Membrane Wastewater Treatment Processes for Improved Nutrient Removal and Degradation of Synthetic Organic Nitrogen Compounds

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A Dissertation

presented to

the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

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by

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DECEMBER 2011

The undersigned, appointed by the dean of the Graduate School,

have examined the dissertation entitled

Membrane Wastewater Treatment Processes for Improved Nutrient Removal and Degradation of Synthetic Organic Nitrogen Compounds

Presented by Zhihua Liang,

A candidate for the degree of

Doctor of Philosophy,

And hereby certify that, in their opinion, it is worthy of acceptance.

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This dissertation is dedicated to my wife and my daughter.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Zhiqiang Hu, my advisor. He provided me with his patience, thought-provoking guidance for the realization of research works presented in this dissertation. I appreciate all his contributions of original suggestions and encouragement to make my Ph.D. experience at the University of Missouri productive and stimulating. I would like to thank my committee members for their guidance, assistance, and support: Professors Thomas Clevenger, Baolin Deng, Kathleen Trauth, and Randall Miles. I would like to gratefully acknowledge their contributions on all of my successful research work.

I also would like to thank Dr. Enos Inniss for bringing us to WEFTEC, MAEEC, and local plants providing me with practical opportunities needed for this research. I would like to thank our group members, Dr. Okkyoung Choi, Shengnan Xu and Shashikanth Gajaraj for their assistance on my experiments. I would like to thank Atreyee Sims, Dr. Chang-Ping Yu, Chiqian Zhang and Tianyu Tang for their cooperation on the molecular biology experiments. I also would like to thank Kevin Stark, Yu Yang, Meng Xu and Dr. Donglei Wu for their looking after my bioreactors during the time I participated in conferences and took professional exams. I express best wishes to former group members, Qian Chen, Hilda F. Khoei, and Jia Guo for their future career.

I would like to express my gratitude to the Department of Civil and Environmental Engineering for granting me the outstanding student award. This was the best encouragement I received during my four years of Ph.D. study at MU and will always be a source of inspiration to me.

I am thankful for my parents and parents-in-law for their understanding and support throughout my Ph.D. study. I am also thankful for my brothers and sisters-in-law for their strong support for my study. I am most deeply grateful to my wife Wei Lian. Her support, understanding, kindness and wisdom helped me accomplish the goals of my four year Ph.D. program.

Finally, I deeply thank my daughter and cute angel, Annie, for the dreams and hopes she brings to me. I experienced the happiest and proudest moments in my life during the past two years being there for her and helping her to grow up. She will inspire me to do my best for future career achievements.

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MEMBRANE WASTEWATER TREATMENT PROCESSES FOR
IMPROVED NUTRIENT REMOVAL AND DEGRADATION
OF SYNTHETIC ORGANIC NITROGEN COMPOUNDS

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ABSTRACT

The eutrophication problem in US wastewater treatment plants becomes more serious with increasing discharge of nutrients from wastewater to water bodies. The US EPA has required more stringent discharge standards on nutrients for higher effluent water quality. The main objective of this research is to investigate cost-effective and efficient environmental technologies such as membrane bioreactors (MBR) and membrane aerated biofilm reactors (MABR) for improved nitrogen removal and recalcitrant organic nitrogen compounds removal.

In this research, the MABR process had demonstrated efficient organic matter and nitrogen removal through simultaneous nitrification and denitrification with low sludge production. The metabolic selection via alternating anoxic/aerobic processes in the modified Ludzack-Ettinger (MLE) type MBR systems resulted in higher bacterial activities and improved nutrient removal than those of integrated fixed-film MBR (IFMBR) systems. The results of a start-up performance study of MBR systems indicated that MBR process configuration and reactor type affect MBR performance, membrane fouling, nutrient removal, and biomass activities. The high biomass MBR systems demonstrated good and stable nitrophenol biodegradation performance with simultaneous nitrogen removal. . The membrane flux and TMP profiles suggested that continuous stirred-tank reactor (CSTR) reactor type MBR appeared to have much better membrane fouling control performance than that of the pseudo-plug-flow-reactor type of MLE-MBR due to the difference of metabolic uncoupling by nitrophenol compounds in MBR systems.

CHAPTER 1

1. Introduction

1.1 Nutrient contamination and treatment needs

The discharge of nutrients from wastewater to water bodies causes a serious eutrophication problem. Nitrogen and phosphorus in the wastewater provoke the excessive growth of microorganisms, including some harmful species such as cyanobacteria, resulting in algal bloom in the lake and estuary areas. The negative impacts of eutrophication include impairment of water quality, alteration of ecological structure and function, and release of biological toxins (Ahn 2006, Dodds 2002, Dodds et al. 2009). The nutrient inflows to the aquatic systems can be from point sources such as wastewater treatment plant effluents or nonpoint sources such as runoff from agricultural, suburb, and urban areas. More stringent discharge standards on nutrients are needed for higher effluent water quality (Henze 2008). For instance, in order to lower algal biomass, as indicated by chlorophyll concentration at the level of less than 200 mg/m^2 in the aquatic systems, the total nitrogen and phosphorus concentrations must be retained below 3 mg/L and 0.4 mg/L, respectively (Dodds and Welch 2000).

Decentralized wastewater treatment systems, also referred to as septic systems, are significant nonpoint nutrient contributors. According to the U.S. Environmental Protection Agency, on-site wastewater treatment systems serve nearly 25 percent of the U.S. population and 40 percent of new development and discharge approximately 4,000 million gallons of wastewater per day (USEPA 2002, 2005). Traditional septic tanks do not removal much organic matter and ammonia.

They provide simple pretreatment with very poor treatment efficiency. Effluents from septic tanks typically contain high concentrations of organic matter (BOD_5 > 100 mg/L) and nitrogen (TN >30 mg/L). Even after soil filtration, organics and nitrogen, especially in the form of nitrate (NO_3^-), can still easily leach into ground and surface water and seriously impact water bodies, resulting in symptoms like low dissolved oxygen (hypoxia), loss of submerged aquatic vegetation, and the occurrence of nuisance and toxic algal blooms.

Conventional nitrogen removal relies on sequential nitrification and denitrification by autotrophic and heterotrophic microorganisms, respectively. Researchers have shown that nitrogen and organic matter can be effectively removed in wastewater treatment plants (WWTPs) using alternating aerobic-anoxic processes such as two stage anoxic/aerobic process with flow recirculation (Modified Ludzack-Ettinger, MLE), four-stage Bardenpho process, and simultaneous nitrification/denitrification (SND) via intermittent aeration in a single biofilm reactor, sequencing batch reactor, or membrane bioreactor (MBR) (Danesh and Oleszkiewicz 1997, Grady et al. 1999, Patel et al. 2005). Technologies for efficient nitrogen removal in septic tanks, however, have been very limited because the sophisticated operational requirements from WWTPs cannot be applied to decentralized systems. Furthermore, conventional nitrogen removal processes require a significant amount of readily biodegradable organic matter, oxygen and resultant high-energy consumption which generates large amounts of sludge. It is therefore necessary to develop cost-effective and efficient environmental technologies to remove nitrogen and organic compounds from septic systems.

Membrane bioreactor (MBR) processes are widely used in municipal and industrial wastewater treatment. For a single reactor membrane bioreactor system to achieve simultaneous nitrification and denitrification, a more efficient MBR process was needed to operate the system at a high organic loading rate (OLR) and low Dissolved Oxygen (DO) concentrations (Baek and Pagilla 2008). By optimizing the ratio of sludge recirculation to the anoxic reactor, the biological nitrogen removal efficiency reached 90% in a MBR system consisting of two anoxic and aerobic reactors in series (Abegglen et al. 2008). In another study of the effect of MBR configuration on nitrogen removal, compared to the AO (anoxic-oxic) process for high organic and nitrogen loading wastewater, the A_2O MBR process achieved higher organic total nitrogen and nitrate nitrogen removal efficiencies of 95%, 95% and 91%, respectively (Kim et al. 2008). Solids retention time (SRT) is the key design and operational parameter in activated sludge processes. In one study of the effect of SRT on the municipal wastewater treatment by pre-denitrification submerged MBR systems, the highest total nitrogen removal was achieved at an SRT of 33.3 days due to higher mixed liquor suspended solids (MLSS) concentration and lower DO concentrations in the mixed liquor recirculation flow (Tan et al. 2008). Studies also indicate the importance of mixed liquor recirculation and DO levels on nitrogen removal and membrane fouling control in pre-denitrification submerged MBR systems since higher aeration rate minimized membrane fouling while lower aeration rate improved total nitrogen removal (Tan and Ng 2008). More studies are needed to determine biomass characteristics, microbial activities and bioreactor configurations on nitrogen removal in the submerged MBR systems.

1.2 Biodegradation of synthetic nitroaromatic compounds

Organic nitrogen species represent an important fraction of effluent total nitrogen in wastewater treatment. One of the organic nitrogen sources is from industrial activities. Thousands of

nitroaromatic compounds produced through many industrial processes of pesticides, dyes, pharmaceuticals and explosives are released into soil and water causing environmental pollution. The physico-chemical methods used to remove these contaminant compounds are often costly and inefficient. Recent research efforts focus on biological methods for degradation and mineralization of nitroaromatic compounds (Jain et al. 2005). Unlike the biogenic organic compounds utilized by microorganisms which are always available as carbon and energy sources, the synthetic nitroaromatic compounds are often not readily biodegradable (Gribble 1992). Few nitro-substituted compounds are found easily biodegradable and most of them are antibiotics (Venulet 1970). The toxicity and low bioavailability of synthesized nitroaromatic compounds could further reduce microbial metabolic activities to degrade these compounds (Peres and Agathos 2000, Rieger et al. 2002).

New bacterial genes, enzymes and metabolic pathways involved, however, have been discovered. Some bacteria can utilize nitroaromatic compounds as nitrogen and carbon sources for their growth. These are sometimes called induced microorganisms, which can produce enzymes capable of nitroaromatic compounds' transformation (Gorontzy et al. 1994, Toze and Zappia 1999). The most common enzymes that can catalyze the transformation of nitroaromatic compounds are various redox enzymes served as nitroreductases. Other important enzymes include monooxygenase which catalyzes the elimination of the nitro group as nitrite and dioxygenase which catalyzes the insertion of two hydroxyl groups with subsequent elimination of the nitro group as nitrite (Jain et al. 2005, Spain 1995). Other partial reduction pathways are transformation to hydroxylamine and subsequent hydrolytic reactions and elimination of ammonia or partial reduction of aromatic group into Meisenheimer complex and subsequent

elimination of the nitro group as nitrite (Spain 1995). The complex and multiple enzymes and pathways let us to consider and explore the biodegradation of mixtures of nitrophenols by biomass at high concentrations in the MBR systems.

1.3 Hypotheses and research overview

Although there are quite a few research efforts on membrane aerated bioreactor (MABR) and membrane bioreactor (MBR) processes, more research on MABR and MBR is needed with a focus on the improved removal of nutrients and organic nitrogen compounds. By exploring new applications of MBR and MABR technologies, the biomass characteristics, and the bioreactor performance at different stages of operation, this dissertation will answer the following questions:

a) Is MABR technology applicable to on-site wastewater treatment systems for nitrogen removal?
b) Do configurations and operations of MBR affect the performance of MBR and nitrogen removal?
c) How will biomass characteristics of MBR systems be adapted to the process with improved nitrogen removal?
d) Does the key design factor, solids retention time (SRT), control the performance of MBR?
e) What will be the effects of high biomass concentration on nitrogen removal in MBR?
f) Can organic nitrogen compounds be degraded by high biomass concentration under cyclic redox conditions?

To answer the above questions, this research presents the following hypotheses:

Hypothesis 1: MABR process can remove nitrogen through simultaneous nitrification and denitrification by creating aerobic/anoxic zones in the wastewater treatment systems.

Hypothesis 2: Improved nitrogen removal in the MBRs can be achieved with metabolic selection via alternating anoxic and aerobic processes.

Hypothesis 3: Alternating anoxic and aerobic processes will result in higher bacterial activities and less biofouling in the MBRs.

Hypothesis 4: High biomass concentration in the MBRs helps degrade synthetic organic nitrogen compounds.

Hypothesis 5: Maintained at high concentrations, biomass in the MBR is less susceptible to nitrophenol inhibition, and therefore, resulting in complete nitrification.

Hypothesis 6: Microbial metabolic uncoupling due to the inhibition of ATP synthesis by nitrophenols reduces membrane fouling in the MBR.

In this thesis, chapter 2 addresses the MABR process that demonstrated efficient organic matter and nitrogen removal through simultaneous nitrification and denitrification. Chapter 3 describes how metabolic selection via alternating anoxic/aerobic processes resulted in higher bacterial activities and improved nutrient removal in MBR systems. The results of chapter 4 are related to start-up of MBR systems, indicating that MBR process configuration and reactor type affect MBR performance, membrane fouling, nutrient removal, and biomass activities. In chapter 5, MBRs with high activated sludge concentrations demonstrated the capability of removing nitrophenols and inorganic nitrogen simultaneously. The metabolic uncoupling effect, which is

correlated with lower concentrations of bound extracellular polymeric substances (EPS) in the biomass of MBR under continuos flow conditions (CSTRs), made the MBR more resistant to membrane fouling compared to that of the MBR operated under anoxic and aerobic conditions.

CHAPTER 2

2. SIMULTANEOUS NITROGEN REMOVAL IN A MEMBRANE AERATED BIOFILM WASTEWATER TREATMENT SYSTEM

Efficient nutrient removal in decentralized wastewater treatment systems is a challenging task. To improve the removal of organic matter and nitrogen from wastewater, one type of bioreactors using membrane-aerated biofilm reactor (MABR) technique was evaluated. During more than 250 days of continuous flow reactor operation, the MABR reactor showed consistently high COD removal (>86%). At an influent NH_4^+-N concentration of 30 mg/L N, the average effluent NH_4^+-N concentrations was 6.2 mg/L N for MABR reactor while the effluent NO_3^--N concentrations were 5.4 mg/L in the MABR reactor. The overall total inorganic N removal efficiencies were 64% for the MABR reactor. At the measured DO concentration of 0.23 mg/L in aerobic/anoxic zone of the MABR, no significant specific oxygen uptake rate due to ammonia oxidation was detected in settled sludge from the MABR reactor. The results suggest that MABR technique has the potential to improve organic and nitrogen removal in decentralized wastewater systems.

2.1 Objectives

More study is needed to investigate the possibility of simultaneous nitrification and denitrification using MABR technique to effectively remove COD and nitrogen from wastewater while minimizing sludge production. The objective of this study was to evaluate the effectiveness of MABR technique in nitrogen removal for small flow wastewater treatment systems (e.g., septic tanks). The results could shed new light on exploring the limits of nutrient

removal technologies for design and operation of a new generation of decentralized wastewater systems or for retrofitting of existing septic tanks.

2.2 Biological nitrogen removal

Biological nitrogen removal can efficiently remove organic nitrogen compounds to harmless nitrogen gas (N_2) and is generally more economical than physicochemical methods (Ahn 2006, Kim et al. 2008). However, conventional biological nitrogen removal processes such as post-denitrification process have the disadvantage of requiring an external carbon source (Downing and Nerenberg 2008). Alternative processes using membrane bioreactor (MBR) and membrane-aerated biofilm reactor (MABR) techniques have the potential to overcome the disadvantage with simultaneous nitrification and denitrification. The characteristics of high biomass concentration in the MBR may allow simultaneous nitrification and denitrification because anoxic zone is formed in the inner side of biomass floc (Sarioglu et al. 2009). In the MABR, the intramembrane oxygen supply structure can support biofilm growth with simultaneous nitrification and denitrification (Li et al. 2008, Syron and Casey 2008).

Simultaneous nitrification and denitrification can accomplish biological nitrogen removal in single sludge process. Compared to conventional biological nitrogen removal with nitrification and denitrification in two separate tanks, simultaneous nitrification and denitrification have advantages such as small footprint to save space and reduce construction cost (Bernat and Wojnowska-Baryla 2007). Another advantage is a reduced demand or need for alkalinity chemicals (Andrade do Canto et al. 2008). Simultaneous nitrification and denitrification can take place inside the activated sludge flocs because of DO concentration gradients in the flocs. High

DO concentrations at the exterior layer of flocs result in aerobic zone for autotrophic nitrification. Due to the limited DO diffusion and high oxygen consumption of nitrifiers, anoxic microzones are developed in the inner rings of the floc, which favors the growth of heterotrophic denitrifiers to convert nitrates produced in exterior layers to nitrogen gas (Holman and Wareham 2005). Heterotrophic denitrifiers have ability to reduce nitrate under microaerobic conditions with low DO concentration 0.8-2.0 mg/L (Bernat and Wojnowska-Baryla 2007). With the co-respiration mechanism of aerobic denitrification, the heterotrophic denitrifiers can simultaneously use oxygen and nitrite/nitrate as electron acceptors. Furthermore, parallel channels of electron transport chains in microorganisms act to simultaneously transfer electron flows to denitrifying enzymes and oxygen-reducing enzymes (Huang and Tseng 2001, Lesley A. Robertson 1988).

In membrane aerated biofilm reactors simultaneous nitrification and denitrification are achieved through the development of oxic and anoxic zones within the biofilm. Unlike the conventional biofilm reactors, the oxygen and nutrients are supplied from two opposite sides of the biofilm in MABR. The loading rates of oxygen and nutrients, reaction kinetics in biofilm, and mass diffusion rates determine the critical interface location between oxic and anoxic zones in the biofilm of MABR. The oxic zone located at the biofilm-membrane interface and anoxic zone exist at biofilm-liquid interface which gives the MABR higher rates of simultaneous nitrification, denitrification and COD removal compared to conventional biofilm reactors (Semmens et al. 2003, Syron and Casey 2008).

2.3 Membrane aerated biofilm reactor

Recent research on membrane aerated biofilm reactor process provides new information of nitrogen removal via simultaneous nitrification and denitrification (Downing and Nerenberg 2008, Lackner et al. 2008, LaPara et al. 2006, Satoh et al. 2004, Terada et al. 2006). MABR provides a counter-diffusion system where oxygen supplied to the base of the biofilm (membrane tubing) diffuses out of the biofilm while the substrate from the bulk liquid phase, such as NH_4^+ and carbon, diffuses into the biofilm. By decoupling between the substrate and oxygen transport, high degradation rates (1-2 kg NH_4-N /m^3/day) with almost complete nitrification and denitrification was achieved in a membrane-tube-module reactor (Walter et al. 2005). In the study of controllable nitrification by variation air supply pressure in MABR, the nitrification rate of 0.38 kg N/m^3/day is reached at the air oxygen supply rate of 1.28 kg O_2/m^3/day. At this rate the nitrifying region was controlled within the membrane-attached biofilm with bulk liquid DO concentration maintained at level of 0 mg/L which is favorable for denitrification activity and is not affected by membrane aerated oxygen supply (Terada et al. 2006). In another study using gas permeable polyurethane hollow fiber membranes to treat synthetic wastewater (Satoh et al. 2004), more than 90% of chemical oxygen demands (COD) and 95% of ammonia in the wastewater were removed at a COD loading rate of 1.0 g-COD/m^2/day and an ammonium-loading rate of 0.5 g-N/m^2/day. In one study on treatment of high-strength nitrogenous wastewater, the MABR accomplished a total organic carbon removal rate of 5.76 g-C/m^2/day and nitrogen removal rate of 4.48 g-N/m^2/day at total organic carbon and nitrogen volumetric loading rates of 1.92 kg-C/m^3/day and 1.20 kg-N/m^3/day, respectively. However, a regular washing procedure is necessary to remove excess biofilm and sludge aggregation for sustaining stable removal efficiency in the MABR (Terada et al. 2003). Similar excess biofilm accumulation deteriorated the process performance resulting in low nitrogen removal in another MABR study (Semmens et

al. 2003). With efficient oxygen transfer through a gas permeable membrane and biofilm thickness control, MABR has a potential to provide a flexible control strategy for efficient nitrogen removal.

2.4 Materials and Methods

A 7.2L membrane-aerated biofilm reactor (MABR) was constructed with two identical vertical cross stands made of bamboo evenly in the central chamber of the reactor. Plastic baffles were used to divide the reactor into anaerobic, aerobic and settling chambers. The reactors consisted of three basins in a series: an anaerobic sedimentation (or influent) chamber, an aerobic/anoxic chamber, and an internal clarifier (Figure 1). The effective volumes of the anaerobic, aerobic/anoxic chambers and the clarifier were 2.1, 3.3 and 1.8 liters, respectively. An array of openings (3) with a diameter of 1 cm was made on the baffle between the anaerobic and aerobic/anoxic chambers to allow water to flow through the system.

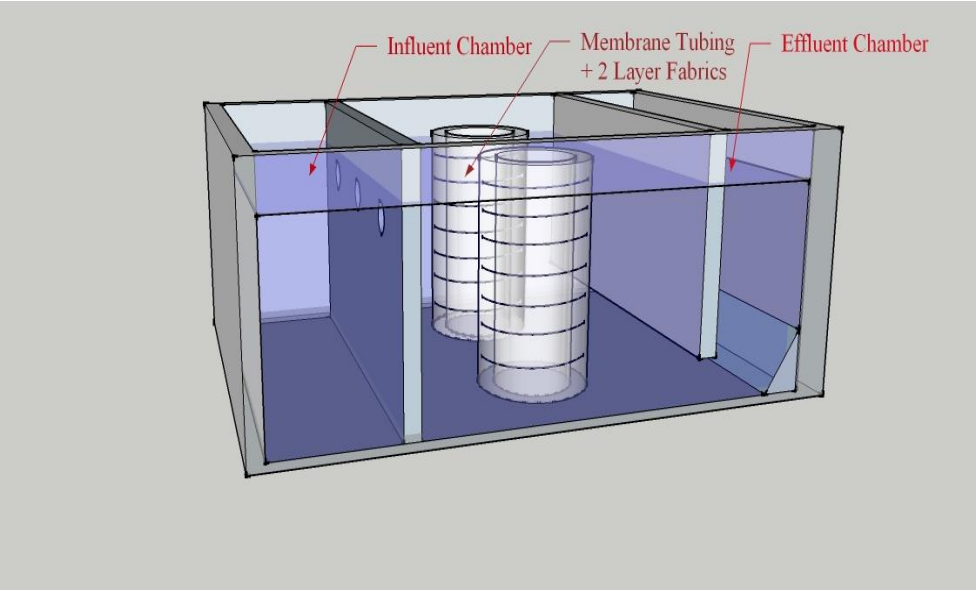


Figure 1. A schematic of MABR based reactor for onsite wastewater treatment. In the MABR, a 7-m long tubing was wrapped around the two identical stands in coils.

In the MABR, the gas-permeable membrane aerated module occupied less than 1.3% of the reactor volume and was made as follows: Two identical vertical stands (6 cm diameter × 16 cm height) made of bamboo were covered with a layer of micro-fibrous non-woven fabric (pore size = 10 μm, Kunin Group Inc., Hampton, NH). A gas-permeable membrane tubing (active length = 7 m, outer diameter = 2.0 mm, inner diameter = 1.5 mm, thickness = 0.24 mm, Silastic® medical grade tubing, Dow Corning) was wrapped around the stands in coils, which yielded a membrane surface area of 0.044 m^2. The open end of the membrane tubing served as the inlet of oxygen while the other end was closed. Pure oxygen was provided at a flow rate of approximately 1 mL/min to the lumen (interior) of the membrane to enable efficient use of oxygen via bubble-less diffusion. The coiled gas-permeable tubing was covered by another layer of non-woven fabric to support biofilm formation. The surface area of the non-woven fabric was 0.060 m^2.

Synthetic wastewater mainly containing sodium acetate with a targeted chemical oxygen demand (COD) concentration of 300 mg/L, 30 mg/L NH_4^+-N and 6 mg/L total P was used throughout the operational period. The synthetic water also contained per liter: 44 mg $MgSO_4$, 140 mg $CaCl_2 \cdot 2H_2O$, 2 mg $FeCl_2 \cdot 4H_2O$, 3.4 mg $MnSO_4 \cdot H_2O$, 1.2 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.8 mg $CuSO_4$, and 1.8 mg $Zn(NO_3)_2 \cdot 6H_2O$ (Table 1). During the shock loading period (from day 89 to day 120), the feed solution was changed to have higher ammonia nitrogen concentrations (target value = 150 mg/L N).

Table 1. Composition of the synthetic wastewater in reactor influent

Compound	Concentrations in reactor influent		
	mg/L	Cations (mM)	Anions (mM)
CH_3COONa	600	7.32 Na^+	7.32CH_3COO^-
$MgSO_4$	44	0.37 Mg^{2+}	0.37 SO_4^{2-}
NH_4Cl	114	2.15 NH_4^{+a}	2.15 Cl^-
$CaCl_2 \cdot 2H_2O$	140	0.96 Ca^{2+}	1.92 Cl^-
Na_2HPO_4	28	0.4 Na^+	0.2 HPO_4^-
$FeCl_2 \cdot 4H_2O$	2	0.01 Fe^{2+}	0.02 Cl^{-b}
$MnSO_4 \cdot H_2O$	3.4	0.02 Mn^{2+}	0.02 SO_4^{2-c}
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1.2	0.006 NH_4^{+a}	0.001 $Mo_7O_{24}^{6-}$
$CuSO_4$	0.8	0.01 Cu^{2+}	0.01 SO_4^{2-c}
$Zn(NO_3)_2 \cdot 6H_2O$	1.8	0.01 Zn^{2+}	0.02 NO_3^-
$Ni(NO_3)_2 \cdot 6H_2O$	0.3	0.001 Ni^{2+}	0.002 NO_3^-

[a]Total NH_4^+= 2.156 mM; [b]Total Cl^-= 4.09 mM; [c]Total SO_4^{2-}= 0.03 mM

The MABR was operated under hydraulic retention time (HRT = 3.6 d or at a flow rate of 2 L/d) and long solids retention time (never wasted unless sludge used for activity measurements). For technical reasons, a constant wastewater flow rate was maintained throughout the study whereas in reality there are minimum and maximum hourly flows of wastewater with instantaneous peak flows in septic tanks each day. Septic systems are often somewhat oversized to accommodate for these fluctuations. The HRT of the MABR reactor was therefore relatively long but it was in the range of design values in Onsite Wastewater Treatment Systems Manual (USEPA 2002). A nitrifying enrichment culture (mainly consisting of *Nitrosospira, Nitrobacter* and *Nitrospira* collected in the lab) and activated sludge from the Columbia Wastewater Treatment Plant (WWTP, Columbia, MO) were used as inoculum. At the start of the MABR reactor experiment, 1 liter of nitrifying enrichment culture from a nitrifying bioreactor in the lab was poured onto the surface of non-woven fabrics. The MABR reactor was run in batch operation mode with effluent fully recycled for the first week before 150 mL of activated sludge from the Columbia WWTP

was added into the aerobic/anoxic chamber of MABR on day 7. The start-up stage of MABR reactor lasted for 30 days.

2.5 Results and Discussion

Organic and nitrogen removal in MABR reactor. During more than 250 days of continuous flow reactor operation, at an average influent COD concentration of 286 ± 57 mg/L (time points n = 68), the effluent COD concentrations were 39 ± 21 mg/L (Figure 4) for the MABR reactor with corresponding COD removal of 86%. For comparison, a typical septic tank removes 30-50% of organic matter in wastewater (USEPA 2002, 2005).

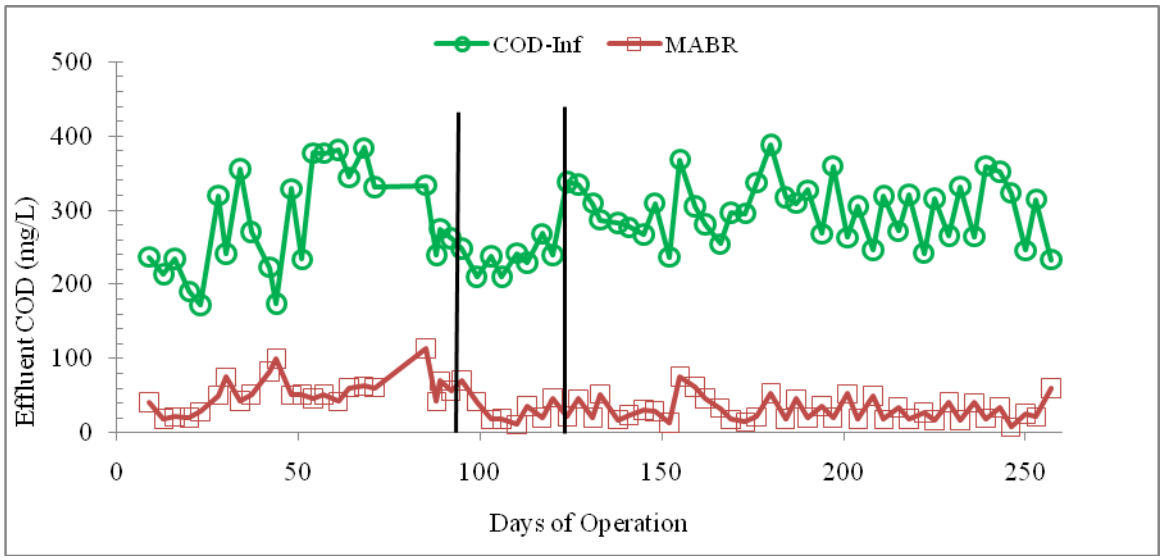


Figure 2. Influent and effluent COD concentrations of the MABR based reactors during the study period. Vertical lines indicate the period of ammonia shock loading.

During the early operating period (from day 16 to day 88) with a typical influent NH_4^+-N concentration of 30 mg/L (ammonia-N loading rate = 11 mg/L/d), more than 90% of ammonia was oxidized in the MABR reactor, demonstrating almost complete nitrification shortly after the start-up period. The effluents of the MABR reactor contained almost non-detectable nitrite and

less than 20 mg/L NO_3^--N (Figure 3), an indication of some level of denitrification in the MABR reactor.

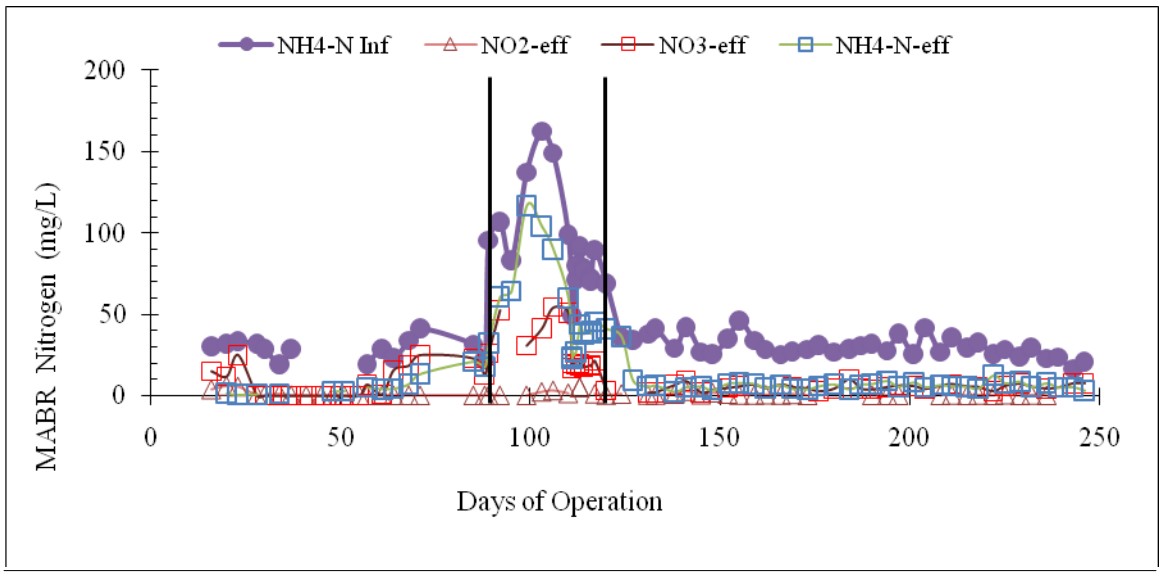


Figure 3. Influent and effluent concentrations of nitrogen species in the MABR during the study period. Vertical lines indicate the period of ammonia shock loading.

A nitrogen shock loading experiment was conducted from day 89 to day 120 to determine the limits of nutrient removal using MABR technique for decentralized wastewater systems. During the period of nitrogen shock loading, COD removal rates for both reactors were not changed because the same organic loading rate was maintained throughout the study. Upon receiving influent containing higher ammonium concentrations (target C_{in} = 150 mg/L NH_4^+-N or ammonia-N loading rate = 55 mg/L/d), significant effluent ammonia accumulation in the MABR was observed (Figure 3). Although effluent nitrate concentrations of MABR increased with increased loading of influent ammonium, it only accounted for less than one-third of the total inorganic N at the high influent ammonium concentrations.

After the shock loading period, the NH_4^+-N loading rates were switched back to original levels (i.e., 30 mg/L N). The MABR reactor had a stable performance on COD and ammonia oxidation for over 100 days. The efficiencies of COD removal and ammonia oxidation in the MABR reactor were 89% and 80%, correspondingly.

The effluent NH_4^+-N concentrations in the MABR (6.2 ± 1.9 mg/L) was relatively higher than conventional effluent of aerated nitrification process whereas the effluent NO_3^--N concentrations in the MABR (5.4 ± 1.9 mg/L) was significantly lower than normal nitrification process effluent. The total inorganic nitrogen (NH_4^+-N + NO_2^--N + NO_3^--N) removal efficiency was 64% for the MABR reactor. High total nitrogen removal in the MABR reactor was attributed to potentially higher level of denitrification developed in the anoxic zone of the reactor because of well controlled, efficient oxygen delivery through gas permeable tubing and immediate oxygen consumption by the biofilm outside the membrane tubing.

The conventional septic tanks should provide at least 24-hrs hydraulics retention time for holding the domestic wastewater produced by a residential household. The remaining volume of the tanks will be used for sludge storage until it is periodically pumped out. For a standard 3600 L septic tank serving for a 4-person size household, the available volume for sludge storage with average daily residential wastewater flow rate of 260L/person/day (USEPA 2002) would be:

Sludge storage volume = 3600L – 260L/person/day × 4 person × 1 day = 2560 L

From USEPA onsite wastewater treatment systems manual, the average influent and effluent of domestic septic tanks total suspended solids concentration were 240 mg/L and 75 mg/L, respectively. The corresponding solids trapping efficiency was 70%. And the total suspended

solids loading rate per person was 75 g/person/day (USEPA 2002). The fraction of volatile suspended solids to the total suspended solids would be the digest decomposition rate of septic tanks and it was 0.7 (Lossing et al. 2010). The amount of sludge retained in the tank would be:

Mass of solids retained = 75 g/person/day × 70% × 0.7 = 36.8 g/person/day

With the assumption that the sludge has 5% solids and the density of water (Lossing et al. 2010), the volume of sludge retained per person daily would be:

Daily volume of sludge = 36.8 g/person/day / (5%) / (1000g/L) = 0. 74 L/person/day

The pumping frequency for 4 people using a 3600L septic tank would be:

2560L/ (4 person) / (0.74 L/person/day) / (365 days/year) = 2.3 years

For MABR operating at steady-state conditions, the thickness of the biofilm was maintained as a constant. The cells generated by organic substrate consumption were then detached from the biofilm surface and dispersed into the liquid phase and then settled at the bottom of MABR reactor. The observed sludge yield coefficient based on the 300 days solids retention time of MABR was calculated as 0.11 day^{-1} using the well-accepted equation (Grady et al. 1999). The daily mass of solids production would be:

Mass of solids produced = 286 mg/L × 0.86 × 0.11 × 260 L/person/day / 1.42

= 4.99 g/person/day

Daily volume of sludge = 4.99 g/person/day / (5%) / (1000 g/L) = 0.10 L/person/day

The pumping frequency for 4 people using a 3600 L MABR tank would be:

2560L/ (4 person) / (0.10 L/person/day) / (365 days/year) = 17.5 years

The pumping cost is the major operating cost of an onsite wastewater treatment system. Less sludge pumping frequency will result in cost savings, minimization of volume of waste sludge and minimum impact on the downstream wastewater treatment system. The typical septic tank pumping cost is estimated to be $150 per pumping. Compared to regular septic tanks, the cost of pumping operation and maintenance cost in the MABR systems would be 7.6 times lower.

CHAPTER 3

3. BIOMASS CHARACTERISTICS OF TWO TYPES OF SUBMERGED MEMBRANE BIOREACTORS FOR NITROGEN REMOVAL FROM WASTEWATER

Biomass characteristics and microbial community diversity between a submerged membrane bioreactor with mixed liquor recirculation (MLE/MBR) and a membrane bioreactor with the addition of integrated fixed biofilm medium (IFMBR) were compared for organic carbon and nitrogen removal from wastewater. The two bench-scale MBRs were continuously operated in parallel at a hydraulic retention time (HRT) of 24 hours and solids retention time (SRT) of 20 days. Both MBRs demonstrated good COD removal efficiencies (> 97.7%) at incremental inflow organic loading rates. The total nitrogen removal efficiencies were 67% for MLE/MBR and 41% for IFMBR. The recirculation of mixed liquor from aerobic zone to anoxic zone in the MLE/MBR resulted in higher microbial activities of heterotrophic (46.96 mg O_2/gVSS-h) and autotrophic bacteria (30.37 mg O_2/gVSS-h) in the MLE/MBR compared to those from IFMBR. Membrane fouling due to bacterial growth was evident in both the reactors. Even though the trans-membrane pressure and flux profiles of MLE/MBR and IFMBR were different, the patterns of total membrane resistance changes had no considerable difference under the same operating conditions. The results suggest that metabolic selection via alternating anoxic/aerobic processes has the potential of having higher bacterial activities and improved nutrient removal in MBR systems.

3.1 Objectives

More information on the sludge characteristics and microbial community structure of the modified MBR systems would help microbial engineers better understand the reactor performance and associated bacterial activity/population and membrane fouling for optimal MBR design and operation. The hypothesis of this study was that metabolic selection via alternating anoxic/aerobic processes (Grady et al., 1999) in the MBR systems resulted in improved nutrient removal, higher bacterial activities and less biofouling. The objective of the study was to compare two modified MBR systems (integrated fixed-film MBR and MLE/MBR) using the same type of membrane module with respect to their system performance (e.g., nitrogen removal), biomass characteristics, microbial activity and nitrifying bacterial community structure at a defined solids retention time (SRT). In addition, the membrane flux and the total membrane resistance of the two types of MBRs at a constant hydraulic retention time (HRT) were compared to assess the extent of biofouling in the two systems.

3.2 Integrated fixed biofilm MBR & conventional MBR

3.2.1 MBR advantages over conventional activated sludge

MBR technology is increasingly applied in wastewater treatment plants as wastewater effluent discharge permits become progressively stringent and the demand for clean water keeps rising. The advantages of using MBR technology for wastewater treatment over conventional activated sludge process include: (1) capability of dealing with high volumetric organic loading rates and the subsequent small reactor volume because of increased biomass concentration in the bioreactor and the absence of secondary clarifier basin (Ben Aim and Semmens 2003, Chu et al. 2008, Huang et al. 2001); (2) improved effluent water quality for water reuse since bacteria and

suspended solids larger than the membrane pore size are retained by membrane and it provides valuable disinfection capacity to achieve up to log 7 total coliforms reduction (Hirani et al. 2010, Krauth and Staab 1993, Le-Clech 2010, Pollice et al. 2008, Rosenberger et al. 2002); (3) complete and stable nitrification owing to the retention of slow-growing nitrifying bacteria at a prolonged solids retention time (SRT) without the need for extended aeration (Davies et al. 1998, Li et al. 2006, Yoon et al. 2004); (4) capability of consistently high removal of organics and nutrients with insensitivity to hydraulic fluctuation when handling wide variation of influent quantity and quality (Chang et al. 2002, Delai Sun et al. 2007, Rosenberger et al. 2002); (5) sludge minimization with low sludge production because of longer SRT resulting in lower waste solids (Hirani et al. 2010, Judd 2008, Le-Clech 2010, Teck et al. 2009); and (6) flexible operation due to separated control of SRT and hydraulic retention time (HRT) without the need of sufficient HRT for the flocs size growth (Judd 2008, Khongnakorn et al. 2007, Teck et al. 2009).

3.2.2 Efforts on improving nitrogen removal in the MBR

In traditional MBRs intensive aeration is carried out to support microbial growth and control membrane fouling. This intensive aeration gives MBRs excellent removal capabilities when dealing with organic matters and ammonia nitrogen. However, the adverse effect of intensive aeration eliminates the anoxic conditions necessary for denitrification and results in poor total nitrogen removal in the MBR systems (Kimura et al. 2008, Patel et al. 2005). Recent research efforts have been conducted to overcome this drawback and improve nitrogen removal with modified reactor configurations. In one MBR configuration study, a baffle was inserted in a membrane bioreactor to divide MBR into separate outer and inner zones in a single tank. With intermittent influent feed and aeration control, alternate aerobic and anoxic conditions were

created. Without mixed liquor recirculation and external carbon source, this MBR system accomplished 77% total nitrogen removal at an HRT of 4.7 hours (Kimura et al. 2008). In another study, the modified Ludzack-Ettinger (MLE) type MBR (MLE/MBR) systems were constructed with mixed liquor recirculated from the aeration tank into the anoxic tank. Through anoxic/aerobic sequencing or simultaneous nitrification/denitrification, more than 70% of total nitrogen in wastewater was removed (Abegglen et al. 2008, Song et al. 2003). The simultaneous nitrification and denitrification also occurred in the aerobic granular sludge membrane bioreactor where glycogen accumulating organism granules varying in size from 0.18 to 0.9 mm were formed in the MBR system. With accumulation of granule-permeated sludge, the MBR exhibited stable total nitrogen removal efficiency of 78% (Wang et al. 2008). In a long term evaluation of system performance of MBR run at a SRT of 40 days, the α ratio of oxygen transfer coefficient in mixed liquor to that in clean water was controlled in the range of 0.2-0.5. With a mean floc size of 135 μm of MBR sludge, 45% of influent total nitrogen was removed with 77% of the removal through simultaneous nitrification and denitrification with the other 23% of nitrogen removal through biomass assimilation (Holakoo et al. 2007).

3.2.3 Effects of MBR configuration on nitrogen removal in the MBR

The effect of bioreactor configurations and operating conditions on the performance and biomass characteristics of MBR systems is a subject to be studied. MLE type MBR systems coupled with ozonation to reduce sludge production present better nutrient removal (70% total N removal efficiency) than without sludge ozonation, possibly due to additional carbon source provided by ozonation for denitrification (Song et al. 2003). In a pilot-scale MBR system, consistently excellent effluent quality was observed in the test range of F/M ratio between 0.34 and 1.41 gCOD/gVSS.d while the membrane fouling rates appeared to be positively correlated with the

F/M ratio and the concentration of soluble microbial products (Trussell et al. 2006). In another study, a moving bed membrane bioreactor (MBMBR) exhibited better total nitrogen removal efficiencies (>70%) than those of a conventional MBR at the same operating conditions although membrane fouling became significant in the MBMBR due to the formation of a thick and dense cake layer on the membrane (Yang et al. 2009).

A recent study of nitrification rates in conventional and membrane-assisted enhanced biological phosphorus removal (EBPR) processes also demonstrated that the specific nitrification activities from the conventional EBPR process can be 15 to 75% greater than those from regular membrane process under identical operating conditions (Monti and Hall 2008). In another study, the specific oxygen uptake rate (SOUR) of the biomass from the submerged microfiltration MBR was 4.3 mg O_2 g^{-1} MLSS h^{-1}, higher than that of nanofiltration MBR (2.9 O_2 g^{-1} MLSS h^{-1}) at the same solids retention time (80 d) although microbial diversity analysis using denaturing gradient gel electrophoresis (DGGE) indicated no significant difference between the MBRs (Choi et al. 2007).

3.3 Materials and Methods

The lab-scale submerged membrane bioreactor having the type of bench scale membrane module with reactor volume (7.2 L) was constructed (Figure 4A). The membrane module used for MLE-MBR was ZeeWeed hollow fiber membrane from Zenon Environmental Systems Inc. The membrane hollow fiber had a nominal pore size of 0.1 μm and a total membrane surface area of 0.047 m^2 per module. MLE-MBR was divided into anoxic, aerobic and settling chambers by glass baffles to introduce alternating anoxic/aerobic conditions. The effective volumes of the anoxic, aerobic chambers and the clarifier were 1.8, 3.6 and 1.8 liters, respectively.

The MLE-MBR was operated in continuously feeding mode at a target SRT of 20 d and a HRT of 1d. The bioreactor was inoculated with 2000 mL seed activated sludge from a local municipal wastewater treatment plant (Columbia, MO). The synthetic wastewater mainly containing nonfat dry milk powder with a chemical oxygen demand (COD) concentration of 500 mg/L, 51.7 mg/L TN, 30 mg/L NH_4^+-N and 6 mg/L total P was used as the influent feed. From day 0 to day 65, the influent COD was maintained at about 300 mg/L to evaluate the treatment of low strength wastewater. The composite feed also contained the following micronutrients/L: 44 mg $MgSO_4$, 14 mg $CaCl_2 \cdot 2H_2O$, 2 mg $FeCl_2 \cdot 4H_2O$, 3.4 mg $MnSO_4 \cdot H_2O$, 1.2 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.8 mg $CuSO_4$, and 1.8 mg $Zn(NO_3)_2 \cdot 6H_2O$. To support microbial growth and control membrane fouling, coarse bubble aeration was provided in the reactors through the membrane module's built-in orifice connected by tubing to an air pump at a constant flow-rate of 8L/min. The permeate was extracted continuously by imposing on the membrane a negative pressure (indicated from trans-membrane pressure, TMP) via a fixed speed suction peristaltic pump.

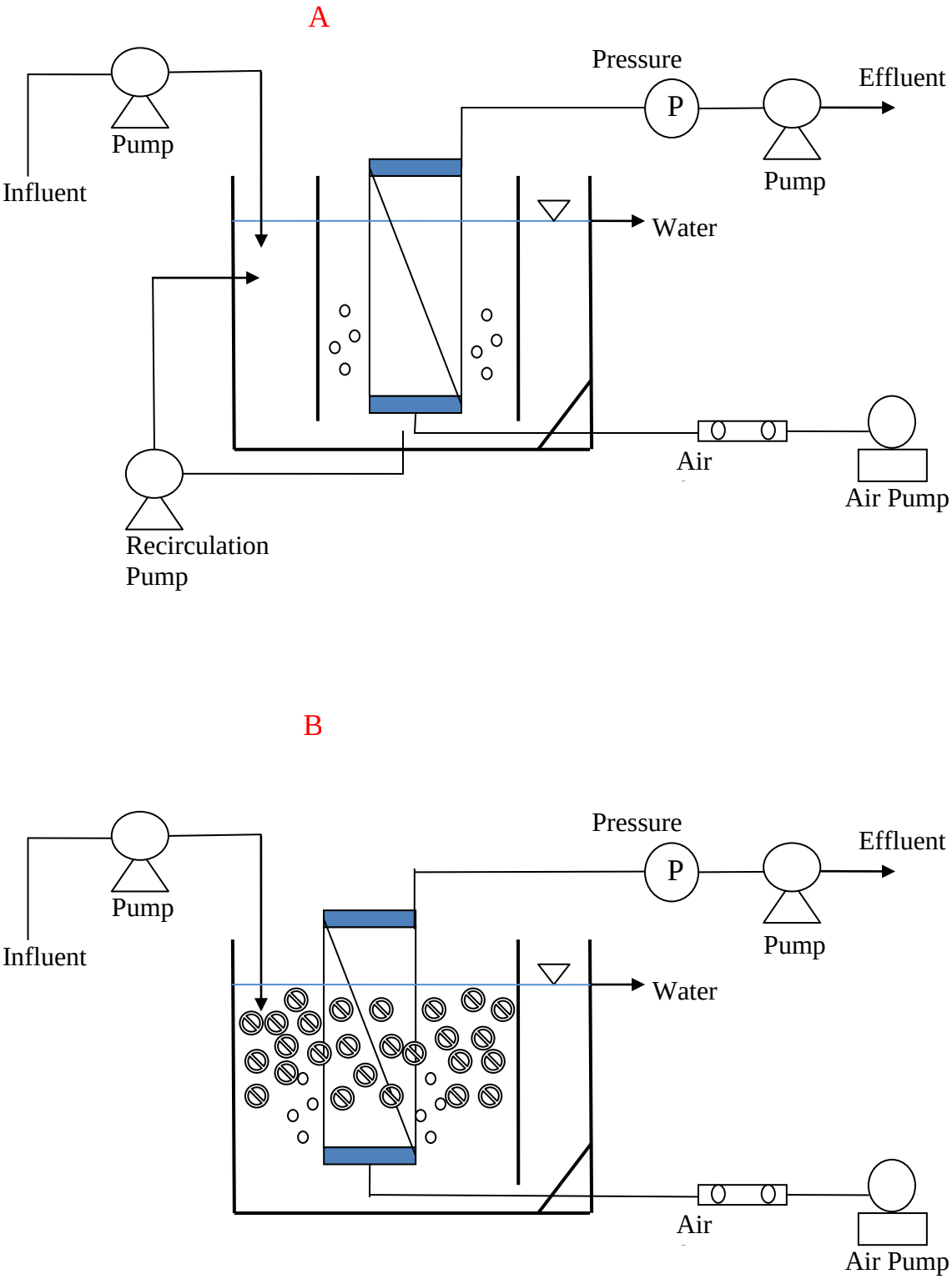


Figure 4. Schematic diagram of the experimental set up: (A) MLE/MBR and (B) IFMBR

The other MBR called integrated fixed-film MBR (IFMBR) (Figure 4B) was constructed with same reactor volume of 7.2 L by submerging same type of membrane module as MLE-MBR into bioreactor. The IFMBR was divided into two chambers by glass baffle: reaction chamber (5.1 L) and settling chamber (2.1 L). A biofilm support plastic medium (AqWise biomass carrier, Siemens Water, WI) was added to the IFMBR to occupy 50% volume of the reaction chamber. The dimension of biomass carrier was 10×10 mm, diameter × height. The density of the carriers was 0.98 g/cm^3 and the effective specific surface area was 600 m^2/m^3. The IFMBR was also operated in a continuous mode with target SRT of 20 d and a HRT of 1d. The bioreactor was inoculated using the same volume seed activated sludge from a local municipal wastewater treatment plant (Columbia, MO). The synthetic wastewater mainly contained nonfat dry milk powder with same formula recipe as feed solution of MLE-MBR.

3.3.1. Biomass microbial activities

The autotrophic and heterotrophic activities of activated sludge in the two MBRs during the study period were determined by batch extant respirometry with specific oxygen uptake rate (SOUR) measurement due to ammonia oxidation and acetate oxidation, respectively (Hu et al., 2002). The brief procedure was as follows: After aliquots (60 mL) of the sludge from the MBRs were aerated with pure oxygen for three minutes, they were transferred to the respirometric bottles to make tightly capped with sealed DO probe. At a predetermined time of 500 to 600 seconds, an aliquot of substrate (10 mg N/L NH_4^+-N or 20 mg/L COD in acetate) was injected with a 10 µL glass syringe. The decrease of the DO concentration in the respirometric bottle due to substrate oxidation was measured by a DO monitor (YSI model 5300A, Yellow Springs, OH) and continuously recorded at 4 Hz by LabView software interfaced computer. The specific oxygen uptake rates were calculated based on a linear regression analysis because a zero-order

reaction was observed for a long period of time. Each SOUR experiment was carried out in at least duplicate.

3.4 Results and Discussion

3.4.1 Reactor performance of MLE/MBR and IFMBR

The influent and effluent COD concentrations in the two MBR systems during the study period were presented in Figure 5. COD removal efficiency was not affected by increasing influent organic loading rate from day 65 onwards. At the average influent COD concentration of 516 ± 80 mg/L, the effluent COD concentrations were 9.3 ± 9.3 mg/L for the MLE/MBR and 12 ± 14 mg/L for the MLE/MBR and IFMBR systems, respectively, with an average COD removal rate of 98.2% and 97.7%, respectively. There was no significant difference of effluent COD concentration between the MLE/MBR and IFMBR system (ANOVA, α=0.05, P-value = 0.43).

The modified MBRs demonstrated excellent NH_4^+-N removals (removal efficiency > 96%). The average effluent NH_4^+-N concentrations of the MLE/MBR and IFMBR were 0.88 mg/L and 1.36 mg/L, respectively There was no significant difference of effluent NH_4^+-N concentration between the two MBRs (P = 0.33). In contrast, the effluent total N concentration of the IFMBR (average value = 30.7 mg/L N) was significantly higher than that of the MLE/MBR (17.0 mg/L N) (ANOVA, α=0.05, P<0.001) (Figure 6). Due to high flow coarse bubble aeration necessary to provide the scouring force for membrane fouling control, no significant biofilm was formed on the surface of plastic media in the aerated reaction chamber. Therefore, limited nitrogen removal (41% total nitrogen removal for influent total nitrogen concentration of 51.7 mg/L) through

simultaneous nitrification and denitrification could be achieved. In contrast, the total nitrogen removal efficiency reached 67% for the MBR reactor, demonstrating the efficiency of anoxic/aerobic sequencing in nitrogen removal.

The effluent water quality of conventional MBR processed for treating municipal wastewater was listed in Table 2. Most MBR process achieved over 90% organics matters removal and ammonia-N removal. Some MBR processes with intermitted aeration accomplished 60% total nitrogen removal efficiency through the serious nitrification and denitrfication. Compared to those MBR processes, proposed IFMBR and MLE-MBR would employ biofilm simultaneous nitrification/denitrification and mixed liquor recirculation configurations to achieve total nitrogen removal.

Table 2. Effluent water quality of MBRs treating municipal wastewater

Influent COD (mg/L)	**Influent NH_3-N (mg/L)**	**Effluent COD (mg/L)**	**Effluent TKN (mg/L)**	**Reference**
150-450	25	20.8	4.9	(Chiemchaisri et al. 1992)
200-300	--	20-30	--	(Visvanathan et al. 1997)
250-550	60-150	30	10	(Chaize and Huyard 1991)
300	39.7	3.1	0.2	(Winnen et al. 1996)
71	35	1.5	1.1	(Kishino et al. 1996)

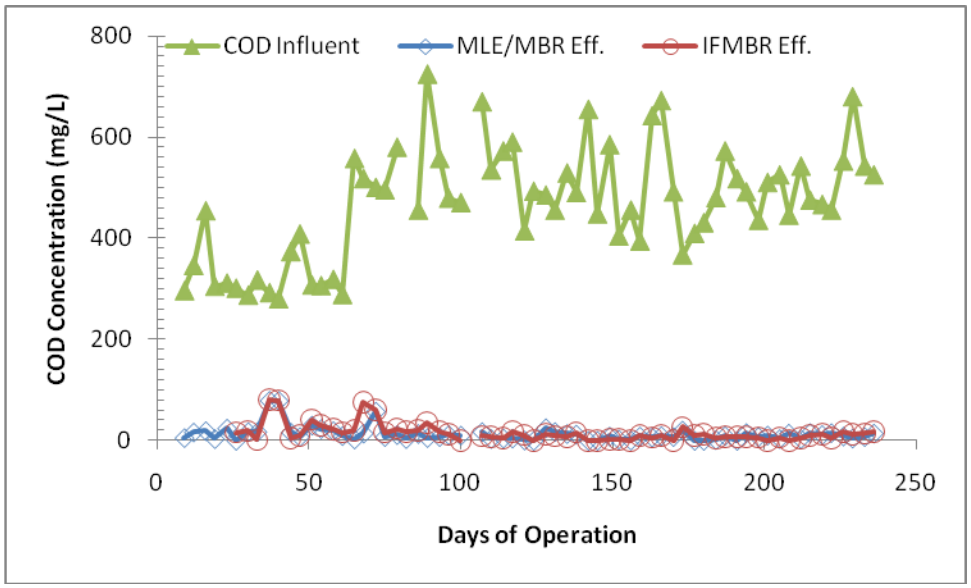


Figure 5. Influent and effluent COD concentrations in MLE/MBR and IFMBR systems

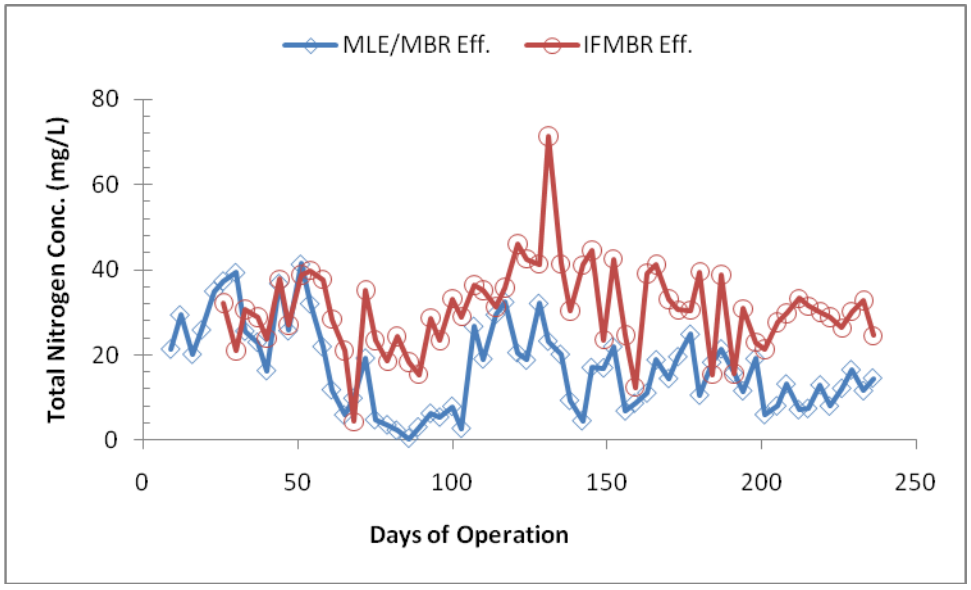


Figure 6. Effluent total nitrogen concentrations of MLE/MBR and IFMBR systems

3.4.2 Microbial activity of MLE/MBR and IFMBR

The heterotrophic microbial growth rates inferred from SOUR measurements were 46.96 ± 7.59 and 30.77 ± 6.58 mg O_2/gVSS-hr for MLE/MBR and IFMBR, respectively. The autotrophic SOURs of biomass in MLE/MBR and IFMBR were 30.37 ± 6.09 mg O_2/gVSS-hr and 22.55 ± 5.86 mg O_2/gVSS-hr, respectively. The SOUR values of both heterotrophic and autotrophic activities were in a relatively normal range compared to previous studies (Yang et al. 2009). The heterotrophic and autotrophic SOUR rates of biomass in the MLE/MBR were statistically higher than those of biomass in the IFMBR. The higher microbial activities of heterotrophic and autotrophic bacteria in the biomass of the MLE/MBR system were attributed to the harsh alternating anoxic/aerobic treatment, which likely selected for populations with inherently faster growth rates (Dytczak et al. 2008).

3.4.3. Membrane flux and total membrane resistance of MLE/MBR and IFMBR

The change of transmembrane pressure (TMP) after membrane cleaning exhibited a similar trend in the MLE/MBR and IFMBR: an initial rapid increase followed by a slow increase of TMP with time (Figure 8A). The TMP rise in the MLE/MBR was faster than that in IFMBR during the initial rapid increase phase. The time required to end the stage of a fast increase of TMP for MLE/MBR (9 hours) was much shorter than that for IFMBR (97 hours). At the end of this phase, the TMP of IFMBR was 1.5 times higher than that of MBR. The change of TMP during membrane operation is commonly attributed to the cake layer formation on the membrane surface (Chang et al. 2002). Both the reactors were designed to have identical suction force at the fixed speed of peristaltic pumps for permeate collection and identical shearing force on the

membrane for fouling control and sludge aeration. Hence, the difference in biomass characteristics (e.g., extracellular polymeric substances or EPS) of the MLE/MBR and IFMBR might account for the large TMP difference because of the cake layer formation on the membrane surface of the MLE/MBR and IFMBR.

Interestingly, the sludge of IFMBR had a better settling property (Figure 7) than that of the MLE/MBR with a corresponding sludge volume index (SVI) value of 75 ml/g (IFMBR) and 113 ml/g (MLE/MBR), indicating that the sludge in both reactors had good settling properties. Microscopic observations confirmed that little filamentous bacteria were detected in both reactors. The better sludge settling property of IFMBR was attributed to the presence of porous support media, which support biofilm formation and provide a settling base to increase the floc size. Further studies are needed to assess the relationship between sludge settling property, EPS concentration, cake layer formation and TMP change at different operating periods.

Not surprisingly, the flux profiles of the MLE/MBR and IFMBR showed the same trend of flux decrease with time (Figure 8B). During the stage of cake layer formation (corresponding to a rapid TMP increase), the fluxes of the MLE/MBR and IFMBR were in the same range. Thereafter, the flux of the MLE/MBR was about 1.5 times lower than that of IFMBR (Figure 8B) most likely because the corresponding TMP of IFMBR was 1.5 times higher than that of MLE/MBR (Figure 8A). Data of the total membrane resistance, defined as the ratio of TMP to flux, was presented in Figure 8C. There was no statistically significant difference of the total membrane resistance between the two types of modified MBRs (ANOVA, $\alpha = 0.05$, p-value = 0.57). As the total resistance consists of intrinsic membrane resistance, reversible cake

resistance and irreversible fouling resistance (Chang et al. 2002), at the beginning of membrane operation the intrinsic membrane resistance was around 1.0E+13 m^{-1} for both the same type of membrane module. The total membrane resistance increased afterwards due to cake formation and biofilm fouling. The patterns of changes in total membrane resistance suggested that under similar operating conditions with the same type of membrane materials the fouling resistance variations were not substantially different between the MLE/MBR and IFMBR, although different stages of cake layer formation existed due to the different biomass characteristics. Action needs to be taken to maintain high membrane flux while minimizing the need for membrane fouling control.

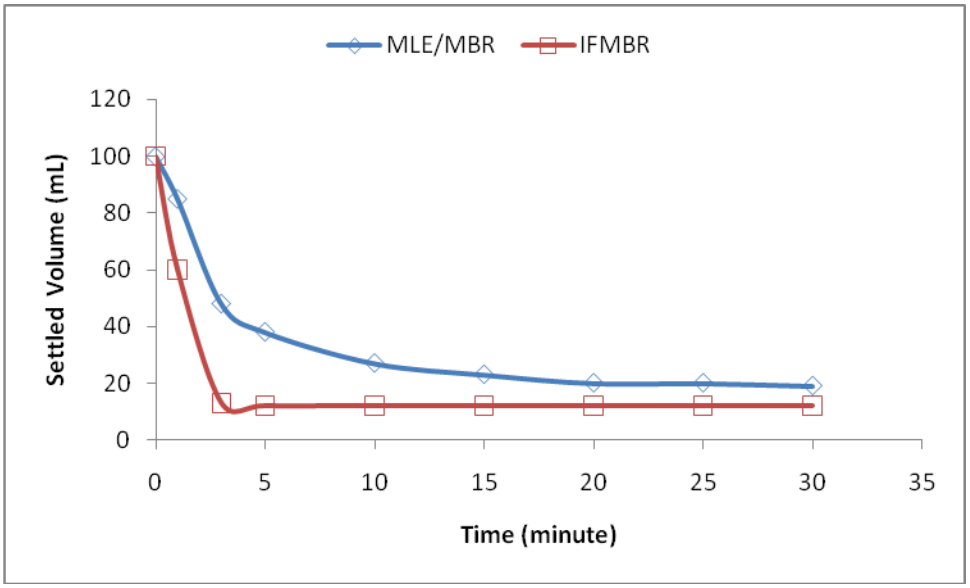
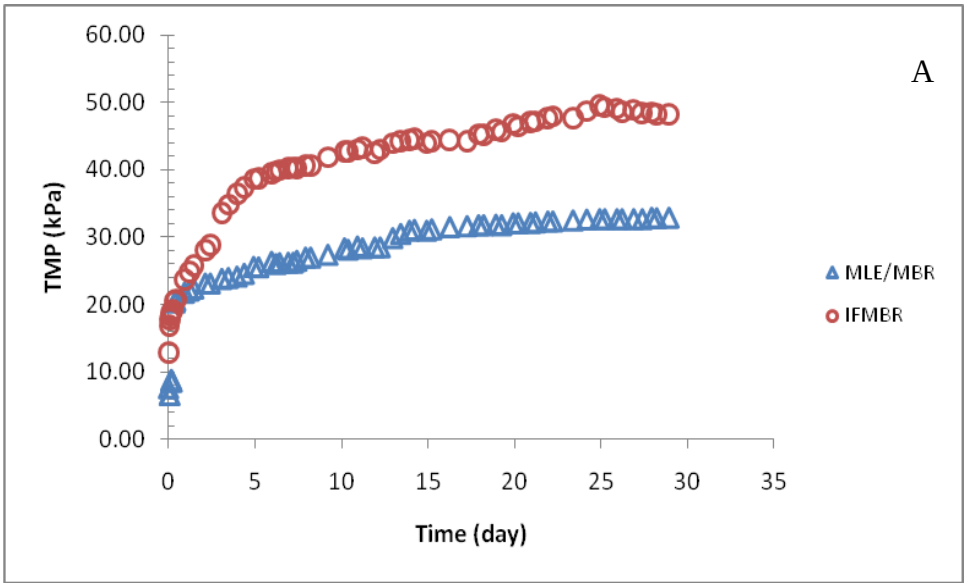


Figure 7. Sludge settling curves from the MLE/MBR and IFMBR systems.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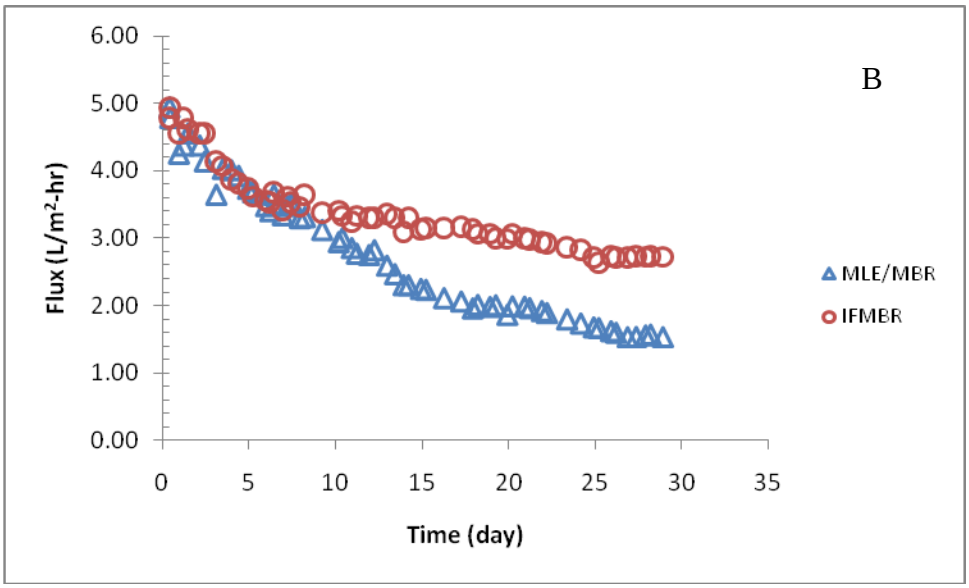
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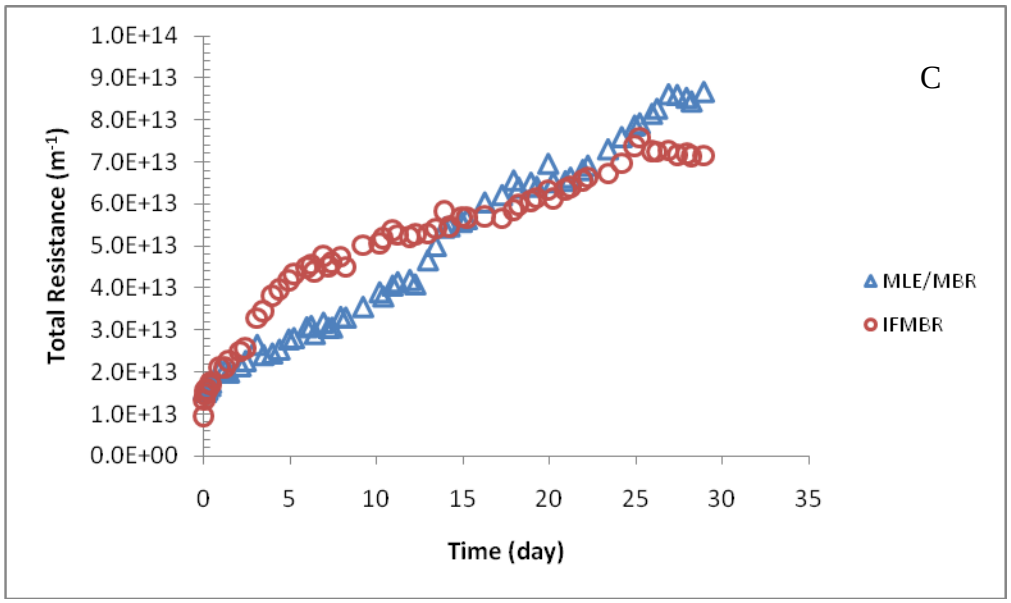


Figure 8. The change of transmembrane pressure (A), flux (B) and total membrane resistance (C) of the same type of membrane module in the MLE/MBR and IFMBR after membrane cleaning

CHAPTER 4

4. COMPARATIVE EVALUATION ON START-UP OF HIGH BIOMASS SUBMERGED MEMBRANE BIOREACTORS

The rising trend of converting conventional activated sludge processes to MBR processes for water reuse requires studying the start-up performance of bioreactors, during which mixed liquid suspended solids concentrations increase significantly. One conventional activated sludge MBR (CAS-MBR) and one modified Luzack–Ettinger (MLE) type MBR (MLE-MBR) with mixed liquor recirculation were evaluated for their performance on organic and nutrient removal, membrane fouling, biomass characteristics and bacterial activities during the start-up period. The two bench-scale MBRs each had an identical reactor volume (7.2 L) and were operated under continuous flow conditions with no sludge wasting during the start-up operation. It took about 130 days for the MBR biomass concentrations to increase from the initial 2500 mg biomass COD/L to a final concentration of 13000 mg/L with net specific biomass growth rates of 0.0125 day^{-1} and 0.0127 day^{-1} for the CAS-MBR and MLE-MBR, respectively. The total nitrogen removal efficiency of the MLE-MBR was 73%, higher than that of the CAS-MBR (44%) while both MBRs had excellent organic removal (> 99%) soon after the start-up operation. Because of alternating anoxic/aerobic operations through mixed liquor recirculation, the biomass of the MLE -MBR exhibited higher heterotrophic and autotrophic bacterial activities and better sludge settling than that of the CAS-MBR. Furthermore, the MLE-MBR experienced less membrane fouling than the conventional MBR. Results of the start-up performance suggest that alternating anoxic/aerobic MBR operations improve wastewater nutrient removal, increase bacterial activities, and reduce membrane fouling.

4.1 Objectives

Currently, most efforts of optimizing operating conditions are dedicated to investigating the MBR performance under the steady state conditions. Little work has been done on the start-up performance in MBRs. There is a big information gap of MBR performance between the start-up operation when the sludge concentrations are low (1500-3000 mg/L) and the steady state operation when the sludge concentrations are maintained high (e.g., > 12000 mg/L) (Ng and Hermanowicz 2005). It is therefore important to investigate the start-up performance in MBRs especially when converting current activated sludge systems to MBRs is necessary. The objective of this study was to evaluate and compare two types of submerged MBR configurations (conventional activated sludge MBR, CAS-MBR and modified Ludzack-Ettinger (MLE) type MBR, MLE-MBR) during the start-up process when the sludge concentrations gradually increased from initial 2000 mg/L to 12000 mg biomass COD/L. We hypothesized that metabolic selection via alternating anoxic/aerobic processes (Grady et al., 1999) in the MBR systems could result in improved nutrient removal, higher bacterial activities, and less membrane fouling. Careful attention was paid to biological nutrient removal efficiency, heterotrophic and autotrophic bacterial activities, sludge settling property, and membrane fouling in the MBRs during the start-up period.

4.2 High sludge concentration submerged MBR

4.2.1 SRT effect on MBR performance

The conventional activated sludge (CAS) process is the most used municipal wastewater treatment technique worldwide. With the increase of water demand and more stringent regulatory requirements, more and more activated sludge wastewater treatment plants are upgrading their facilities to improve treatment capacity for water reuse. However, due to the limited available space and the upper limit for volumetric organic loading rate in existing activated sludge systems, it is very challenging for the current WWTPs to meet the requirements. The membrane bioreactor technology is the most cost-effective and sustainable solution due to its advantages over the CAS process including a smaller footprint (without the need for clarifiers) and a higher applicable organic loading rate, accompanied by higher biomass concentration (Falk et al. 2009, Fenu et al. 2010, Meng et al. 2009, Verrecht et al. 2010, Yeon et al. 2009).

As the value of the MBR process receives more and more recognition and acceptance, there have been many research efforts devoted to bioreactor operating conditions and biomass characterization to achieve best performance (Delai Sun et al. 2007, Meng et al. 2009, Rosenberger et al. 2002, Tan and Ng 2008). These include, but are not limited to, solids retention time (SRT) (Ersu et al. 2010, Kimura et al. 2009, Laera et al. 2007), recirculation ratio (An et al. 2009, Bekir Ersu et al. 2008, Ersu et al. 2008, Tan and Ng 2008), sludge property characterization (Hwang et al. 2008, Liang et al. 2010, Okamura et al. 2010), powdered activated

carbon addition (Remy et al. 2009), and membrane fouling control (Jiang et al. 2010, Jin et al. 2010, Menniti and Morgenroth 2010, Zhang et al. 2010).

Solids retention time is the key design factor in activated sludge systems including MBRs. Although optimal operating conditions such as SRTs have yet to be clearly defined in MBRs, the membrane system can be operated at a much higher SRT than in the CAS system without affecting the biodegradation capacity (Pollice et al. 2008). While the specific maximum bacterial growth rates generally decreased as the SRTs increased, this did not affect organic degradation performance or effluent quality with the help of high biomass concentrations (Pollice et al. 2008). High SRTs also result in reduced sludge production in the MBRs. For instance, at a prolonged SRT of 300 days, the observed sludge yield and endogenous decay rate in the MBRs were 0.115 g VSS/gCOD and 0.024 day^{-1}, respectively, half the reported lower values in the traditional CAS systems (Teck et al., 2009). Furthermore, since the concentration of soluble microbial products (SMP) that affect membrane fouling generally decreased with increasing SRT (Meng et al. 2009), it appears preferable to run MBRs at relatively high SRTs (e.g., SRT = 50 d) to control SMP concentration and improve organic and nutrient removal (Ersu et al. 2010).

4.2.2 Effect of recirculation on MBR performance and sludge properties

Internal recirculation of mixed liquor from aerobic zone to anoxic zone is an essential step for high performance of biological nutrient removal in MBR systems (Ersu et al. 2008). Increasing recirculation ratio up to 3 resulted in the maximum total nitrogen removal efficiency at an aeration rate of 10 L air/min with effective membrane fouling control. Further denitrification with the increase of recirculation ratio was prohibited by organic carbon availability and the

presence of dissolved oxygen (DO) in the mixed liquor. A lower aeration rate of 2.5 L air/min could improve total nitrogen removal efficiency at the expense of accelerated membrane fouling (Tan and Ng 2008).

In a study of the relationship between sludge properties and membrane fouling in MBRs, the dewaterability and filterability of sludge in MBRs were evaluated by the measurements of specific resistance to filtration and sludge volume index (SVI) (Khongnakorn et al. 2007). The results indicated that the dewaterability of sludge in the MBR was comparable to that in the CAS system. The capillary suction time of sludge in the MBR correlated with the amount of SMP and associated filamentous bacteria was found to be a good indicator of membrane fouling potential (Pan et al. 2010). Within the SMP, the fraction of utilization associated products (UAP), due to their higher percentage of low molecular weight molecules, presented the highest specific cake resistance and appeared to have the highest fouling potential (Jiang et al. 2010).

4.3 Materials and Methods

Two bench scale submerged membrane bioreactors equipped with same type ZeeWeed hollow fiber membrane module from GE Water & Process Technologies were set up. The membrane modules had a nominal pore size of 0.1 μm and a total effective surface area of 0.047 m^2. Both membrane reactors had same total reactor volume (7.2 L) except different reactor configurations in that MLE/MBR reactor had a baffle and a recirculation flow (Figure 9). The MLE/MBR was divided into anoxic and aerobic chambers by a glass baffles with recycle flow of mixed liquor from aerobic chamber to anoxic chamber. The effective volumes of the anoxic and aerobic chambers were 1.8 and 5.4 liters, respectively. The other MBR was conventional completed mixed type MBR without recirculation (CAS-MBR). The MBRs were fed with synthetic

municipal wastewater by a continuously running pump at a constant rate. Each MBR's total volume was maintained at a constant volume by two upper and lower level sensors for control of water level in MBR tanks in which the upper level sensor will start the permeate pump and lower level sensor will turn off the permeate pump. The suction peristaltic pump after membrane module acted as the permeate pump to produce relative vacuum pressure for driving force of permeate flow through membrane. One online digital pressure gauge measured the negative pressure as transmembrane pressure (TMP). The speed of permeate pump was set up at the permeate flux level and was greater than the continuously influent flow rate. So the permeate pump was intermittently turned on and off by upper and lower water level sensors to keep reactor volume constant. The air pump supplied compressed air flow to the built-in orifices at the bottom of each membrane module at constant flow rate of 8L/min to provide all necessary aerations and limit the membrane-fouling rate. The TMP and flux of membrane modules were closed monitored during the whole start-up period. When TMP increased dramatically in a short time and TMP level exceeded the predefined TMP value, the membrane module was taken out for physical clear water flush cleaning to resume the clean membrane module TMP.

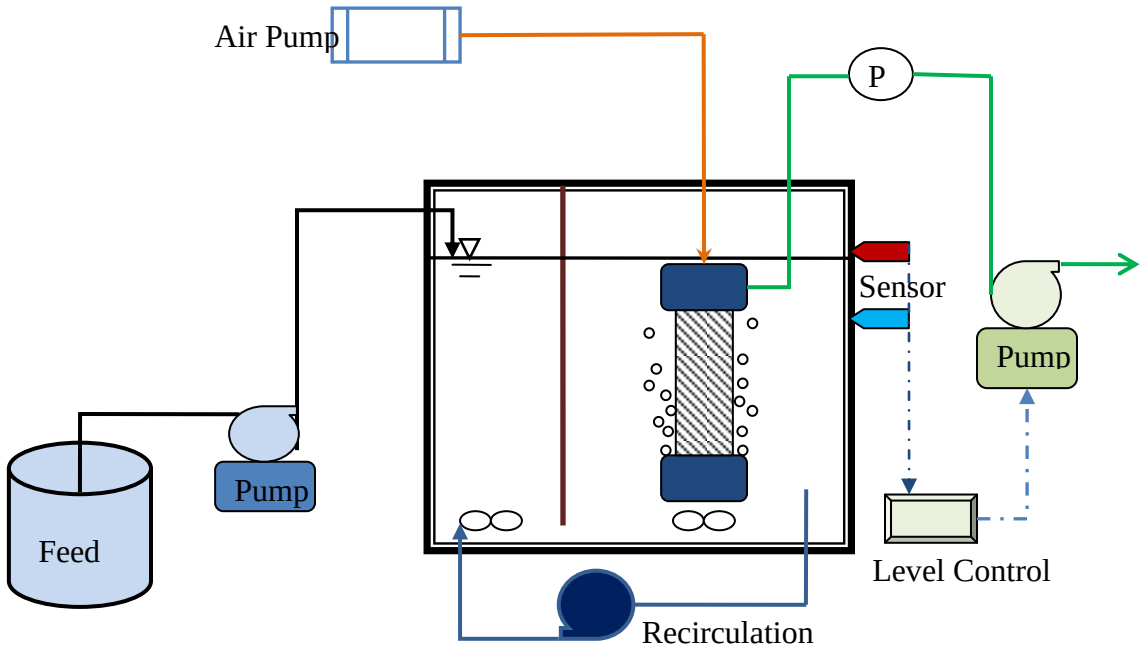


Figure 9. A schematic of a bench scale submerged MLE-MBR. P stands for a transmembrane pressure device.

The inoculation sludge of 8 L volume was taken from a local municipal wastewater treatment plant (Columbia, MO) and acclimated to the synthetic wastewater for one week before transferred to two MBR tanks. The starting mixed liquor suspended solids (MLSS) concentration level of both MBR reactors was 2000 mg/L. During the start-up period of MBR reactors there is no wasted activated sludge until the MLSS concentration level reaches more than 12000 mg/L. The fixed synthetic wastewater feeding rate made the target hydraulic retention time (HRT) of both MBRs as 1 day. The synthetic wastewater feed solutions for MBRs were mainly composed of nonfat dry milk powder as organic carbon source with a chemical oxygen demand (COD) concentration of 500 mg/L. The other major components of synthetic wastewater were 51.7 mg/L total nitrogen (TN), 30 mg/L NH_4^+-N and 6 mg/L total P. The micronutrient components in feed solution were as follows: 44 mg/L $MgSO_4$, 14 mg/L $CaCl_2 \cdot 2H_2O$, 2 mg/L $FeCl_2 \cdot 4H_2O$, 3.4 mg/L

$MnSO_4 \cdot H_2O$, 1.2 mg/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.8 mg/L $CuSO_4$, and 1.8 mg/L $Zn(NO_3)_2 \cdot 6H_2O$.

4.4 Results and Discussion

4.4.1 Organic matter removal in high biomass MBRs

The ANOVA analysis results indicated that there was no significant difference of effluent COD concentration between the CAS-MBR and MLE-MBR systems throughout the start-up period ($\alpha = 0.05$, $P = 0.34$). Figure 10 shows the effluent COD concentrations from the two types of MBRs when the influent COD concentrations were kept relatively constant at 548 ± 8 mg/L during the start-up period. The effluent COD concentrations in both the MBRs gradually decreased from the highest concentration of 26 mg/L at the beginning to lowest concentration on day 67, corresponding to average COD removal efficiencies of 95% and 99.5%, respectively. In the meantime, the biomass concentrations in the MBRs increased from initial 2500 to about 6000 mg COD /L. Before day 67, the average effluent COD concentrations were 13.2 ± 6.7 mg/L for the CAS-MBR and 9.9 ± 7.6 mg/L for the MLE-MBR reactors. As the biomass concentration continued to increase after day 67, the effluent COD concentrations CAS-MBR and MLE-MBR were reduced to 3.6 ± 2.9 mg/L and 2.8 ± 2.6 mg/L, respectively.

The effluent COD concentrations generally decreased as the biomass concentration increased in the MBRs. This is consistent with the popular concept that effluent COD concentration decreases as the SRT increases (Grady et al. 1999). Unlike the unified model predicting that effluent COD is sensitive to the influent COD but less sensitive to SRT (Kiser et al. 2010), this dissertation's research indicated that soluble microbial products (SMP), which could significantly contribute to effluent COD, might not be released as much as predicted (Kiser et al. 2010) or could be mostly

degraded at higher biomass concentrations (Masse et al. 2006) (Figure 10). Although SMP formation was not the main objective in this study, further study is needed to determine the fraction of different components in SMP at various SRTs or biomass concentrations and make correlations and predictions.

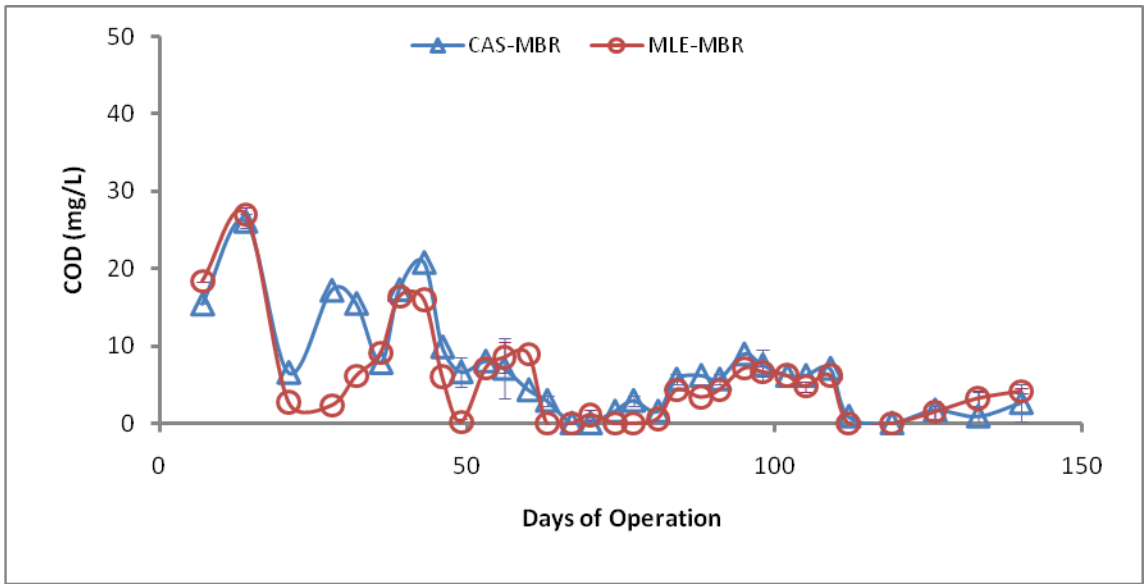
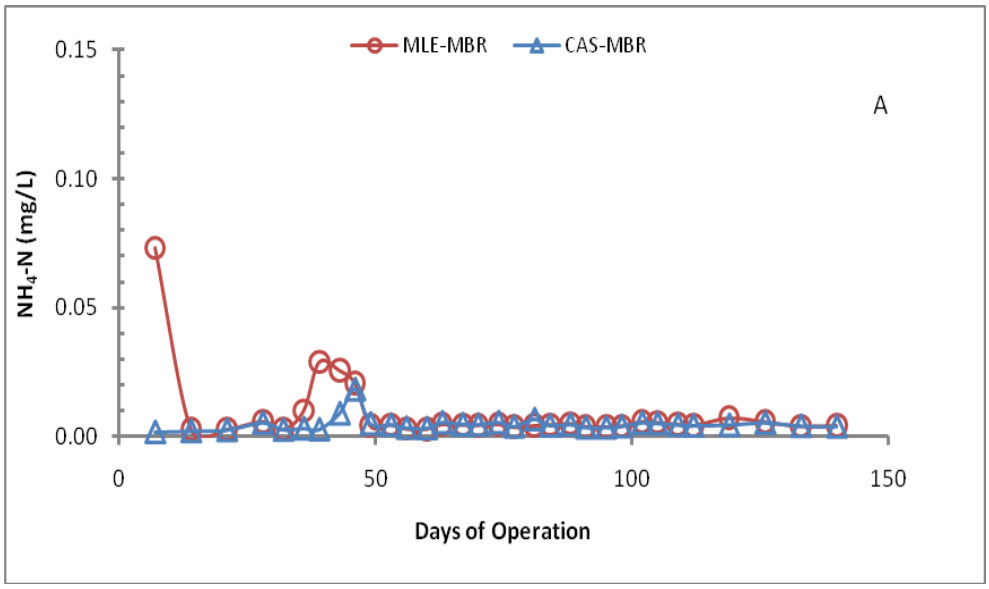


Figure 10. Effluent COD concentrations of the MBRs during the start up period. Error bars represent the range of the data points.

4.4.2 Nitrogen removal in high biomass MBRs

Both the CAS-MBR and MLE-MBR systems exhibited excellent NH_4^+-N removal rates of more than 99.9% with the highest effluent NH_4^+-N concentration observed at 0.073 mg/L. At day 50, the effluent NH_4^+-N concentrations were close to the detection limit in both the systems (Figure 11A), indicating excellent nitrification as biomass concentration continued to increase. The effluent NH_4^+-N concentrations in the MLE-MBR system appeared to be higher than that of CAS-MBR system at the beginning of the MBR operation. This was consistent and reflected the observation of effluent NO_2^--N accumulation at the concentration of 3.5 mg/L (Figure 11C).

During the first 50 days of operation, the average effluent NO_2^--N concnetration was 0.99 mg/L NO_2^--N in the MLE-MBR, higher than that of 0.16 mg/L NO_2^--N in CAS-MBR system (Figure 11C), suggesting the potential minor impact on effluent water quality by using the MLE process during the transision period. While there were a few spikes of nitrite after day 50 due to mixing or other operating issues, both the CAS-MBR and MLE-MBR displayed essentially identical average concentrations of NH_4^+-N and NO_2^--N with ANOVA P-values of 0.11, 0.22, respectively. On the other hand, the effluent nitrate profiles were quite different between the MBRs as described in Figure 11.



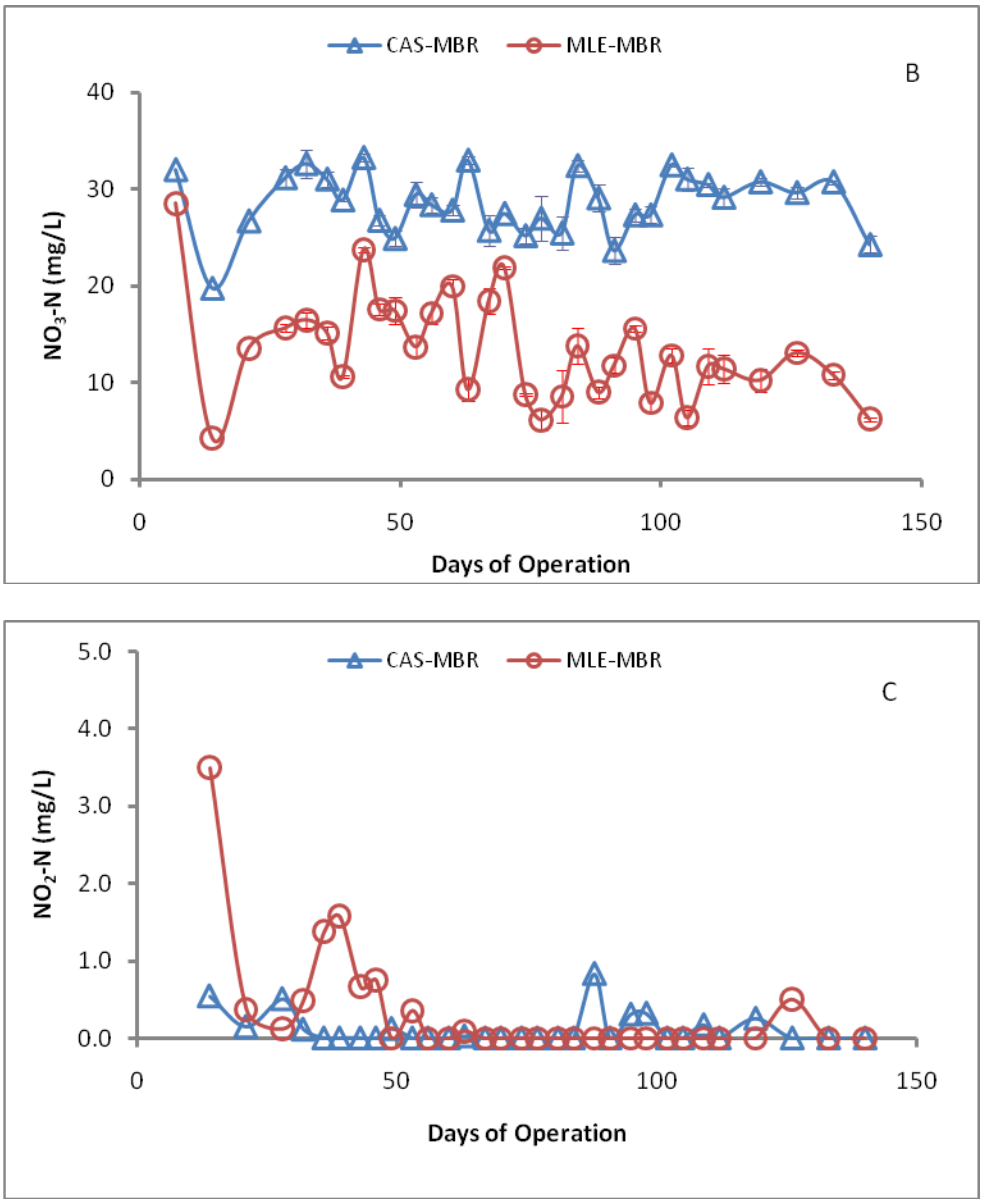


Figure 11. Effluent NH4+-N (A), NO3--N (B), NO2--N (C) concentrations of the MBRs during the startup period. Error bars represent the range of the data points.

Both MBRs started at the same nitrate concnetration of 30 mg/L at the beginning of the start-up. As biomass concentrations continued to increase, the effluent nitrate concentrations in the MLE-MBR were lower than in the CAS-MBR due to recirculation of mixed liquor from the aerobic chamber to anoxic chamber in the MLE-MBR (Figure 11B). Furthermore, if we divided the

whole start-up period into two half-periods, the effluent average NO_3^--N concentration from days 70 to 140 in the MLE-MBR was 10.3 ± 2.8 mg/L, lower than 16.5 ± 5.7 mg/L at the first half period before day 70. The substantial lower nitrate level in the second half start-up period was possibly due to a more favorable anoxic pre-denitrification condition developed with recirculation of mixed liquor as the biomass concentrations continued to increase. It was observed that the average DO concentrations in the anoxic chamber were 1.1 mg/L and 0.35 mg/L before and after days 70, respectively. Without considering the concentration of residual organic nitrogen in effluent, the average total nitrogen removal efficiency in the MLE-MBR was 73% compared to the influent total N concentration. For comparison, the average total nitrogen removal efficiency in the CAS-MBR was only 44%.

4.4.3. Biomass concentration changes over time during the start-up period

The biomass concentrations in both the MBRs were around 2500 mg COD/L at the beginning of MBR operation. With continuous feeding of organic matter at an average COD concentration of 548 ± 8 mg/L and no sludge wasting from both the MBRs, the biomass concentration in the MBRs gradually increased. It took 130 days for the biomass concentrations in the MBRs to reach a level of around 12000 mg COD/L. Paired comparison analysis of biomass concentrations in the CAS-MBR and MLE-MBR reactors indicated that there was no significant difference between

biomass concentration in the CAS-MBR and MLE-MBR (ANOVA, $\alpha = 0.05$, $P = 0.38$).

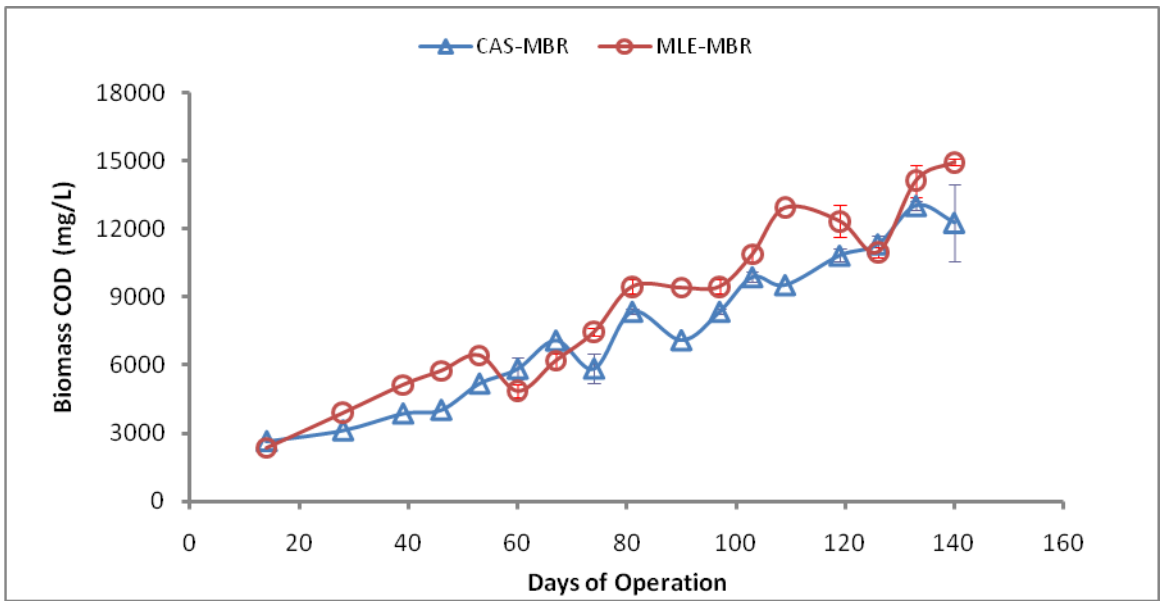


Figure 12. Biomass profiles of the two types of MBRs during the start-up period. No biomass was wasted until day 120.

The average measured observed biomass yield coefficients in the CAS-MBR and MLE-MBR were 0.09 ± 0.02 gVSS/gCOD and 0.12 ± 0.02 gVSS/gCOD, respectively. There was no significant difference of the observed yield coefficient between the CAS-MBR and MLE-MBR (P=0.22). The observed biomass yield coefficients in the MBRs were in the same range of reported values in the literature (e.g., Y_{max} = 0.115 gVSS/gCOD) (Delai Sun et al. 2007).

With no sludge wasting from the MBRs except withdrawing 100 mL of mixed liquor for bacterial activity measurements, an exponential biomass growth model based on mass balance and the assumption of completely mixing conditions in the MBRs were established. The model equation fitted well with the biomass concentration data with R-square values of 0.95 and 0.91 for the CAS-MBR and MLE-MBR, respectively. The best-fitted exponential model equations

predicted the net specific biomass growth rates in the CAS-MBR and MLE-MBR were 0.0125 day^{-1} and 0.0127 day^{-1}, respectively.

4.4.4 Bacterial activity in high biomass MBRs

The heterotrophic bacterial activities inferred from biomass SOUR were 6.1 ± 2.2 and 20.8 ± 2.7 mg O_2/gVSS-hr for the CAS-MBR and MLE-MBR, respectively (Figure 13A). The autotrophic SOURs in the CAS-MBR and MLE-MBR were 7.1 ± 0.8 mg O_2/gVSS-hr and 9.4 ± 1.1 mg O_2/gVSS-hr, respectively (Figure 13B). The measured microbial activities in both the CAS-MBR and MLE-MBR were consistent with data reported in the literature (Pollice et al. 2008). In general, the specific bacterial growth rates decreased as the SRTs increased (Pollice et al. 2008). This was validated from our recent study of similar MBR reactors run at lower SRTs (= 20 d) where the bacterial activities were 120 % to 230 % higher than the ones measured in this study (Liang et al. 2010). Furthermore, the statistical analysis indicated that the heterotrophic bacterial activities were higher in the MLE-MBR than that in the CAS-MBR (ANOVA, $\alpha = 0.05$, $P < 0.0001$).

The autotrophic activities were also statistically higher in the MLE-MBR (ANOVA, $\alpha = 0.05$, $P < 0.005$). The results of higher microbial activities in the MLE-MBR than in the CAS-MBR were consistent with the results under shorter SRT conditions (Liang et al. 2010), indicating that metabolic selection via alternating anoxic/aerobic processes (Grady et al. 1999) in the MBR systems could result in improved nutrient removal and higher bacterial activities.

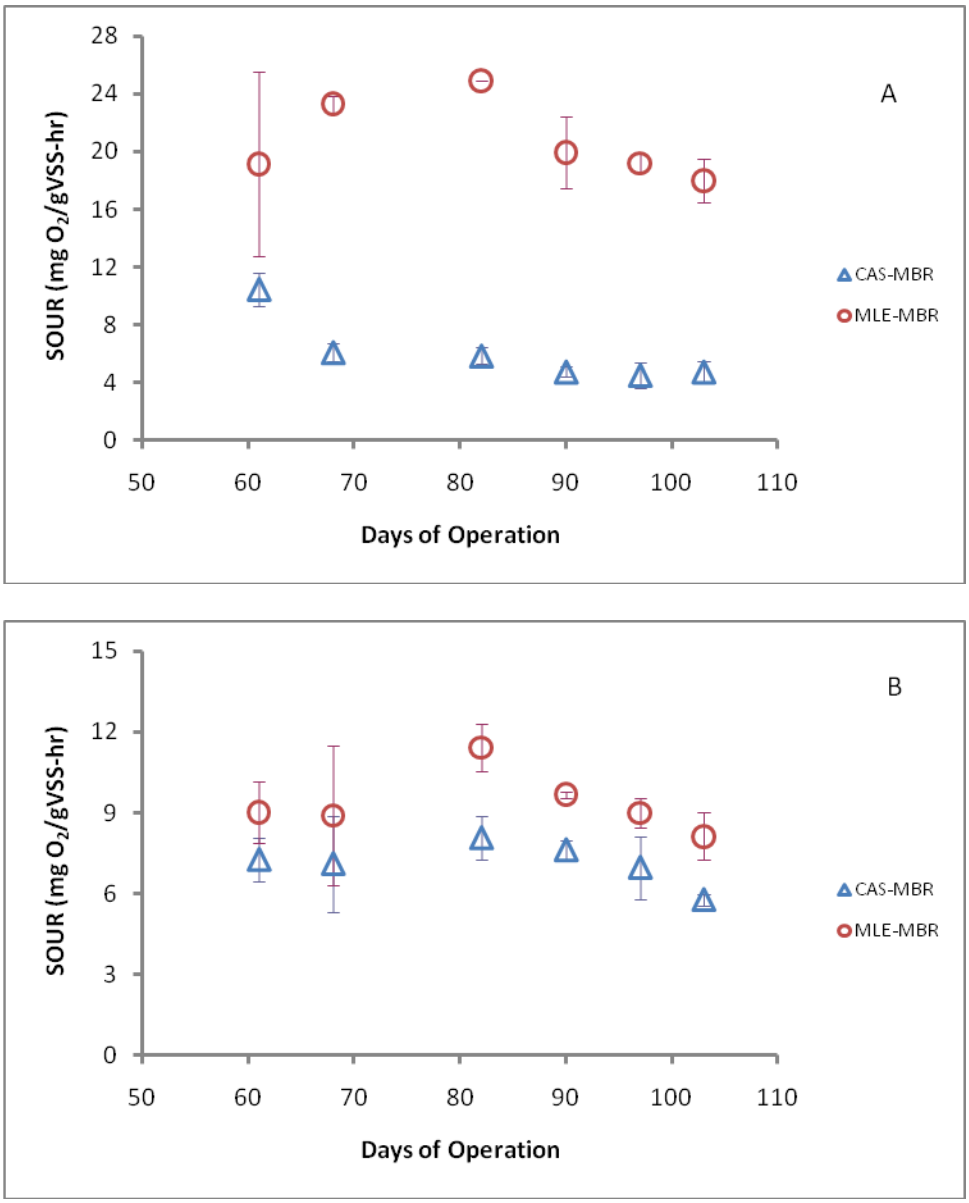


Figure 13. Heterotrophic (A) and autotrophic (B) bacterial activities inferred from the SOUR data in the MBRs during the start-up period.

4.4.5 Membrane fouling trends between high biomass MBRs

Figure 14A presents the profiles of membrane flux from the CAS-MBR and MLE-MBR under the constant flux operating conditions during the start-up period. At the beginning the membrane flux from the CAS-MBR was almost double the value of the MLE-MBR. This was manually corrected thereafter so that the flux was maintained relatively constant between the MBRs

throughout the study. Correspondingly, the TMP profiles of the CAS-MBR and MLE-MBR developed differently (Figure 14B). The TMP in the CAS-MBR decreased very slowly while the TMP in the MLE-MBR experienced rapid rise in short time. The rising TMP trend in the MLE-MBR at the begining was reversed at day 6 and gradually decreased with the help of continuous coarse air bubble scouring . Such trends could also be due to the phenomena that the longer pump-off cycle time under the higer water flux conditions reduced the cake layer formation and thus mimimized membrane fouling.

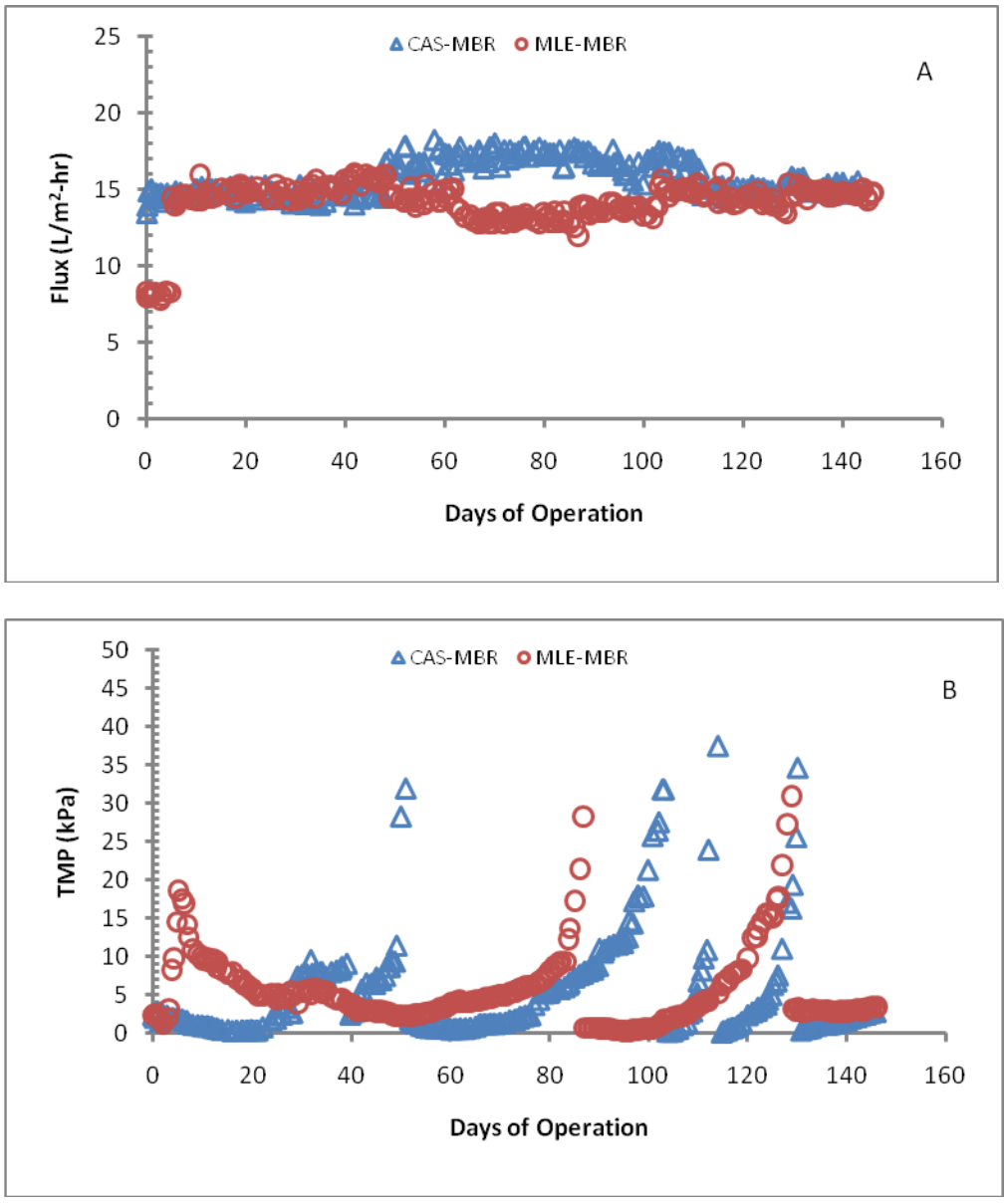


Figure 14. Permeate flux (A) and transmembrane pressure (B) profiles of the MBRs during the start-up period.

Afterwards, the shapes of the TMP profiles in the CAS-MBR and MLE-MBR were generally similar, each ending with a TMP jump before the membrane module was taken out of the tank for physical cleaning. The observed thick biomass layers built onto the membrane module surface indicated that the TMP jump was related to the formation of cake layer biofilm structure

(Zhang et al. 2006). However, the time interval to reach the TMP jump or between each membrane cleaning operation was different between the MBRs. It took 87 days for the MLE-MBR to be cleaned the first time while the cleaning had to be taken after day 52 for the CAS-MBR. The longer time interval of physical membrane cleaning in the MLE-MBR than in the CAS-MBR might be related to lower SMP release from the MLE-MBR (Barker and Stuckey 1999). On the other hand, higher biomass SVI values in the CAS-MBR with potentially higher fractions of filamentous bacteria could result in higher bound extracellular polymetric substances (EPS) release which in turn cause higher TMP (Meng et al. 2006). During the start-up period, the CAS-MBR membrane module experienced four times the number of TMP jumps compared to the only two cleaning events necessary in the MLE-MBR. As the biomass concentration increased, the accumulation of irreversible membrane fouling materials such as SMP could not be removed by physical cleaning. Therefore, the time interval between the two TMP jumps in the CAS-MBR and MLE-MBR became shorter, as was confirmed in a previous study (Trussell et al. 2007).

4.4.6 Sludge settling property of the high biomass MBRs

After 30 days of operating, the sludge settling property inferred from the SVI measurements was recorded on a regular basis. At the beginning, the SVI in the MLE-MBR was higher than in the CAS-MBR. However, after day 50, the average SVI values in the MLE-MBR and CAS-MBR were 102 ± 14 and 175 ± 17 mL/g, respectively. Increasing SRTs in conventional MBR could result in sludge having high SVI values and high viscosity with poor settling property (Teck et al. 2009). The relatively high SVI value in the CAS-MBR was consistent with observations in a previous study of conventional MBR (Ng and Hermanowicz 2005), suggesting that the metabolic

selection via alternating anoxic/aerobic processes (Grady et al. 1999) in the MBR systems could result in improved sludge settling.

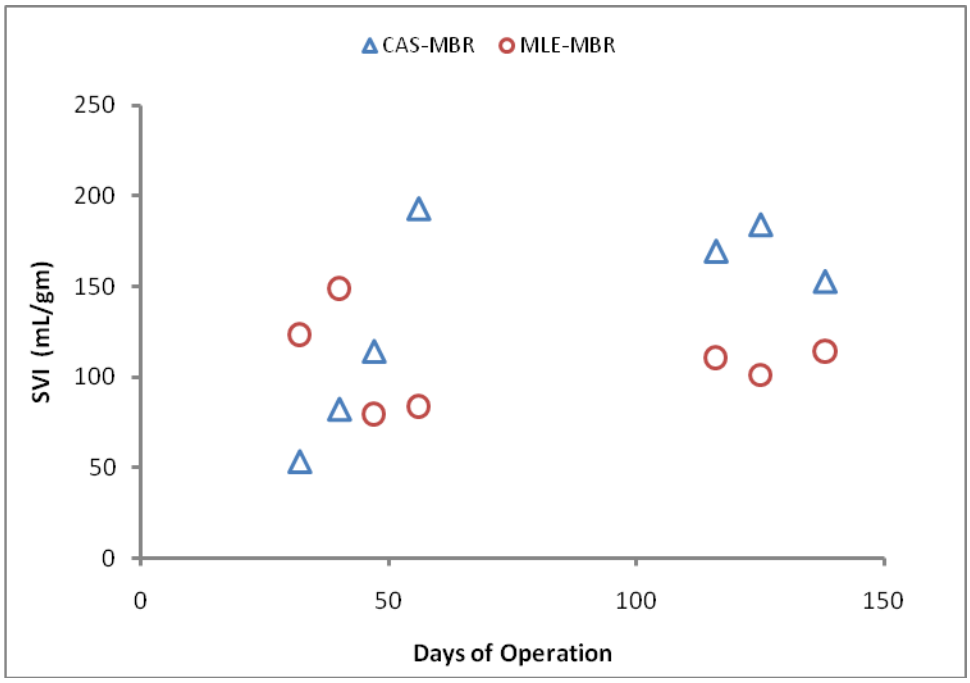


Figure 15. Sludge volume index of the MBRs during the start-up period.

CHAPTER 5

5. BIODEGRADATION OF NITROPHENOL COMPOUNDS AND THE MEMBRANE FOULING TRENDS IN TWO TYPES OF MEMBRANE BIOREACTORS

Phenolic compounds are commonly used in industry and discharged into water. The objectives of this study were to:

1) evaluate the ability of submerged membrane bioreactors (MBRs) to simultaneously remove nitrophenol compounds containing 2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol and inorganic nitrogen from wastewater and
2) determine the membrane fouling trends in two types of MBR configurations: CAS-MBR (conventional activated sludge in a continuous stirred tank reactor) and MLE-MBR (modified Ludzack Ettinger).

The mixed liquor suspended solids (MLSS) concentrations in the MBRs were maintained between 8200 and 9900 mg L^{-1} in the MBRs. Synthetic wastewater containing four nitrophenol compounds was continuously dosed in the MBRs at influent concentrations of 0.05 mM each. The average nitrophenol removal efficiencies ranged from 87% to 94% by the MBRs while the total inorganic nitrogen removal efficiencies were 52% and 75% for CAS-MBR and MLE-MBR, respectively. The metabolic uncoupling by nitrophenols inferred from the adenosine triphosphate (ATP) assays demonstrated the lower ATP yield of 0.062 mg ATP/g VSS and bound EPS level of 20.5 mg/g VSS of the biomass from the CAS-MBR compared to those from the MLE-MBR. These results suggest that MBRs with high activated sludge concentrations are effective in removing nitrophenols and inorganic nitrogen simultaneously. The metabolic uncoupling effect and lower bound EPS level of the biomass from the CAS-MBR indicatethat

the CAS-MBR configuration is superior to the MLE-MBR with better membrane fouling control and potentially increased resistance to shock loads of industrial wastewate containing nitrophenols.

5.1 Introduction

Nitrophenol compounds are used as intermediates for the production of pharmaceuticals, pesticides, dyestuffs, pigments, rubber chemicals and lumber preservatives (Uberoi and Bhattacharya 1997). Among the nitrophenol compounds, the annual productions of 4-nitrophenol and 2-nitrophenol are 41 million and 15 million pounds, respectively. Furthermore, 3-nitrophenol and 2,4-dinitrophenol have an approximate annual production level of 1 million pounds (US-EPA 1980). Because of their solubility and stability in water, they are often present in industrial wastewater. Mononitrophenols can also enter into water through atmospheric droplets by photochemical reactions and into soil and groundwater from hydrolysis of pesticides and herbicides (Kahru et al. 2002, Labana et al. 2005, Morville et al. 2006). Because of their potential carcinogenic risk, the US Environmental Protection Agency (US EPA) has listed 2-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol on the "Priority Pollutants List" (EPA 2009).

To remove nitrophenols from industrial wastewater, physical-chemical treatment methods including solvent extraction and activated carbon adsorption have been applied (Nevskaia et al. 2004, Nouri and Haghseresht 2004, Pan et al. 2007, Yu et al. 2010). These removal approaches transfer the nitrophenols from liquid phase to solid phase or from a dilute to concentrated phase. They do not convert nitrophenol compounds into harmless substances. Although advanced chemical oxidation processes can degrade the nitrophenol compounds, the transformation is

often incomplete and could produce intermediates that have similar toxic characteristics to the parent chemicals. Furthermore, the expense of advanced chemical oxidation processes impedes their broad applications. As an alternative approach, biological degradation can accomplish complete mineralization of nitrophenol compounds with relative low capital and operating costs (Grady 1990, Sarfaraz et al. 2004).

Among the nitrophenol compounds, 4-nitrophenol has received extensive attention because it has the highest demand and production (Bhatti et al. 2002, Liu et al. 2007, Tomei et al. 2008). Extensive studies on 4-nitrophenol biodegradation with sequencing batch reactor (SBR) have been conducted due to the compound's dynamic operating conditions and optimal biodegradation environments (Martin-Hernandez et al. 2009, Tomei and Annesini 2005, 2008, Tomei et al. 2004, Tomei et al. 2003, Tomei et al. 2008, Yi et al. 2006). In a recent study, the previously acclimated biomass was used in a SBR with integrated aerobic-anoxic cycling to remove 4-nitrophenol which was fed to the reactor as the sole carbon source. With complete denitrification developed three weeks after the start-up procedure, the average effluent 4-nitrophenol concentration was less than 1 mg/L and the maximum specific removal rate of 4-nitrophenol was 6.99 mg 4NP/mgVSS/day (Tomei and Annesini 2005). In another SBR study on 4-nitrophenol biodegradation, the aerobic granules were developed to improve sludge settle ability. Although a lower maximum specific removal rate was observed at 0.876 mg 4NP/mg VSS/day, the high volumetric 4-nitrophenol removal rate of 256 mg 4NP/L/h was achieved at a 4NP loading rate of 0.6 kg/m^3/day due to the high biomass content in aerobic granules (Yi et al. 2006). Complete 4-nitrophenol removal was achieved in an aerobic SBR for treatment of high-strength 4-nitrophenol wastewater. Depending on 4-nitrophenol feeding strategy in the SBR

reactor, K-strategist microorganisms (which were capable of surviving long periods on limited substrate) demonstrated a higher substrate inhibition constant (Martin-Hernandez et al. 2009), indicating more adaptability to 4-nitrophenol inhibition.

Previous studies on biodegradation kinetics of 4-nitrophenol removal by SBR were mostly described with the Haldane kinetics model which proved suitable for describing kinetics of phenolic compound biodegradation (Brown et al. 1990, Rozich and Gaudy Jr 1985). In both kinetics study of 4-nitrophenol biodegradation with or without biogenic substrate, the concentration profiles show the trends of substrate inhibition kinetics with the removal rate increasing as the substrate concentration decreased. The maximum specific removal rates calculated from the Haldane model with 4-nitrophenol as the sole carbon source or with the presence of a biogenic source indicated no significant differences (Tomei et al. 2003). In the study of SBR filling time effect and biomass concentration on 4-nitrophenol biodegradation kinetics, it was observed that longer aerated feeds will enhance the biodegradation while reducing the toxic effect of nitrophenol compounds on biomass activity (Tomei et al. 2004).

Nitrophenols are believed to be effective chemical uncouplers, which dissociate the anabolism from catabolism. In the presence of a chemical uncoupler, the energy generation from substrate consumption is more excessive than the ATP synthesis due to the inhibited anabolism caused by uncouplers. The excessive energy generated dissipated by wasting the oxidative phosphorylation driving force (Low et al. 2000, Ye et al. 2003). Among the nitrophenols, 4-nitrophenol and 2,4-dinitrophenol were found to be the most effective uncouplers in uncoupled metabolism and resulted in the most effective biomass yield reduction in activated sludge systems (Ye et al.

2003). Furthermore, 2-nitrophenol and 3-nitrophenol also significantly inhibit ATP generations in cynobacterium, while green algae culture and the inhibitory effect increased with increasing nitrophenol concentration (Umamaheswari and Venkateswarlu 2004). Furthermore, 2,4-dinitrophenol inhibited denitrification with a 50% denitrification inhibition threshold value of 0.5 mM (Walter 1978).

Membrane bioreactor (MBR) technologies have become increasing widespread applications in municipal and industrial wastewater treatment. The average annual market growth rate of 10.9% for MBR was significantly faster than other wastewater treatment technologies such as SBR or biological aerated filters (BAF) (Judd 2008). The applications of MBR technology are predicted to double every seven years. Compared to SBR, MBR has several advantages: (1) MBRs require much smaller footprint than SBRs because the mixed liquor suspended solids (MLSS) concentration in the MBRs are several folds higher than that in the SBRs (Ben Aim and Semmens 2003, Chu et al. 2008, Huang et al. 2001); (2) MBRs have a superior effluent water quality than SBRs because solids separation in the SBRs is dependent on gravity settling while MBRs use membrane for solids separation (Hirani et al. 2010, Krauth and Staab 1993, Le-Clech 2010, Pollice et al. 2008, Rosenberger et al. 2002); (3) Because MBR can operate under much longer SRT, the system can substantially reduce sludge production more than with the SBR system (Judd 2008, Le-Clech 2010, Teck et al. 2009).

However, membrane fouling caused by high MLSS concentration is a major concern in MBR applications. The extracellular polymeric substances (EPS), produced by activated sludge, play an important role in cake layer formation on membrane surface and in biofouling (Ramesh et al.

2007). Generally, the specific cake layer resistance increased as bound EPS level increased (Ahmed et al. 2007). While bound EPS was fractionized into loosely bound EPS and tightly bound EPS, it was the loosely bound EPS that caused the fouling problem in MBRs (Ramesh et al. 2006). The loosely bound EPS was found to correlate with the performance of flocculation and sedimentation process as well as the dewaterbility of activated sludge (Li and Yang 2007). Many research efforts have been focused on the operation parameters of MBR such as SRT, HRT, organic loading rate, and aeration intensity to control bound EPS in MBRs (Ahmed et al. 2007, Hong et al. 2007, Ji and Zhou 2006, Menniti and Morgenroth 2010). However, very few studies have investigated the bound EPS level in the presence of chemical uncouplers such as nitrophenols in the MBRs.

Also, little is known about the biodegradation of nitrophenol compounds mixed in the MBR systems. The objectives of this thesis study were to evaluate the biodegradation of mixed nitrophenol compounds in modified Ludzack-Ettinger (MLE) type and continuous stirred tank reactor (CSTR) type membrane bioreactors. Because MLE type MBR is popular in wastewater treatment with potential nutrient removal, while CSTRs are more resistant to toxic shock loading, it is necessary to assess and compare the system performance between the two types of MBRs. Furthermore, the metabolic uncoupling effect of nitrophenols on membrane fouling control was also investigated. Previous studies on metabolic uncoupling in the activated sludge process indicate that nitrophenols have the potential to control biofouling in MBRs (Liu 2000, Low et al. 2000).

5.2 Materials and Methods

5.2.1. Membrane bioreactors and operation

Two lab-scale submerged MBR systems were operated in parallel during the study. Each was equipped with a submerged ZeeWeed hollow fiber membrane module (GE Water & Process Technologies, Trevose, PA). The membrane module was made of polyvinylidene fluoride (PVDF) with a nominal pore size of 0.1 μm and a total effective filtration area of 0.047 m^2. For MLE-MBR, the system was composed of the sequential anoxic and aerobic chambers separated by a glass baffle, which provided alternative anoxic and aerobic conditions. There was a recirculation at a flow rate equal to the influent flow from aerobic chamber to anoxic chamber. The CAS-MBR was a completed mixed aerobic reactor where the membrane module was immersed. The total reactor volume for both MBRs was 7.2 L while the effective volumes of the anoxic and aerobic chambers in the MLE-MBR were 1.8 and 5.4 liters, respectively.

The MBRs were fed continuously with a synthetic wastewater at a target hydraulic retention time (HRT) of 1 d. The wastewater was mainly composed of nonfat dry milk powder as an organic carbon source with a chemical oxygen demand (COD) concentration of about 500 mg/L. Other major components of the synthetic wastewater included 51.7 mg/L total nitrogen (TN), 30 mg/L NH_4^+-N, and 6 mg/L total P. The micronutrients in the feed solution contained the following: 44 mg/L $MgSO_4$, 14 mg/L $CaCl_2 \cdot 2H_2O$, 2 mg/L $FeCl_2 \cdot 4H_2O$, 3.4 mg/L $MnSO_4 \cdot H_2O$, 1.2 mg/L $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.8 mg/L $CuSO_4$, and 1.8 mg/L $Zn(NO_3)_2 \cdot 6H_2O$.

The mixed liquor volume in each MBR was maintained relatively constant by the upper and lower water level sensors (Cole-Palmer, Vernon Hills, Illinois) in the MBR. The volume

difference between the upper and lower water level was less than 5% of the total mixed liquor volume in the MBR. An air pump supplied compressed air to the built-in orifices at the bottom of each membrane module at a constant air flow rate of 8 L/min to support aerobic biodegradation and control membrane fouling. The mixed liquor suspended solids (MLSS) concentration in both MBRs was maintained between 8200 and 9900 mg/L. During this study period, aliquots of sludge were wasted daily to maintain relatively constant SRTs of about 110 days in both the MBRs.

For each MBR system, a nitrophenol mixture containing 2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol was separately dosed at a final concentration of 50 µM each in the influent. The mixed nitrophenol feed solution was combined with synthetic wastewater flow at the entrance of the influent channels located at the aerobic CSTR chamber and anoxic chamber for the CAS-MBR and MLE-MBR, respectively. Before nitrophenol loading, the CAS-MBR and MLE-MBR systems had already operated for about 300 days and steady-state conditions were assumed based on organic matters and total nitrogen water quality data.

5.2.2. Membrane fouling monitoring

The transmembrane pressure (TMP) and permeate flux of each membrane module were closed monitored throughout the study period. The membrane flux was maintained relatively constant by adjusting the speed of the permeate pump. When the TMP increased dramatically in a short period of time and the TMP level exceeded the predefined TMP value of 45.5 kPa, the membrane module was taken out from the MBR for physical cleaning by rinsing with distilled water for 0.5 h before it was submerged in the mixed liquor in the MBR.

5.2.3. Adenosine 5'-triphosphate measurement

Prior to ATP assays, aliquots (5 mL) of MLSS taken from the MBR, were centrifuged at 4000 × g for 5 minutes. The supernatant from each sample was discarded and 5 mL 2% Trichloroacetic acid (Metcalf and Eddy) reagent was immediately added to the remaining biosolids in a 15 mL tube. The sample was mixed well by vortex before it was incubated with continuous mixing at room temperature for 20 minutes. After centrifuging for 10 minutes, 0.25 mL supernatant was mixed with 0.5 mL 0.1 M Tris-Acetate-EDTA (TAE) buffer and the pH of the suspension was adjusted to 7.8 by 1 M sodium hydroxide solution before ATP assays.

When ATP is the limiting component in the luciferase reaction, the intensity of the emitted luminance is proportional to ATP concentration. Hence, ATP was determined by a luciferin-luciferase bioluminescence method with the EnzyLight bioluminescent ATP Assay Kit (BioAssay Systems, Hayward, CA, USA) and a microreader (VICTOR^3, PerkinElmer, Shelton, USA). Briefly, an aliquot (100 µl) of the sample was added to each well in a 96-well plate and

then a 90 µL reconstituted reagent was also added to each well. The reconstituted reagent is a mixture containing 95 µL assay buffer, 1 µL substrate and 1 µL ATP enzyme according to the manufacturer's manual. A series of ATP standards prepared by dilution were also mixed with 90 µL reconstituted reagent to build an ATP standard curve by plotting the intensity of the emitted light with ATP concentration. After gently mixing, samples and standards in the 96-well plate were incubated for 10 minutes at room temperature. The sample luminescence light emission (in Counts Per Second, CPS) was recorded every 5–10 seconds by a microreader (VICTOR^3, PerkinElmer, Shelton, USA). The ATP content measurement was carried out in triplicate.

5.2.4. EPS measurement

Bound EPS measurements were performed according to previous studies (Hwang et al. 2007, Jorand et al. 1994, Zhang et al. 1998) with minor modification. Aliquots (50 mL) of MLSS from the MBRs were centrifuged at 8200 × g for 5 minutes to separate the soluble EPS from bound EPS (Teck et al. 2009). The supernatant was discarded and pellets resuspended in a 50 mL solution containing 8.5% sodium chloride and 0.22% formaldehyde. The suspension was held in an ice bath and sonicated at a power output of 40 W for 5 minutes. After centrifugation at 20000 × g and 4 ^{o}C for 30 minutes, the EPS concentration in the supernatant was analyzed for total polysaccharides and proteins, while the sum of the total polysaccharides and proteins was reported as the bound EPS. Polysaccharides were determined by the phenol-sulfuric acid method with glucose used as a standard (Dubois et al. 1956). Proteins were quantified by a modified micro pyrogallol red method using a total protein assay kit (Sigma-Aldrich) containing standards of human serum albumin in saline with 0.1% sodium azide.

5.2.5. Biomass microbial activities

The autotrophic and heterotrophic activities of activated sludge in the two MBRs during the study period were determined by batch extant respirometry by specific oxygen uptake rate (SOUR) measurement due to ammonia oxidation and acetate oxidation, respectively (Hu et al. 2002). The brief procedure was as follows: After aliquots (60 mL) of the sludge from the MBRs were aerated with pure oxygen for three minutes, they were transferred to the respirometric bottles, which were tightly capped after insertion of a DO probe with no headspace. At a predetermined time of 500 to 600 seconds, an aliquot of substrate (10 mg N/L NH_4^+-N or 20 mg/L COD in acetate) was injected with a 10 µL glass syringe. The decrease of the DO concentration in the respirometric bottle due to substrate oxidation was measured by a DO monitor (YSI model 5300A, Yellow Springs, OH) and continuously recorded at 4 Hz by LabView software interfaced with a computer. The specific oxygen uptake rates were calculated based on a linear regression analysis because a zero-order reaction was observed for a long period of time. Each SOUR experiment was carried out in duplicate as a minimum requirement for the procedure.

5.2.6. Nitrophenol measurement and other chemical analysis

The 2-nitrophenol, 3-nitrophenol and 4-nitrophenol used in this study were 99% analytical reagents (Acros Organics) and 2,4-dinitrophenol was 99% analytical reagents (Sigma-Aldrich). The concentrations of nitrophenol compounds were determined by the high-performance liquid chromatographic system (Shimadzu, LC-2010A, Japan) equipped with a UV-VIS detector and an automatic sample injection device with a loop volume of 10 µL. All nitrophenol compounds were detected at a wavelength of 254 nm. A reverse-phase chromatographic column (150 × 4.6

mm, Epic C18, ES Industries, NJ, USA) packed with C18 sorbent (particle size = 5 μm) was run at a flow rate of 1.0 mL/min for chemical separation. The mobile phase consisted of 10 mM sodium 1-octanesulfonate (99%, Alfa Aesar) and 10 mM citric acid of high-purity grade, while and acetonitrile (HPLC grade, Fisher Scientific) served as a buffer. The ratio of mobile phase to acetonitrile buffer was 60:40. Before HPLC analysis, the nitrophenol samples were filtered through the 0.45 μm pore size syringe nylon membrane filters. All nitrophenol samples were measured in triplicate.

The influent and effluent (permeate) water quality parameters such as NH_4^+-N, NO_3^--N, NO_2^--N, COD in the MBRs were measured in duplicate according to Standard Methods (APHA 1998).

5.3 Results and Discussion

5.3.1 Nitrophenol compound removal in the MBRs

Figure 16 and Figure 17 show the nitrophenol concentration profiles in the effluents of CAS-MBR and MLE-MBR At the same influent concentrations of 2-nitrophenol, 3-nitrophenol, 4-nitrophenol (7.0 mg/L each) and 2,4-dinitrophenol at 9.0 mg/L, the effluent nitrophenol concentration profiles in both MBRs appeared to have the similar trends. At the beginning of continuous loading of nitrophenolic compounds, the effluent 2-NP concentrations were always low at around 1.0 mg/L. However, the concentrations of 3-NP, 4-NP and 2,4-DNP in both MBRs increased exponentially (The average R-square values of fitted exponential model were 0.99 and 0.98 for CAS-MBR and MLE-MBR). On the fifth day the concentrations of 4-NP reached

peaks of 6.0 mg/L and 5.0 mg/L in the CAS-MBR and MLE-MBR, respectively. On the eighth day, the concentrations of 3-NP and 2,4-DNP approached the peak concentrations of 4.9 mg/L and 6.0 mg/L, respectively in the CAS-MBR, while these peak concentrations in the MLE-MBR were 5.0 mg/L and 5.2 mg/L, respectively. The peak concentrations of 3-NP, 4-NP and 2,4-DNP were lower than the cooresponding influent concentrations and suggested incomplete degradation of nitrophenolic compounds by the unacclimated sludge in the MBRs and pososible adsorption of these compounds to the sludge. The unacclimated sludge in both MBRs were able to rapidly develop the biodegradation pathway to remove 2-NP at the very beginning. Furthermore, the peak concentrations of 4-NP and 2,4-DNP from the MLE-MBR were around 1.0 mg/L lower ($p < 0.03$) than those from the CAS-MBR which indicated the advantage of plug flow and potentially increased bacterial activities (Liang et al. 2010) in the MLE-MBR.

From day 15 onward, the average effluent concentraions of 2-NP, 3-NP, 4-NP, and 2,4-NP were 0.63 ±0.29 mg/L, 0.35 ±0.10 mg/L, 0.77 ±0.32 mg/Lmg/L, and 0.68 ±12 mg/L in the CAS-MBR. For comparison, the average effluent concentraions of 2-NP, 3-NP, 4-NP, and 2,4-NP were 0.49 ±0.30 mg/L, 0.38 ±0.06 mg/L, 0.83 ±0.28 mg/Lmg/L, and 0.63 ±0.10 mg/L in the MLE-MBR. Statistical analysis indicated that there was no significant difference of the average effluent nitrophenol concentrations between the MBRs (p value = 0.856). This profile demonstrated the good nitrophenol removal efficiencies of both MBRs. The average nitrophenol removal efficiencies ranged from 87% to 94% by the MBRs. Specifically, the average removal efficiencies of 2-NP, 3-NP and 4-NP were 90%, 94%, 88%, respectively in CAS-MBR and 92%, 93%, 87%, respectively in MLE-MBR. The 2,4-DNP removal efficiency was 91% in the CAS-MBR and 92% in the MLE-MBR.

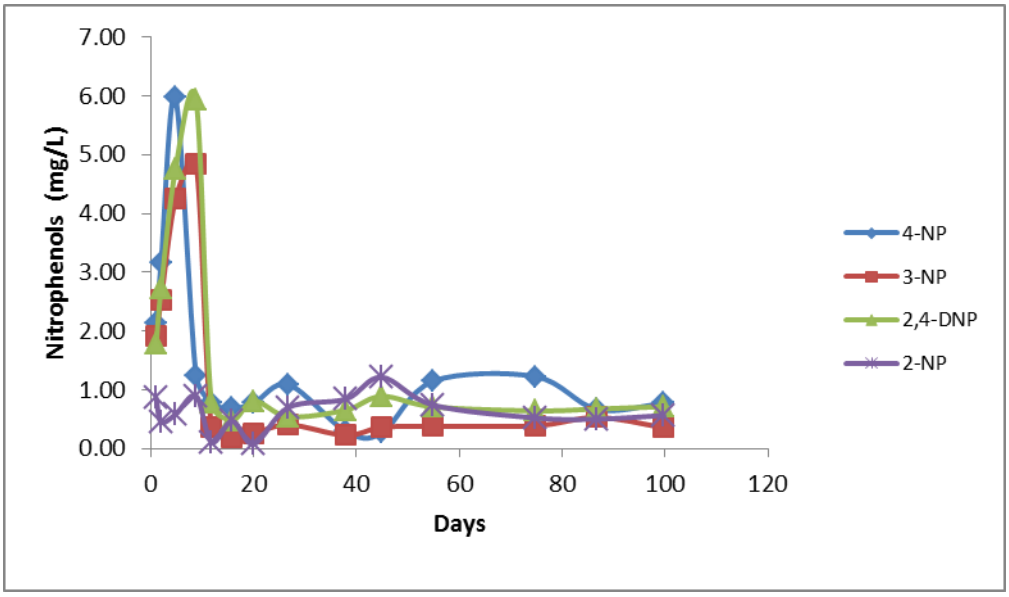


Figure 16. Effluent nitrophenol concentrations of CAS-MBR

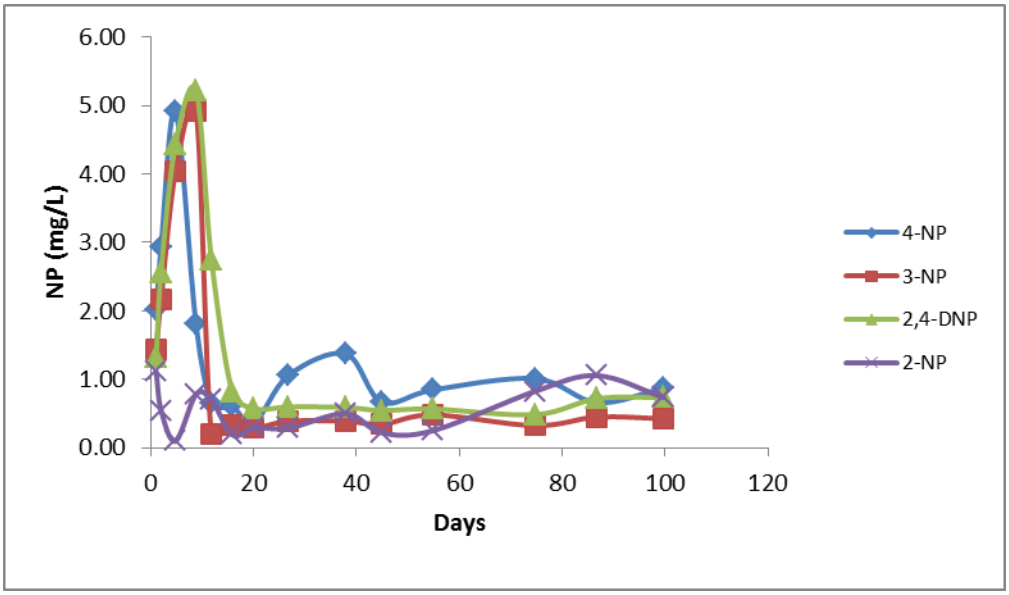


Figure 17. Effluent nitrophenol concentrations of MLE-MBR

5.3.2 Batch studies of nitrophenol biodegradation by biomass from the MBRs

To evaluate the effects of sludge acclimation on nitrophenol biodegradation, The biodegradation rates of nitrophenolic compounds by the biomass from the MBRs were investigated in a series of batch experiments. The initial concentrations of four nitrophenol compounds by the biomass from the CAS-MBR and MLE-MBR were 35 mg/L, 18 mg/L, 20 mg/L, and 18 mg/L for 2-NP, 3-NP, 4-NP, and 2,4-DNP, respectively. All nitrophenol degradation profiles followed the first-order reaction kinetics. For the boimass from the CAS-MBR and MLE-MBR without previous exposure to nitrophenols, the four nitrophenol compounds exhibited different biodegradation behaviors. Surprisingly, 3-NP showed maximum degradation rate by the biomass from the CAS-MBR and MLE-MBR, while the biodegradation of 2-NP, 4-NP, and 2,4-DNP were relatively slow (<40% nitrophenols removal after 24 hours). After two months of sludge acclimation to nitrophenols, the biodegradation rates of 3-NP, 4-NP, and 2,4-DNP were significantly increased. For instance, more than 95% of 3-NP was degraded by the biomass from the MLE-MBR after 24 hours of reaction. Batch studies indicated that biodegradation of nitrophenols by unacclimated sludge from the CAS-MBR follows a first-order reaction kinetics with k values of 0.48, 2.64, 0.17, and 2.20 d^{-1} for 2-NP, 3-NP, 4-NP, and 2,4-DNP, respectively. After two months of acclimation, the k values increased to 1.23, 9.61, 4.73, and 5.04 d^{-1} for 2-NP, 3-NP, 4-NP, and 2,4-DNP, respectively. For comparison, the k values of first-order degradation of nitrophenols by unacclimated sludge from the MLE-MBR were 0.56, 1.77, 0.46, and 0.29 d^{-1} for 2-NP, 3-NP, 4-NP, and 2,4-DNP, respectively. After two months of acclimation, the k values increased to 0.95, 7.95, 3.41, and 3.95 d^{-1} correspondingly.

As an exception the 2-NP biodegradation in biomass from the MLE-MBR after two months acclimation did not show a significant difference from that study of biomass without acclimation (p value = 0.084). With the least sum of error model analysis, the biodegradation rate constants of four nitrophenol compounds for biomass from MLE-MBR and CAS-MBR were calculated and shown in Figure 18 and Figure 19, respectively. The 3-NP showed maximum degradation rate constants before and after acclimation. After the acclimation the biodegradation rate constants for both MBRs demonstrated the same order of 3-NP > 2,4-DNP > 4-NP > 2-NP, while before acclimation, the biodegradation rate constants order for CAS-MBR was 3-NP > 2,4-DNP > 2-NP > 4-NP and that order for MLE-MBR was 3-NP > 2-NP > 4-NP > 2,4-DNP.

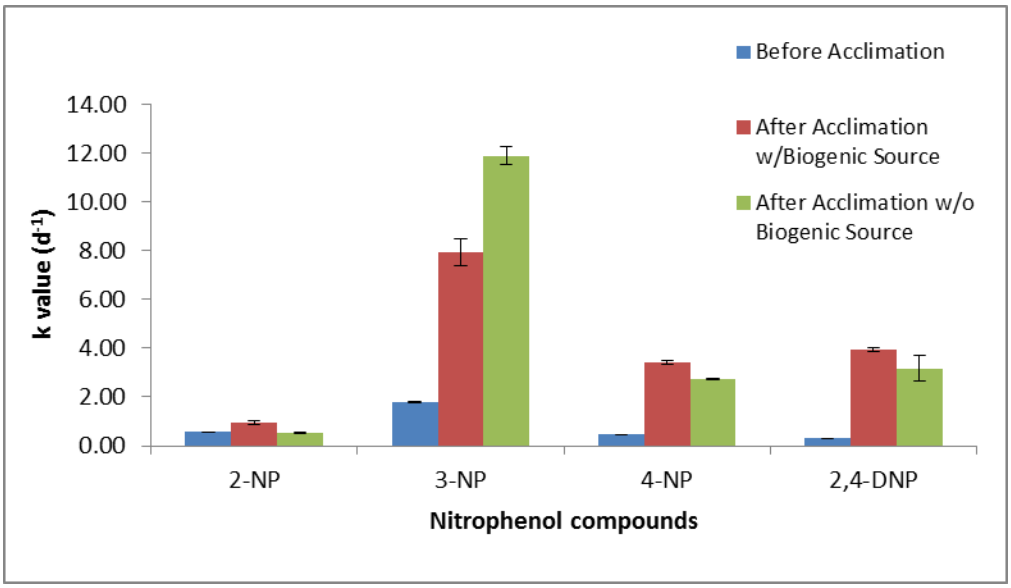


Figure 18. Rate constants of nitrophenol biodegradation batch study with biomass from MLE-MBR. Error bars represent the range of the data points.

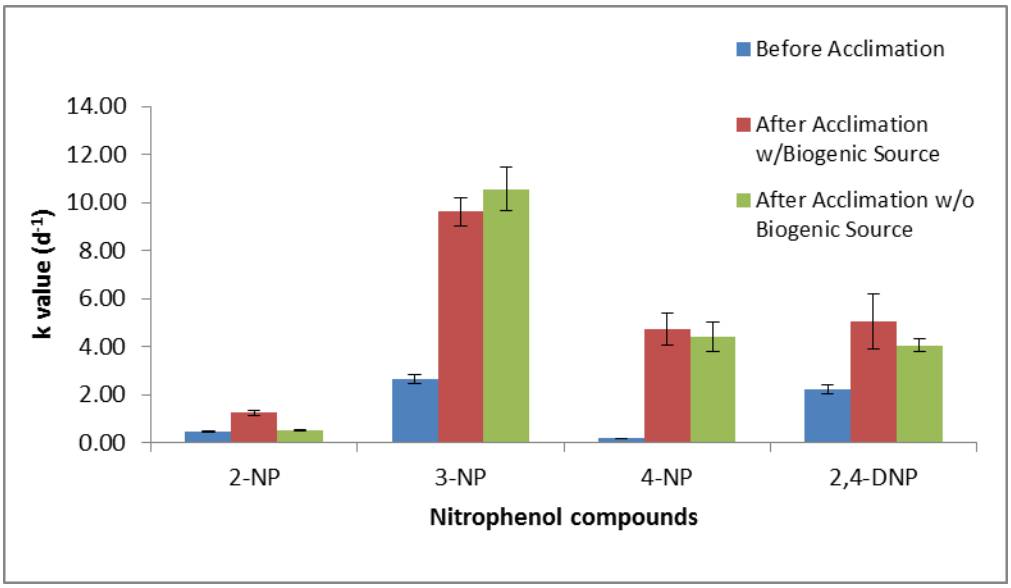


Figure 19. Rate constants of nitrophenol biodegradation batch study with biomass from CAS-MBR. Error bars represent the range of the data points.

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The ANOVA analysis results of biodegradation rate constants for four nitrophenol compounds before and after acclimation were listed in Table 3. All biodegradation rate constants were significantly increased after acclimation by at least two and half times except 2-NP biodegradation by the biomass from the MLE-MBR. Before acclimation the biomass from CAS-MBR and MLE-MBR demonstrated different biodegradation rate constants order in four nitrophenol compounds. This suggested that the difference of biomass characteristics of the CAS-MBR and MLE-MBR ressult in different responses of induced microbial selections in response to nitrophenol exposure. After two months of sludge acclimation, the same biodegradation rate constant order for four nitrophenol compounds indicate possibly similar development of metabolic pathways and transformation enzymes for both MBRs. The 3-NP had the highest degradation rate constant for both MBRs, suggesting that the special enzyme of 3-NP monooxygenase in the first step of biodegradation pathway may possibly have a much higher enzyme activity than that of 4-NP monooxygenase and 2-NP monooxygenase. The rate constant

of 4-NP was higher than that of 2-NP indicating that para aromatic ring position to the OH group was more easily attacked by the enzyme than the ortho position to the OH group. The fact that there was no significant difference of 2-NP biodegradation before and after acclimation in biomass from MLE-MBR suggested that anoxic/aerobic metabolic selection in the MLE-MBR made it easy for the 2-NP biodegradation pathway to develop with possible release of the consititute enzymes responsible for 2-NP degradation.

The effect of biogenic organic sources on nitrohenol biodegradations was also investigated. The biodegradation rate constants of biomass from CAS-MBR and MLE-MBR (with or without the presence of biogenic substrate) are shown in Figure 18 and Figure 19. The ANOVA analysis results of the effect of biogenic substrate source on nitrophenol biodegradation rate constants are shown in Table 4. For the CAS-MBR biomass, there was no significant difference in the biogenic substrate effect on biodegradation rate constants of 3-NP, 4-NP, and 2,4-DNP. On the contrary, the biodegradation rate constant of 2-NP which the presence of biogenic source had significantly increased the rate constant. For the MLE-MBR biomass, the presence of biogenic substrate sources had significant effect on boidegradation rate constants of 2-NP, 3-NP, and 4-NP but hadno significant effect on 2,4-DNP biodegradation. This result suggested that although after acclimation, both MBRs biomass developed similar biodegradation pathway and enzymes fornitrophenol degradation, the difference of biomass characteristics between CAS-MBR and MLE-MBR made different responses to the organic carbon sources. It is worth noting that the presence of biogenic substrate source significantly decreased the biodegradation rate constant of 3-NP in biomass of MLE-MBR while the biodegradation rate constant of 3-NP in biomass of CAS-MBR was also decreased but not significantly ($p = 0.209$). This means that 3-NP

biodegradation enzymes including 3-NP monooxygenase involve special enzymes and the presence of external biogenic source may compete out the co-metabolic enzyme sources for 3-NP biodegradation.

Table 3. ANOVA on biodegradation rate constants of CAS-MBR and MLE-MBR before and after biomass acclimation

	CAS-MBR				MLE-MBR			
	2-NP	3-NP	4-NP	2,4-DNP	2-NP	3-NP	4-NP	2,4-DNP
F-test	116.39	382.63	137.08	17.29	5.25	2318.7	10632.6	95.1
P-value	0.0004	4.028E-05	0.0003	0.014	0.084	1.11E-06	5.3E-08	0.0006
Difference ratio	0.388	0.275	0.036	0.437	1.076	0.149	0.169	0.091

Table 4. ANOVA on effect of biogenic source on biodegradation rate constants of CAS-MBR and MLE-MBR after biomass acclimation

	CAS-MBR				MLE-MBR			
	2-NP	3-NP	4-NP	2,4-DNP	2-NP	3-NP	4-NP	2,4-DNP
F-test	101.688	2.238	0.371	2.093	102.01	109.07	228.70	6.69
P-value	0.001	0.209	0.575	0.221	0.0005	0.0005	0.0001	0.0609
Difference ratio	2.353	0.910	1.073	1.248	1.842	0.669	1.244	1.244

Table 5. ANOVA on comparison of biodegradation rate constants of individual and mixture nitrophenol before biomass acclimation

	CAS-MBR				MLE-MBR			
	2-NP	3-NP	4-NP	2,4-DNP	2-NP	3-NP	4-NP	2,4-DNP
F-test	275.65	60.15	4.51	286.19	2493.2	292.3	1862.7	24148.3
P-value	7.71E-05	0.0015	0.1010	7.2E-05	9.6E-07	6.9E-05	1.7E-06	1.0E-08
Difference ratio	2.520	1.464	0.938	7.197	3.699	1.218	1.894	3.473

Figure 20 and Figure 21 show the comparison of biodegradation rate constants of four nitrophenol compounds in the batch studies of individual nitrophenol and mixed nitrophenols by the same biomass from the CAS-MBR and MLE-MBR before acclimation. The ANOVA analysis results in Table 5 show that the biodegradation rate constants were significantly decreased in the nitrophenol mixture compared to those in individual nitrophenol batch studies for all nitrophenol compounds except 4-NP biodegradation by the biomass of CAS-MBR. The request of different kinds of enzymes in the mixed nitrophenol batch study for four nitrophenol compounds showed that biodegradation will strive for the limited biomass resource. This competition for a limited biomass resource may cause the reduction of biodegradation rate in the mixed nitrophenol biodegradation compared to individual nitrophenol biodegradation.

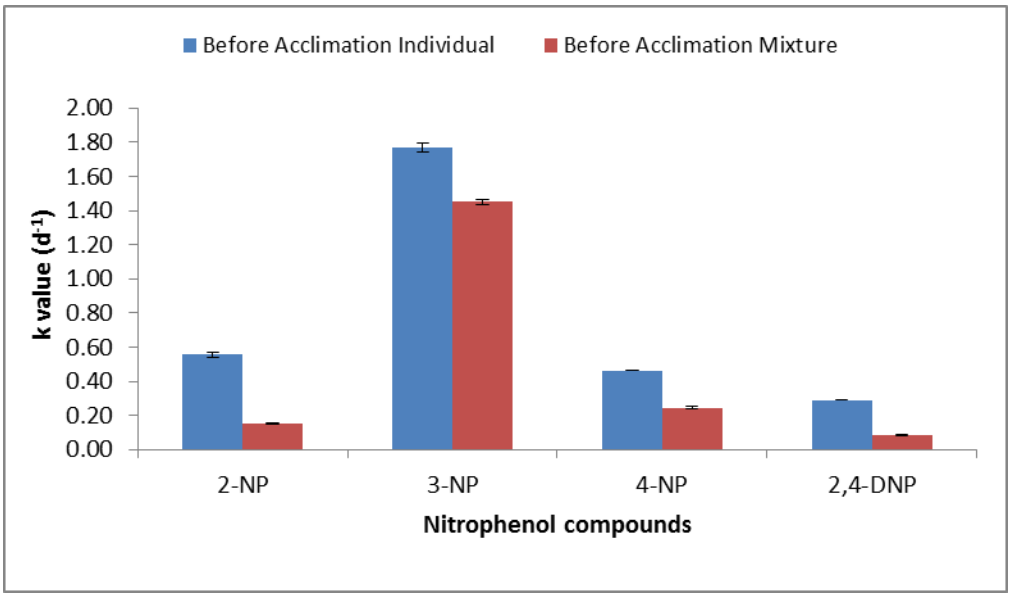

Figure 20. Comparison of rate constants of nitrophenol biodegradation batch study with biomass from MLE-MBR between individual and mixed nitrophenolic compounds. Error bars represent the range of the data points.

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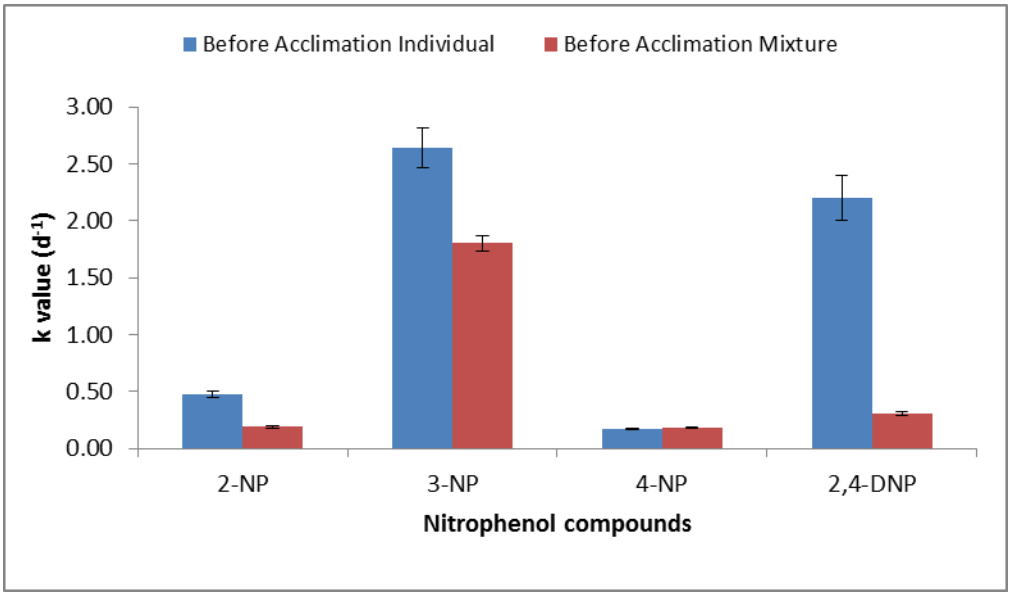

Figure 21. Comparison of rate constants of nitrophenol biodegradation batch study with biomass from CAS-MBR between individual and mixed nitrophenol compounds. Error bars represent the range of the data points.

5.3.3 Organic matter and nitrogen removals in the MBRs

Before nitrophenol loading, two MBRs were continuously operated for a long period. The MBRs have excellent COD removal efficiencies and the effluent COD concentrations were maintained at the level of 5.0 mg/L. After nitrophenol loading, the effluent COD concentrations reached peaks of 33.9 and 27.6 mg/L in the CAS-MBR and MLE-MBR, respectively. The difference between the peak COD concentration before and after nitrophenol loading indicated that at the beginning of the nitrophenol loading, the activated sludge in the MBRs were not able to develop nitrophenol biodegradation pathways and high residual nitrophenol concentrations in the effluent contributed to the peak COD concentration of MBRs effluent. The heterotrophic activities were not changed before and after nitrophenol loading (9.48 mg O_2/gVSS-hr and 9.51 mg O_2/gVSS-hr, respectively). With gradual acclimation and more specific nitrophenol degrading enzymes being developed, the effluent COD concentrations gradually decreased. In the mean time the

amount of intermediate of nitrophenol biodegradation was also gradually decreased (indicated from the HPLC profiles, data not shown). On day 70, the effluent COD concentrations of both MBRs were even lower than the effluent COD concentrations before nitrophenol loading.

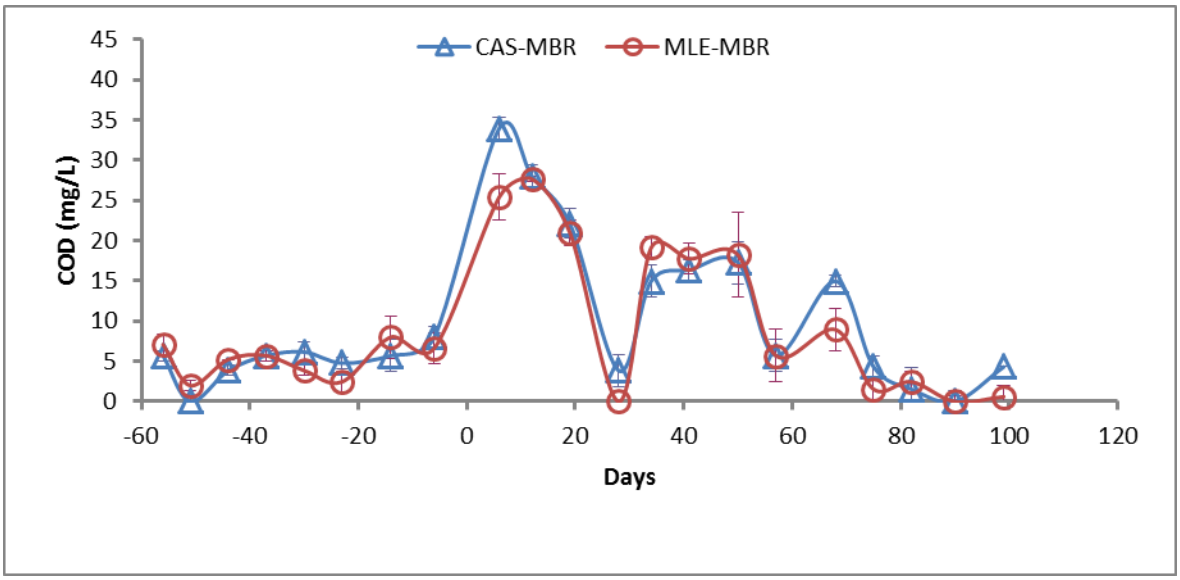

Figure 22. Effluent COD concentrations of CAS-MBR and MLE-MBR before and after nitrophenol loading. Error bars represent the range of the data points.

As shown in Figure 24, two MBRs achieved complete nitrification before nitrophenol loading. After nitrophenol loading, due to the toxic effect of nitrophenols, the nitrification processes in MBRs were inhibited resulting in significant increases of effleunt ammonia nitrogen concentrations. The autotrophic activities before and after nitrophenol loading were 4.88 mg O_2/gVSS-hr and 4.62 mg O_2/gVSS-hr, respectively. After day 15, the effluent ammonia nitrogen concentrations were all below the level of 1.0 mg/L, indicating nitrification processes were completely resumed. From day 20 onward, the effluent ammonia nitrogen concentrations of MBRs were below the detection limit (0.01 mg/L) as those presented before loading. At the beginning of nitrophenol loading when ammonia oxidation was inhibited in the MBRs, the effluent nitrate nitrogen concentrations dropped to below the detection limit (Figure 24). Shortly after nitrophenol loading, the effluent nitrite nitrogen concentrations exhibited peak

concentration. After the nitrificaiton was completely recovered, the nitrite nitrogen concentrations of MBRs were resumed to below detection limit to th same status as that before nitrophenol loading (Figure 25). Due to the high concentrations of biomass in the MBRs, both reactors resumed the complete nitrification from day 20 onward. The corresponding total inorganic nitrogen removal efficiencies for CAS-MBR and MLE-MBR after nitrification and denitrification recovery were 52% and 75%, respectively.

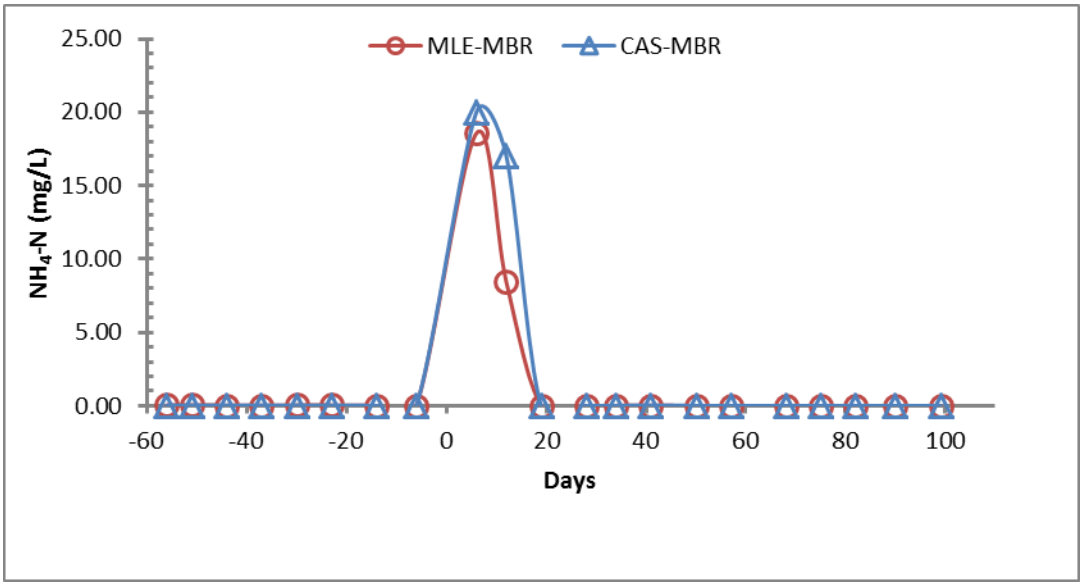

Figure 23. Effluent ammonium nitrogen concentrations of CAS-MBR and MLE-MBR before and after nitrophenol loading.

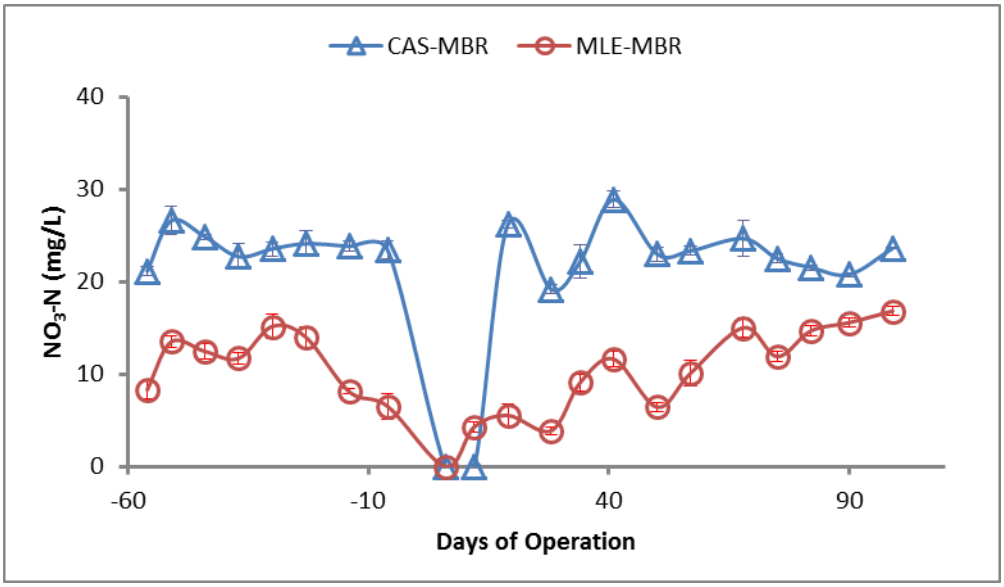

Figure 24. Effluent nitrate nitrogen concentrations of CAS-MBR and MLE-MBR before and after nitrophenol loading. Error bars represent the range of the data points.

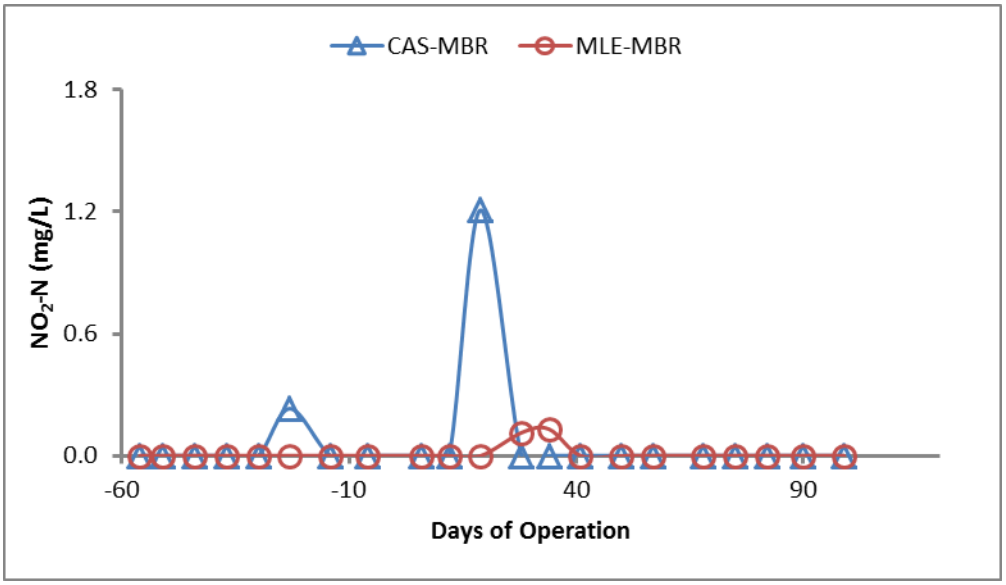

Figure 25. Effluent nitrite nitrogen concentrations of CAS-MBR and MLE-MBR before and after nitrophenol loading.

5.3.4 Biomass reduction after nitrophenol loading

With defined SRTs, the biomass concentrations in both MBRs were kept at relatively constant at 12000 mg/L biomass COD for the CAS-MBR and MLE-MBR, respectively, before nitrophenol

loading. After the continuous nitrophenol loading, the biomass concnetration in the CAS-MBR was gradually decreased while the biomass concentration in the MLE-MBR was maintained at the same level as at the beginning but started to decrease after day 34. The metabolic uncoupling effects of nitrophenols caused reduced biomass production and a lower sludge yield in activated sludge processes (Liu 2000, Low et al. 2000, Ye et al. 2003). The presence of anoxic zone in the MLE-MBR may dampen or postpone the metabolic uncoupling effect acting on the biomass of aerobic zone in the MLE-MBR.

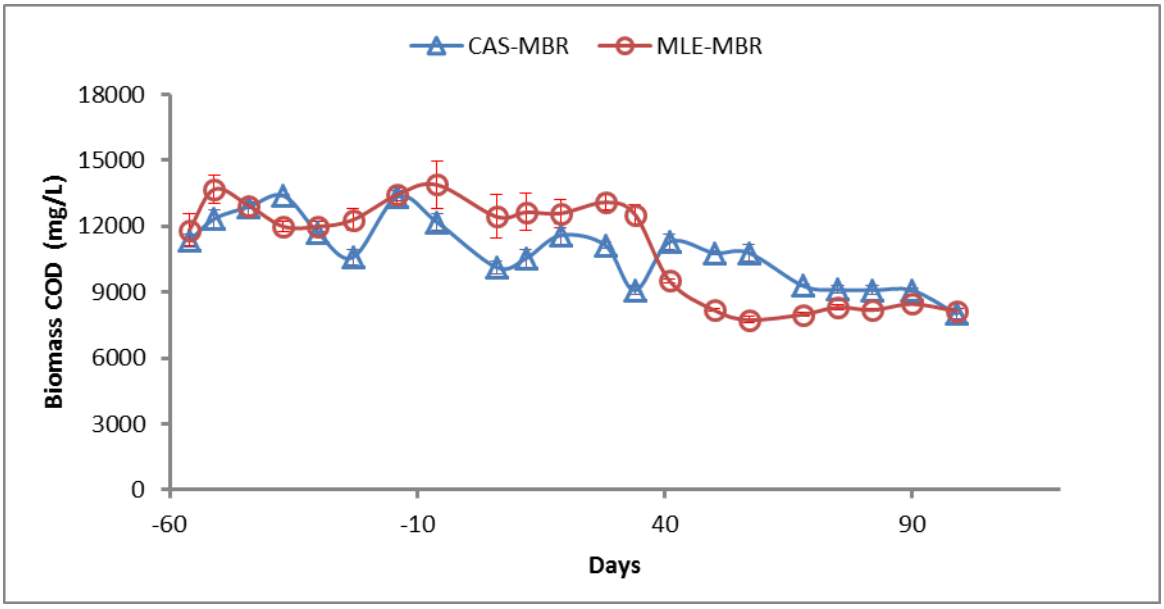

Figure 26. Biomass profiles of CAS-MBR and MLE-MBR before and after nitrophenol loading. Error bars represent the range of the data points.

5.3.5 Membrane fouling trends before and after nitrophenol loading

According to the manufacturer's manual, when the TMP of membrane module increased to 45 kPa, the membrane module was taken out of the MBR tank for physical cleaning. As shown in Figure 28, the membrane modules of CAS-MBR and MLE-MBR experienced with the same physical membrane cleaning procedures and started operation at the same time before the

nitrophenol loading. At the moment of nitrophenol loading, the TMP of CAS-MBR jumped. Therefore, the module was taken out for physical cleaning. The time interval of membrane cleaning for the CAS-MBR before nitrophenol loading was 20 days while no physical cleaning was needed in the MLE-MBR. However, just 10 days after the nitrophenol loading, the MLE-MBR was experienced with a TMP jump and membrane cleaning was necessary. The MLE-MBR membrane module required a total of four physical membrane cleanings after loading. The average time interval of membrane cleaning of the MLE-MBR module after nitrophenol loading was 22 days. For comparison, the TMP of CAS-MBR was gradually increased to 19 kPa on day 7. Then it began to decrease gradually and remained at about 10 kPa for a long time period. Until day 100, it required membrane physical cleaning. As presented in Figure 27, the CAS-MBR was well maintained within the constant flux operation mode. While in the MLE-MBR, as the TMP increased, the flux decreased. This indicated that substantial cake layer formed in the MLE-MBR.

The difference of TMP profiles between the CAS-MBR and MLE-MBR after nitrophenol loading demonstrated that the CAS-MBR has a better performance of membrane fouling control than MLE-MBR to some extent. A recent study demonstrated that metabolic uncoupling effect on ATP synthesis inhibiton could be beneficial to membrane biofouling control (Xu and Liu 2011). The ATP levels of biomass in the MBRs after nitrophenol loading were significantly lower than those of biomass without exposure to nitrophenol compounds (Figure 29). This result suggested that nitrophenol compounds effectively inhibited the ATP synthesis through metabolic uncoupling. Before nitrophenol dosing, the average biomass specific ATP was 0.304 mg ATP/g VSS; however, the average specific ATP yields of CAS-MBR biomass after

nitrophenol dosing was 0.062 mg ATP/g VSS. For comparison, two months after dosing, the biomass in the anoxic zone and aerobic zone of MLE-MBR had specific ATP value of 0.052 mg ATP/g VSS and 0.092 mg ATP/g VSS, respectively. Because the location of nitrophenol feed in the CAS-MBR was directed to the aeration basin where the membrane module submerged, the membrane module of CAS-MBR experienced an immediate effect of nitrophenol metabolic uncoupling. While in the MLE-MBR, the anoxic zone biomass served as a buffer to toxic nitrophenol loading so the biomass in the aerobic zone of MLE-MBR had a much higher specific ATP level than that in the anoxic zone ($p < 0.001$). The specific ATP level in the CAS-MBR was 50% lower than that in the aerobic zone of MLE-MBR suggesting that the CAS-MBR configuration has a much better membrane fouling control performance than the MLE-MBR.

EPS content in activated sludge is related to membrane fouling (Al-Halbouni et al. 2009, Al-Halbouni et al. 2008, Lyko et al. 2008, Rosenberger et al. 2006). As presented in Figure 30, bound EPS level of biomass in the CAS-MBR was 26.98 mg/g VSS, higher than that in MLE-MBR before nitrophenol loading ($p < 0.01$). After nitrophenol loading, the bound EPS level in the CAS-MBR was decreased. For comparison, the average bound EPS level of biomass in the aerobic zone of MLE-MBR was 30.65 mg/g VSS higher than the bound EPS level of the CAS-MBR, which was correlated with the higher specific ATP content of the aerobic zone biomass in the MLE-MBR. It is well known that high content of bound EPS results in an increase of cake layer resistence (Ahmed et al. 2007). Due to the higher bound EPS level and higher specific ATP content of aerobic biomass in the MLE-MBR, it exhibited significant membrane fouling problem.

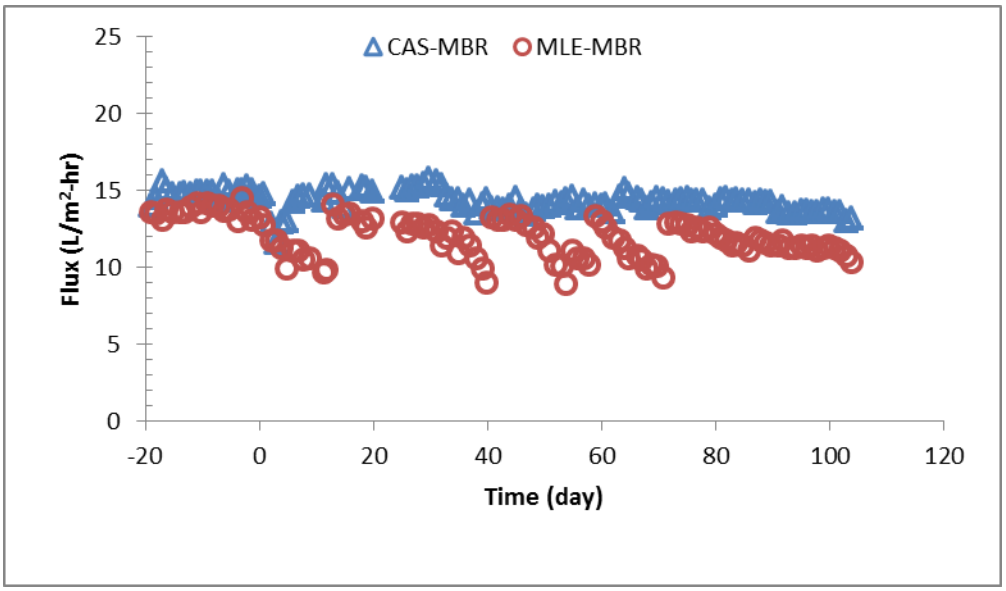

Figure 27. Permeate flux profiles of CAS-MBR and MLE-MBR before and after nitrophenol loading.

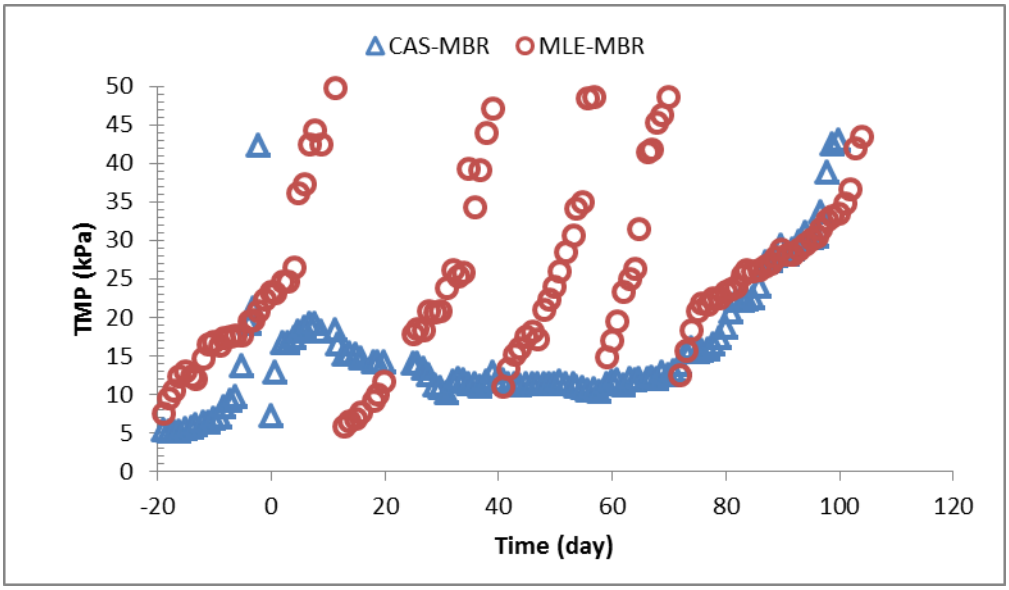

Figure 28. Transmembrane pressure profiles of CAS-MBR and MLE-MBR before and after nitrophenol loading.

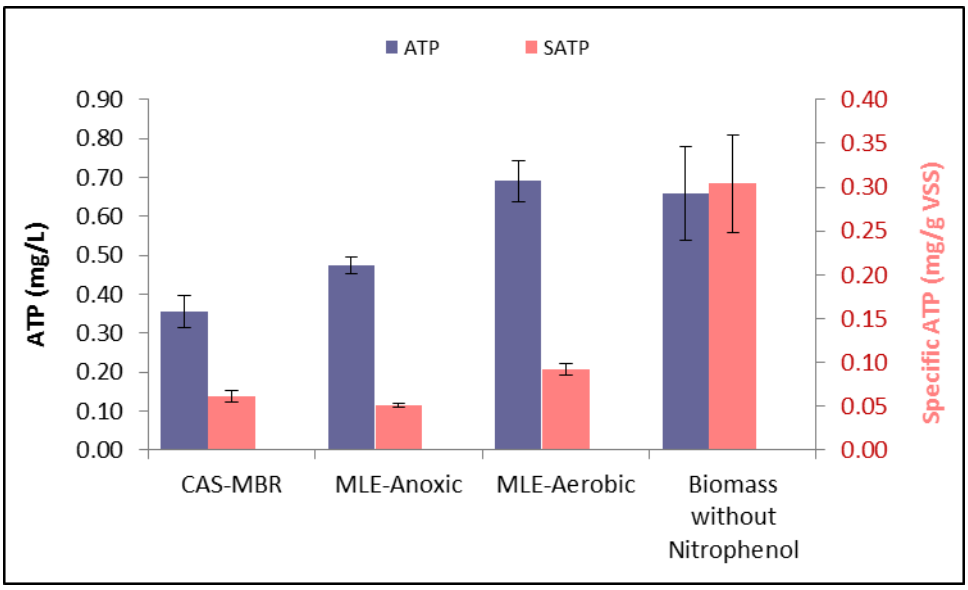

Figure 29. Biomass ATP levels in the CAS-MBR and MLE-MBR before and after nitrophenol exposure.

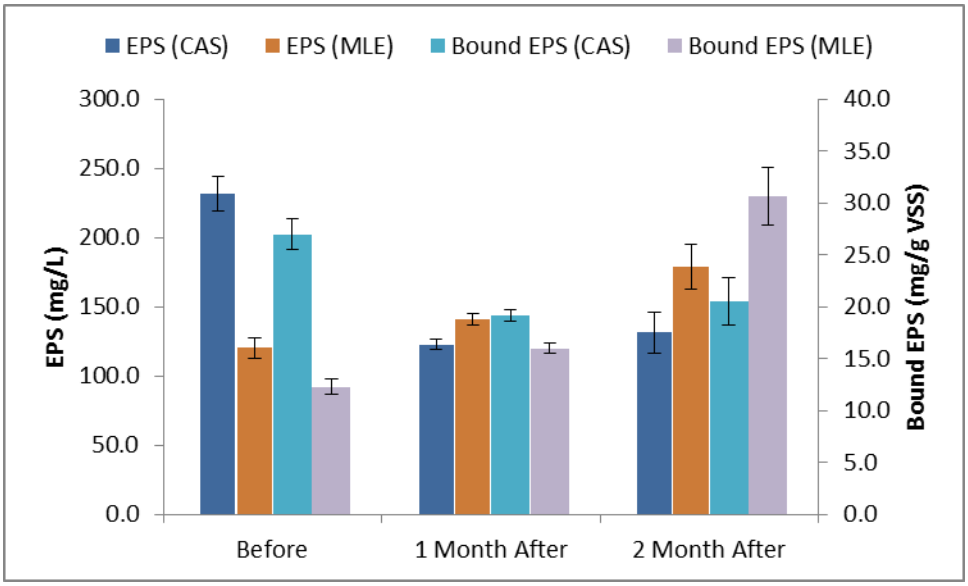

Figure 30. Biomass EPS content of CAS-MBR and MLE-MBR before and after nitrophenol loading.

5.4 Conclusions

The biodegradation of mixture nitrophenol compounds and their kinetics in modified Ludzack-Ettinger (MLE) type and conventional activated sludge (CAS) membrane bioreactors were evaluated in the batch and continuous flow systems. The metabolic uncoupling effect of nitrophenols on membrane fouling control in the two types of MBRs was also investigated. The following conclusions were made:

● All four nitrophenol compounds were biodegradable by biomass in the CAS-MBR and MLE-MBR. The average nitrophenol removal efficiencies ranged from 85% to 91% by the MBRs. The MBRs achieved simutanenous nitrophenol and nitrogen removal with total inorganic N removal efficiencies of 52% and 75% for CAS-MBR and MLE-MBR , respectively.

● The nitrophenol metabolic uncouplingas indicated by ATP production and the lower bound EPS level in the biomass of CAS-MBR suggest that the CAS-MBR configuration has a much better membrane fouling control performance than the MLE-MBR after nitrophenol loading.

CHAPTER 6

6. SUMMARY

The membrane-aerated biofilm reactor (MABR) as an efficient biological nutrient removal technique used in onsite wastewater treatment systems was evaluated in this research. A 7.2 L MABR reactor was constructed and continuously operated for more than 250 days. The reactor demonstrated consistently high organic matter removal efficiency. With the measured DO concentration in anoxic zone of MABR as 0.23 mg/L and aerobic biofilm region close to membrane, the efficient development of simultaneous nitrification and denitrification gives the overall total inorganic nitrogen removal a 64% efficiency rate. . The microbial activity analysis on settled sludge in the MABR reactor showed no nitrifying activity. This confirmed that the nitrification process was established in the aerobic biofilm region close to membrane where is rich in DO concentration. The low sludge production rate of the MABR system put the sludge pumping frequency at only one eighth of the regular onsite wastewater treatment system serving the same size household.

To better understand the biomass characteristics and microbial community diversity of a submerged membrane bioreactor with mixed liquor recirculation (MLE/MBR) and a membrane bioreactor with the addition of integrated fixed biofilm medium (IFMBR), two bench-scale MBRs were continuously operated in parallel with a hydraulic retention time (HRT) of 24 hours and solids retention time (SRT) of 20 days. Both MBRs demonstrated no significant difference on efficient removal of organic matters. MLE/MBR achieved over 25 percent higher total nitrogen removal efficiency than IFMBR. The recirculation of mixed liquor from aerobic zone to

anoxic zone in the MLE/MBR resulted in higher microbial activities of heterotrophic (46.96 mg O_2/gVSS-h) and autotrophic bacteria (30.37 mg O_2/gVSS-h) in the MLE/MBR compared to IFMBR. The alternating anoxic/aerobic conditions in the MLE/MBR resulted in more diversity for nitrifying bacterial community that those in IFMBR. Two different configuration MBRs demonstrated similar total membrane resistance change patterns with the same operation parameters.

The start-up performances of one conventional MBR and one MLE type MBR with mixed liquor recirculation on organic and nutrient removal, membrane fouling, biomass characteristics and microbial activities were presented when MBR processes were used to upgrade conventional activated sludge processes with biomass concentration increasing from 2000 mg/L to 12000 mg/L. Two water level sensors in MBRs control the constant mixed liquor volume of MBR tanks. It takes around 130 days for MBR biomass concentration to increase from 2500 mg/L to 13000 mg/L with specific sludge growth rates of 0.0125 day^{-1}. The MLE/MBR configuration with mixed liquor recirculation achieved higher total nitrogen removal efficiency than conventional MBR. Meanwhile MLE type MBR demonstrated higher heterotrophic and autotrophic microbial activities, lower SVI value, and less severe extent membrane fouling than conventional MBR with double stable filtration time.

To remove recalcitrant organic nitrogen chemicals of nitrophenols, the biodegradation of four nitrophenol compounds in MBR reactors and their impact on the membrane fouling control performance of MBR were evaluated. The average SRT of two different reactor type MBRs was 106 days with mixed liquor suspended solids (MLSS) concentrations in MBR ranged from 8200

to 9900 mg L^{-1}. The influent concentrations of 2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol were 0.05 mM each. After short biomass acclimation to nitrophenol loadings, both MBRs exhibited good and stable nitrophenol biodegradation performance with the average removal efficiencies of 2-nitrophenol, 3-nitrophenol, 4-nitrophenol and 2,4-dinitrophenol at 90%, 94%, 88%, and 91% respectively, in both MBRs. The total nitrogen removal efficiencies for CAS-MBR and MLE-MBR were 52% and 75%, respectively. The membrane flux and TMP study results suggested that continuous stirred-tank reactor (CSTR) reactor type MBR appeared to have much better membrane fouling control performance than that of pseudo-plug-flow-reactor type of MLE-MBR due to the difference of metabolic uncoupling by nitrophenol compounds in the MBRs.

CHAPTER 7

7. FUTURE RESEARCH

Future study will continue to investigate the mechanisms of metabolic uncoupling effect of nitrophenols on biofilm formation and membrane fouling control in the MBR process. The energy uncoupling effect of nitrophenols has demonstrated efficient reduction in the ATP synthesis resulting in reduced membrane fouling in this study. But how the uncoupling effect and ATP synthesis inhibition affects biofilm formation on the membrane module surface needs to be further determined. Very few studies have addressed the biofilm development mechanism under the long-term exposure of nitrophenols at low concentrations in the MBR systems for industrial wastewater treatment. Hence, more research is needed to investigate the importance of long-term performance of MBR systems treating nitrophenols at low concentrations. The floc size distribution of activated sludge in the MBR system may be also related to the membrane fouling. Nitrophenol compounds have been found helpful in forming granule sludge in the activated sludge system (Yi et al. 2006). More study is needed to determine the effect of nitrophenol compounds on the floc size distribution and its relationship to membrane fouling control of MBR systems.

Other factors involved in the biofilm formation and biofouling in the MBR systems should be considered. For example, with wide application of silver nanoparticles, it is worthwhile to determine the effect of long-term exposure of silver nanoparticles at low concentrations on the biofouling control.

Additional studies on organic nitrogen removal in the MABR and MBR systems could provide new information of the "limit of technology" nutrient removal. Organic nitrogen is an important part of total nitrogen in domestic wastewater. For MABR and MBR systems which are used for domestic wastewater treatment, the fate and transport of organic nitrogen in the start-up and steady state operations of these systems can ultimately help optimize the design and operation of nutrient removal processes in wastewater treatment facilities.

Appendices

A: Additional relevant scholar work

Liang, Z., Das, A., and Hu, Z. Q. (2010) Bacterial response to a shock load of nanosilver in an activated sludge treatment system. ***Water Research***, **44**, 5432-5438. (Paper attached)

B: Selected data of batch studies and bioreactor biomass

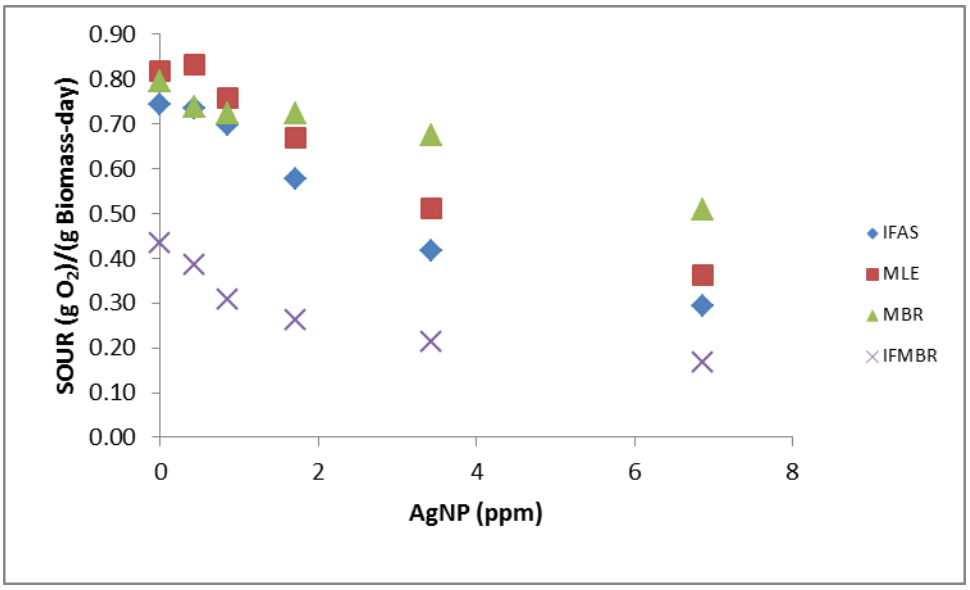

Figure 31. Effect of silver nanoparticles on the hetrotrophic microbial activities of biomass in four bioreactors

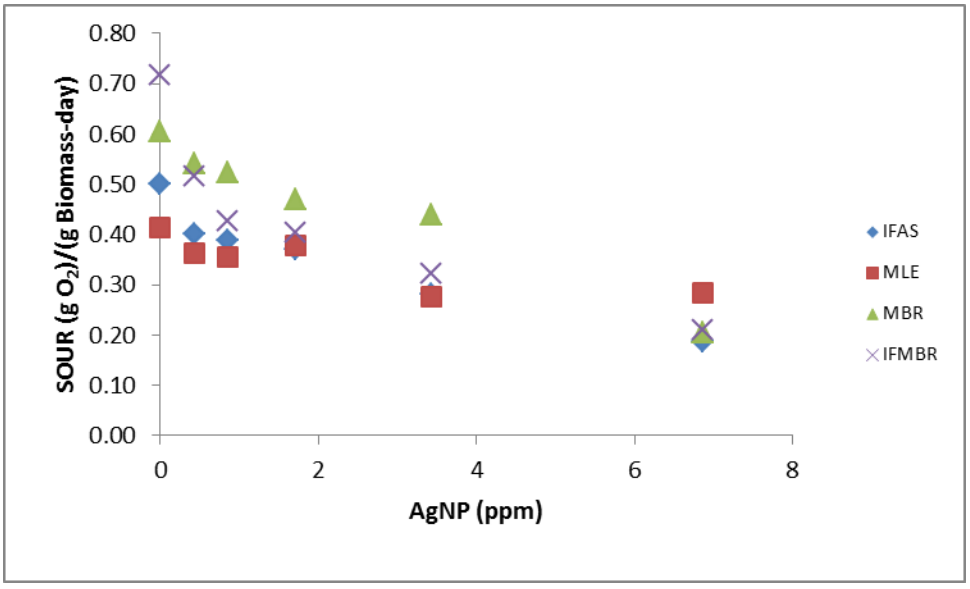

Figure 32. Effect of silver nanoparticles on the autotrophic microbial activities of biomass in four bioreactors

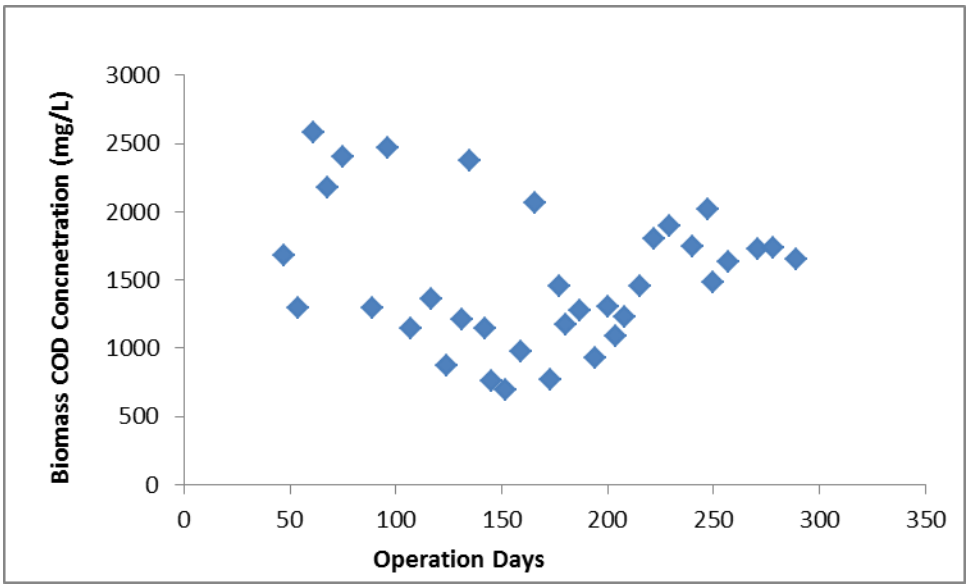

Figure 33. Biomass COD concentration in IFAS bioreactor during the operation period

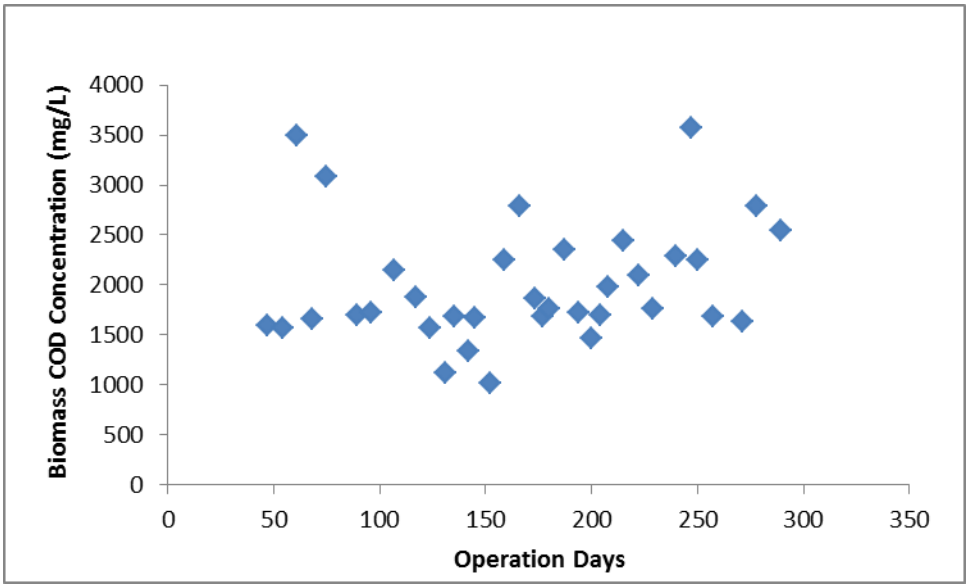

Figure 34. Biomass COD concentration in MLE bioreactor during the operation period

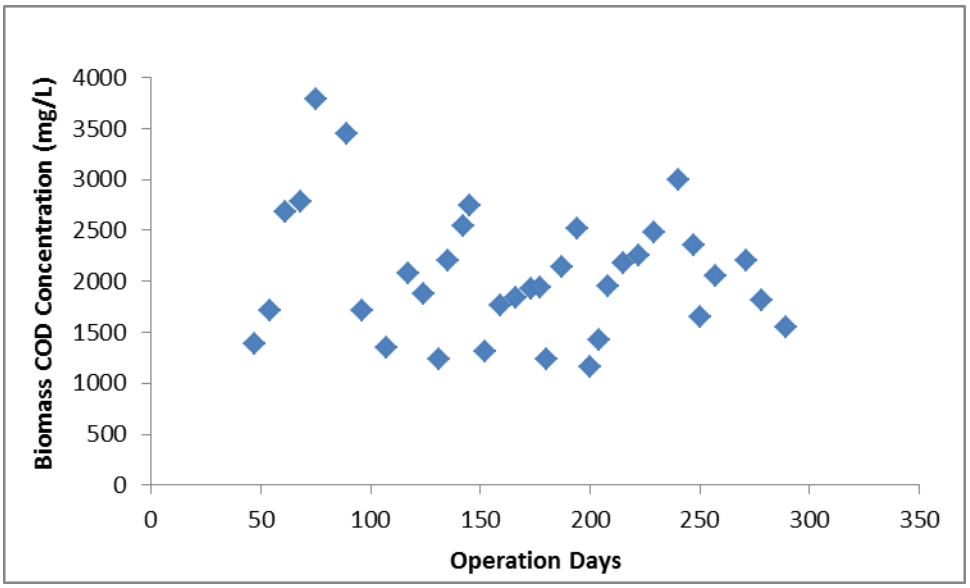

Figure 35. Biomass COD concentration in MBR bioreactor during the operation period

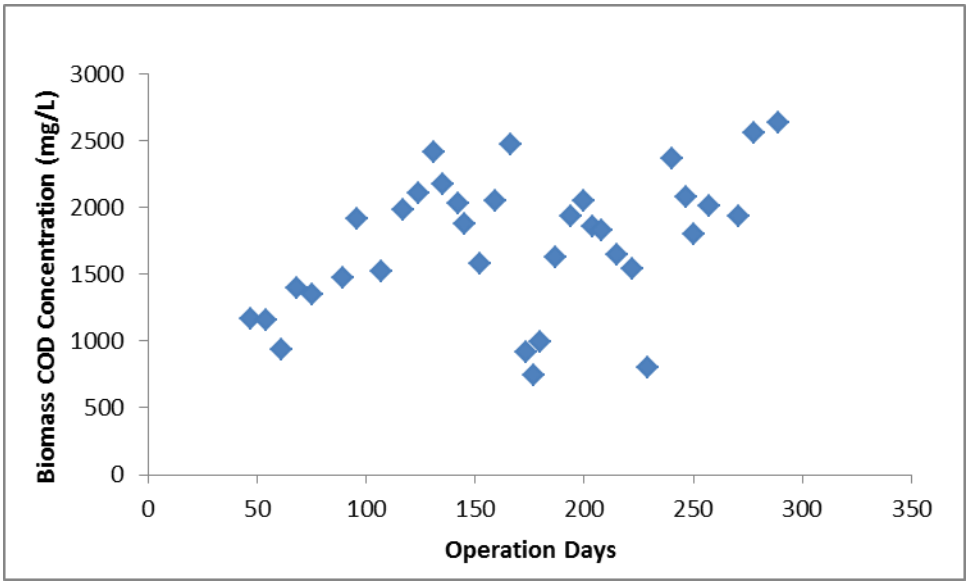

Figure 36. Biomass COD concentration in IFMBR bioreactor during the operation period

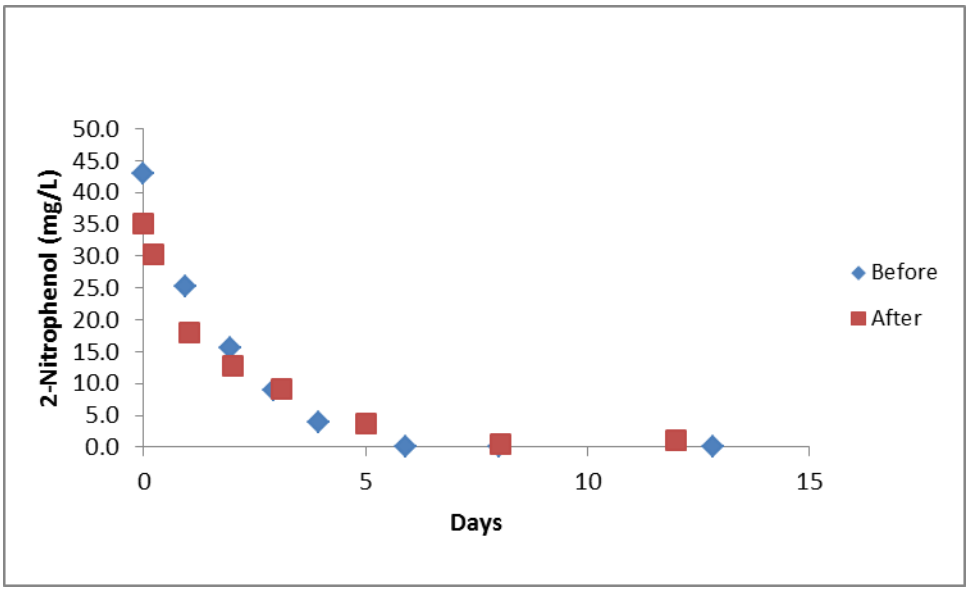

Figure 37. 2-Nitrophenol biodegradation batch study of biomass from MLE-MBR before and after acclimation to nitrophenols loading

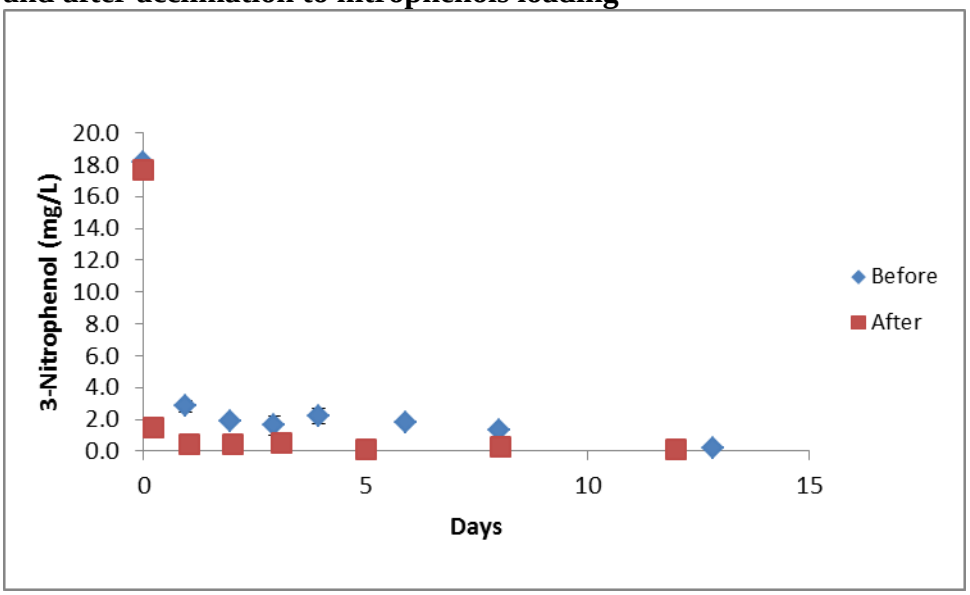

Figure 38. 3-Nitrophenol biodegradation batch study of biomass from MLE-MBR before and after acclimation to nitrophenols loading

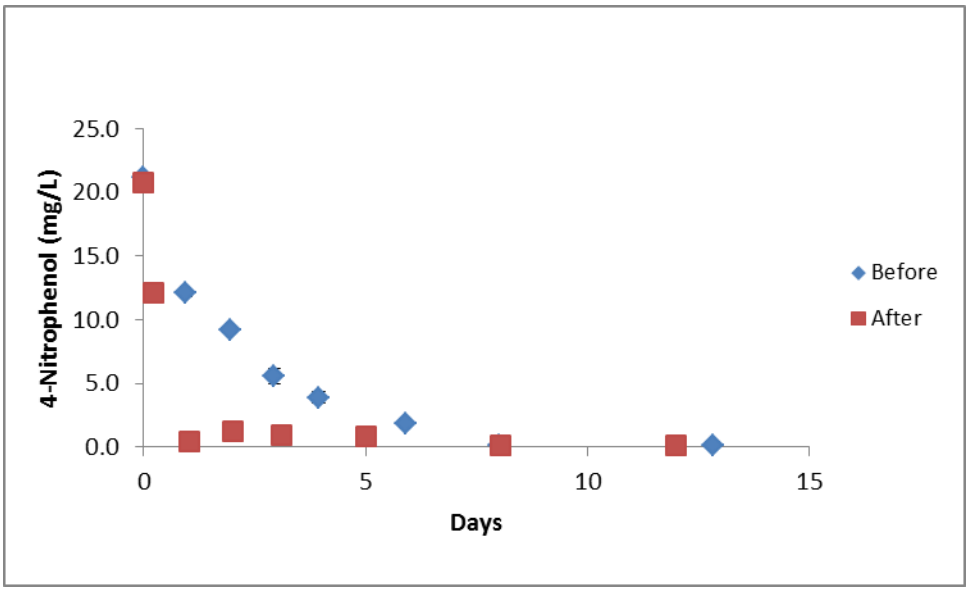

Figure 39. 4-Nitrophenol biodegradation batch study of biomass from MLE-MBR before and after acclimation to nitrophenols loading

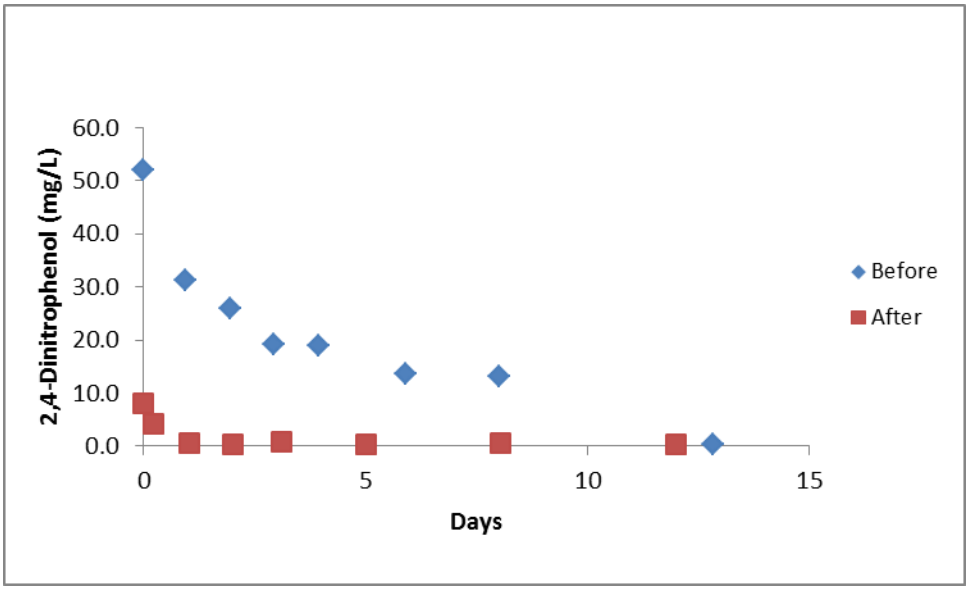

Figure 40. 2,4-Dinitrophenol biodegradation batch study of biomass from MLE-MBR before and after acclimation to nitrophenols loading

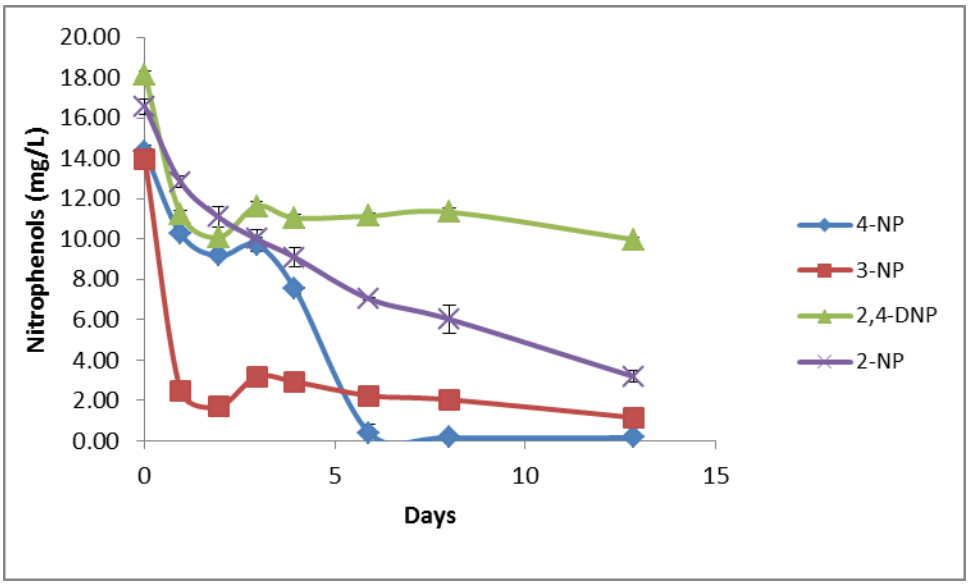

Figure 41. Mixture nitrophenols biodegradation batch study of biomass from MLE-MBR before acclimation to nitrophenols loading

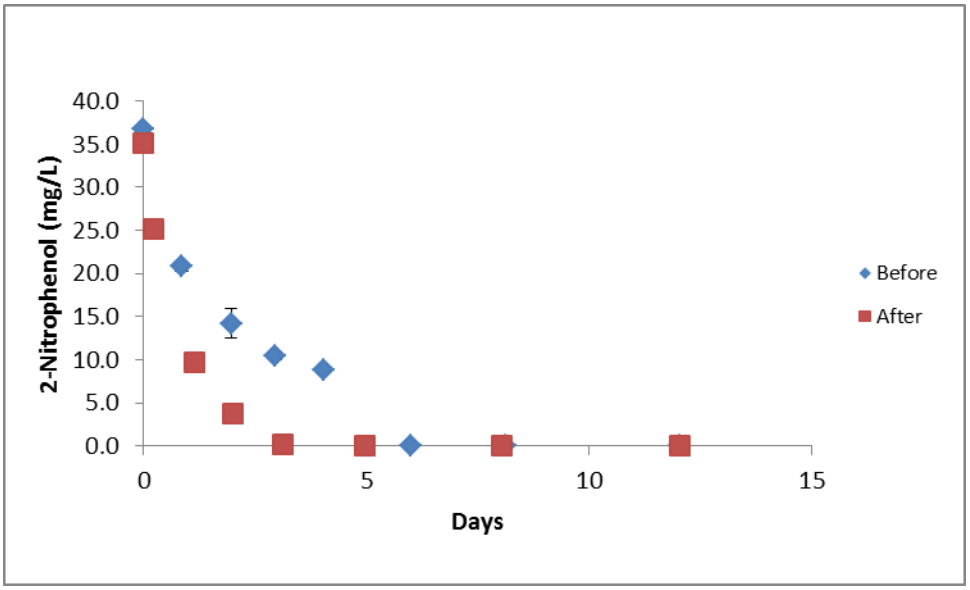

Figure 42. 2-Nitrophenol biodegradation batch study of biomass from CAS-MBR before and after acclimation to nitrophenols loading

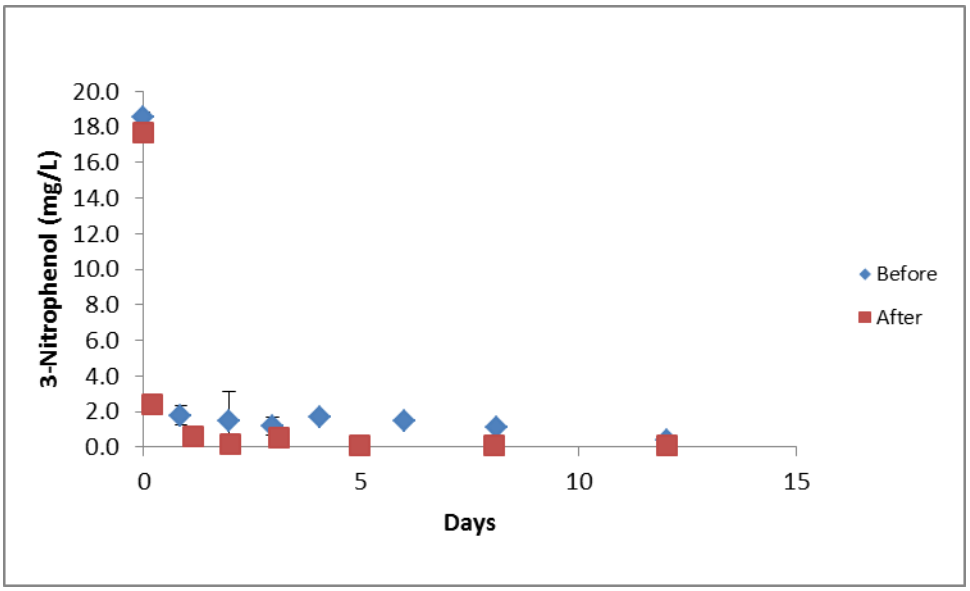

Figure 43. 3-Nitrophenol biodegradation batch study of biomass from CAS-MBR before and after acclimation to nitrophenols loading

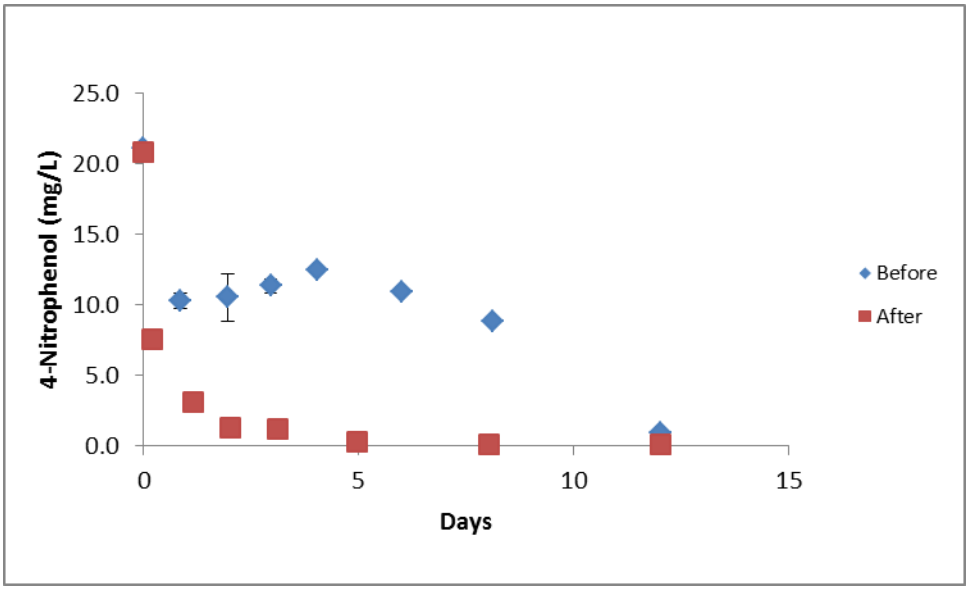

Figure 44. 4-Nitrophenol biodegradation batch study of biomass from CAS-MBR before and after acclimation to nitrophenols loading

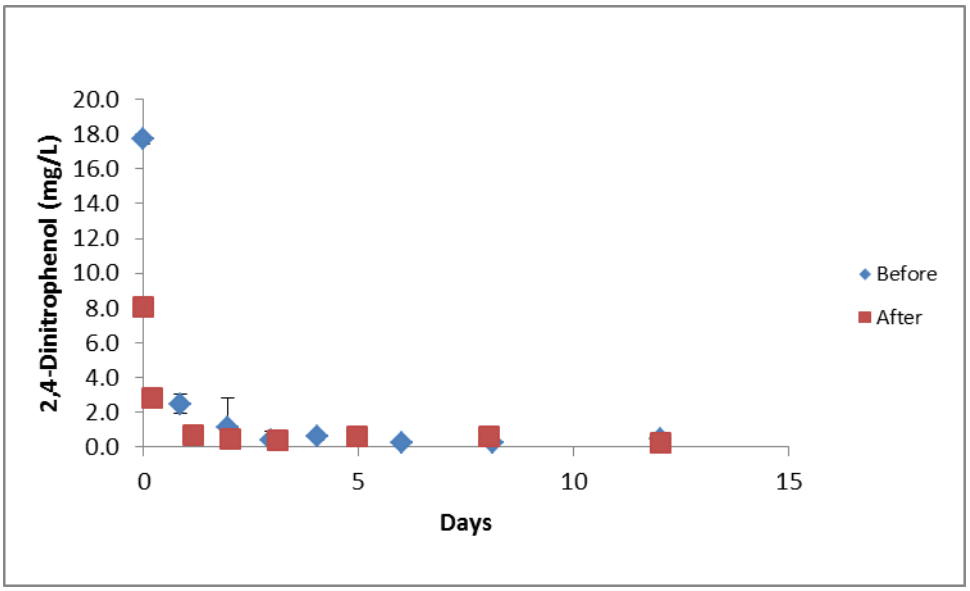

Figure 45. 2,4-Dinitrophenol biodegradation batch study of biomass from CAS-MBR before and after acclimation to nitrophenols loading

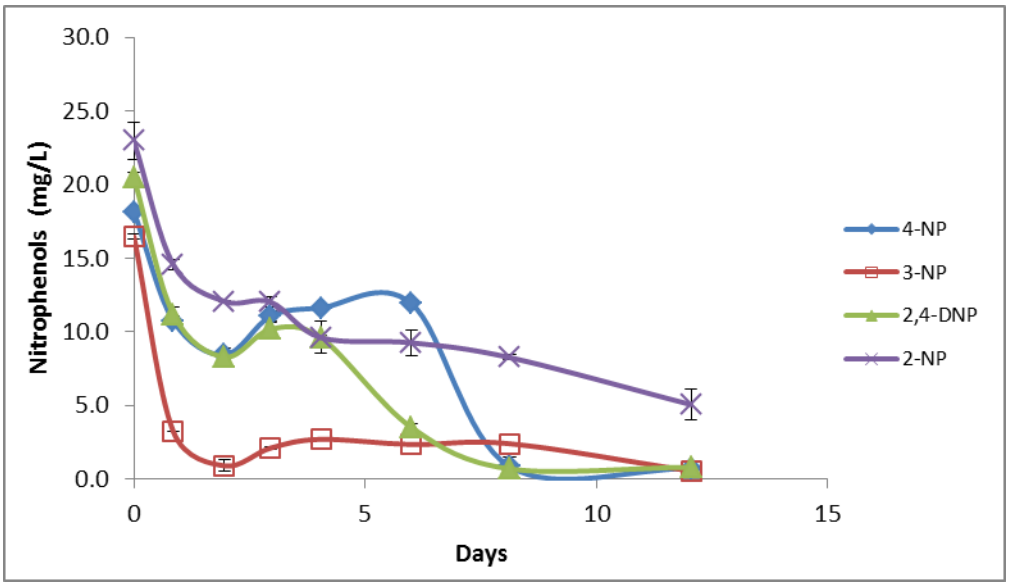

Figure 46. Mixture nitrophenols biodegradation batch study of biomass from CAS-MBR before acclimation to nitrophenols loading

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VITA

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Bacterial response to a shock load of nanosilver in an activated sludge treatment system

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ARTICLE INFO

Article history:
Received 20 March 2010
Received in revised form
14 June 2010
Accepted 22 June 2010
Available online 17 July 2010

Keywords:
Nanosilver
Shock load
Wastewater treatment
Nitrogen removal
Nitrifying community structure

ABSTRACT

The growing release of nanosilver into sewage systems has increased the concerns on the potential adverse impacts of silver nanoparticles (AgNPs) in wastewater treatment plants. The inhibitory effects of nanosilver on wastewater treatment and the response of activated sludge bacteria to the shock loading of AgNPs were evaluated in a Modified Ludzack–Ettinger (MLE) activated sludge treatment system. Before shock-loading experiments, batch extant respirometric assays determined that at 1 mg/L of total Ag, nitrification inhibitions by AgNPs (average size = 1–29 nm) and Ag^+ ions were 41.4% and 13.5%, respectively, indicating that nanosilver was more toxic to nitrifying bacteria in activated sludge than silver ions. After a 12-h period of nanosilver shock loading to reach a final peak silver concentration of 0.75 mg/L in the MLE system, the total silver concentration in the mixed liquor decreased exponentially. A continuous flow-through model predicted that the silver in the activated sludge system would be washed out 25 days after the shock loading. Meanwhile, a prolonged period of nitrification inhibition (>1 month, the highest degree of inhibition = 46.5%) and increase of ammonia/nitrite concentration in wastewater effluent were observed. However, nanosilver exposure did not affect the growth of heterotrophs responsible for organic matter removal. Microbial community structure analysis indicated that the ammonium-oxidizing bacteria and nitrite-oxidizing bacteria, *Nitrospira*, had experienced population decrease while *Nitrobacter* was washed out after the shock loading.

1. Introduction

Silver nanoparticles (AgNPs, nanosilver) have acquired a variety of applications in consumer products because of their antimicrobial properties (Duran et al., 2007; Holley et al., 2002; Kim et al., 2007; Morones et al., 2005; Markarian, 2006). With expanding use of nanosilver products, the release of nanosilver particles into sewage collection systems and wastewater treatment plants (WWTPs) becomes a growing concern. Several recent research results confirm the release of AgNPs into water from nanosilver-embedded sock fabrics after washing (Benn and Westerhoff, 2008; Geranio et al., 2009). It has been predicted the amount of silver released into wastewater from silver-containing products would reach a maximum of 410 tons per year for European countries alone in 2010 (Blaser et al., 2008).

Nanosilver inhibits bacterial growth (Panacek et al., 2006; Sondi and Salopek-Sondi, 2004; Yoon et al., 2007). In our recent batch study, autotrophic nitrifying bacterial growth was inhibited by about 80% at 1 mg/L of silver nanoparticles (average size = 14 ± 6 nm) (Choi et al., 2008). Nanoparticles are able to attach to cell membranes, resulting in changes of membrane permeability and redox cycling in the cytosol, accumulation of intracellular radicals, and dissipation of the

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0043-1354/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2010.06.060

proton motive force for ATP synthesis. Each of these has been reported as a possible mechanism of nanosilver toxicity (Lok et al., 2006; Morones et al., 2005; Nel et al., 2006; Sondi and Salopek-Sondi, 2004). Smaller particles (<10 nm) may enter the bacterial cell directly to cause further damage by interfering with DNA and protein synthesis (Morones et al., 2005).

The antibacterial properties of nanosilver suggest the potential adverse impact of nanosilver in wastewater treatment. Healthy growth of heterotrophic and autotrophic microorganisms in activated sludge of has also been revised is crucial to the removal of organic matter and nutrients from wastewater. The toxicity of heavy metals to activated sludge has been well documented (Hu et al., 2004; Tsa et al., 2006). Furthermore, we have demonstrated that the short-term batch assays often underestimate observed metal toxicity in the continuous flow systems due to slow kinetics of metal internalization and an exacerbation effect on bacteria due to continued metal exposure (Hu et al., 2004). At present, however, little is known on how the discharge of silver nanoparticles affects bacterial growth and the properties of activated sludge in WWTPs.

Although the predicted silver concentration in sewage treatment plants is from 2 to 18 μg/L (Blaser et al., 2008), some WWTPs have experienced high concentration (e.g., 105 μg/L of total silver) in the wastewater influent receiving periodic silver discharge from the industry (Shafer et al., 1998). Therefore, it is necessary to evaluate the effects of AgNPs at a higher concentration range on bacterial growth and bacterial community structure in activated sludge treatment systems. The autotrophic nitrifying bacteria in activated sludge are especially sensitive to inhibition by chemical toxicants including nanosilver (Choi et al., 2008; Hu et al., 2002). The objective of this study was to evaluate the potential negative impact of AgNPs on wastewater treatment and to determine the bacterial response (e.g., changes of bacterial activity and community structure) to a shock load of AgNPs. A modified Ludzack–Ettinger (MLE) process was selected because it is one of the most commonly used biological nutrient removal (BNR) processes for organic and nitrogen removal in activated sludge wastewater treatment systems (Metcalf and Eddy, 2003; Ryu and Lee, 2009).

2. Materials and methods

2.1. Nanosilver synthesis and characterization

Stock suspensions of silver nanoparticles were made from 0.7 mM $AgNO_3$ (EM Science) by adding sodium borohydride ($NaBH_4$, Sigma) at the final concentration of 0.7 mM with polyvinyl alcohol (PVA) as a capping agent. Sodium borohydride was directly added to a 0.06% (wt) PVA solution, and silver nitrate was then rapidly injected into the vigorously stirred solution at room temperature. To dissolve polyvinyl alcohol, the PVA solution was heated to 100 °C and cooled down to room temperature before use. The freshly prepared nanosilver suspensions contained almost 100% of Ag in the form of AgNPs (Choi et al., 2008; Choi and Hu, 2008). The nanosilver suspensions having an average particle size of 21 nm (via transmission electron microscopy or TEM) were well characterized as described earlier (Choi et al., 2008; Choi and Hu, 2008). The results of nanoparticle distribution analysis by the TEM were generally consistent with those by dynamic light scattering (DLS) (primary particle size = 29 nm).

2.2. MLE bioreactor

A lab-scale MLE bioreactor with a total reactor volume of 7.2 L was constructed. The MLE bioreactor was divided into anoxic, aerobic and settling chambers by glass baffles. The effective volumes of the anoxic, aerobic and settling zones were 1.8, 3.6 and 1.8 l, respectively. The wastewater influent was fed to the anoxic chamber continuously by a fixed speed peristaltic pump. An internal mixed liquor recirculation flow from the aerobic zone to anoxic zone was provided at 100% of influent flow rate. The dissolved oxygen (DO) concentration level in the aerobic zone was controlled around 3 mg/L using a fine bubble diffuser in order to keep the DO level in the anoxic zone below 0.5 mg/L. The bioreactor was operated at a HRT of 24 h and a solids retention time (SRT) of 20 days. At the beginning of reactor operation, a total of 2000 mL mixed liquor taken from the aeration basin of a local municipal WWTP (Columbia, MO) was added as an inoculum to the MLE bioreactor. Synthetic influent wastewater was prepared using nonfat dry milk powder with a target chemical oxygen demand (COD) concentration of 500 mg/L, 50 mg/L TN, 30 mg/L NH_4^+-N and 6 mg/L total P. The synthetic feed also contained the following micronutrients per liter: 44 mg MgSO4, 14 mg $CaCl_2 \cdot 2H_2O$, 2 mg $FeCl_2 \cdot 4H_2O$, 3.4 mg $MnSO_4 \cdot H_2O$, 1.2 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.8 mg $CuSO_4$, and 1.8 mg $Zn(NO_3)_2 \cdot 6H_2O$. Before the nanosilver shock-loading experiment, the MLE bioreactor was run for more than 300 days with the average reactor biomass concentration of 2045 ± 550 mg/L biomass COD.

2.3. Heterotrophic and autotrophic bacterial activities

The autotrophic and heterotrophic activities of the sludge in the MLE bioreactor before and after nanosilver shock loading were inferred from the specific oxygen uptake rates (SOUR) due to ammonia oxidation and acetate oxidation, respectively, using batch extant respirometry (Hu et al., 2002). Briefly, aliquots (60 mL) of the sludge collected from the MLE bioreactor were aerated with pure oxygen before being transferred to the respirometric bottles, and then tightly capped with no headspace. At a predetermined time (e.g., between 500 and 600 s), an aliquot of substrate (e.g., 10 mg N/L NH_4^+-N or 20 mg/L COD in acetate) was injected using a 10-μL glass syringe. A decrease of the DO level in the respirometric bottles due to substrate oxidation was measured by a DO probe (YSI model 5300A, Yellow Springs, OH) and continuously monitored at 4 Hz by an interfaced computer. Oxygen uptake rates were calculated based on a linear regression analysis because zero-order reactions were observed for a long period of time. The zero-order reactions were ascertained in separate experiments since the rates were independent of substrate concentration at the tested NH_4^+-N or acetate concentrations. All SOUR experiments were carried out in at least duplicate trials.

Prior to the nanosilver shock-loading experiment in the MLE bioreactor, short-term nitrification inhibitions by silver

ions and AgNPs were measured for the selection of the target final peak concentration of AgNPs in the continuous flow reactor. The respirometry based nitrification inhibition assays (Hu et al., 2002) were carried out the same as described above, except that aliquots of the sludge collected from the MLE reactor before nanosilver shock loading were spiked with 1 mg/L of silver ions or AgNPs before the assay was initiated. Nitrification inhibition was determined from four replicate samples by calculating the difference between the measured specific oxygen uptake rates in the absence and presence of silver.

2.4. Nanosilver shock loading to the MLE bioreactor

The freshly prepared nanosilver stock suspension was separately and continuously fed to the MLE bioreactor for 12 h at a constant nanosilver loading rate with a target final silver peak concentration of ca. 1 mg/L. During and after shock loading of silver nanoparticles the wastewater effluent samples of the MLE bioreactor were collected for NH_4^+-N, NO_3-N, NO_2-N, and COD analysis. Before and after shock loading, aliquots of mixed liquor were taken from the aeration zone once a week to determine autotrophic and heterotrophic bacterial activities using extant respirometry.

Total silver and soluble silver species (after passing through a 1000 Da molecular weight cut-off membrane) were determined during and after the AgNP shock loading. To determine the partitioning of silver between biomass and liquid phases, aliquots (20 mL) of the mixed liquor were taken from the aeration chamber of the MLE bioreactor and separated by centrifugation at 2500 g for 5 min. The supernatant was removed and biomass pellets were collected to determine the total silver concentration in the biomass phase. For silver in wastewater effluent, both total and soluble silver concentrations were measured. Aliquots of the effluent water samples were filtered through the 1000 Da molecular weight cut-off (MWCO) ultrafiltration membrane (Millipore MA) by a stirred ultrafiltration cell unit (Amicon 8050, Millipore MA) and the filtrates were used to determine the concentration of soluble silver species in wastewater effluent.

A mass balance-based mathematic model was developed to predict the dynamics of total silver concentration in the sludge of the continuously flow MLE bioreactor. The detailed information about the model of metal dynamics in a continuous flow reactor is available elsewhere (Hu et al., 2004). Equation (1) was used to predict total silver concentrations in the sludge during the shock-loading period and equation (2) was developed to determine the remaining silver concentrations in the sludge after the shock loading.

$$C = \left(\frac{Q_m C_m}{Q_{in}+Q_m}\right)\left(\frac{K_P X_R}{1+K_P X_R}\right)\left(1-\exp\left(\frac{-(Q_{in}+Q_m)t}{V}\right)\right) \quad (1)$$

where C = total silver concentration in the sludge of MLE, mg Ag/L; C_m = nanosilver concentration in the stock suspension, mg Ag/L; Q_{in} = flow rate of influent to the MLE reactor, L/day; Q_m = flow rate of a separate feed containing nanosilver stock only, L/day; X_R = biomass concentration in MLE reactor, g/L; K_P = silver sludge–liquid partition coefficient, L/g; t = time elapse from the start of nanosilver shock loading, day; V = mixed liquor volume of MLE reactor, L.

$$C = C_0 \exp\left(-\left(\frac{Q_{in}+Q_W K_P X_R}{1+K_p X_R}\right)\frac{(t-0.5)}{V}\right) \quad (2)$$

where C_0 = total silver concentration in the sludge at the completion of shock loading (t = 0.5 day), mg Ag/L; Q_w = flow rate of the wasted sludge from the aeration basin of the MLE reactor, L/day.

2.5. Nitrifying bacterial community structure analysis

Terminal Restriction Fragment Length Polymorphism (T-RFLP) was used to analyze nitrifying bacterial community in the MLE bioreactor based on the known 16S rRNA genes of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria as described earlier (Siripong and Rittmann, 2007). DNA was extracted from a 5 mL sample of mixed liquor in the bioreactor using Ultraclean Soil DNA Isolation Kit (Carlsbad, CA). All primers were synthesized by Integrated DNA Technologies (Coralville, IA). A fluorescent dye, 6-FAM, was incorporated into the DNA fragment in fluorescence-labeled primers. Considering the low concentration of DNA from the nitrifiers, we amplified DNA from the 16S rRNA genes of ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) by nested PCR, using the universal primers 11f and 1492r to produce an initial increase in template concentration. This was followed by the specific amplification of the nitrifier genes (Siripong and Rittmann, 2007).

The universal amplification of the 16S rRNA gene was conducted using a PCR DNA thermocycler (Eppendorf, Westbury, NY), followed by purification of the PCR amplicons using the Wizard SV Gel and PCR Clean-Up system (Madison, WI). The thermal profile used for the universal amplification was: 5 min at 95 °C; 35 cycles of 30 s at 95 °C, 30 s at 55 °C, and 45 s at 72 °C; and a final elongation for 10 min at 72 °C. The thermal profile used for the nitrifier-specific amplification was: 5 min at 95 °C; 35 cycles of 90 s at 95 °C, 30 s at 60 °C, and 90 s at 72 °C; and a final elongation for 10 min at 72 °C. The PCR products were purified again and digested with MspI restriction endonuclease (Promega, Madison, WI) at 37 °C for 3 h as described earlier (Siripong and Rittmann, 2007). The digested PCR products were run through an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA) in the DNA Core Facility at the University of Missouri. An internal lane standard that ranges from 20 to 600 bases (Genescan 600 LIZ) was added to each sample for precise sizing of each fragment by adjusting for lane to lane loading variation.

2.6. Analytical methods

Ammonia nitrogen (NH_4^+-N), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), biomass concentration and wastewater COD were measured in duplicate using Standard Methods (APHA, 1998). Silver concentration after microwave digestion using HNO_3 at a temperature of 160 °C was measured by an inductively coupled plasma-optical emission spectrometer (ICP-OES) (Model Vista-MPX CCD simultaneous ICP-OES, Varian Inc., CA) with a detection limit of 0.05 mg/L, according to EPA method 3015.

3. Results and discussion

3.1. Nitrification inhibition by AgNPs and Ag^+ ions from batch assays

Nitrification inhibition by AgNPs was first determined by batch respirometric assays to select the target peak silver concentration in the MLE reactor. Furthermore, the difference of inhibition between silver ions and AgNPs was confirmed. At 1 mg/L of total silver, nitrification inhibitions were found to be $41.4 \pm 13\%$ and $13.5 \pm 6.7\%$ by AgNPs and silver ions, respectively (Fig. 1). The results were consistent with an earlier study (Choi et al., 2008) demonstrating that AgNPs were more toxic than silver ions, although the inhibitions by both species were lower in the activated sludge system compared to those using the enriched nitrifying bacteria (Choi et al., 2008).

3.2. Dynamics of silver concentration in the MLE bioreactor

AgNPs at a nominal analytical concentration of about 20 mg/L were dosed to the MLE reactor for 12 h at a constant flow rate of 0.58 L/day to achieve a target peak silver concentration of ca. 1 mg/L at the completion of nanosilver shock loading. The measured maximum total silver concentration in mixed liquor of the MLE was 0.75 mg Ag/L, which was observed right after shock loading (Fig. 2). Thereafter, the total silver concentration in the sludge decreased exponentially. However, the concentrations remained detectable 20 days after the nanosilver shock loading (Fig. 2). The total silver concentrations of the sludge were 0.273, 0.183 and 0.106 mg Ag/L on day 15, 19, and 21, respectively. For comparison, in the next 10 days after shock loading, the average concentrations of total silver and free silver species in the wastewater effluent samples were 0.034 ± 0.008 mg/L, and 0.042 ± 0.006 mg/L, respectively, both of which were close to the instrument detection limit. While silver complexed with humic acid or other organic acids presented in the sludge could contribute to soluble silver in the reactor, the fraction of silver in water (liquid phase) was always low (Fig. 2). These results suggested that majority of AgNPs present in the MLE reactor existed in the sludge phase, supporting the earlier findings that more than 90% of AgNPs or Ag ions are typically associated with biomass (Benn and Westerhoff, 2008; Shafer et al., 1998). Based on the total silver concentrations in the sludge and in the wastewater effluent, the calculated silver sludge–liquid partition coefficient was 7.1 ± 1.8 L/g. The similarity of the total silver concentrations in wastewater effluent samples with and without ultrafiltration suggested that the silver species in wastewater effluent were all in the soluble form.

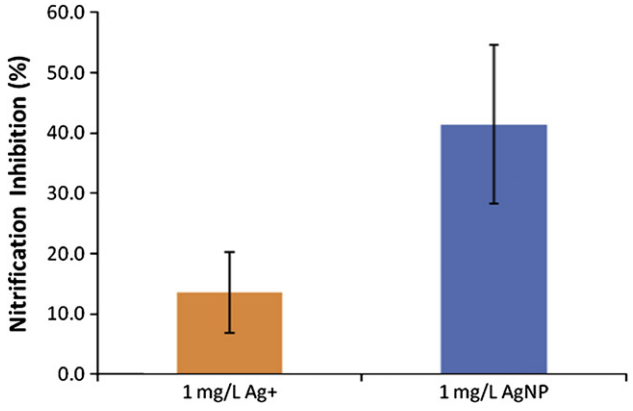

Fig. 1 – Nitrification inhibition inferred from batch respirometric assays by AgNPs and Ag^+ ions at 1 mg/L. Error bars represent one standard error of the mean ($n = 4$).

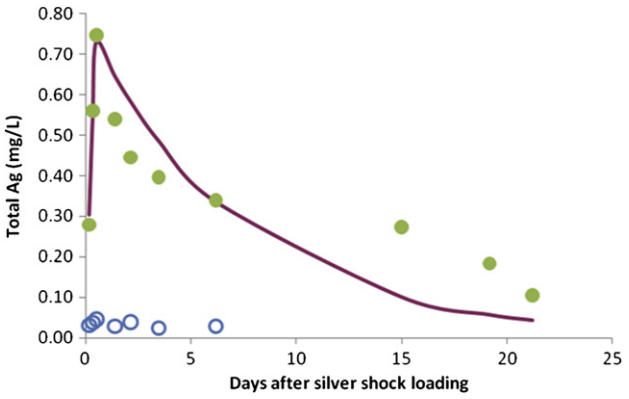

Fig. 2 – Dynamics of measured silver concentration (●) in the sludge and in the effluent (○) of the MLE reactor during and after 12-h AgNP shock load. The continuous line represents the predicted silver concentrations using Equations (1) and (2).

By incorporating the assumed linear sludge–liquid partitioning, the mass balance solutions adequately described the dynamics of silver in the MLE reactor (Fig. 2). Best-fit curves using the least square error (LSE) technique yielded the estimate K_p value of 7.4 L/g in the MLE reactor, confirming strong adsorption of silver to activate sludge (Benn and Westerhoff, 2008; Shafer et al., 1998). The model predicted further that after 25 days the silver in the sludge phase would be washed out through the continuous flow system and the wastage of the sludge.

3.3. Effluent water quality change after nanosilver shock loading

The effluent COD concentrations of the MLE bioreactor were routinely monitored before and after the shock load (Fig. 3). There was no significant difference between the effluent COD concentrations before and after silver shock load (ANOVA, $\alpha = 0.05$, P-value $= 0.94$). The 20-d average wastewater effluent COD concentrations before and after nanosilver shock load were 10.2 ± 4.4 mg/L and 10.2 ± 3.1 mg/L, respectively. This result suggested that 12-h shock load of silver nanoparticles with the final peak silver concentration of 0.75 mg/L in the sludge did not affect organic removal, as was confirmed from the heterotrophic activity measurements of the sludge described in the following section (Fig. 4).

An apparent increase of effluent NH_4^+-N concentration right after the initiation of shock loading was observed. The effluent NH_4^+-N reached a maximum of 0.99 mg/L and the effluent ammonia nitrogen water quality appeared to recover

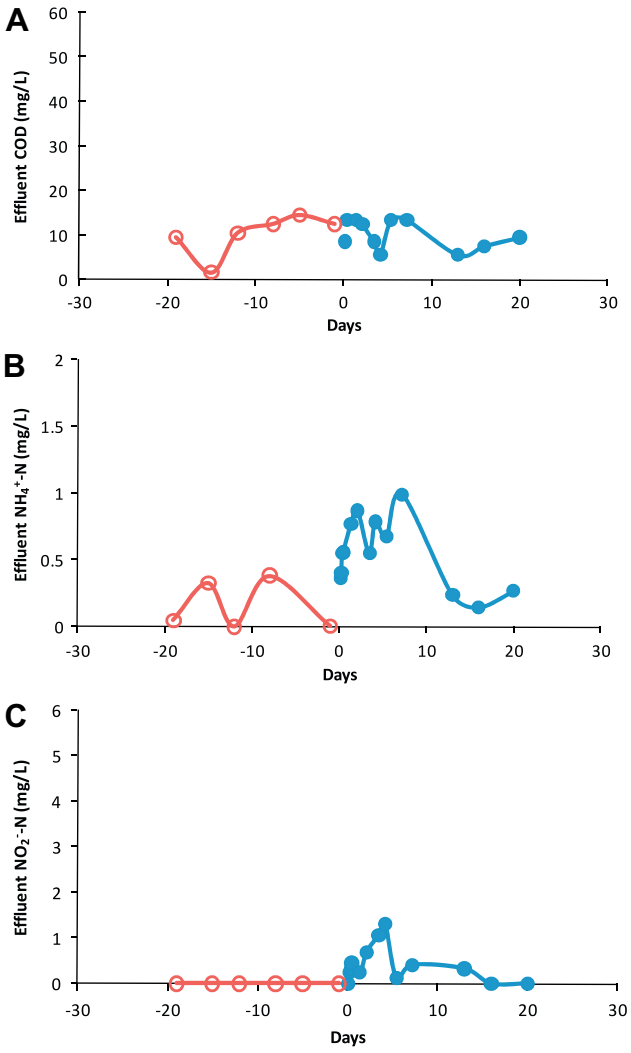

Fig. 3 – Effluent water quality before (○) and after (●) nanosilver shock loading: COD (A), NH_4^+-N (B), NO_2^--N(C) concentrations.

16 days after the shock loading. There was a significant difference between the average effluent NO_2-N concentrations before and after nanosilver shock loading (ANOVA, $\alpha = 0.05$, P-value $= 0.006$). The NO_2-N effluent concentration started to increase during the silver shock-loading period (Fig. 3). The effluent nitrite concentration reached the maximum at 1.31 mg N/L four days after shock loading. Similar to the trend of increase of NH_4^+-N in wastewater effluent, the accumulation of NO_2-N in the effluent lasted for about 16 days, an indication of nitrification inhibition. The difference of the changes between the effluent nitrite and ammonia concentration right after the initiation of shock loading indicated that complex, dynamic changes and recovery of nitrifying bacterial activities in MLE bioreactor.

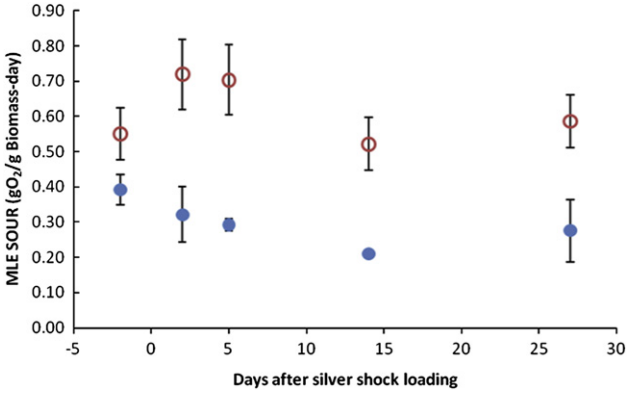

Fig. 4 – The autotrophic (●) and heterotrophic (○) activities of the sludge inferred from SOUR measurements before and after the AgNP shock loading in the MLE reactor.

3.4. *Bacterial activities before and after shock loading*

The changes of bacterial activities in response to nanosilver shock loading were inferred from the extant respirometic assays as shown in Fig. 4. The heterotrophic SOUR values of the sludge before and after the shock loading were similar at an average value of 0.63 ± 0.09 g O_2/g biomass-day. Hence, the 12-h shock-loading event with the peak silver concentration of

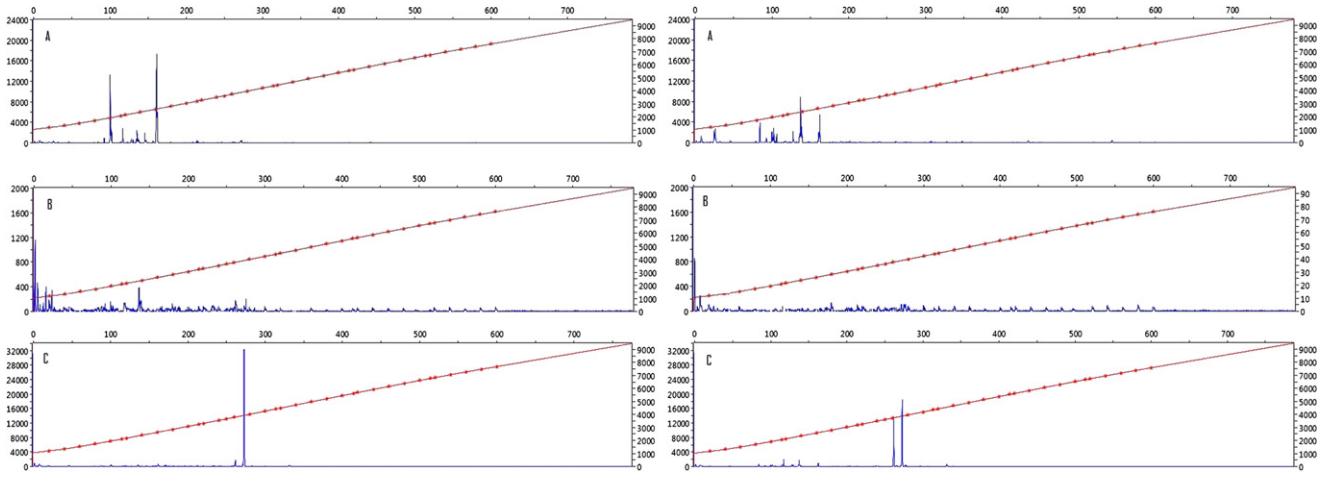

Fig. 5 – Before nanosilver shock loading (left panels): *Nitrosomonas eutropaea* (161 bps) (A) was the dominant genus of ammonia-oxidizing bacteria (AOB) while NOB such as *Nitrobacter* (136 bps) (B) and *Nitrospira* (129, 189, 262 bps) (C) were present in the MLE. After shock loading (right panels): The population of *Nitrosomonas eutropaea* (161 bps) (A) inferred from the peak intensity decreased while *Nitrobacter* was not detected (B) and the population of *Nitrospira* (262 bps) (C) decreased in the MLE. The red line (with scale of Y axis on the right) denotes the fluorescence intensity of each known fragment for the determination of the expected fragment size.

0.75 mg/L in the sludge did not inhibit heterotrophic activities, consistent with the water quality data showing low effluent COD concentrations (Fig. 3). However, the autotrophic SOUR values were significantly lower than those before silver shock loading (Fig. 4). The maximum inhibition on autotrophic growth was 46.5%, which was observed at day 14 after shock load. Even after a prolonged period (>25 days) when almost all silver in the sludge was washed out, the autotrophic activities did not recover fully. The inhibition results were generally in agreement with effluent ammonia and nitrite data after the shock loading (Fig. 3). The results were also consistent with that from the batch experiment before the shock load (Fig. 1), although it was demonstrated again that the short-term batch assays generally underestimate observed bacterial growth inhibition in the continuous flow systems due to slow kinetics of metal internalization and an exacerbation effect on bacteria due to continued metal exposure (Hu et al., 2004).

The measured nitrification inhibition in the MLE reactor by silver nanoparticles in this study was only half of the inhibition by the same nanosilver dosed to the enriched nitrifying system (Choi et al., 2008). The difference could be due to factors such as bacterial concentration and nanoparticle aggregation in the presence of extracellular polymeric substances (EPS). For instance, due to the composition of high protein concentration and protein to carbohydrate ratio (Leppard et al., 2003), nano-scale agglomerations of silver were confined almost entirely to EPS matrices of activated sludge (Leppard et al., 2003). EPS from activated sludge have high biosorption properties so that it could act as a protective barrier for bacteria cells (Comte et al., 2008).

3.5. *Nitrifying bacterial community structure change after nanosilver shock loading*

T-RFLP analysis indicated the difference of nitrifying bacterial community structure in the MLE reactor before and after the 12-h nanosilver shock loading. Before the shock loading event, *Nitrosomonas* was the dominant genus of ammonia-oxidizing bacteria while *Nitrospira* and *Nitrobacter* species were dominant among nitrite-oxidizing bacteria (Fig. 5, left panels). After the shock loading, in contrast, there was a substantial decrease in the AOB population as indicated from the change of peak intensity of the *Nitrosomonas* (Fig. 5, right panel A). *Nitrobacter* population was not detected (Fig. 5, right panel B) and *Nitrospira* peak size was reduced considerably (Fig. 5, right panel C). The decrease of population size in *Nitrosomonas* and *Nitrospira* and non-detection of *Nitrobacter* species could account for relatively slow recovery of AOB/NOB activities and a prelonged period of ammonium and nitrite accumulation (Fig. 3) due to nanosilver inhibiton. It appeared, therefore, that the higher nitrifying activities might be correlated with more diversity of nitrifying bacterial populations in the MLE system.

4. Conclusions

The bacterial responses to a 12-h shock load of nanosilver were evaluated in this study by monitoring the changes of wastewater effluent water quality, heterotrophic and autotrophic bacterial activities and nitrifying bacterial community structure. The following conclusions were made:

- Nanosilver was more toxic to nitrifying bacteria than silver ions. At a total Ag concentration of 1 mg/L, nitrifying activated sludge was inhibited by 41.4% and 13.5% for AgNPs and Ag^+ ions, respectively.
- A 12-h period of AgNP shock loading to the MLE activated sludge system resulted in the peak silver concentration of 0.75 mg Ag/L in the sludge. The continuous flow-through model predicted that the silver in the activated sludge phase would be washed out 25 days after the shock loading.

- After the shock loading, a prolonged period of nitrification inhibition with the maximum inhibition of 46.5% along with ammonia and nitrite accumulation in wastewater effluent was observed. For comparison, the shock-loading event did not affect heterotrophic activity and organic matter removal.
- Nitrification inhibition after nanosilver shock loading was consistent with a shift or loss of nitrifying population in the MLE reactor. The ammonium-oxidizing bacteria and nitrite-oxidizing bacteria, *Nitrospira*, experienced the decrease of population size, while *Nitrobacter* was totally washed out after the shock loading.

Acknowledgment

This research was funded in part by the Water Environment Research Foundation (WERF) and the National Science Foundation under Grant No. 0650943.

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