L) Profiles

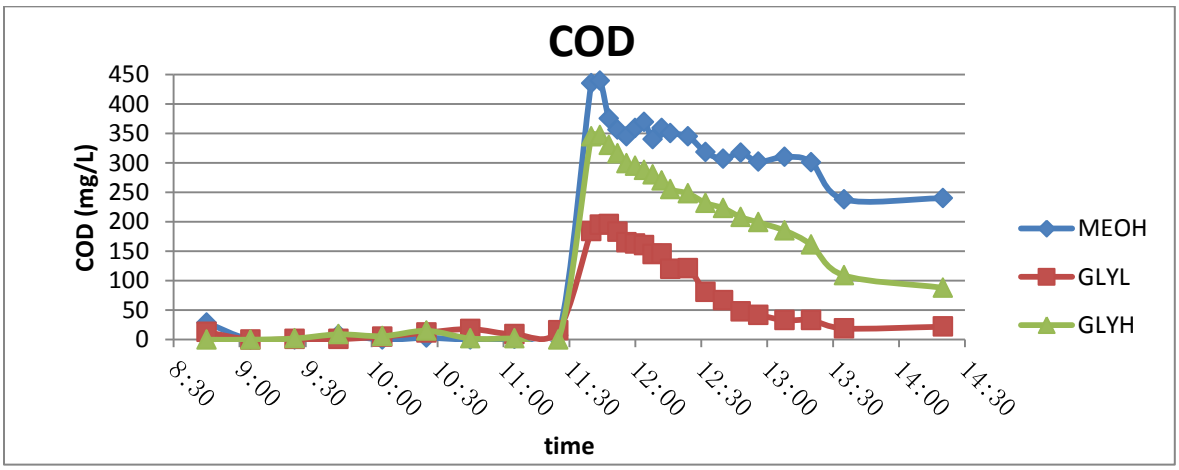


Fig. 6-21 3/14 COD (mg/L) Profiles

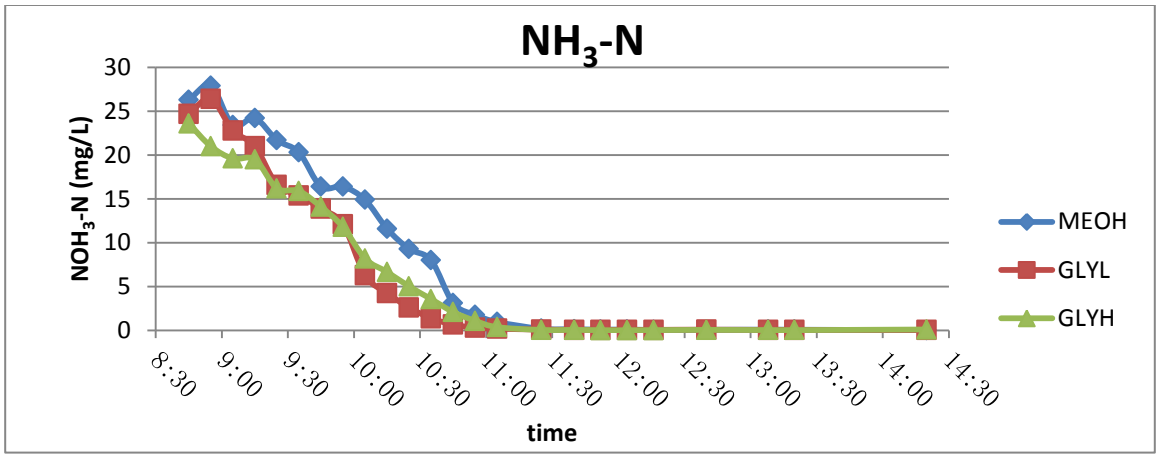


Fig. 6-22 3/14 NH_3-N (mg/L) Profiles

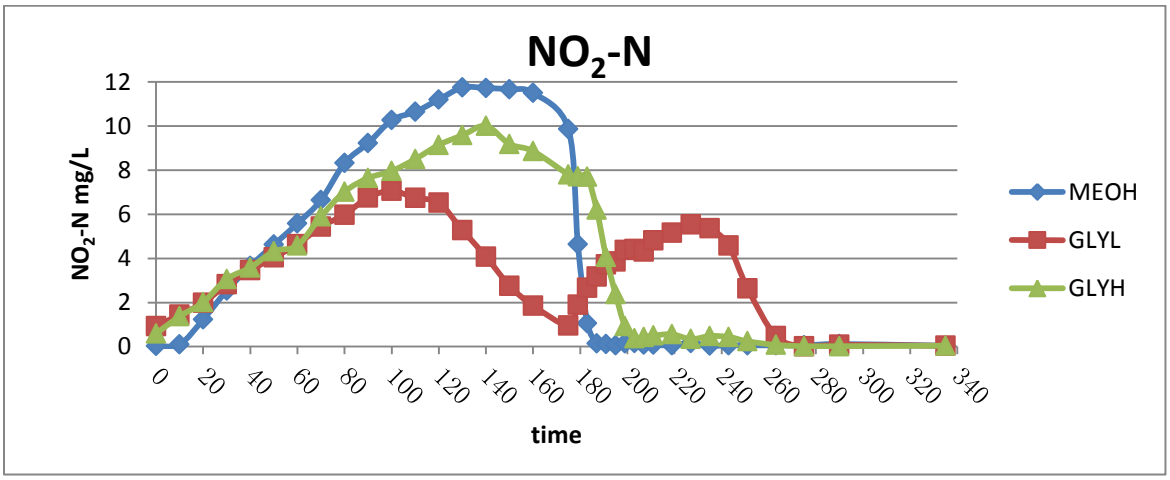


Fig. 6-23 3/14 NO_2-N (mg/L) Profiles

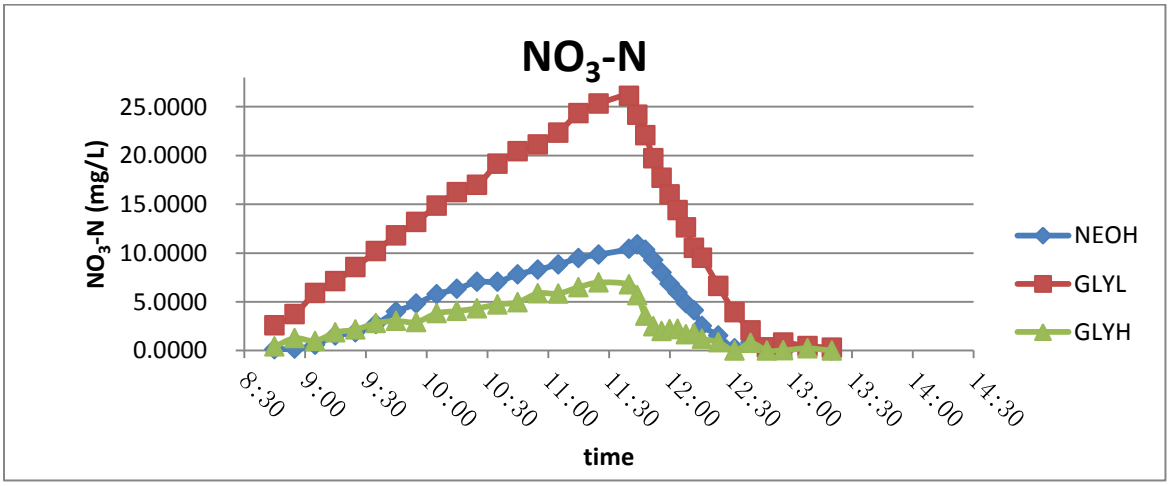


Fig. 6-24 3/14 NO_3-N (mg/L) Profiles

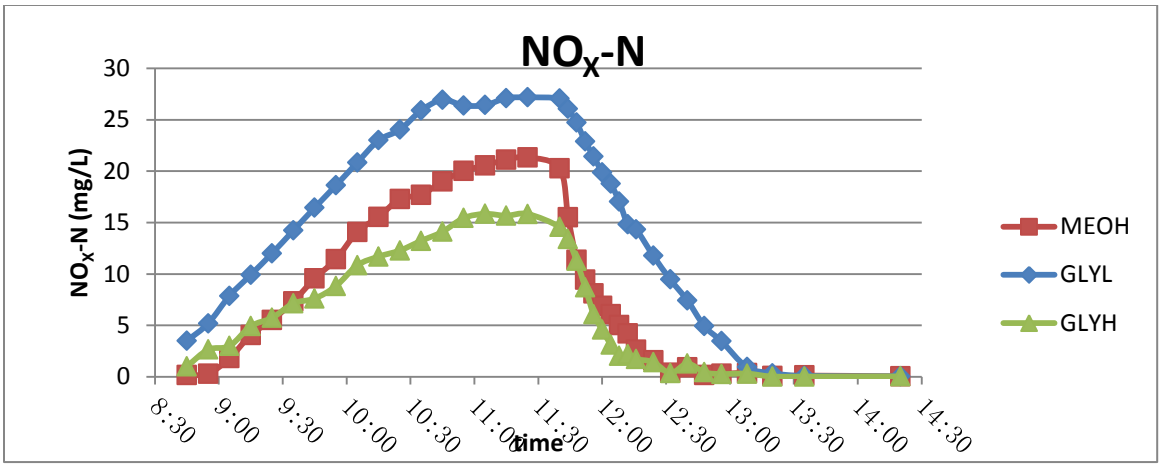


Fig. 6-25 3/14 NO_x-N (mg/L) Profiles

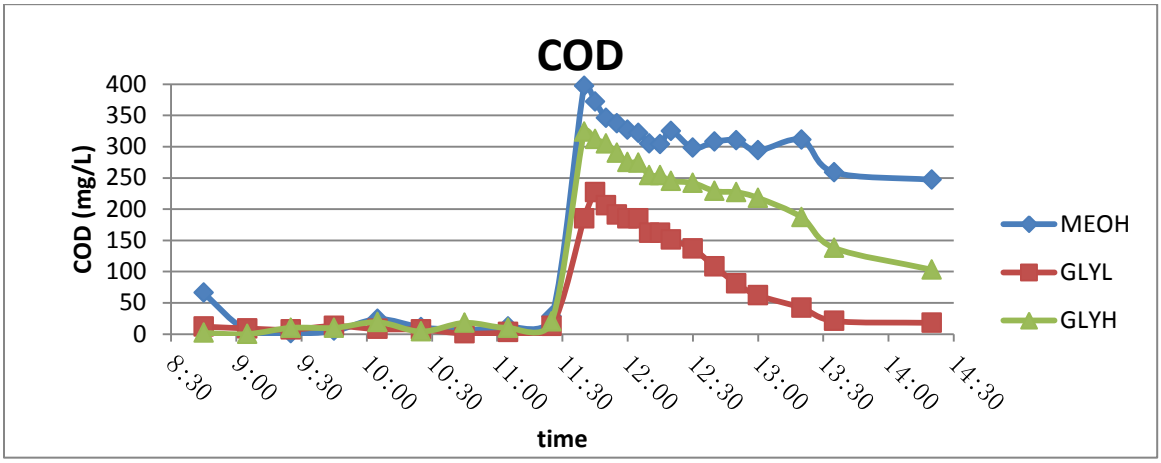
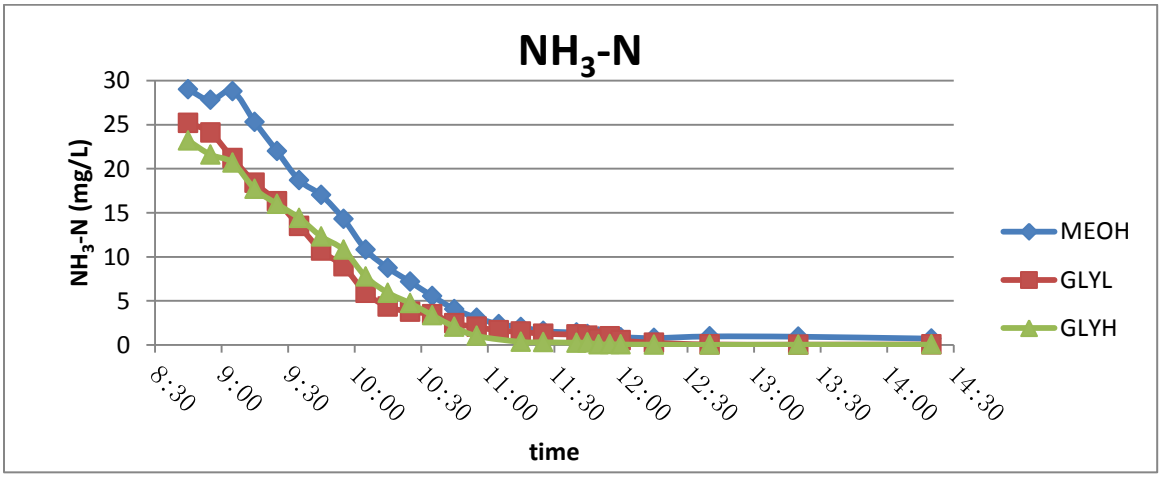
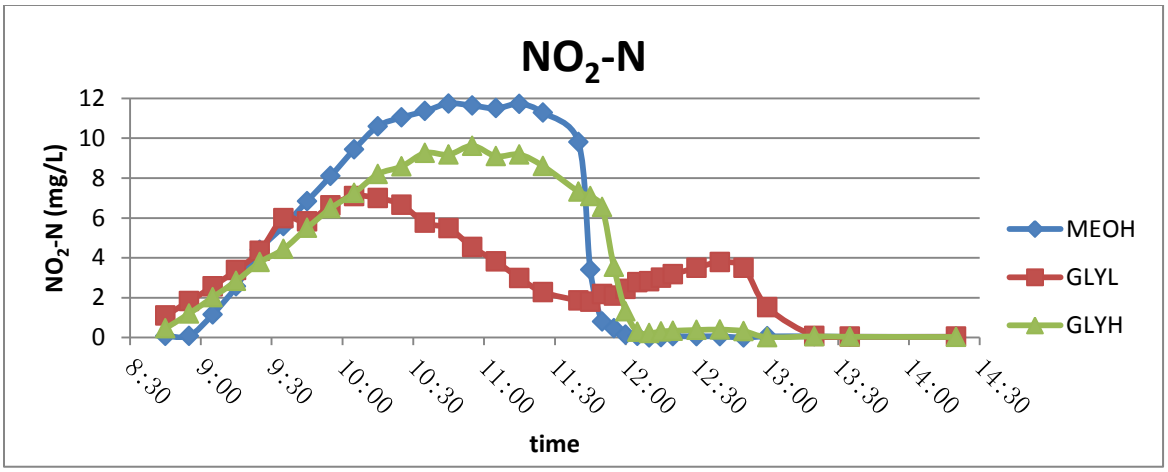


Fig. 6-26 3/17 COD (mg/L) Profiles



1283 Fig. 6-27 3/17 NH_3-N (mg/L) Profiles



1284 Fig. 6-28 3/17 NO_2-N (mg/L) Profiles

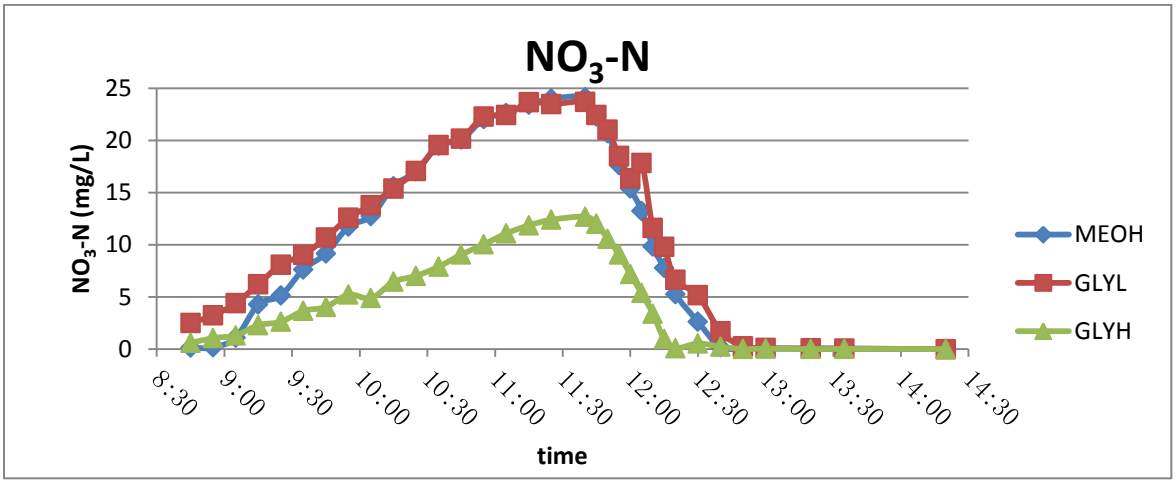
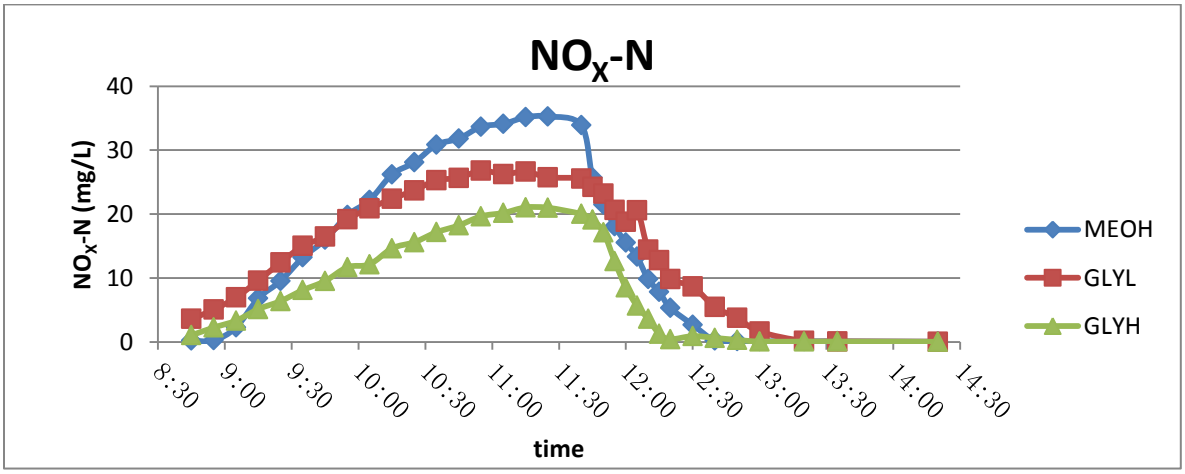


Fig. 6-29 3/17 NO_3-N (mg/L) Profiles



1285 Fig. 6-30 3/17 NO_x-N (mg/L) Profiles

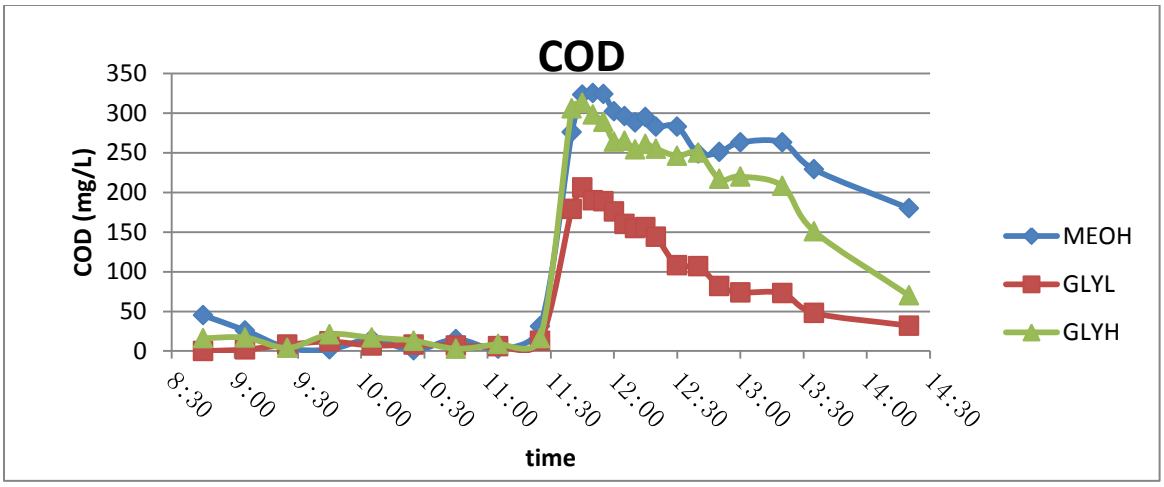
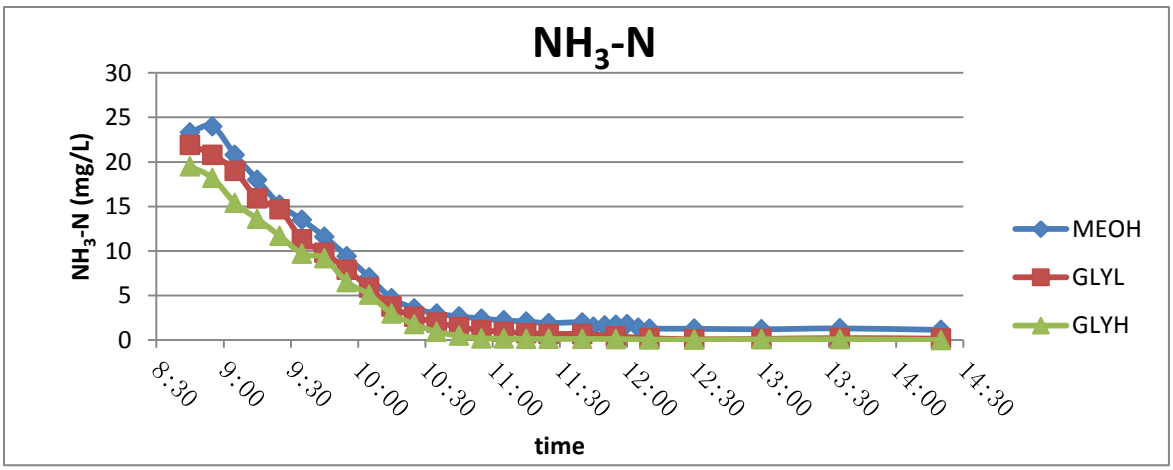
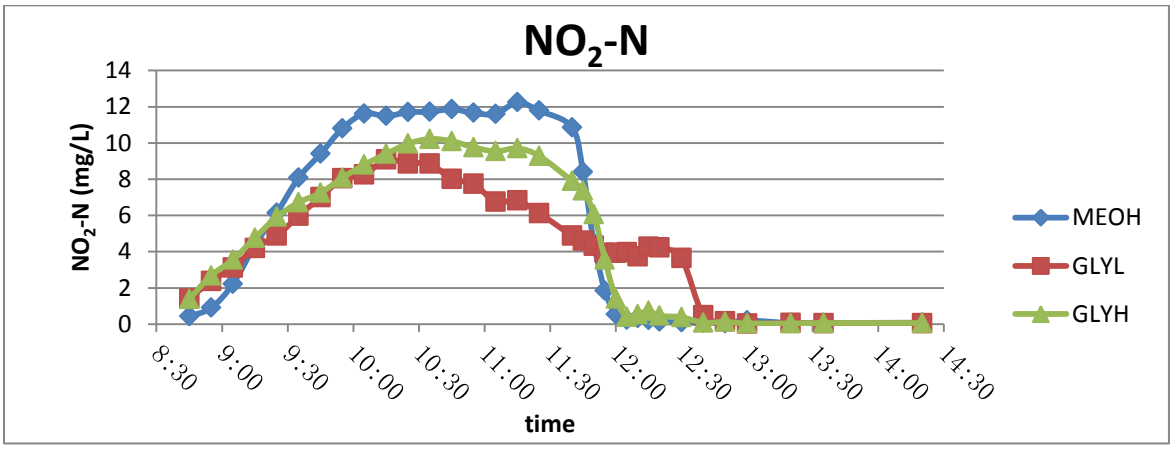


Fig. 6-31 4/2 COD (mg/L) Profiles



1286 Fig. 6-32 4/2 NH_3-N (mg/L) Profiles



1287 Fig. 6-33 4/2 NO_2-N (mg/L) Profiles

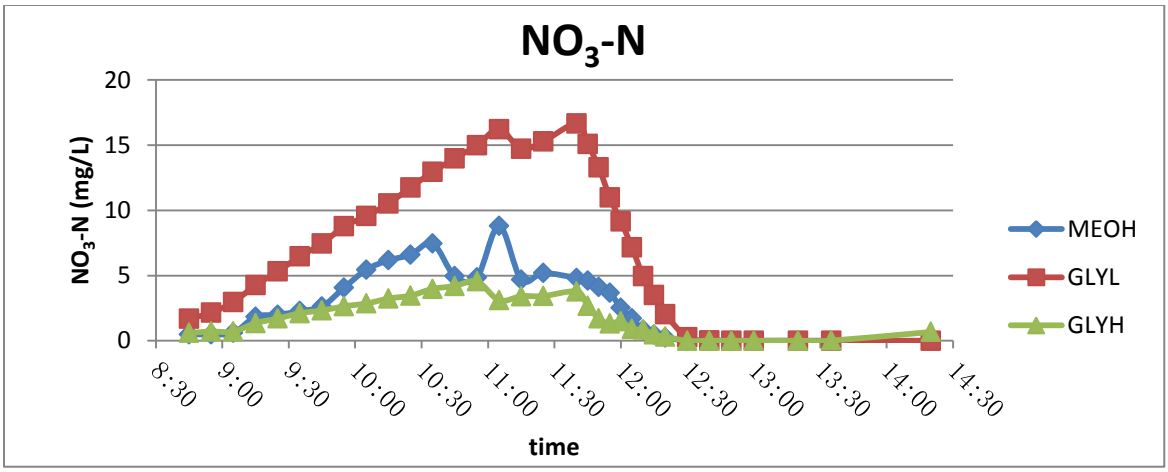


Fig. 6-34 4/2 NO_3-N (mg/L) Profiles

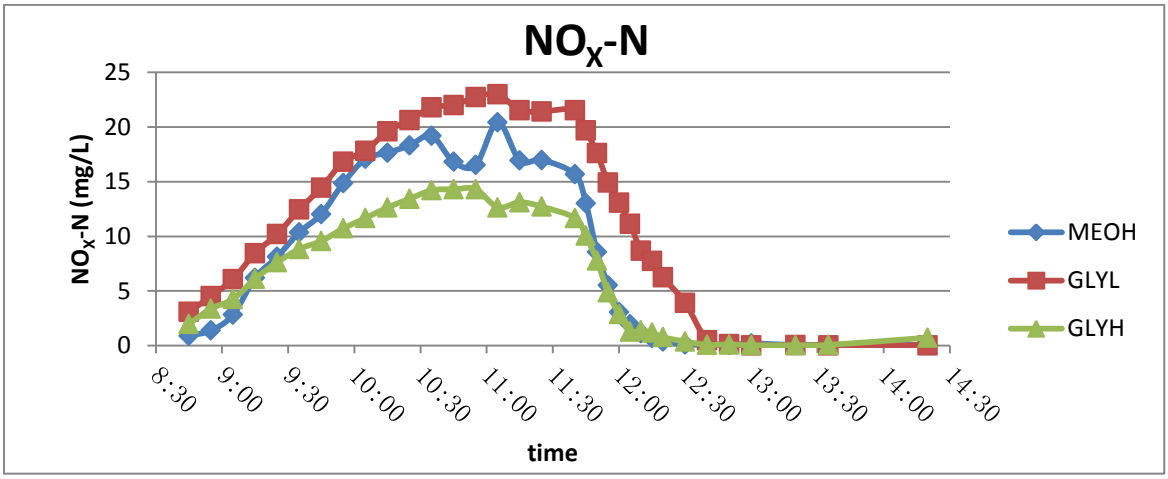


Fig. 6-35 4/2 NO_x-N (mg/L) Profiles

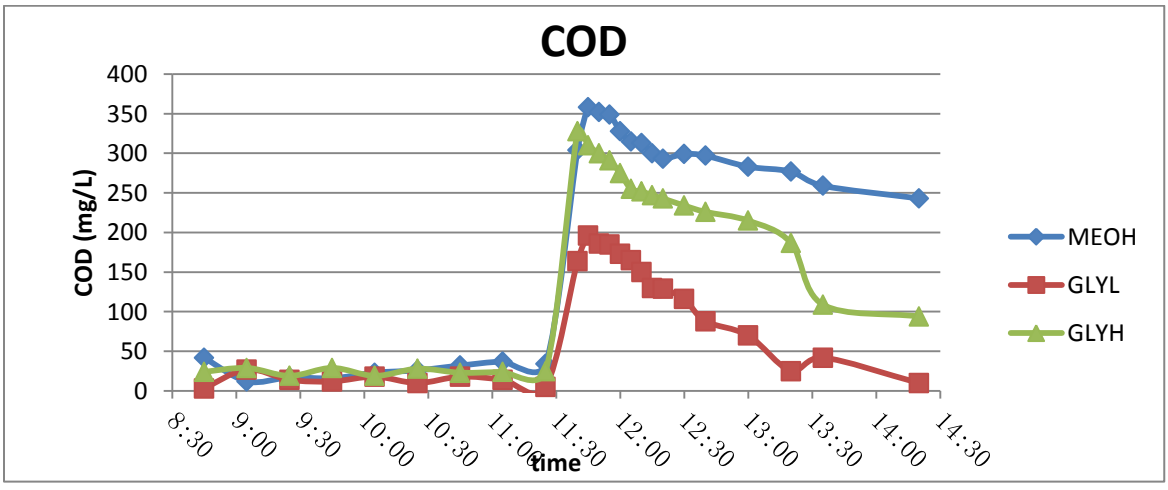


Fig. 6-36 4/5 COD (mg/L) Profiles

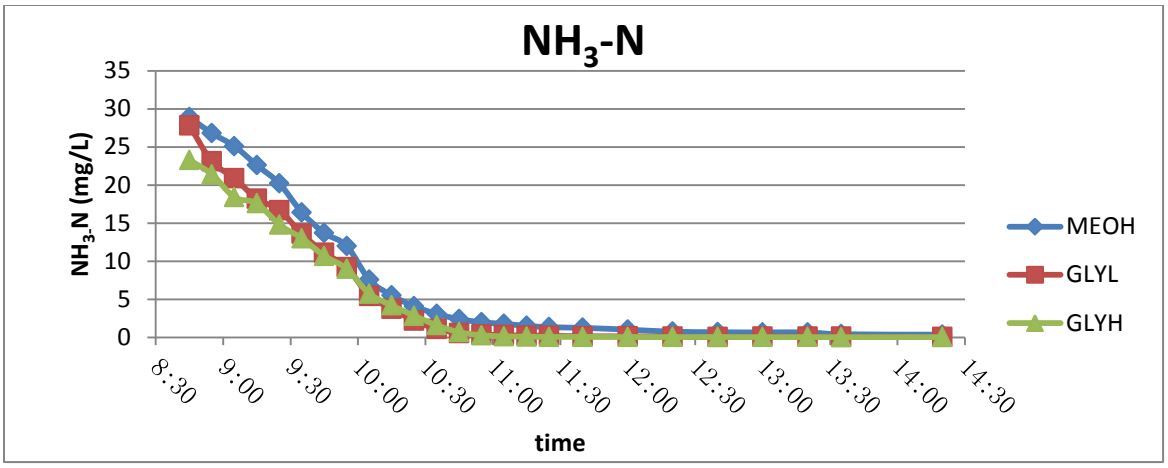


Fig. 6-37 4/5 NH_3-N (mg/L) Profiles

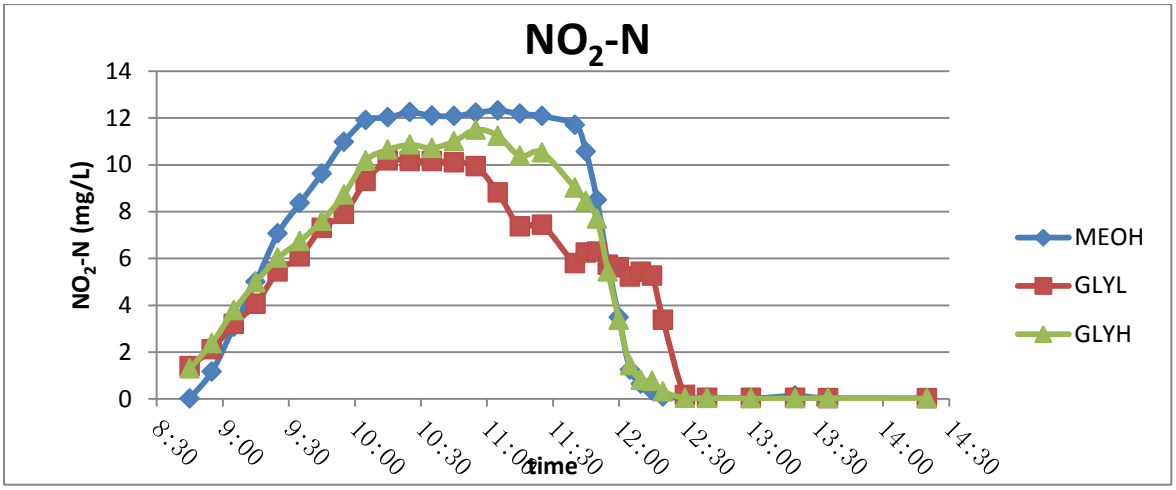


Fig. 6-38 4/5 NO_2-N (mg/L) Profiles

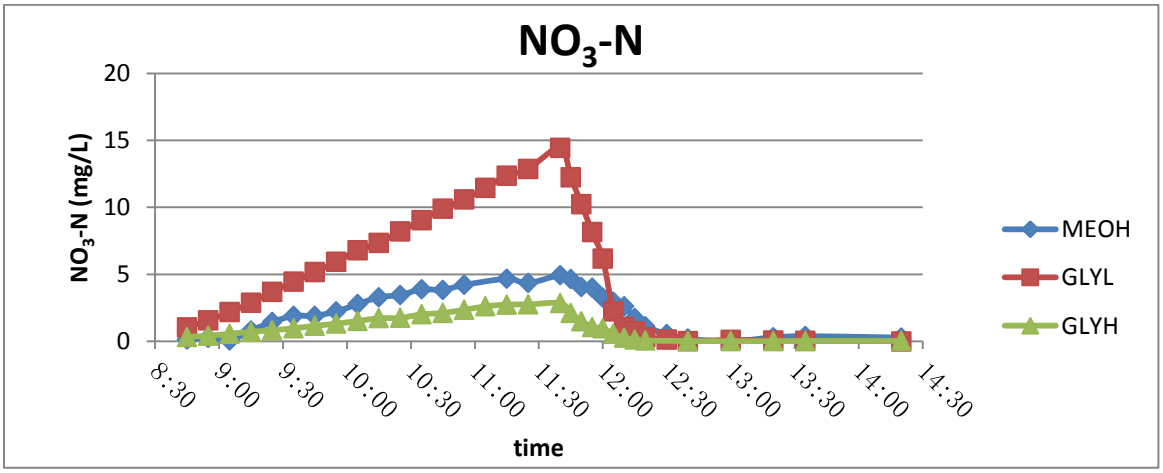


Fig. 6-39 4/5 NO_3-N (mg/L) Profiles

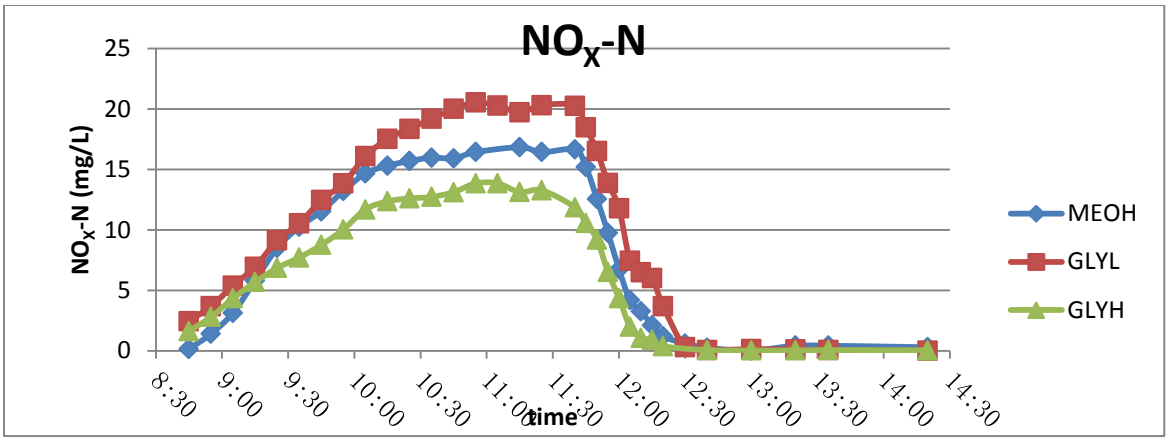


Fig. 6-40 4/5 NO_x-N (mg/L) Profiles

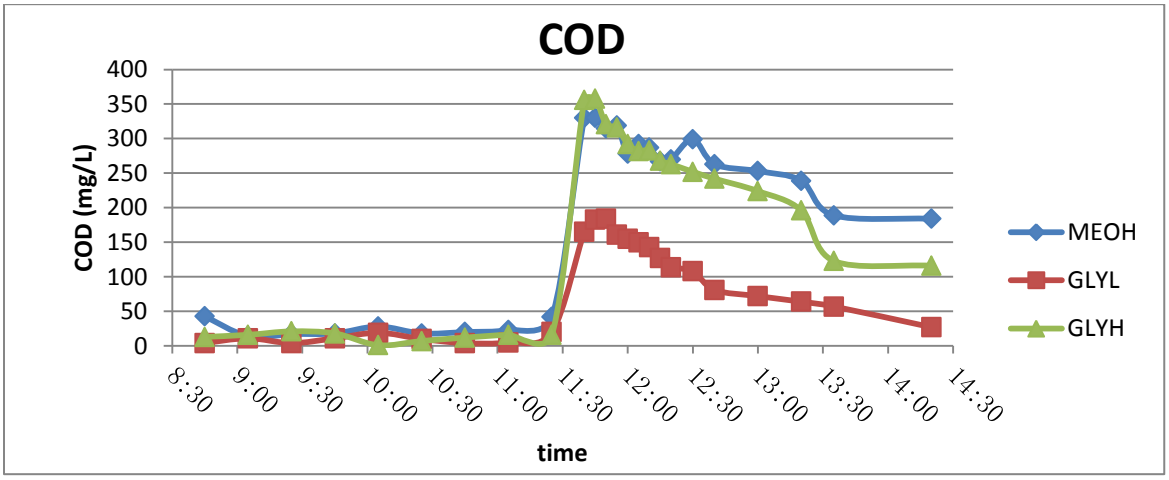


Fig. 6-41 4/8 COD (mg/L) Profiles

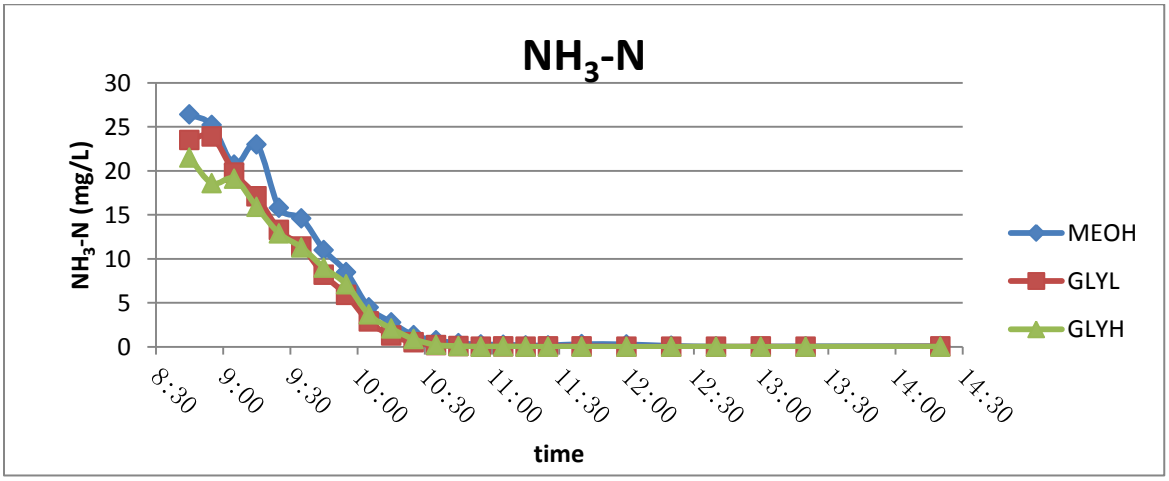


Fig. 6-42 4/8 NH3-N (mg/L) Profiles

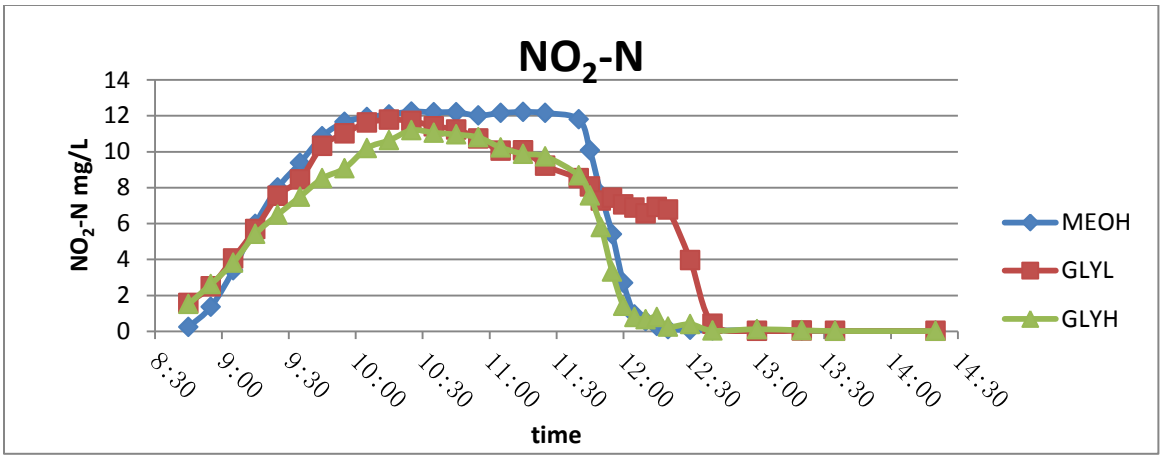


Fig. 6-43 4/8 NO_2-N (mg/L) Profiles

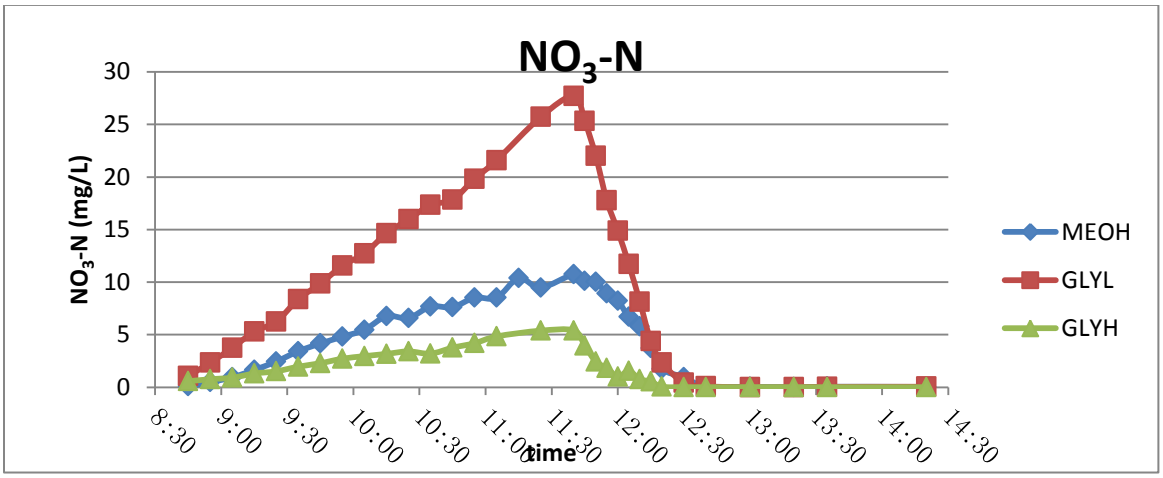


Fig. 6-44 4/8 NO_3-N (mg/L) Profiles

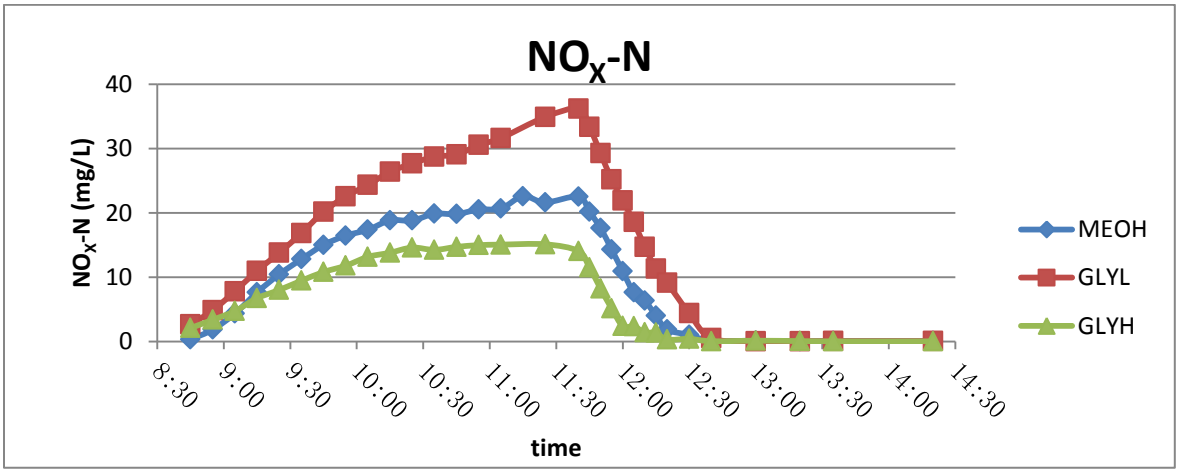


Fig. 6-45 4/8 NO_x-N (mg/L) Profiles