博士学位論文

Nitrification of High-Strength Ammonium Landfill Leachate for Improvement of River Water Quality in Malaysia

(マレーシアにおける高濃度アンモニア埋立地滲出水の硝化と河川水質浄化)

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LIST OF ABBREVIATIONS

MSW	Municipal solid waste
AOB	Ammonia-oxidizing bacteria
NOB	Nitrite-oxidizing bacteria
FA	Free ammonia
FNA	Free nitrous acid
SBR	Sequencing batch reactor
ANNAMOX	Anaerobic ammonium oxidation
SHARON	Single-reactor high activity ammonia removal over nitrite
INWQS	Interim National Water Quality Standard Class IIA/IIB river for Malaysia
NLR	Nitrogen loading rate (kg $N\text{-}NH_4^+/m^3$/day)
OLR	Organic loading rate (kg BOD $/m^3$/day)
HRT	Hydraulic retention time (day)
SRT	Solid retention time (day)
MLSS	Mixed liquor suspended solid (mg/L)
TKN	Total Kjeldahl nitrogen (mg/L)
$N\text{-}NH_4^+$	Ammonium nitrogen (mg/L)
$N\text{-}NO_3^-$	Nitrate nitrogen (mg/L)
$N\text{-}NO_2^-$	Nitrite nitrogen (mg/L)
TOC	Total organic carbon (mg/L)
DOC	Dissolved organic carbon (mg/L)
BOD_5	Biochemical oxygen demand (5-day incubation) (mg/L)
COD	Chemical oxygen demand (mg/L)
TSS	Total suspended solid (mg/L)
VSS	Volatile suspended solid (mg/L)
SVI	Sludge volume index (mL/g VSS)
vvm	Volume of air per working volume per minute (L/L/min)
MPN	Most probable number
CFU	Colony formation unit

SEM	Scanning electron microscopy
FISH	Fluorescence *in situ* hybridization
FITC	Fluorescein iso-thiocyanate

ABSTRACT

The mature landfill leachate is characterized by high-strength ammonium, which leads to difficulties in reducing the ammonium concentration in the wastewater discharge to the permissible limit (10 mg/L) using the existing biological treatment of sequencing batch reactors (SBRs). The challenge of the nitrogen removal via nitrification of high-strength ammonium landfill leachate is substrate inhibition, particularly in the form of free ammonia (FA) and free nitrous acid (FNA) in ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). The problem is more severe, as 43% of the landfills are not well designed and not properly equipped with leachate control mechanism facilities. In particular, this type of landfill exposed the river water to the risk of ammonium contamination from the landfill leachate. Therefore, there is an urgent need to improve the existing leachate management at landfills.

Prior to the nitrification study, leachate characteristics and the presence of inorganic nitrogen in the rivers receiving landfill leachate from three different types of landfills in Selangor state, Malaysia were assessed throughout a year to determine the impact of landfill leachate on river water chemistry. In response to the results of the water quality study, a nitrification-activated sludge system has been developed for high-strength ammonium synthetic wastewater, which serves as a reference before treatment with the actual landfill leachate. The system was operated under controlled conditions that favor nitrification and was started in the fed-batch mode of operation to prevent inhibitory effects of FA and FNA on nitrifiers. As the heterotrophs could also inhibit the nitrification performance, the organic carbon removal was monitored during the nitrification of mature landfill leachate. A molecular technique, fluorescence *in situ* hybridization (FISH), was used to identify both the microbial populations as well as the localization of the nitrifiers in the sludge floc complex community.

The background, scope and objectives of the study are described in the introduction, Chapter 1.

In Chapter 2, leachates from three different types of landfills, namely active uncontrolled, active controlled and closed controlled, were characterized, and their relationships with river water chemistry were examined each month for a year. The influence of leachate on river water chemistry from each type of landfill depended on many factors, including the presence of a leachate control mechanism, leachate characteristics, precipitation, surface run-off and the applied treatment. The impact of leachate from an active uncontrolled landfill was the highest, as the organic content, $N\text{-}NH_4^+$ Cd and Mn levels appeared high in the river. At the same time, influences of leachate were also observed from both types of controlled landfills in the form of inorganic nitrogen ($N\text{-}NH_4^+$, $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$) and heavy metals (Fe, Cr, Ni and Mn). Improper treatment practice led to high levels of some contaminants in the stream near the closed controlled landfill.

In Chapter 3, the feasibility of a nitrifying activated sludge system to completely nitrify synthetic mature landfill leachate with $N\text{-}NH_4^+$ concentration of 1452 mg/L was tested. The process started with a nitrogen loading rate (NLR) of 0.4 $kgN\text{-}NH_4^+/m^3/day$ in a fed-batch mode to avoid any accumulation of the FA and FNA in the system, and the NLR was subsequently gradually increased. Complete nitrification was achieved with a very high ammonium removal percentage (~100%). The maximum specific and volumetric nitrification rates obtained were 0.49 $gN\text{-}NH_4^+/g$ VSS/day and 3.0 $kgN\text{-}NH_4^+/m^3/day$, respectively, which were higher than those reported previously for ammonium-rich removal using an activated sludge system. The nitrifying sludge exhibited good settling characteristics of up to 36 mL/g VSS and a long solid retention time (SRT) of more than 53 days, which contributed to the success of the nitrification process. The coexistence and synthropic association of the AOB and NOB were observed using the FISH technique, which supported the results on complete nitrification obtained in the system. These findings would be of prominent importance for further treatment of actual landfill leachate.

In Chapter 4, nitrification of mature sanitary landfill leachate with high-strength $N\text{-}NH_4^+$ (1080-2350 mg/L) was performed in a 10 L continuous nitrification-activated

sludge reactor. During the entire period of study, dissolved oxygen and pH were maintained at a minimum of 2.0 mg/L and 7.4-7.6, respectively. The nitrification system was acclimatized with synthetic leachate for about 13 days before being fed with actual mature leachate. Successful nitrification was achieved with an approximately complete ammonium removal (99%) and a 96% conversion of $N\text{-}NH_4^+$ to $N\text{-}NO_3^-$. At the same time, BOD removal of 85 to 95% and COD removal of 38-57% were accomplished. The maximum volumetric and specific nitrification rates obtained were 2.56 $kgN\text{-}NH_4^+/m^3$/day and 0.23 g $N\text{-}NH_4^+$/g VSS/day, respectively, at a HRT of 12.7 h and SRT of 50 days. Incomplete nitrification of 3.14 kg $N\text{-}NH_4^+/m^3$/day with up to 460 mg/L of $N\text{-}NO_2^-$ built up in the system was encountered when operating at higher (NLRs). The inhibitory effect of FNA on nitrifiers rather than interspecies competition between heterotrophs and nitrifiers was believed to trigger the accumulation of $N\text{-}NO_2^-$. Results from FISH experiments, which revealed the disintegration of some AOB cell aggregates into single cells, further supported the inhibitory effect mentioned above. During the complete nitrification, the AOB and NOB were found in almost similar percentages, while the number of the AOB and NOB decreased and the heterotrophs dominated for the duration of the incomplete nitrification.

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Municipal solid waste as a source of nitrogen in landfill leachate

Nitrogen is an essential element required by all organisms. Although it is abundant on earth, only < 2% is accessible to living organisms. The rest is tied up either in the form of igneous and sedimentary rock (20%) or in the atmosphere (78%). Atmospheric nitrogen exists as triple-bonded N_2, which is the most stable form of nitrogen. High amounts of energy are required to break the triple bond through the process known as nitrogen fixation to make it available to living organisms. Naturally, the process is carried out by biological nitrogen fixation and atmospheric fixation through lightning. However, only a small portion of microorganisms is able to utilize nitrogen by biological nitrogen fixation. Hence, the available nitrogen for living organisms is mainly in the form of ammonia and nitrate. In living organisms, this inorganic nitrogen is either assimilated into amino acids in the form of structural proteins, globular proteins, conjugated proteins, or it is incorporated as nucleic acids.

With the growing human population, the demand for resources in the form of food and energy in particular has increased for survival and the improvement of quality of life. Because most of the food produced will end up as solid waste or sewage, the amount of bioavailable forms of nitrogen, such as ammonia and nitrate, is increased in the landfill. The existence of nitrogen in solid waste has important implications on the management of nitrogen in landfills. In a study on MSW composition in Kuala Lumpur City, a high amount of food waste was observed, which accounted for about 60% of the total waste (Kathirvale et al., 2003). The total nitrogen content analyzed from this MSW was 1.26% of the total solid content. In Malaysia, about 6 million tons of MSW is being produced annually, and 95% of the collected waste is disposed through landfill systems (Idris et al., 2004; MHLG, 2005). Thus, on the basis of total nitrogen measured (1.26%), about 0.07 million tons of nitrogen are disposed to landfills annually.

Landfills remain the most economic and attractive mean of waste disposal in Malaysia as a result of the increase in the amount of waste produced. The landfills have evolved from open dumps to the fully engineered sanitary landfills, with a distribution among them of about 43% and 6.3%, respectively. Generally, most of the landfills are operated under anaerobic conditions. During the anaerobic decomposition of waste, the organic carbon present in MSW is converted to carbon dioxide and methane. Meanwhile, the organic nitrogen, particularly in the form of protein, is hydrolyzed into simpler compounds, such as amino acids. The amino acids are then degraded to other substances, including volatile fatty acids, carbon dioxide, ammonium and hydrogen sulfide (Jokela and Rintala, 2003). Even though the anaerobic ammonium oxidation (anammox) pathway has been discovered, the oxidation of ammonium into dinitrogen gas requires nitrite as an electron acceptor under anoxic conditions (Strous et al., 1998). As no further degradation pathways are available for ammonium under anaerobic conditions, in the absence of nitrite, the ammonium concentration will remain high in the landfill leachate either as ammonia or ammonium ions.

1.1.2 The need to control nitrogen

The open dumps, which are still mostly observed, tend to pose environmental hazards to the ecosystem. Due to financial constraints, ex-mining ponds are often used to dispose waste in a manner in which very limited measures are taken to control operation, particularly in relation to the environmental impacts of landfills (i.e., without bottom liners and leachate collection systems). Therefore, leachate, which is generated by landfills and contains various types of pollutants, such as organic contents, inorganic components, heavy metals and xenobiotic organic compounds (Christensen et al., 2001), may enter the ground water or nearby river water. The ammonium that is part of the leachate constituent could possess a threat to aquatic ecosystems and consequently to human health. The problems encountered as a result of ammonium wastewater discharged into the rivers are as follows: (1) there is a decrease in oxygen concentration due to the oxidation of ammonium to nitrite and nitrate by nitrifiers, (2) more ammonia, which is toxic to some types of aquatic life, is produced since the chemical equilibrium of ammonia and ammonium is temperature- and pH-dependent, (3) eutrophication occurs, as ammonia and nitrate stimulates the growth of algae, (4) methemoglobinemia occurs among infants due to the high concentrations of nitrate and nitrite in drinking water, and (5) carcinogenic nitrosamines potentially form from nitrite (Wiesmann, 1994). For these reasons, ammonia/ammonium and nitrate have to be removed from landfill leachate before it is discharged into the river system.

The minimum permissible limit for the discharge of landfill leachate into inland waters is laid down by the Environmental Quality (Sewage and Industrial Effluents) Regulations, 1979. The N-NH_3 concentration imposed for sanitary landfills must be lower than 10 mg/L (Appendix A). With the stringent discharge limit imposed, the landfill operators have to seek a better solution for improvement of the existing leachate treatment system. This is in particular due to the high concentrations of N-NH_4^+ constituents in leachate, mainly from the mature landfills, rendering the treatment system with a low N-NH_4^+ removal efficiency. Biological systems, namely sequencing batch reactors (SBRs), are the most commonly used treatments for removing ammonium from landfill leachate. This biological method allows nitrification of ammonium to nitrite and subsequently to nitrate. The following process is denitrification, where nitrate is reduced to nitrogen gas under anoxic conditions. Since parameter of nitrate is not included in the landfill leachate discharge standard, elimination of inorganic nitrogen is focused only on ammonium. The direct biological methods, such as SBR, may not be able to completely remove high-strength ammonium as a result of substrate inhibition on the responsible microorganisms (nitrifiers). In response to this problem, nitrification with fed-batch and continuous modes of operation might be applicable as substrate concentrations can be controlled, thus reducing the inhibition effect on nitrifiers.

1.1.3 Nitrification

Nitrification is the two-step biological conversion of ammonia to nitrite and subsequently to nitrate under aerobic conditions. This biological conversion involves two important groups of bacteria: ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (WEF, 2005). The most known genera of AOB and NOB that carry out ammonia oxidation are *Nitrosomonas* and *Nitrobacter*, respectively (Ahn, 2006). However, other *Betaproteobacteria*, including *Nitrosococcus*, *Nitrosospira*, *Nitrosolobus* and *Nitrosovibrio,* are also able to oxidize ammonium to nitrite (Watson et al., 1989). Meanwhile, the *Nitrospira*, *Nitrospina* and *Nitrococcus* genera are involved in the nitrite oxidation step (Daims et al., 2001). The stoichiometric equations that define the oxidation of ammonium to nitrite and subsequently to nitrate by *Nitrosomonas* and *Nitrobacter* are shown in Equation 1 and Equation 2, respectively.

$$NH_4^+ + 1.5O_2 \xrightarrow{Nitrosomonas} NO_2^- + 2H^+ + H_2O \tag{1}$$

$$NO_2^- + 0.5O_2 \xrightarrow{Nitrobacter} NO_3^- \tag{2}$$

In contrast with heterotrophs, which use carbon sources from organic carbon compounds, the AOB and NOB are autotrophs, which take inorganic carbon sources from carbon dioxide. Hence, the nitrifiers are slow growers with biomass yields of about 0.06 to 0.20 g VSS/g N-NH_4^+ oxidized (WEF, 2005). The nitrification destroys alkalinity as a result of the hydrogen ions produced; thus, about 7.14 g of alkalinity (as $CaCO_3$)/g N-NH_4^+ is required for a sufficient process. However, severe pH decrease can occur due to the acid produced through nitrification. Therefore, an alkalinity additive, i.e., bicarbonate solution, is necessary to maintain an optimum pH of 7.5-8.0 for growth of nitrifiers. The nitrifiers are obligate aerobes, which are only able to function in the presence of oxygen. About 4.57 g O_2/ g N-NH_4^+ are used for this process. However, it is generally accepted that nitrification is not limited at a dissolved oxygen concentration higher than 2.0 mg/L (WEF, 2005). Temperature plays an important role in nitrification. Even though nitrification has been shown to occur in wastewater at temperatures between 4 and 45 °C, the optimum growth for nitrifiers is in the range of 25-30 °C (Watson et al., 1989). At this temperature range, the maximum growth rate of NOB is higher than that of the AOB thus, nitrite does not accumulate in the nitrification system. In contrast, at higher temperatures (30-40 °C), the AOB grows faster than the NOB, which results in the build-up of nitrite (Volcke, et al., 2006). The AOB and NOB are known to be susceptible to inhibition from various organic and inorganic compounds (Wiszniowski et al., 2006; WEF, 2005). However, the two most important and often discussed are un-ionized ammonia [free ammonia, NH_3, (FA)] and un-ionized nitrous acid [free nitrous acid, HNO_2, (FNA)] (Isaka, 2007; Kim et al., 2006; Anthonisen et al., 1976). The equilibrium of both ammonia and nitrous acid is pH-dependent (Equation 3 and Equation 4, respectively). At a higher pH, the concentration of FA increases, and about 10-150 mg/L and 0.1 to 1.0 mg/L of FA can inhibit AOB and NOB, respectively. In contrast, FNA concentration increases at lower pH, and the inhibition on both of the nitrifiers is initiated at an FNA level of 0.22 to 2.8 mg/L (Anthonisen et al., 1976).

$$NH_4^+ + OH^- \leftrightarrow NH_3 + H_2O \tag{3}$$

$$H^+ + NO_2^- \leftrightarrow HNO_2 \tag{4}$$

Besides the FA and FNA inhibition, the presence of biodegradable organic compounds also affects nitrification. High concentrations of organic substances in wastewater create competition for oxygen between heterotrophs and nitrifiers. Because of the higher growth rate of the former (4.8 day^{-1}), nitrifiers with growth rate of 0.54-1.08 day^{-1} are often out-competed (Sozen et al., 1998; Okabe at al., 1996). Therefore, a BOD:TKN less than 2 is usually required to maintain a stable nitrification (WEF, 2005).

1.1.4 Why nitrification for treatment of high-strength ammonium from landfill leachate?

Biological nitrogen removal through nitrification and denitrification is commonly practiced for removal of low ammonium-containing wastewater. Therefore, this method could be a potential method for treating high-strength ammonium from landfill leachate from the environmental and economical points-of-view. The nitrification-activated sludge system used may offer several advantages. One of these advantages includes, through the fed-batch mode of operation at the beginning of the process, the dilution of high concentrations of N-NH_4^+ in the aeration reactor, thus resulting in less of an inhibition effect on nitrification. This is because the liquid volume in the reactor will increase linearly with time as the N-NH_4^+ is added into the reactor. At the time when the biomass concentration is equal to the maximum biomass concentration and the dilution rate is less than μ_{max}, the substrate (N-NH_4^+) will be consumed as fast as it enters the system, and the input of the N-NH_4^+ into the reactor will be equal to the N-NH_4^+ consumption (Kargi et al., 2003). Second, the continuous feeding performed after the fed-batch mode of operation allows control of substrate (N-NH_4^+) loading into the reactor. Therefore, the maximum nitrification rate and the lowest hydraulic retention time (HRT) for the system can be obtained. Third, the higher solid retention time (SRT) of 50 days is chosen to provide sufficient growth for such slow growing nitrifiers. Fourth, the characteristics of mature landfill leachate with a lower BOD:TKN ratio may favor the growth of nitrifiers in the system. However, lower biodegradable compounds in leachate may affect the following treatment of denitrification. These operational and process improvements might increase ammonium removal efficiency as compared with the existing SBR ponding system. In addition, the low excess sludge that is generated by this process due to the slow growth of the nitrifiers may reduce the environmental and cost burdens of further disposal of sludge.

Besides the biological treatment, ammonium removal from landfill leachate can also be accomplished by physico-chemical methods. Due to the high level of N-NH_4^+ in mature landfill leachate, the physico-chemical technologies, such as air stripping, chemical precipitation, activated carbon adsorption, reverse osmosis and ion exchange, are often preferred either as individually or in combination with biological processes for high-strength ammonium removal from landfill leachate (Kurniawan et al., 2006). However, the physico-chemical approach is limited by high cost due to the expensive chemicals and adsorbents used. Furthermore, environmental impacts, i.e., the need of further disposal of the sludge from chemical precipitation and the release of NH_3 gas in ammonia stripping, should be taken into consideration. Therefore, the physico-chemical pre-treatment can be eliminated if nitrification is applied to remove high-strength ammonium from mature landfill leachate.

1.2 Scope of thesis

Prior to the nitrification study, landfill leachate characteristics and the presence of three important parameters in leachate, particularly the organic carbon, inorganic nitrogen and heavy metals in the rivers receiving landfill leachate, were investigated throughout a year. Three different types of landfills, namely active uncontrolled (landfills operated without leachate management facilities such as bottom liners, leachate collection and treatment systems), active controlled (fully engineered sanitary landfills with appropriate leachate management facilities) and closed controlled landfills in Selangor State, Malaysia were selected as the study areas. Characterization of leachate is important, as the concentrations of the substances in the leachate are dependent on the biochemical decomposition of the waste in landfills. Young leachate mainly in the acidogenic phase has large amounts of degradable organic substances and heavy metals. Mature landfill leachate undergoes the methanogenic phase, which is normally characterized by a lower BOD:COD ratio and heavy metals but with a high concentration of $N\text{-}NH_4^+$.

By monitoring these substances in the rivers, both downstream and upstream, in the vicinity of the landfills, the current status of the river water quality due to the point sources of pollution from landfill leachate can be determined. As very limited data on the impact of leachate, particularly in the form of inorganic nitrogen from controlled and uncontrolled landfills, on river water can be obtained for Malaysia, this information is useful for landfill management purposes, especially to improve the existing leachate treatment system, primarily for ammonium removal.

In response to the results obtained through the water quality study, nitrification of high-strength ammonium of synthetic leachate, which mimicked to those of the mature landfill leachate (closed controlled landfill), was conducted using a nitrification-activated sludge system under controlled pH and dissolved oxygen conditions. This study served as a reference prior to treatment of actual mature landfill leachate. The nitrification acclimatization during the fed-batch operation was well studied by determination of the substrate inhibition effect, particularly the FA and FNA, using the HNO_2 and $N\text{-}NH_3$

equilibrium relationships from Anthonisen et al. (1976). During the entire periods of study, nitrification performance and biomass changes of the system were monitored. The maximum volumetric and specific nitrification rates, biomass yield, HRT and SRT were determined for the system.

The feasibility study for obtaining complete nitrification of high-strength ammonium from mature landfill leachate was performed using a similar system as used for the synthetic leachate. The nitrification system was acclimatized with synthetic leachate prior to the treatment of actual landfill leachate. Throughout the study, nitrification performance as well as the organic carbon removal (BOD and COD) were monitored. As a comparison, the performance parameters obtained, including the maximum volumetric and specific nitrification rates, biomass yield, and HRT, were compared with those derived from the study using synthetic leachate and the other study using high-strength ammonium wastewater. To obtain a better understanding of the co-existence of the AOB, NOB and heterotrophs in the system, culture-based (MPN and plate count) and molecular (fluorescence *in situ* hybridization (FISH)) techniques were performed. The 16S rRNA-targeted oligonucleotide probes were used for identifying almost all of the total bacteria, ammonia-oxidizing betaproteobacteria and nitrite-oxidizing bacteria.

1.3 Objectives

The objectives of this research are as follows:

1) To determine the presence of inorganic nitrogen, organic carbon and heavy metals in rivers receiving leachate from controlled and uncontrolled municipal solid waste landfills.
2) To determine the feasibility of obtaining complete nitrification of high-strength ammonium from mature landfill leachate.
3) To identify microbial population contributions to the nitrification of high-strength ammonium using the fluorescence *in situ* hybridization (FISH) technique.