The Effect of Landfill Leachate on Nitrification Inhibition of Activated Sludge

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Water and Environmental Engineering

Master's Project

STUDENT REPORT

AALBORG UNIVERSITY

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Master's thesis in Water and Environmental Engineering

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ABSTRACT

While increasing complex wastewater produced in modern society, the technology of treatment is demanded improvement to be suitable sustainable development, especially in nitrification processes, which plays an important role in wastewater treatment plant. However, microorganism, nitrification process relied, is sensitive to some toxicant affecting quality of outlet water. The main objective of this study is collection and conclusion of literature about NI (nitrification inhibition), and controlled experiment on landfill leachate causing NI degree by assay type - ammonia conversion rate. The controlled experiment on ATU (N-Allylthiourea) is to prove the effectivity of this assay method. In literature review, it's considered sources of NI, assay types and nitrifier resistance. They showed this batch of landfill leachate couldn't cause NI. It's suggestion that classification and limitation of wastewater sources guarantee security of microorganism.

I takt med at den komplekse spildevandsproduktion i det moderne samfund stiger, kræves der forbedringer af behandlingsteknologien for at sikre bæredygtig udvikling, især inden for nitrifikationsprocesser, som spiller en vigtig rolle i spildevandsrensningsanlæg. Imidlertid er mikroorganismer, som er baseret på nitrifikationsprocessen, følsomme over for visse giftige stoffer, der påvirker kvaliteten af udløbsvandet. Hovedformålet med denne undersøgelse er at indsamle og konkludere litteratur om NI (nitrifikationshæmning) og kontrollerede eksperimenter med lossepladsperkolat, der forårsager NI-graden, efter analysetype - ammoniakkonverteringshastighed. Det kontrollerede eksperiment på ATU (N-Allylthiourea) har til formål at bevise effektiviteten af denne analysemetode. I litteraturgennemgangen er kilder til NI, analysetyper og nitrifierresistens overvejet. De viste, at denne batch af lossepladsperkolat ikke kunne forårsage NI. Det foreslås, at klassificering og begrænsning af spildevandskilder garanterer mikroorganismernes sikkerhed.

ACKNOWLEDGEMENTS

This is my major master project created from October 1^{th} 2024 to June 1^{th} 2025. The project main theme is about nitrification inhibition in wastewater bio-treatment. This project includes two major part: literature review and controlled experiments. The literature review is summarized abundant results from studies about nitrification inhibition. In controlled experiments, landfill leachate and ATU (N-Allylthiourea) are as toxicant.

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ABBREVIATIONS

List of all abbreviations in alphabetic order:

- **Abs** Wavelength absorption by spectrophotometer
- **AOB** Ammonia oxidizing bacteria
- **ATU** Chemical, N-Allylthiourea
- **AUR** Ammonia utilization rate
- **BOD_5** Biochemical oxygen demand over 5 days
- **COD** Chemical oxygen demand
- **CSTR** Continuous flow stirred reactors
- **EC** Effective concentration for nitrification inhibition
- **EC_{50}** Effective concentration of 50% nitrification inhibition
- **MLSS** Mixed liquor suspended solids
- **NI** Nitrification inhibition
- **NOB** Nitrite oxidizing bacteria
- **PNS** Potassium nitrate solution
- **STD** Standard deviation
- **TKN** Total Kjeldahl nitrogen
- **TN** Total nitrogen
- **TSS** Total suspended solid
- **WWTPs** Wastewater treatment plants

CHAPTER

ONE

INTRODUCTION

1.1 Background

1.1.1 Wastewater influences human society

Wastewater is an inseparable part of our daily lives, yet it is often ignored by the public. Its unpleasant odor and murky appearance are often avoided by people without second thought. However, a few people realize that the huge efforts are required to transport and treat wastewater.

The wastewater contains a lot of harmful substances, such as bacteria, virus, toxic organic matters and heavy metals etc [Henze et al., 2008]. While the environment has an own ability to recover, excessive untreated wastewater, especially from industrial sources, can overwhelm this resilience. In severe cases, it leads to ecological collapse, mass wildlife deaths, and even contamination of drinking water supplies. The damage exceeds the environmental recover ability. Airborne viruses from wastewater can infiltrate the human respiratory system, causing disease outbreaks. There are a lot of real global disasters serving as grim reminders. For example, severe eutrophication in Taihu, Zhejiang, resulted from industrial wastewater discharge, contaminating the region's raw potable water in 2007. In 1956, Japan witnessed the devastating Minamata disease outbreak, killing over 900 people due to methylmercury pollution from a chemical factory[1].

Therefore, ignoring wastewater is no longer an option in the society. It's a ticking time bomb that threatens environmental sustainability and public healthy. Urgent action is needed to globally enforce and enhance stricter regulations of wastewater discharge with rapid development of industry. Moreover, sustainable wastewater management and treatment processes are needed to be globally promoted, which is crucial to preventing further irreversible damage.

[1] https://www.env.go.jp/en/chemi/hs/minamata2002/summary.html

1.1.2 Characteristics of various types of wastewater

Types of wastewater can be classified by source as stormwater runoff, municipal wastewater, industrial wastewater, agricultural wastewater and landfill leachate [Vymazal, 2009; Henze et al., 2008]. The level of pollution in wastewater is often characterized by two key indicators: BOD_5 (biochemical oxygen demand over five days) and COD (chemical oxygen demand) [Zarka et al., 2014; Sikosana et al., 2019]. BOD_5 and COD are determined the difficulty of treatment and varied depending on waste water types, which are crucial for implementing effective treatment solutions and preventing irreversible environmental damage.

While often perceived as harmless, stormwater runoff carries pollutants from urban surfaces into natural waterways. Though it contains fewer harmful substances, improper management can still contribute to water contamination. Some studies recommended that retention ponds can effectively treat stormwater runoff [Tixier et al., 2011; Ayub et al., 2005].

Municipal wastewater is loaded with solids and nutrients, achieving its treatment essential for good quality in receiving waters and for potential reuse [Dai et al., 2024]. Some studies showed the range of BOD_5 is from 200 to 600mg/L and the ratio of BOD_5/COD is from 0.5 to 1 in raw municipal wastewater generally [Zarka et al., 2014; Henze et al., 2008]. This range of ratio is indicated municipal wastewater can be treated by biological treatment easily.

Industrial wastewater can be one of the most hazardous types due to its highly variable and often toxic composition. The ranges of BOD_5 and COD vary significantly depending on the type of industrial wastewater. Some industrial wastewater requires specialized pretreatment before integration with municipal wastewater systems, when COD level is high - sometimes exceeding 5000mg/L [Dai et al., 2024; Paśmionka et al., 2022]. Some untreated industrial discharge with hazardous toxicity would lead to severe environmental degradation, long-term ecosystem damage, and even human health crises.

Agricultural wastewater contains harmful substances or conditions such as high levels of COD, salinity, and ammonia [Dai et al., 2024]. Yang et al. (2022) said that raw livestock wastewater can have COD concentrations ranging from 2,143 to 4,580 mg/L and ammonia levels between 556 and 950 mg/L. One of the most alarming issues is the presence of massive amounts of antibiotics, which disrupt bacterial ecosystems, encourage drug-resistant bacteria, and pose a direct threat to environmental balance. In Europe, antibiotic residues in treated wastewater exceed safety limits [Rodriguez-Mozaz et al., 2020]. Additionally, agricultural wastewater contains harmful material including heavy metals, residual pharmaceuticals and pesticide residues.

Among all wastewater types, landfill leachate is unpredictable and difficult to treat due to its extreme variations in composition. It contains COD levels ranging from 440 to 10,750 mg/L, BOD_5 between 50 and 6,380 mg/L, ammonia concentrations of 415 to 3,000 mg/L, and a pH range of 6.5 to 8.5 [Paśmionka et al., 2022; Y. M. Kim et al., 2008; L. Wang et al., 2014; Aktaş et al., 2001]. The fluctuating quality of landfill leachate would cause significant environmental hazard. Therefore, it demands advanced treatment technologies to prevent groundwater and soil contamination. The details of landfill leachate are referred Subsection 1.1.5.

1.1.3 Development of wastewater treatment plant

Before 1800s, people discharge wastewater without any sewer system. At that time, the environment could handle a limited amount of contaminants due to the low level of economic activity. However, this also caused discomfort and sanitation issues. In the 1800s, simple wastewater collection systems began to appear in Europe, marking the end of the "Sanitary Dark Ages" [Gray, 2004; Riffat, 2012b]. The primary goal of these early sewage systems aimed to prevent the spread of viruses and bacteria that threatened human health.

As wastewater has become more complex and harmful, simple collection systems have evolved into extensive networks and large-scale treatment facilities to keep pace with rapid societal development. Some countries have even implemented policies requiring the reuse of treated wastewater for farmland irrigation and construction. The first WWTP (wastewater treatment plant) was Blackburn Meadows WWTP, built in South Yorkshire in 1886, as part of efforts to prevent a cholera pandemic at the time[1]. With the development of technology, some WWTPs aim to reuse treated wastewater. For example, treated wastewater was reused for irrigation in Israel reaching 90% [2]. In Europe, LIFE projects, aiming to zero pollution, are promoting wastewater regeneration used to irrigation [3]. Meanwhile, Singapore has adopted advanced technologies in wastewater treatment, achieving 40% water reuse in non-potable applications[4].

Wastewater treatment methods can be categorized into physical, biological, and chemical treatments [Ahmed et al., 2021; Tchobanoglous et al., 2014a]. Fig.1.1.1 presented general processes in WWTP.

Physical treatment

Physical treatment in wastewater treatment can remove solid, colloidal particles and a significant portion of BOD through processes such as screening and sedimentation. It includes screening, sedimentation, flotation, filtration and absorption etc. [Tchobanoglous et al., 2014a; Riffat, 2012b]. In a WWTP, screening and

[1]https://www.shepherdgilmour.co.uk/projects/blackburn-meadows-waste-water-treatment-works

[2]https://www.fluencecorp.com/israel-leads-world-in-water-recycling/

[3]https://cinea.ec.europa.eu/news-events/news/bringing-urban-wastewater-back-life-2025-01-24_en

[4]https://www.voanews.com/a/east-asia-pacific_singapore-turns-sewage-clean-drinkable-water-meeting-40-demand/6209374.html

primary sedimentation are positioned at the beginning of the process, which can lower the oxygen demand in the downstream biological process tanks. Flocculation, filtration, and flotation are applied in advanced treatment processes after biological treatment to further improve the quality of the effluent. Moreover, UV disinfection, which uses radiation to prevent viruses and microorganisms from reproducing, is sometimes used at the final stage of the treatment process.

Chemical treatment

Chemical treatment includes processes such as pH adjustment, precipitation, coagulation, disinfection with specific chemical [Tchobanoglous et al., 2014a; Riffat, 2012b]. The precipitation process is applied in some types of industrial wastewater containing abundant metals generally [Tchobanoglous et al., 2014c]. The coagulation process is applied in advanced treatment for potable reuse to further reduce turbidity. The precipitation and coagulation processes can be combined with sedimentation process. Activated carbon adsorption is used to remove persistent organic and inorganic compounds to achieve reuse quality of wastewater [Riffat, 2012c]. Disinfection process is applied with oxidant such as chlorine, hypochlorite and ozone near the outlet.

Biological treatment

Biological treatment is used to remove degradable organics and nutrients, such as nitrogen and phosphorus by microorganism. It's located between primary physical treatment and secondary clarifier generally. There are two types of biological treatment classified on microorganism growth: suspended growth process and attached growth process [Tchobanoglous et al., 2014b; Riffat, 2012a]. The most common suspended growth process is the activated sludge process, which offers flexibility in adjusting wastewater loading. However, it requires integration with sludge treatment. Attached growth process, on the other hand, generates less excess sludge than suspended growth process but requires more maintenance due to biofilm clogging. The biofilm process is a typical example of attached growth process.

Moreover, biological treatment is classified on oxygen existence: aerobic process, anoxic process and anaerobic process [Tchobanoglous et al., 2014b]. They can be applied in both types of microorganism growth. In domestic wastewater biological treatment, the main targets are nitrogen and phosphorus removal. The anoxic-aerobic process (Fig.1.1.2 A.), arranged anoxic and aerobic zones sequentially, is effective on nitrogen removal, since it can achieve nitrification and denitrification [Riffat, 2012c]. The anaerobic-aerobic process (Fig.1.1.2 B.) is effective on phosphorous removal. The anaerobic-anoxic-oxic process (Fig.1.1.3), arranged anaerobic, anoxic and aerobic zones sequentially, is applied in nitrogen and phosphorous removal [Riffat, 2012c].

Biological treatment can be used to treat industrial wastewater with high COD. Some studies have shown that aerobic systems are suitable for wastewater with COD levels below 1,000 mg/L, while anaerobic systems are more effective for COD levels exceeding 4,000 mg/L [Sikosana et al., 2019]. Aerobic fluidized bed and up-flow anaerobic sludge bed are commonly used in treating industrial wastewater [He et al., 2022; Sikosana et al., 2019]. These system utilize fluidized bed technology which is an advanced form of attached growth process. The hydraulic surface loading of fluidized bed reactor can exceed 1,000 $m^3/(m^2 \bullet h)$, significantly outperforming conventional attached growth process systems. This high efficiency makes fluidized bed reactor particularly effective for treating high COD wastewater [Bello et al., 2017; Nelson et al., 2017; Sridang et al., 2024].

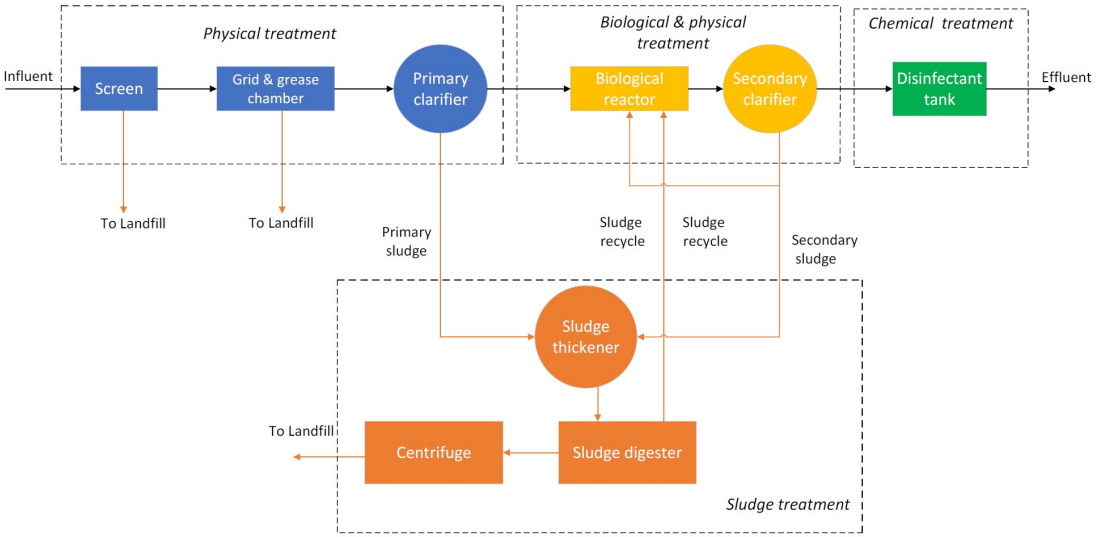

Fig. 1.1.1. The flow chart of general wastewater treatment in WWTP (black arrow: wastewater flow; brown arrow: sludge flow)

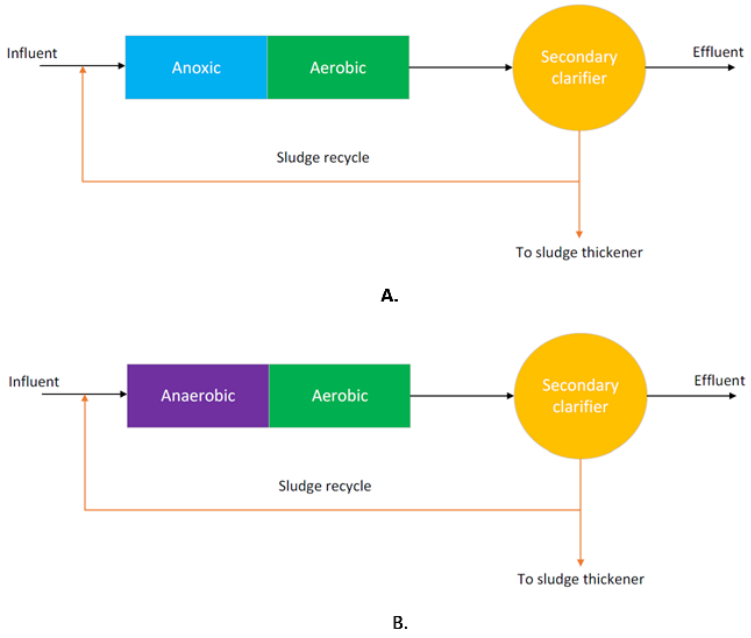

Fig. 1.1.2. The flow chart of anoxic-aerobic process and anaerobic-aerobic process (A. Anoxic-aerobic process; B. Anaerobic-aerobic process; black arrow: wastewater flow; brown arrow: sludge flow)

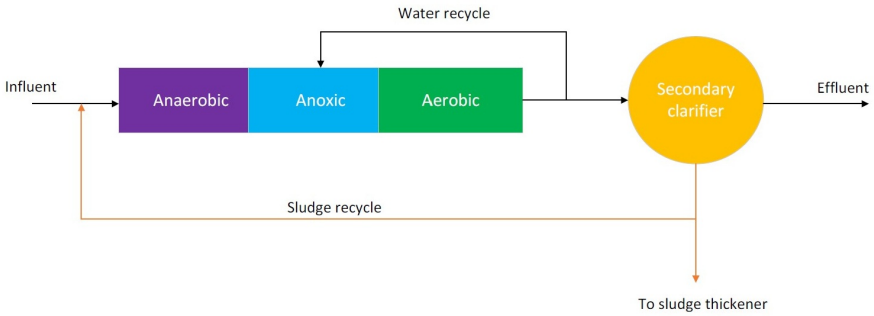

Fig. 1.1.3. The flow chart of anaerobic-anoxic-oxic process (black arrow: wastewater flow; brown arrow: sludge flow)

1.1.4 Necessity of biological treatment process

Harmful impacts of nutrients discharge

Nitrogen and phosphorous are major components of most wastewater. Wastewater with high nitrogen and phosphorus content but low turbidity can still be harmful to the environment when discharged. Eutrophication occurs when a water body becomes overly enriched with nutrients [Art, 1993], often leading to excessive growth of algae [Diatta et al., 2020; Liang et al., 2024]. With eutrophication and sunlight, phytoplankton reproduces and consumes oxygen in the water. As a result, hypoxic conditions develop, causing phytoplankton to die in the middle layers of the water body. As dead phytoplankton decomposing, they release organic matter that consumes oxygen in the water [Akinnawo et al., 2023; Masram et al., 2020; Ngatia et al., 2019]. Therefore, severe eutrophication leads to a decline in fish and aquatic life, followed by the production of harmful substances. This zone is called dead zone. Even though, the study reported that eutrophication significantly increases greenhouse gas emission, as methane forming under anaerobic conditions and leading high contents of organic matter [Yi Li et al., 2021]. Numerous cases of eutrophication disasters have been reported worldwide, and their occurrence increasing over time continuously [Glibert et al., 2005]. In the United States, eutrophication in the Mississippi River has created a dead zone in the Gulf of Mexico[1]. In Yunnan Province, China, eutrophication in the Nine Large Lakes has affected drinking water sources [Zhang et al., 2023]. The famous large lakes are eutrophication in the world such as Lake Erie, Lake Victoria and Tai Lake[2].

Effective nitrogen and phosphorus treatment in wastewater is crucial to protect environment. Phosphorus removal can be achieved by biological and chemical treatment. However, nitrogen removal can only be achieved through biological treatment alone. This is the reason why biological treatment processes is a crucial part of WWTP.

Essential of maintaining microbial active

Maintaining healthy microbial populations is crucial for biological treatment operation and ensuring high quality effluent. However, this is not an easy task, as microorganisms are highly sensitive, fragile, and difficult to control. Cases of complete bacterial die-offs have occurred due to inflow of toxic substances to the WWTP. Numerous factors can lead to microorganism inactivation or death. Except severe toxicity, the certain types of wastewater, such as high COD, low nutrients or extreme temperature etc., can cause microorganism inactivation. Therefore, adjusting the carbon source content is essential.

[1] https://serc.carleton.edu/integrate/teaching_materials/food_supply/student_materials/1113

[2] https://www.wri.org/research/eutrophication-sources-and-drivers-nutrient-pollution

One parameter for assessing wastewater biodegradability is the BOD_5/COD ratio. A ratio greater than 0.3 indicates that the wastewater is biodegradable, while a lower ratio suggests otherwise. Moreover, the content of volatile fat acid and humic and fulvic acid serves as an indicator of the biodegradability of organic matter. These acids are production of solid decomposition generally. A high volatile fat acid content suggests that the organic matter is easily biodegradable, whereas a high humic and fulvic acid content indicates that it is more resistant to biodegradation. Therefore, monitoring and analyzing the qualities of raw wastewater are keys to determine its biodegradability.

Nitrification is a crucial process in WWTP

The nitrification and denitrification processes are essential for nitrogen removal. Nitrification is the first step and serves as the foundation for denitrification. Therefore, nitrification plays an important role in biological treatment processes. Nitrifiers are the initial agents of nitrification process. Providing a safe and suitable environment for nitrifiers is key to maintaining their activity. NI (nitrification inhibition) can cause nitrifiers to lose their activity or even die. NI would cause several serious issues in WWTP. When partial nitrifiers become inactive, the treatment capacity of the WWTP decreases, resulting in exceeding discharge permits. When wastewater treatment fails, large amounts of ammonia and viruses can be released into receiving waters, potentially causing serious diseases in humans [Pijnacker et al., 2024; Berg et al., 2023]. Moreover, over ammonia and organic matter accumulates in the effluent causing environmental harm [Trávníček et al., 2022]. Therefore, maintaining healthy and active nitrifiers can prevent numerous serious incidents.

NI has been extensively studied in wastewater researches for many years, and its achievements have been applied in engineering. Research on NI has evolved from simple observational studies to deep investigations of microbial genes and protein characteristics, providing a more detailed and complex understanding of its sources and mechanisms.

1.1.5 Characteristics of landfill leachate

When rainwater passes through solid waste in a landfill, landfill leachate is generated and collected through the landfill collection system, as shown in Fig.1.1.4. Landfill leachate is considered a highly toxic liquid threatening public health and demand treatment as domestic wastewater. In some cases, specialized treatment is required. In England, leachate from 17 landfills has been identified as potentially carcinogenic, posing a threat to drinking water sources[1].

Municipal solid waste landfill leachate can be classified on its composition [Carey et al., 2013] or, more commonly, by its age. The leachate is typically categorized into three age groups: recent leachate (less than 5 years old), intermediate leachate (5 to 10 years old), old leachate (more than 10 years old) [Mojiri et al., 2021; Renou et al., 2008; K. Wang et al., 2018]. The composition of leachate changes over time and can be categorized into four phases: aerobic phase (a few days to weeks), acidogenic phase (a few months to 5 years), methanogenic phase (5 to 30 years), and stabilization phase (30 to 100 years) [Kylefors et al., 1997; Kjeldsen et al., 2002].

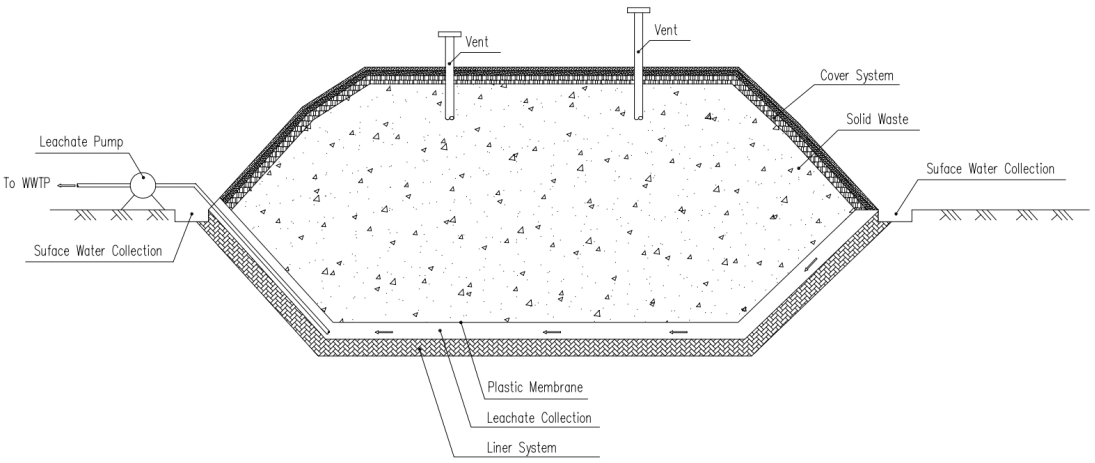

Fig. 1.1.4. Sectional drawing of landfill

[1]https://www.theguardian.com/environment/2024/feb/01/seventeen-landfills-in-england-make-toxic-liquid-hazardous-to-drinking-water

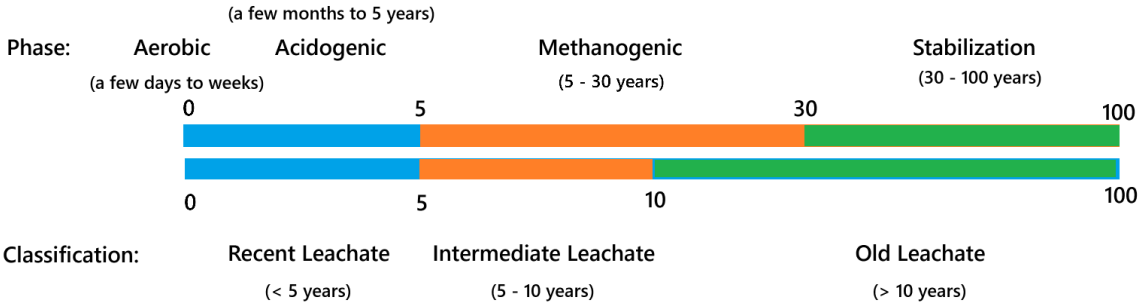

Fig. 1.1.5. The time axis about phase and classification of landfill leachate

The characteristics of four phases are significantly different. In aerobic phase, leachate is rich in oxygen and supporting aerobic microbial activity. In acidogenic phase, large amounts of organic matter and metals are released, causing decreasing in pH and increasing in VFA (volatile fat acid) and organic matter. In methanogenic phase, VFA is converted into methane and carbon dioxide, leading to a rise in pH until stable. In stabilization phase, leachate becomes more stable, with slow degradation and reduced pollutant levels [Kylefors et al., 1997; Kjeldsen et al., 2002].

The classifications of municipal solid waste landfill leachate by ages are strongly different in biodegradability. Although the COD of leachate decreases by its ages, the BOD_5/COD ratio also declines. Studies indicate that in old leachate, the BOD_5/COD ratio ranges from 0.1 to 0.3, while in recent leachate, it exceeds 0.3 [Renou et al., 2008; Kjeldsen et al., 2002; Tonjes et al., 2013; K. Wang et al., 2018]. Additionally, the contents of VFA and HFA (humic and fulvic acid) varies with leachate age. Recent leachate contains approximately 80% VFA, intermediate leachate contains 5–30% VFA, and old leachate consists of 80% HFA [Mojiri et al., 2021; Renou et al., 2008]. These findings are confirmed that the difficulty of treating landfill leachate is increasing by it ages.

The toxic components in municipal solid waste landfill leachate include organic substances and inorganic substances. Construction and demolition landfill leachate is characterized by a high pH (above 6.5) and contains abundant metal ions such as Fe, Zn, Al, and As [Mocová et al., 2019; Weber et al., 2002]. The main treatment methods are physical, chemical, and biological processes. Biological treatment is effective for recent leachate. However, for intermediate and old leachate with low BOD_5, biological treatments alone is insufficient. Instead, a combination of physical and chemical treatments is required. Several studies have focused on treating low biodegradability leachate. Abdel-Shafy et al. (2024) recommended Fenton process, a chemical process effective in increasing BOD_5/COD ratio. Sossou et al. (2024) suggested using coagulation and flocculation before biological treatment to reduce toxicity. Renou et al. (2008) compared various treatment methods and recommended advanced physical treatments such as nanofiltration and reverse osmosis. J. Wang et al. (2024) also recommended advanced physical treatments, including reverse osmosis, nanofiltration, and ultrafiltration, which can remove up to 99% of contaminants.

1.2 Problem statements and objectives

Environmental pollution is a serious issue in modern life. The discharge of untreated wastewater contributes significantly to pollution, particularly due to the presence of toxic substances. This pollution disrupts ecosystem balance and poses risks to human health. To support sustainable societal development, wastewater treatment is essential. As a result, various types of WWTPs have been constructed around urban areas.

The biological treatment process plays a crucial role in nutrient removal, directly affecting effluent quality. Microorganisms, particularly nitrifiers, are essential for this process and are among the most important bacteria in wastewater treatment. However, nitrifiers are at the same time the most sensitive group of organism in a treatment plant. Besides, excessive toxicity in raw wastewater can inactivate or even kill microorganisms. The landfill leachate is considered as one of serious toxicants. When nitrifiers lose their activity, ammonia accumulates in the effluent. Therefore, studying the causes and mechanisms of NI is a critical for nitrifiers protection. Moreover, it has significance for raising the levels of application and operation in WWTPs.

This project aims to explore the following key questions:

- What are the biological mechanisms behind NI?
- Which are specific substances in wastewater cause NI?
- What methods are used to assess NI in activated sludge from aerator?
- What are the resistance of nitrifiers and their applications?
- What methods can be used to confirm that NI is caused by landfill leachate?
- What methods are available to assess or verify NI?

The objectives of this research is to determine whether landfill leachate causes nitrification inhibition and analyze experimental results. To achieve this objectives, it is essential to demonstrate the effectiveness of the method. Additionally, literature review of NI theory is significant for results analysis.

CHAPTER

TWO

LITERATURE REVIEW

2.1 Mechanisms of nitrification inhibition

The nitrification process converts ammonia into nitrate, but it does not occur in a single step. Instead, it consists of two sequential stages:

- Ammonium is oxidized to nitrite:

$$NH_4^+ + \frac{3}{2}O_2 \rightarrow NO_2^- + H_2O \tag{2.1}$$

- Nitrite is oxidized to nitrate:

$$NO_2^- + \frac{1}{2}O_2 \rightarrow NO_3^- \tag{2.2}$$

Stoichiometrically, the oxygen demand for two steps are 3.43 mgO_2/mgN and 1.14 mgO_2/mgN respectively. Both steps depend on the activity of specific microorganisms. The first step of nitrification is carried out by AOB (ammonia oxidizing bacteria), while the second step is performed by NOB (nitrite oxidizing bacteria). Table 2.1.1 lists the gene names and groups associated with these bacteria. This kind of nitrification process belongs autotrophic nitrification [Prosser, 2007; Gao et al., 2023].

Table 2.1.1: List of AOB and NOB

AOB	Phylogenetic Group	NOB	Phylogenetic Group
Nitrosomonas	Beta	*Nitrobacter*	Alpha
Nitrosococcus	Gamma	*Nitrospina*	Delta
Nitrosospira	Beta	*Nitrococcus*	Gamma
		Nitrospira	Nitrospirota
		Nitrolancea	Chloroflexi

The stage at which NI occurs can be identified through process kinetics. Numerous studies have examined substances that cause NI at each step of the process [Hockenbury et al., 1977; Faust et al., 2023; Lee et al., 1997]. If NI occurs in the first step, ammonia levels remain unchanged, and neither nitrite nor nitrate concentrations increase. This means that inhibition in the first step will also affect the second step. Conversely, if NI occurs in the second step, ammonia decreases, nitrite increases but nitrate does not increase, indicating that the nitrification process is halted at this stage.

There are two types of NI: reversible NI and irreversible NI. In reversible NI, enzymatic activity can be restored once the inhibitors are removed. This type of inhibition can be further categorized as competitive inhibition, noncompetitive inhibition, and anticompetitive inhibition, based on the relationship between NI severity and substrate concentration [Orozco et al., 2008; Beg et al., 1983]. It's summarized these classifications as following contents:

- Competitive inhibition: NI decreases as substrate concentration increases.
- Anticompetitive inhibition: NI increases as substrate concentration increases.
- Noncompetitive inhibition: NI remains unaffected by substrate concentration.

Irreversible NI occurs when enzymes become denatured. For example, McCarty et al. (1999) found that oxidized acetylenes can bind to enzymes, leading to irreversible NI. This type of inhibition is commonly applied in agriculture to retain fertilizer in the soil [Garrido et al., 2000; R. Liu et al., 2017]. Since irreversible NI is not the focus of this study, the discussion will be limited to reversible NI.

2.2 Conditions causing nitrification inhibition

Many factors can cause nitrification inhibition (NI), including excessive concentrations of heavy metals and specific organic compounds, high salinity, and deviations from the optimal pH and temperature range. Numerous studies have identified NI inducing concentrations through extensive experimentation. Their findings provide valuable guidance for establishing local wastewater discharge standards.

A commonly used metric to assess NI is EC_{50}, which represents the effective concentration at which 50% inhibition occurs. In generally, this inhibition is reversible. A lower EC_{50} indicates higher toxicity. However, EC_{50} values can vary significantly across different studies. This variability doesn't imply that the results are inaccurate; rather, it reflects the unpredictability and adaptability of microorganisms (see Subsection 2.5). Other factors influencing EC_{50} results include differences in assay methods (see Subsection 2.4), reactor types, sludge sources, and temperature or pH conditions.

2.2.1 Heavy metal ions

It's well known that heavy metals have harmful effects on microorganism. Most of heavy metal ions in raw wastewater come from metal manufacturing. Despite strict regulations and standards in some countries, completely eliminating heavy metal contamination is difficult. Table 2.2.2 summarizes studies on NI causing by metals.

A careful comparison of different studies reveals significant variations in EC_{50} values. For example, Juliastuti et al. (2003a) and Juliastuti et al. (2003b) reported EC_{50}s of Cu^{2+} are below 1mg/L, whereas Gernaey et al. (1997) found EC_{50} is 173mg/L. These discrepancies highlight the complexity of microbial responses to heavy metals. When ignoring exact concentration and only focusing on ranking, the following results are concluded:

- Cu^{2+} is more toxic than Zn^{2+} [Juliastuti et al., 2003a; Juliastuti et al., 2003b; Madoni et al., 1999; Çeçen et al., 2010b; Shi et al., 2024].
- Cu^{2+} is more toxic than Ni^{2+} [Lee et al. (1997); Shi et al. (2024)], but Çeçen et al. (2010b) reported the opposite.
- Cd^{2+} is more toxic than Cu^{2+} [Lee et al., 1997; Madoni et al., 1999].
- Zn^{2+} is more toxic than Cd^{2+} and Ni^{2+} [Hu et al., 2004].

Metals with EC_{50}s values below 1mg/L include Cu, Zn, and Ag [Juliastuti et al., 2003a; Juliastuti et al., 2003b; Çeçen et al., 2010a]. Pb does not reach 50% NI, so its EC_{50} cannot be determined, but it can be absorbed by bacteria [You et al., 2009; Çeçen et al., 2010a]. In summary, excluding radioactive elements, the heavy metals most responsible for NI are Ag, Hg, Cd, Cu, Ni, and Zn. Table 2.2.1 presents typical concentrations of metals in raw wastewater. In domestic wastewater, Fe, Cu and Zn are the most prevalent.

Table 2.2.1: Typical metals concentrations in raw wastewater (unit: mg/L)

Ref. Type	Gray (2004) Domestic	Sörme et al. (2002) Domestic	Gray (2004) Run off
Fe	0.15-1.3	-	5-440
Cu	0-0.88	0.078	0.007-2.55
Cd	0-0.05	0.00023	-
Cr	0-0.4	0.004	0.018-1
Mn	0.01-0.2	-	-
Zn	0.05-0.84	0.15	1-15
Ni	0-0.33	0.0062	0.02-1.5
Co	0-0.02	-	-
Pb	0.01-1.78	0.0036	-
Hg	-	0.0001	0.029

Table 2.2.2: The summaries of studies about metal causing NI

Reference	Assay Type	Summary
You et al., 2009	AUR	The study compared NI caused by Ni, Cd and Pb in activated sludge from two processes SBR and A_2O. The decreasing order of toxicity is Ni >Cd >Pb in both processes. EC_{80} of Ni is 5 mg/L in A_2O process.
Juliastuti et al., 2003a Juliastuti et al., 2003b	OUR	The study showed EC_{50} of Zn^{2+} and Cu^{2+} are 0.35-0.5 mg/L and 0.08-0.1 mg/L respectively in batch reactor. The toxicity of Cu^{2+} is higher than Zn^{2+}.
Radniecki et al., 2011	AUR	The study said EC_{50} of Ag^{+} is 0.008 mg/L in nitrification inhibition of *Nitrosomonas europaea*.
Lee et al., 1997	AUR	The study compared NI caused by Cu and Ni in CSTR. The toxicity of Cu is higher than Ni. The concentration of Cu start IN at 30 mg/L.
Gernaey et al., 1997	OUR	The study showed EC_{50} of Cu^{2+} and Cd^{2+} are 173 mg/L and 8.3 mg/L respectively. The toxicity of Cd^{2+} is higher than Cu^{2+}.
Hu et al., 2004	OUR	The study compared NI caused by Zn, Ni and Cd in two processes batch equilibrium and continuous flow reactor. There is no obvious difference in continuous flow reactor. But in batch equilibrium, the decreasing NI order is Zn >Cd >Ni.
Madoni et al., 1999	AUR & OUR	The study showed toxicity decreasing orders: Cd >Cu >Zn and Pb >Cr. Cr and Zn are similar on toxicity in measuring by both.
Insel et al., 2006	OUR	The study showed EC_{50} of Cr^{6+} and Ni^{2+} are 60 mg/L and 33 mg/L respectively. The toxicity of Ni^{2+} is higher than Cr^{6+}.
Çeçen et al., 2010b	OUR & CO_2 production	The study showed measuring by OUR toxicity decreasing orders: Ni >Zn >Cu >Co, measuring by CO_2 production toxicity decreasing orders: Ni >Cu >Zn >Co. EC_{50} of Ni is 5-7 mg/L.
Çeçen et al., 2010a	OUR & CO_2 production	The study indicated EC_{50} of Cd, Pb, Hg, Ag and Cr in measuring by OUR and CO_2 production. The result of decreasing toxicity order is Ag >Hg >Cd >Cr^{3+} = Cr^{6+}. EC_{50} of Ag is 0.33 mg/L.
Shi et al., 2024	Molecular Assay	The study indicated EC_{50} of Cu^{2+}, Ni^{2+} and Zn^{2+} are 4.89 mg/L, 5.42 mg/L and 21.26 mg/L respectively affecting on TAC-1 activity of heterotrophic nitrification bacteria.

2.2.2 Chemical compounds

The main toxic chemical compounds in wastewater originate from chemical industries and agriculture. Many of these compounds negatively affect nitrification, and extensive research has been conducted to assess their impact. Walker et al. (1990) and Hockenbury et al. (1977) compiled a vast amount of EC_{50} data for NI substances. Table 2.2.3 and Table 2.2.4 are listed EC_{50} extracted from the literature. In Table 2.2.4, EC_{50}s in one chemical compound have large differences depending on different assay types.

The chemical is hypertoxic when EC_{50}s are less than 0.1mg/L (see Table 2.2.3). Most of these belong aromatic hydrocarbons. NPE (nonylphenol ethoxylate) is the most toxic chemical as table showed. NPE is widely used in laundry production[1], so it's most probably contained in wastewater. In Denmark, NPE can't be placed on market and used greater than 0.1% concentration[2]. TAA (thioacetamid) is the second most toxic in table. However, TAA isn't always appeared in daily life and it's always used in medical research because of its carcinogenic. Dimethyl yellow is a familiar acid-base indicator in chemical laboratory. Cyanide doesn't belong to organic matter, but it's highly toxic and frequently-used in manufacture. Free cyanide is much more toxic than complex cyanides [Neufeld et al., 1986]. The cultured cyanide-degrading bacteria can be used in cyanide treatment of wastewater. Han et al. (2014) showed that wastewater can be recovered 95% after 10 days through this cyanide treatment. Phenol is also frequently-used in manufacture, but its toxicity is weaker than cyanide [Neufeld et al., 1986; Inglezakis et al., 2017]. Phenol can be biodegraded through phenol bacteria in concentration of phenol ranging from 100mg/L to 2500mg/L [Amor et al., 2005; Gu et al., 2016].

In summary, aromatic hydrocarbons, cyanide and phenol-based compounds are among the most concerning pollutants for nitrification inhibition in wastewater treatment.

Biological nitrification inhibitors

While NI is a major challenge in WWTPs, it has significant applications in agriculture and scientific research. The common BNIs (biological nitrification inhibitors) are chlorate, ATU (allylthiourea), phenol, DCD (dicyandiamide), TCMP (2-chloro-6 (trichloromet-hyl) pyridine or Nitrapyrin) and DMPP (3,4-dimethylpyrazole phosphate).

BNIs are widely used in farmland to retain nitrogen fertilizers by inhibiting ammonia oxidation to nitrate [Shen et al., 2013; Nardi et al., 2020]. A lot of studies focus on identifying effective BNIs for improving soil nitrogen retention. Moreover, BNIs are also widely used for BOD_5 test of research in laboratory [Young et al., 1973]. Since ammonia oxidation consumes oxygen, BNIs prevent this process, ensuring

[1]https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-nonylphenols-and-nonylphenol-ethoxylates

[2]https://eng.mst.dk/chemicals/chemicals-in-products/chemical-legislation/fact-sheets-on-legislation/fact-sheet-nonylphenol-and-nonylphenol-ethoxylates

that BOD_5 values reflect only organic matter degradation. Young et al. (1973) recommended TCMP for long-term BOD_5 tests due to its extended effectiveness compared to ATU. The other studies found that TCMP and ATU could remove antibiotics reaching over 90% in livestock wastewater treatment, and author preferred TCMP because of its more effective NI function [Yang et al., 2022; Yang et al., 2023]. Despite its negative impact in WWTPs, nitrification inhibition is a valuable tool in agriculture, laboratory research, and wastewater treatment.

2.2.3 Other substances and conditions

Beyond heavy metal ions and toxic chemical compounds, several environmental factors and naturally occurring substances can also cause NI. These factors are often overlooked but play a significant role in microbial activity. Variations in these conditions contribute to differences in sludge bacteria vitality across different location of WWTPs. This subsection discussed free ammonia, salinity, pH and temperature effect on NI. Unlike large differences in results of NI caused by heavy metals and chemical compounds (see Subsection 2.2.1 and 2.2.2), the results in this subsection have narrow differences. These substances and conditions influence microbial health and nitrification efficiency, making them crucial considerations in wastewater treatment.

Free ammonia

Ammonia concentration plays a crucial role in NI [Y. M. Kim et al., 2008]. There are two forms ammonia existing in wastewater: ionized-ammonia (NH_4^+-N) and free ammonia (NH_3-N). The forms are depending on pH and temperature in water body. Effler et al. (1990) found that FA is significant more toxic than ionized-ammonia, especially in aquatic ecosystems. In wastewater treatment, Free ammonia can cause NI at low concentration. More precisely, NOB is highly sensitive to free ammonia, showing inhibition at 3–15mg/L [Pourbavarsad et al., 2022; Sun et al., 2021; Vel M Vadivelu et al., 2007; Xiao et al., 2025]. D. J. Kim et al. (2006) said free ammonia at 0.7mg/L can reach 50% inhibition on NOB activity. However, AOB is more tolerant and its inhibition starts at 210mg/L free ammonia [Q. Wang et al., 2017]. These findings confirm that NOB is more sensitive to FA than AOB. For the same reason of biological nitrification inhibitors, free ammonia is used for controlling nitrification process stop at first step. Because of its selective toxicity, free ammonia is used to control nitrification, particularly in short-cut nitrogen removal processes, where nitrification is intentionally stopped at the first step to optimize denitrification [Luo et al., 2023].

Free nitrous acid

Nitrite is an intermediate product in the nitrification process. However, free nitrous acid is recognized as a key inhibitor in this process. The studies have showed that free nitrous acid is significantly more toxic than free ammonia [Park et al., 2009; Yucan Liu et al., 2023]. It has negative effect on both types of nitrifiers - AOB and NOB [C. Jiang et al., 2022; Duan et al., 2020]. Free nitrous acid begins to inhibit AOB at concentrations as low as 0.018 mg/L [Yucan Liu et al., 2023], with complete inhibition occurring at 0.4 mg/L [Vel Murugan Vadivelu et

al., 2007]. For NOB, inhibition starts at just 0.011 mg/L, with total inhibition observed at 0.02 mg/L [Vel Murugan Vadivelu et al., 2006; Zhou et al., 2011].

Salinity

Salinity is often an overlooked factor in NI. However, research has shown that increased salinity can significantly impact nitrifying bacteria. Dinçer et al. (1999) showed that wastewater with 2%-3% salinity could begin to cause NI. Chhetri et al. (2022) showed that over 30% salinity could cause NI completely and below 1% salinity couldn't cause any. Comparing AOB and NOB, survival rate of NOB is less than 1% at over 2% salinity, but survival rate of AOB is 50% [Ye Liu et al., 2009]. It means that NOB is more sensitive for high salt concentration than AOB in wastewater [Ye Liu et al., 2009; Jeong et al., 2018; Xiao et al., 2025]. This sensitivity difference suggests that salinity stress primarily inhibits the second step of nitrification rather than the first step.

pH and temperature

The nitrifiers can tolerate pH from 5.0 to 9.7 [Yue et al., 2023]. The optimal values of pH for AOB and NOB are 7.0-8.0 and 7.5-8.0 respectively [USEPA, 2002; Antoniou et al., 1990]. When pH above 8, it begins to cause damage on bacterial, and total inhibition happens at pH above 9.3 [Yue et al., 2023; Skadsen et al., 2002]. Beside, Faust et al. (2023) found that nitrite accumulation would happen when pH is outside the range from 5.8 to 6.7.

When the temperature is over 30°C or below 16°C, the rate of nitrification begin to decrease. Total NI happens at temperature over 45°C [Neufeld et al., 1986; Gnida et al., 2016]. The study recommended that optimum temperature range is from 15°C to 25°C [Antoniou et al., 1990]. When low temperatures are unavoidable, especially in northern regions, certain measures can be implemented in WWTPs, such as adjusting the BOD:N:P ratio of raw wastewater, increasing retention time, and cultivating selective nitrifiers[1].

These studies indicate that both extreme pH and temperature deviations can lead to significant nitrification inhibition, affecting wastewater treatment efficiency.

Cocktail effect

The cocktail effect on NI means that combination of substances can be highly harmful to nitrifiers, even when each individual substance is present at low, non-toxic levels[2]. You et al. (2009) demonstrated that the combined toxicity of Cd, Ni, and Pb on nitrifiers in activated sludge was greater than the toxicity of each metal alone at the same concentration. Besides, Wium-Andersen et al. (2011) found that Cu and Zn among 6 heavy metals have a greater toxic effect on NI than PAHs in stormwater. Nguyen et al. (2023) found that the cocktail effect of oxytetracycline and sulfamethoxazole on NI was stable and irreversible, whereas the inhibition caused by oxytetracycline alone was reversible.

[1] https://www.ebsbiowizard.com/articles/how-to-prepare-your-wwtp-for-cold-weather/
[2] https://www.eea.europa.eu/highlights/more-action-needed-to-tackle

Table 2.2.3: Selective chemical compounds causing NI

Chemical Compound	Assay Type	EC_{50} (mg/L)	Reference
2-chloro-6 (trichloromethyl) pyridine	BLA	0.17-0.95	[4]
(TCMP) *	AUR	11	[5]
4-Nitrophenol	ASRI	59	[6]
	Microtox	9.4	[6]
	AUR	59.94	[7]
	NUR	30.73	[7]
Allylthiourea (ATU) *	BLA	0.02-0.06	[4]
	AUR	1.2	[6]
Benzene	Microtox	102.98	[6]
Chlorobenzene	Microtox	11.25	[6]
Cyanide #	BLA	0.078	[3]
	OUR	0.18	[7]
	AUR	0.93	[7]
	NUR	1.36	[7]
Dicyandiamide (DCD) *	BLA	332.87-823.14	[4]
Ethanol	AUR	4100	[5]
	EC20	30000	[6]
Ethylbenzene	OUR	8-10	[1], [2]
Formaldehyde	EC20	2	[6]
	Microtox	7.44	[6]
	AUR	42	[8]
Methanol	BLA	103.49-142.26	[4]
	AUR	160	[5]
	AUR	225	[8]
	EC20	12000	[6]
Phenol	OUR	3-4	[1], [2]
	OUR	2.67	[3]
	Microtox	21.07	[6]
	AUR	28.08	[7]

1. Reference [1]. Juliastuti et al., 2003a; [2]. Juliastuti et al., 2003b; [3]. Gernaey et al., 1997; [4]. Iizumi et al., 1998; [5]. Walker et al., 1990; [6]. Hockenbury et al., 1977; [7] Inglezakis et al., 2017; [8]. Chhetri et al., 2022
2. Assay types: (1) ASRI: activated sludge respiration inhibition; (2) Microtox: 30 minutes Microtox; (3) AUR: ammonia utilization rate; (4) OUR: oxygen utilization rate; (5) NUR: nitrite utilization rate; (6) BLA: bioluminescence assay; (7) EC20: Toxi-Chromotest;
3. "*" is used for common nitrification inhibitors;
4. Except for "#", all chemical compounds belong to organic matter.

Table 2.2.4: EC_{50} of chemical compound less than 1 mg/L

Chemical Compound	EC_{50} (mg/L)	Reference
(EC_{50} <0.1 mg/L)		
1,4-dinitrobenzene	0.0945	[6]
4-benzoyl-N,N-dimethylaniline	0.068	[6]
Allylthiourea (ATU)	0.02-0.06	[4]
Benzylisothiocyanate (BITC)	0.0103	[6]
Cyanide #	0.078	[3]
Dimethyl yellow	0.0192	[6]
Methyl isobutyl ketone (MIBK)	0.08	[6]
Nonylphenol ethoxylate (NPE)	0.0003	[6]
Phenylhydrazine	0.06	[6]
Thioacetamide (TAA)	0.003-0.03	[4]
Thiosemicarbazide	0.08-0.33	[4]
Sodium linear alkylbenzene sulfonate (LAS)	0.056	[6]
(0.1 mg/L $\leq EC_{50}$ <1 mg/L)		
2,3,4,5-tetrachlorophenol (2,3,4,5-TCP)	0.4	[6]
3,5-dichlorophenol (3,5-DCP)	0.51	[3]
4-aminobenzylcyanide	0.3636	[6]
4-aminodiphenylamine (4-ADPA)	0.3276	[6]
4-benzylphenol	0.2485	[6]
4-chlorobenzylchloride	0.5757	[6]
4-chlorobenzylmercaptan	0.4892	[6]
4-chlorophenyl isothiocyanate	0.3636	[6]
4-cyanoaniline	0.1873	[6]
4-cyanobiphenyl	0.1834	[6]
4-(dimethylamino)benzonitrile	0.1678	[6]
4-ethylaniline	0.2106	[6]
4-hydroxy benzotrifluoride	0.3235	[6]
4-iodophenol	0.2526	[6]
4-methoxyazobenzene	0.1114	[6]
4-nitro-diphenylamine	0.7259	[6]
4-nitrobenzenebulfonamide	0.6106	[6]
4-nitrodiphenylamine	0.7259	[6]
4-phenoxyaniline	0.5594	[6]
4-phenylazophenol	0.9272	[6]
4-trifluoromethylaniline	0.9489	[6]
8-hydroxyquinoline	0.25-0.29	[4]
Allylsulfide	0.15-0.21	[4]
Benzalkonium chloride (BKC)	0.25	[6]
Benzil	0.6349	[6]
Benzylthiocyanate	0.4016	[6]

Chemical Compound	EC_{50} (mg/L)	Reference
Cetyltrimethylammonium chloride (CTAC)	0.59	[6]
Chlorobenzene (PhCl)	0.25	[1], [2]
Diphenylmercury	0.1349	[6]
Hydrazobenzene	0.9894	[6]
L-Histidine	0.5	[5]
Methomyl	0.9	[6]
Nitrapyrin	0.17-0.95	[4]
Patulin	0.5	[6]
Quinone	0.32-0.54	[6]
Thiophenol	0.8752	[6]
Thiosemicarbazide	0.9	[5]
Trichloroethylene (TCE)	0.75	[1], [2]

1. Reference [1]. Juliastuti et al., 2003a; [2]. Juliastuti et al., 2003b; [3]. Gernaey et al., 1997; [4]. Iizumi et al., 1998; [5]. Walker et al., 1990; [6]. Hockenbury et al., 1977;
2. Except for "#", all chemical compounds belong to organic matter.

2.3 Toxicity of landfill leachate

In Subsection 1.1.5, it is indicated that municipal solid waste landfill leachate develops through four phases: the aerobic phase, acidogenic phase, methanogenic phaseb and stabilization phase, with biodegradability decreasing in that order. Table 2.3.1 presents the main substances found in landfill leachate during the acidogenic and methanogenic phases, respectively. The concentration of metals is higher in the acidogenic phase compared to the methanogenic phase. However, when comparing the BOD_5/COD ratios and volatile fatty acid content in both phases, the acidogenic phase shows higher values. In particular, the volatile fatty acid content in the acidogenic phase is significantly greater than in the methanogenic phase, indicating that biodegradability is higher in the acidogenic phase. Additionally, the pH value in the methanogenic phase exceeds the optimal range for nitrification, as discussed in Subsection 2.2.3. According to Yun Li et al. (2017), the first step of the nitrification process occurs at a slower rate in old leachate comparing to more recent leachate. Recent leachate may cause NI due to its high metal concentrations, while old leachate contributes to NI as a result of its low biodegradability and elevated pH. Furthermore, Kylefors et al. (1997) reported that phenol concentrations in landfill leachate can reach up to 44 mg/L, which exceeds the EC_{50} value listed in Table 2.2.3.

In construction and demolition waste landfill leachate, the pH value can reach up to 7.6, comparable to the methanogenic phase of municipal solid waste, as shown in Table 2.3.2. Ca and S ions are predominant, and other metal ions are also present at high levels particularly As, which reaches 0.148 mg/L. Therefore, this type of landfill leachate causes NI due to its high pH, high concentrations of specific heavy metals, or cocktail effect.

Table 2.3.1: Municipal solid waste landfill leachate substances in acidogenic phase and methanogenic phase

		Acidogenic phase				Methanogenic phase			
	Unit	[1]	[2]	[3]	Average	[1]	[2]	[3]	Average
pH	-	6.73	5.5	6.1	6.11	7.52	7.1	8	7.54
COD	mg/L	36817	43300	6000	28705.67	2307	2200	3000	2502.33
BOD_5	mg/L	18632	38500	4000	20377.33	374	980	180	511.33
B/C	-	0.51	0.89	0.67	0.69	0.16	0.45	0.06	0.22
VFA	mg/L	8197	7860	-	8028.50	18	1.1	-	9.55
NH_4-N	mg/L	922	610	740	757.33	889	335	740	654.67
S	mg/L	676	160	500	445.33	67	23	80	56.67
P	mg/L	5	43	6	18.00	4.3	19	6	9.77
Mg	mg/L	384	170	470	341.33	250	90	180	173.33
Al	mg/L	-	0.27	-	0.27	-	2.56	-	2.56
Cr	mg/L	0.13	-	0.28	0.21	0.09	-	0.28	0.19
Mn	mg/L	32.94	67.5	25	41.81	0.46	0.78	25	8.75
Fe	mg/L	653.8	650	780	694.60	27.4	0.04	15	14.15
Ni	mg/L	0.42	-	0.17	0.30	0.17	-	0.17	0.17
Cu	mg/L	0.13	58	0.065	19.40	0.13	0.69	0.065	0.30
Zn	mg/L	17.7	26	5	16.23	1.14	1.96	0.6	1.23
As	mg/L	0.024	0.083	-	0.05	0.034	0.04	-	0.04
Hg	mg/L	0.0004	-	-	0.0004	0.0002	-	-	0.0002
Pb	mg/L	0.28	0.01	0.09	0.13	0.2	0.37	0.09	0.22
Co	mg/L	-	0.74	0.05	0.40	-	0.1	0.05	0.08

1. Reference [1]. Carey et al., 2013; [2]. Kylefors et al., 1997; [3]. Kjeldsen et al., 2002;
2. B/C: the ratio BOD_5/COD; VFA: volatile fat acid.

Table 2.3.2: Comparison of different types waste of landfill leachate substances

	Unit	C&D	MSW	
			AP	MP
pH		6.45-7.6	6.11	7.54
Ca	mg/L	15.28-2608	1200	60
S	mg/L	11.7-1700	445.33	56.67
Fe	mg/L	0.05-275	694.6	14.15
Mg	mg/L	15-280	341.33	173.33
As	mg/L	0.0014-0.148	0.05	0.04
Cr	mg/L	0.00178	0.21	0.19
Mn	mg/L	0.02-76	41.81	8.75
Pb	mg/L	0.0049-1.18	0.13	0.22

1. C&D: construction and demolition waste; MSW: municipal solid waste; AP: acidogenic phase; MP: methanogenic phase;
2. The data of C&D is referred Weber et al. (2002). The data of AP and MP are the averages of data referred Carey et al. (2013), Kylefors et al. (1997), Kjeldsen et al. (2002).

2.4 Assay types for nitrification inhibition

NI assay can be classified as: respirometry assay, nitrification inhibition assay, bioluminescence assay and molecular assay [X. Li et al., 2016; Ren, 2004]. In Table 2.2.2 and Table 2.2.3, the EC_{50}s in one materials with different assay types have big differences.

Respirometry assay is OUR (oxygen uptake rate) measurement for microorganism [X. Li et al., 2016; Jubany et al., 2005]. Nitrification inhibition assay is AUR (ammonia utilization rate) or NUR (nitrite utilization rate) for microorganism [X. Li et al., 2016; Inglezakis et al., 2017]. ISO 9509[1] provided standard operation manual for AUR measurement. The above assays are standard methods that can be used to detect partial NI in both pure and mixed cultures [Grunditz et al., 2001; X. Li et al., 2016].

Bioluminescence assay is utilized luminescent bacteria to indicate the toxicity level of water. The Microtox® system measures the light intensity emitted by specific luminescent indicator bacteria to assess bacterial stress [Ren, 2004; X. Li et al., 2016]. It approved by US EPA as a reliable toxicity test [James et al., 2003]. Bioluminescence assay provides greater accuracy than respirometry or nitrification assays, as it directly measures bacterial survival.

[1]Water quality - Toxicity test for assessing the inhibition of nitrification of activated sludge microorganism (ISO 9509:2006)

Molecular assay identifies NI at a molecular level by detecting specific bacterial proteins or genes. X. Li et al. (2016) introduced detection of polymerase chain reaction (PCR) and quantitative PCR (qPCR) to analyze bacterial gene expression. A.J.Duncan et al. (2000) introduced detection of GroEL protein, which indicates bacterial stress response. Molecular assay provides a highly specific assessment of microbial activity, independent of external environmental factors.

Each assay method offers unique advantages, making them suitable for different research applications in wastewater treatment and nitrification inhibition studies.

2.5 Resistance of nitrifiers

In Section 2.1, reversible NI is introduced, indicating that nitrification recovers once the toxicant is removed. However, nitrifiers can also recover even when the toxicant is not removed, a phenomenon known as resistance.

Nitrifiers exhibit remarkable adaptability to NI, demonstrating resistance to high concentrations of toxic substances and extreme environmental conditions. Several studies have shown that NOB can develop resistance to high levels of FA by transforming into more adaptable NOB species [S. Li et al., 2020a; S. Li et al., 2020b; H. Jiang et al., 2021]. S. Li et al. (2020b) reported that the NOB changed the forms from *Nitrospira* to *Candidatus Nitrotoga* when free ammonia concentration is above 220 mg/L. H. Jiang et al. (2021) found that NOB changed from *Nitrospira* to *Nitrolancea* when free ammonia is raising from 37.9 to 715.3 mg/L. Similarly, AOB can also develop resistance through repeated ammonia loading shocks. Cho et al. (2016) found that AOB, after three cycles of ammonia shock loading (TKN: 120-180 mg/L), gradually adapt and regain nitrification ability by a lot of AOB shifting to *N. aestuarii*, which is a species more tolerate to high FA environment. Cao et al. (2022) even demonstrated that, after six cycles of extreme ammonia loading (TKN: 1928 mg/L), AOB showed similar adaptive responses. Most nitrifiers resistance mechanisms arise from microbial variability and adaptation. Similar adaptations occur under other extreme conditions, such as low dissolved oxygen, extreme temperatures, and nitrite limitations, as summarized in Table 2.5.1 .

To enhance microbial resistance, cultivating specific and well-adapted nitrifiers based on the composition of raw wastewater is an effective approach. Alternatively, subjecting microorganisms to continuous shocks can stimulate bacterial evolution, leading to increased resilience. Mpongwana et al. (2022) recommended and compared several methods for accelerating the recovery of biological treatment in heavy metal-contaminated wastewater. It's concluded that external field energy was the most effective in accelerating the recovery process, and chelating agents negatively impacted bacterial function and overall treatment efficiency while useful in removing heavy metals.

Table 2.5.1: Resistance of nitrifiers in toxicity

Nitrifier	Type	Toxicity	Reference
N. aestuarii	AOB	high ammonia	[3]
Nitrosomonas	AOB	high FA and salinity	[5],[3],[6]
Nitrosomonas eutropha	AOB	high salinity	[4]
Ca. Nitrotoga	NOB	chemical inhibitors and high FA	[1]
Nitrotoga	NOB	low temperature and high FA	[2]
Nitrobacter	NOB	high FA	[2]
Nitrolancea	NOB	high temperature	[1]
Nitrospira	NOB	low DO and low nitrite	[1], [2]
Nl. hollandica	NOB	high temperature	[1]

1. Reference [1]. Su et al., 2023; [2]. S. Li et al., 2024; [3]. Cho et al., 2016; [4]. Jeong et al., 2018; [5]. Xiao et al., 2025; [6]. Cao et al., 2022.

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CHAPTER
THREE

METHODS

3.1 Development of the methods

To verify the real effect on NI, the controlled experiment is an effective method to be conducted for quantitative analysis. In this study, the controlled group consists of activated sludge with nutrients, while the experimental group consists of activated sludge with both nutrients and a toxicant. The volume of activated sludge and concentration of nutrient remain constant in both groups. The assay types of this study used observation of ammonia conversion rate.

The selection of toxicant is an important consideration. Landfill leachate is suspected to be a toxicant that contributes to NI in WWTPs. It is one of the selected toxicants in the controlled experiments. The control variable is the percentage of landfill leachate in activated sludge. As demonstrated in Subsection 1.1.5, the biodegradability of municipal solid waste landfill leachate in activated sludge depends on its age. The recent landfill leachate is biodegradable, whereas the old landfill leachate is not. The construction and demolition waste landfill leachate contains high alkalinity and a lot of heavy metals. Therefore, the NI effect of landfill leachate is unpredictable. To enable comparison, ATU is selected as the standard reference for NI to demonstrate the availability of this method. ATU is considered a common biological nitrification inhibitors, as mentioned in Subsection 2.2.2. The control variable in this study is the concentration of ATU in the activated sludge. Given its strong effect on NI, the concentration of ATU is controlled within a range of 0.125–2 mg/L.

3.2 Experimental layout

Two controlled experiments are conducted to examine the effects of two toxicants in activated sludge. Controlled experiment I examines on ATU in order to prove availability of this method. Controlled experiment II examines landfill leachate by the same method. The landfill leachate samples are collected from Rærup Deponi, a construction and demolition waste landfill located north of Aalborg. The toxicant dosages differ for each experiment: landfill leachate is tested in varying volumes, while ATU is tested at different concentrations. The substrate is ammonium chloride at a concentration of 20mg N/L. The volume of activated sludge in each flask is 1L mixed liquid, though MLSS (mixed liquor suspended solids) is not measured. It is assumed that MLSS remains equal across all flasks. Each controlled experiment consists of one control group and three experimental groups to minimize deviations caused by uneven aeration. The final result for each experimental condition is taken as the average of the three experimental groups. All sludge samples used in the experiments come from the same batch. Before conducting the experiments, the following preparations are required: sludge sampling, sludge preservation, and standard curve calibration. Each controlled experiment involves aerating the samples for two hours, with extraction of tube samples every 10 minutes. The nitrate concentration of the samples is then measured using a spectrophotometer. The experiments take place in a laboratory at Thomas Manns Vej 23, Aalborg, from October 2024 to January 2025. The indoor temperature is maintained between 20-23°C.

Fig. 3.2.1. The location of sludge sampling

3.3 Preparation

3.3.1 Sludge sampling and preservation

The first step of the experiment is sludge sampling. The experimental sludge is collected from the aeration tank (Fig.3.2.1) at Renseanlæg Vest, a domestic WWTP located in western Aalborg. The aeration tank operates with intermittent aeration based on the ammonia concentration in the tank. To ensure maximum mixing with sampled sludge and substrate, sampling must be conducted during the aeration phase. After collection, the sludge was stored in the laboratory at a temperature below 5°C to keep the microorganisms in a dormant state. Additionally, the sludge was used in experiments within 15 days of sampling to maintain its viability. In this study, the mixing nitrifiers are applied and ignored partial process of nitrification.

3.3.2 Standard curve

A spectrophotometer is general machine which is used to measure the specific chemical concentration of a solution indirectly. For nitrate detection, the spectrophotometer measured the absorbance difference between wavelengths at 220nm and 275nm. Generally, an increasing absorbance difference indicated a higher nitrate concentration. To ensure accurate measurements, standard potassium nitrate solution was prepared at concentrations ranging from 1 to 7mg N/L. The potassium nitrate stock was dried in an oven at 105°C for 24 hours before use. The solvent for standard solution was outlet water from Renseanlæg Vest, collected at the same time as the sludge samples. In the laboratory, the outlet water was filtered through a 1.2μm filter to reduce turbidity. For accuracy, three sets of standard solution (1–7mg N/L) were measured each time to determine their corresponding absorbance values. After measurement, a regression line was established between concentration and the average absorbance values. When applying a quadratic regression model, the R-squared value typically exceeded 98%, ensuring high accuracy. The regression line was then used to calculate the nitrate concentration of sample solutions based on their absorbance difference. Establishing a standard curve was a crucial preparation step for the controlled experiments.

3.4 Experimental process

3.4.1 Controlled experiment I

Five sets of controlled experiments were conducted with ATU dosages of 0.125mg/L, 0.25mg/L, 0.5mg/L, 1mg/L, and 2mg/L. The substrate - 1mL of ammonium chloride solution was added to each group, creating a nutrient environment of 20mg N/L. The next step involved aerating each flask while collecting 10mL tube samples every 10 minutes over a 2-hour period. The aeration system consisted of an air pump and a bent steel pipe with uniformly distributed aeration holes to ensure efficient oxygenation. Each tube sample was centrifuged using a 5430 centrifuge at 7,000 rpm for 6 minutes and then filtered through a 1.2μm filter. The filtered supernatant was collected, and its nitrate concentration was measured using a

spectrophotometer at absorbance wavelengths of 220nm and 275nm, following the principle outlined in Subsection 3.3.2. The experimental process for one set of experiments is illustrated in Fig.3.5.1.

3.4.2 Controlled experiment II

Three sets of controlled experiments were conducted with landfill leachate dosages of 100mL, 200mL, and 300mL, respectively. Unlike controlled experiment I, the activated sludge in each flask was allowed to settle for 2 hours to extract an equal amount of supernatant, ensuring that the total volume remains 1L before dosing landfill leachate. After the substrate was dosed, the subsequent procedures followed the same steps as in Controlled Experiment I.

3.5 Data treatment

In this study, the assay type is ammonia conversion rate, which is equal concentration of nitrate production tested. R_{CN} (Eq.3.1) measures the rate of nitrate production, reflecting the speed of the reaction per minute. ΔR_{CN} (Eq.3.2) compares the values of R_{CN} in both groups, where a value above zero indicates nitrification inhibition. The degree of NI is determined by comparing the nitrate production concentrations between the control and experimental groups, as shown in Eq.3.3. The nitrate production concentration for the experimental group is calculated as the average of three replicates.

$$R_{CN} = \frac{\Delta CN}{10min} \tag{3.1}$$

$$\Delta R_{CN} = R_{CNC} - R_{CNE} \tag{3.2}$$

$$NI\% = \frac{C_c - C_E}{C_c} \times 100\% \tag{3.3}$$

Where:

R_{CN}	The rate of ammonia conversion [mg N/(L• min)];
ΔCN	Ammonia conversion in 10min [mg N/L];
ΔR_{CN}	The difference of rates for nitrate production [mg N/(L• min)];
R_{CNC}	The rate of nitrate production for controlled group [mg N/(L• min)];
R_{CNE}	The rate of nitrate production for experimental group [mg N/(L• min)];
NI%	The percentage of nitrification inhibition;
C_c	The concentration of nitrate production for controlled group [mg N/L];
C_E	The concentration of nitrate production for experimental group [mg N/L].

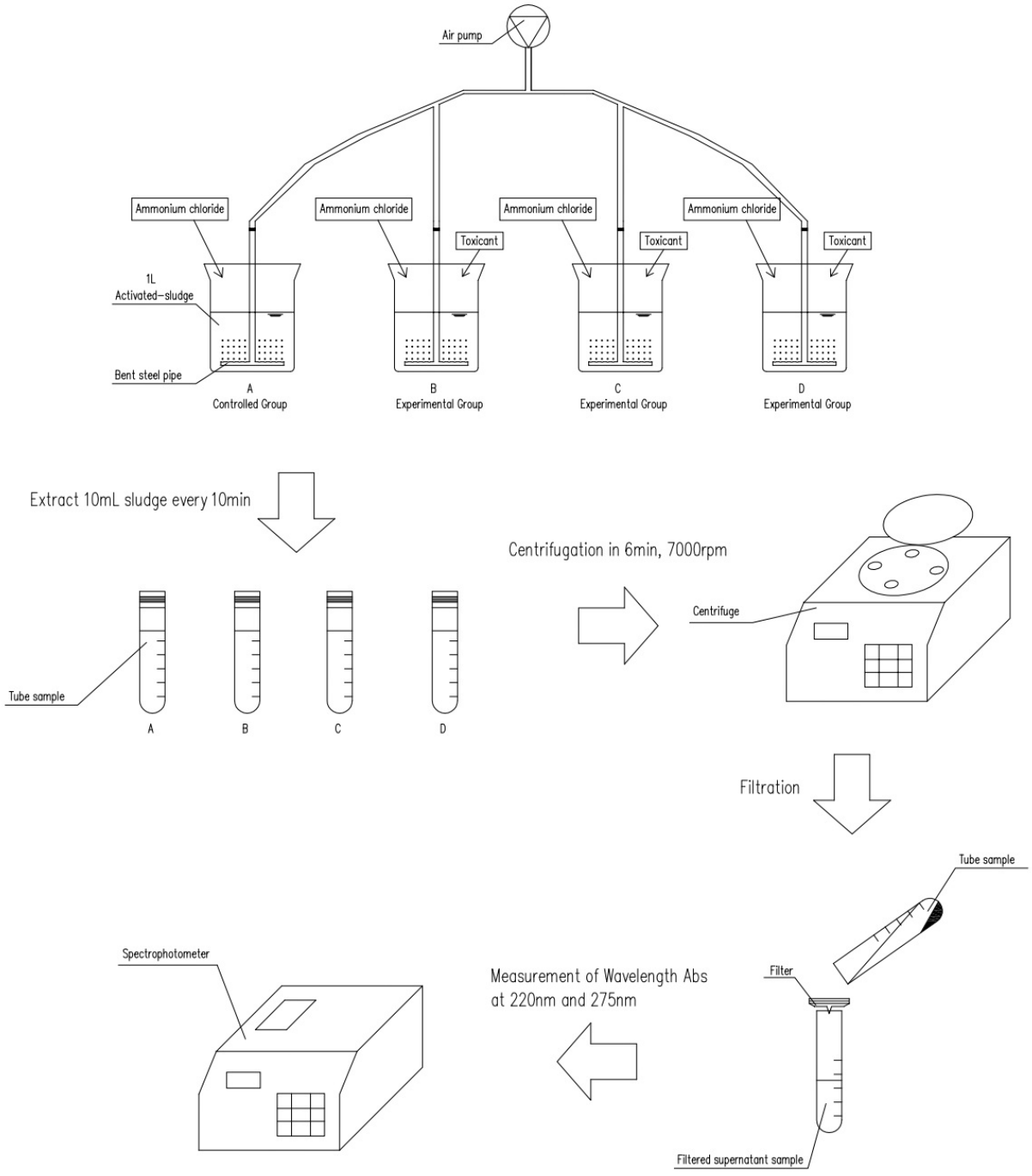

Fig. 3.5.1. The sketch about one set of experimental process

CHAPTER

FOUR

RESULTS AND DISCUSSION

4.1 Results

This section presents the statistical results of the two controlled experiments for ATU and landfill leachate respectively. The raw data and data processing details are provided in Table A1 and Table B1. The comparative curves for detailed R_{CN} of ATU are presented in Fig.A1 and Fig.A2. The comparative curves for detailed R_{CN} of landfill leachate is presented in Fig.B1.

4.1.1 Controlled experiment I

The experimental results showed that ATU exhibited a clear and consistent NI effect in the experiments, aligning with expectations. Moreover, the NI ability of ATU remained stable, as indicated by the low standard deviation values in Table 4.1.1. Inhibition began within the first 20 minutes, with 2mg/L and 1mg/L ATU showing effects as early as 10 minutes, as illustrated in Fig. 4.1.1. When ATU concentrations exceeded 0.5mg/L, NI percentages surpassed 50%.

As shown in Fig.4.1.2, except for ΔR_{CN} at 0.25mg/L and 0.125mg/L ATU, which were slightly below zero, all other ΔR_{CN} values remained above zero. Nitrification inhibition was consistently observed at ATU concentrations above 0.5mg/L. The only unexpected result was that ΔR_{CN} at 1mg/L ATU was slightly higher than at 2mg/L ATU. Additional, inhibition at 1mg/L ATU started slightly earlier than at 2mg/L ATU.

4.1.1.1 Effective concentration of ATU

The EC (effective concentration) of ATU was calculated in this study, as the results of Controlled Experiment II aligned with expectations. Statistical information on EC from 30 to 120 minutes is presented in Fig.4.1.3, including average, median, maximum, minimum, and STD (standard deviation). Additionally, NI effects remained stable during this period, as shown in Fig.4.1.1. The EC results at each NI degree, derived from averages and 95% confidence intervals, are summarized in Table 4.1.2. The 95% confidence intervals fell within the standard deviation range of the original data, as illustrated in Fig.4.1.4. Total inhibition occurred at an ATU concentration of 2.18 ± 0.03 mg/L (18.78 ± 0.26 μM) ATU. EC_{50}, a common toxicity indicator, was determined to be 0.41 ± 0.03 mg/L (3.55 ± 0.23 μM) in this study.

Table 4.1.1: The statistics of NI% and ΔR_{CN} caused by ATU

ATU concentration (mg/L)	2	1	0.5	0.25	0.125
NI%					
Average	93%	72%	62%	28%	16%
Maximum	98%	86%	68%	42%	26%
STD	0.126	0.152	0.046	0.06	0.068
ΔR_{CN} (mg N•L^{-1}•min^{-1})					
Average	0.11	0.13	0.08	0.03	0.02
Maximum	0.19	0.22	0.25	0.12	0.14
STD	0.069	0.056	0.07	0.037	0.064

Table 4.1.2: The values of EC for ATU

NI%	EC		NI%	EC	
	mg/L	μM		mg/L	μM
10	0.12 ± 0.01	1.03 ± 0.07	60	0.49 ± 0.02	4.20 ± 0.21
20	0.17 ± 0.05	1.42 ± 0.39	70	0.76 ± 0.12	6.56 ± 1.05
30	0.26 ± 0.03	2.26 ± 0.26	80	1.13 ± 0.22	9.69 ± 1.87
40	0.34 ± 0.03	2.91 ± 0.25	90	1.66 ± 0.12	14.24 ± 1.03
50	0.41 ± 0.03	3.55 ± 0.23	100	2.18 ± 0.03	18.78 ± 0.26

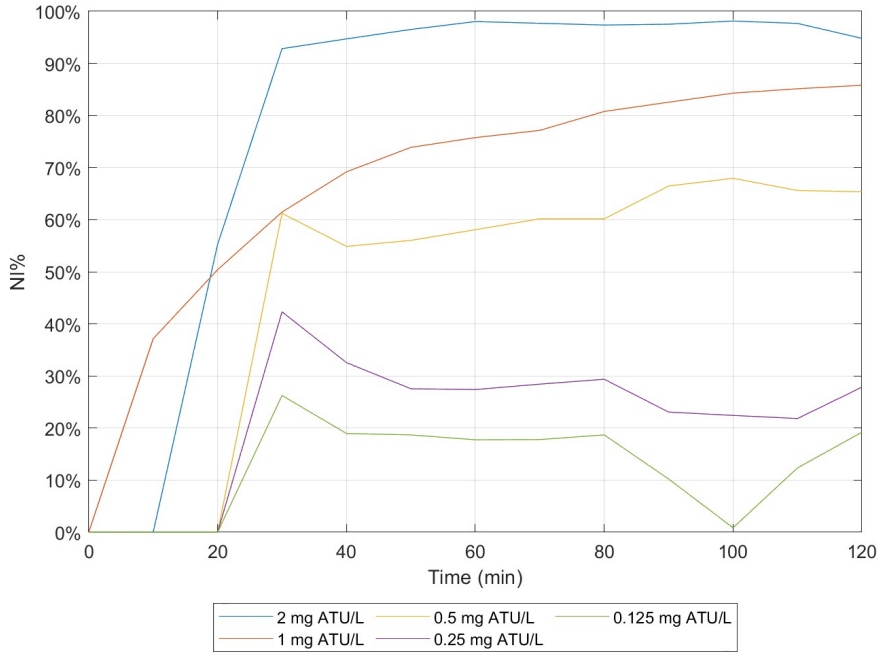

Fig. 4.1.1. Different content of ATU causing NI degree by time

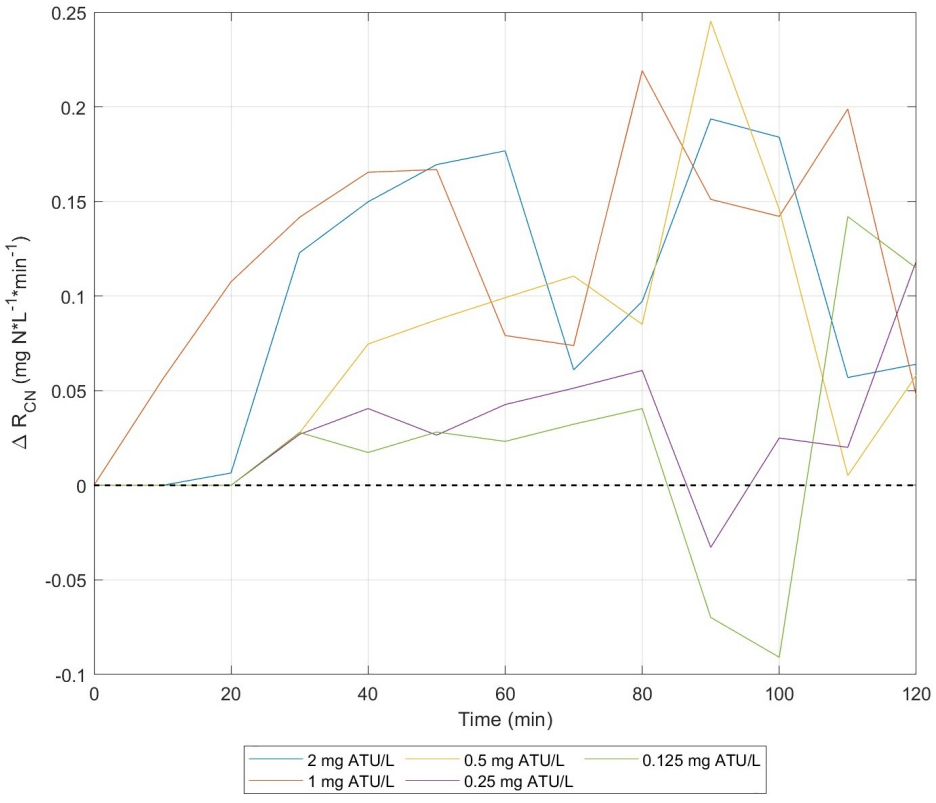

Fig. 4.1.2. The variety of ΔR_{CN} by different content of ATU

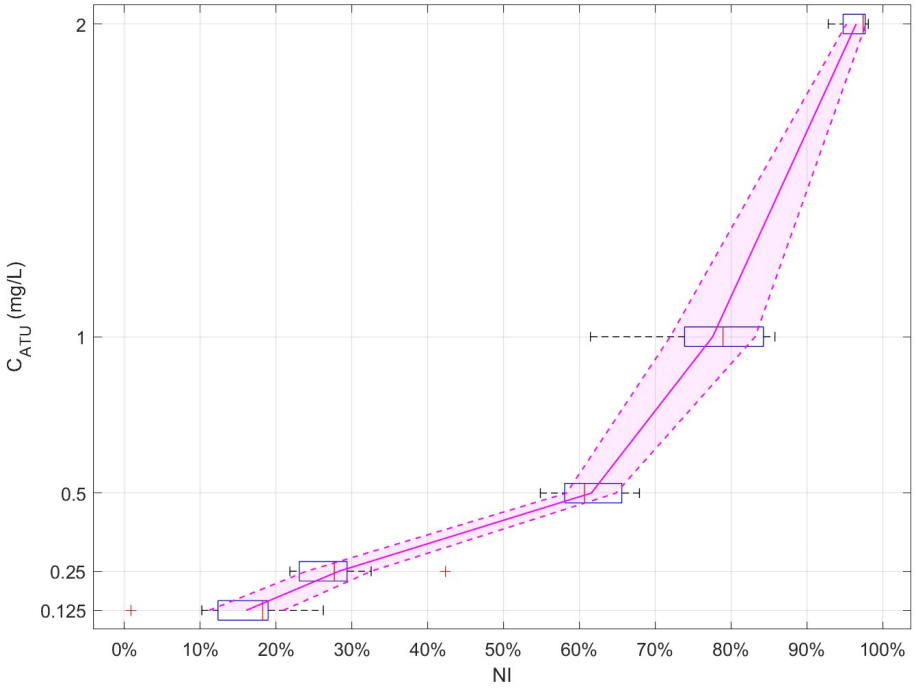

Fig. 4.1.3. The box and line plot of NI% and C_{ATU} (full line: the average; dashed line: STD for experimental data)

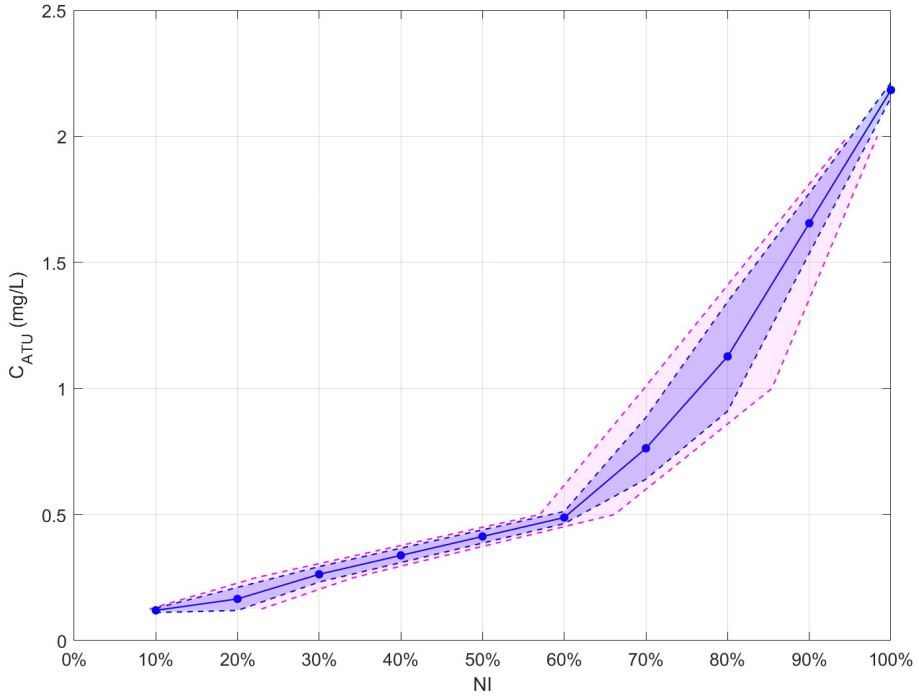

Fig. 4.1.4. The curve of ECs with error (blue line: the average; blue area: the range of EC errors; pink area: the range of STD for experimental data)

4.1.2 Controlled experiment II

It was initially assumed that a higher landfill leachate content would result in a higher degree of NI, even without experimental data. However, the experimental results contradicted this expectation.

Fig.4.1.5 summarizes the NI% caused by different landfill leachate content. The results show that 10% landfill leachate did not cause any inhibition, while 20% and 30% landfill leachate led to inhibition at 110 minutes. However, at 120 minutes, nitrate production in the experimental groups exceeded that of the control group across all three conditions. Surprisingly, 20% landfill leachate resulted in a higher NI degree than 30% landfill leachate, as shown by the average and maximum NI% values in Table 4.1.3. Although 20% landfill leachate caused an NI degree exceeding 50% at the beginning, the effect was short-lived. Overall, none of the three experiments achieved an average NI of 50%. In contrast, 10% landfill leachate accelerated the nitrification process after 60 minutes.

As shown in Fig.4.1.6, the ΔR_{CN} values for 10% landfill leachate were consistently below zero, confirming its accelerating effect. Both 20% and 30% landfill leachate lost their inhibitory effects after 80 minutes. Overall, the ΔR_{CN} values for the three experiments showed minimal differences in terms of their averages and maximums.

Table 4.1.3: The statistics of NI% and ΔR_{CN} caused by landfill leachate

Landfill leachate content	NI%			ΔR_{CN} (mg N•L^{-1}•min^{-1})		
	10%	20%	30%	10%	20%	30%
Average	-2%	31%	24%	-0.01	-0.01	0.00
Maximum	7%	89%	39%	0.12	0.15	0.12
STD	0.047	0.253	0.15	0.073	0.115	0.13

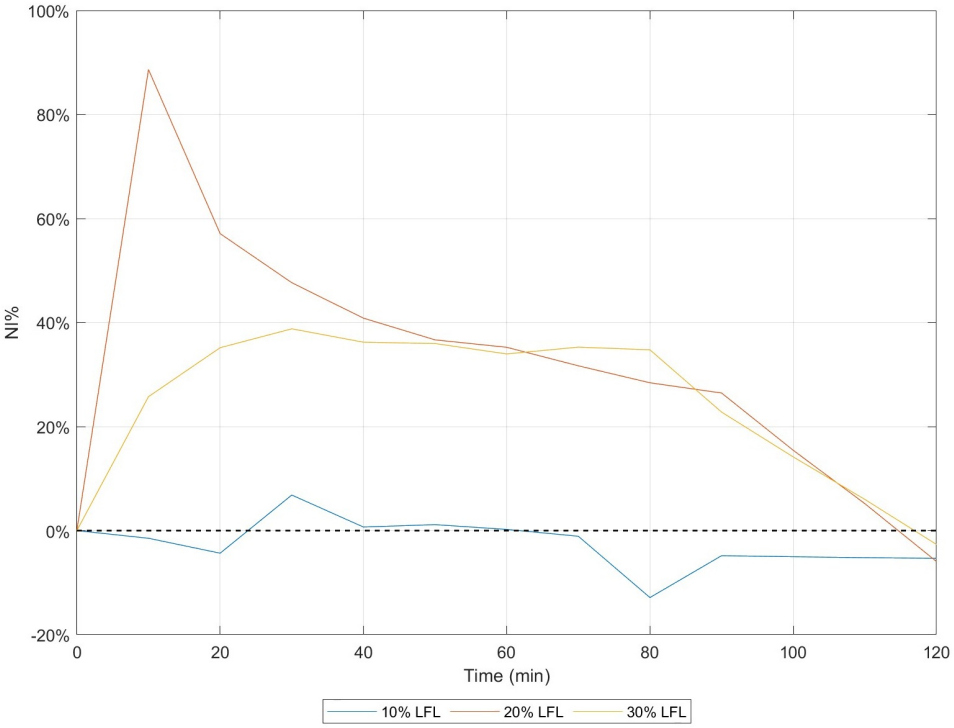

Fig. 4.1.5. Different content of landfill leachate causing NI% by time

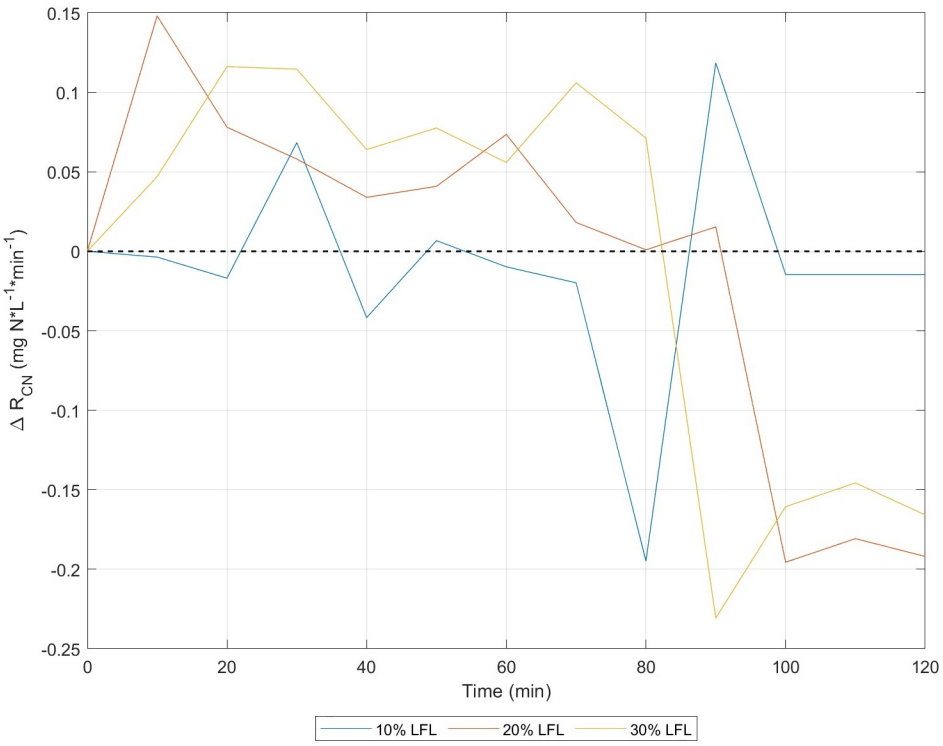

Fig. 4.1.6. The variety of ΔR_{CN} by different content of landfill leachate

4.2 Discussion

4.2.1 Controlled experiment I

Controlled experiment I for ATU is designed to prove the effectiveness of the method. The experimental results demonstrated that NI effects caused by ATU were more distinct and exhibited a clear dose. The results allowed for the determination of ATU's effective concentrations at various NI levels, as shown in Table 4.1.2 showed. Additionally, EC_{50} is widely used to express the toxicity of chemicals, as referenced in Table 2.2.2, 2.2.3 and 2.2.4. Table 4.2.1 provides a comparison of ATU causing NI effects observed in this study with those reported in selected literature. The EC_{50} value obtained in this study falls within the medium range. Young et al. (1973) found that total inhibition occurred at 2mg/L ATU, which closely aligns with the results of this study. The average EC_{50} from Table 4.2.1 is 0.61±0.49 mg/L. Therefore, this method can be applied to test landfill leachate toxicity on NI.

Despite the overall completeness of this experiment, several limitations remain. First, MLSS (mixed liquor suspended solids), a key indicator of sludge concentration, wasn't measured before the experiment. This omission is a major factor contributing to differences in EC_{50} values. Second, this experimental process didn't distinguish whether ATU primarily inhibits AOB or NOB. Previous studies by Iizumi et al. (1998) and Hooper et al. (1973) suggest that ATU mainly affects AOB nitrification. Investigating this distinction would provide a more comprehensive understanding of ATU's inhibitory effects. Third, increasing the number of sampling batches could enhance the accuracy of results. However, conducting more batches would be highly time consuming. The available assay types are introduced in Section 2.4. Lastly, experimenting with multiple sludge batches would improve the statistical reliability of the data by expanding the sample size.

Although ATU is not widely used in urban catchment, it is frequently applied in farmland and chemical laboratories. Chemicals with similar functions include TCMP and DMPP, as discussed in Subsection 2.2.2. Notably, ATU demonstrated a stable ability to cause NI throughout this experiment.

Table 4.2.1: The comparison of NI caused by ATU from studies

Study	NI%	EC (mg/L)	Assay type
This study	50	0.41	Ammonia conversion
	100	2.18	Ammonia conversion
Young et al. (1973)	100	2	OUR
Iizumi et al. (1998)	50	0.21	Bioluminescence in 1 min
	50	0.15	Bioluminescence in 5 min
	50	1.07	AUR
Hockenbury et al. (1977) & Hooper et al. (1973)	50	1.2	AUR

4.2.2 Controlled experiment II

The results in Controlled experiment I had proved the effectiveness of this method. However, the results in Controlled experiment II appeared to be no significant NI overall, based on the values in Table 4.1.3. However, this conclusion is not entirely rigorous. A more detailed analysis of the curves in Fig.4.1.5 and Fig.4.1.6 shows that 20% and 30% landfill leachate caused over 20% NI before 80 minutes. After 80 minutes, the nitrification rates increased sharply, even surpassing those of the control group. This suggests that this particular batch of landfill leachate does not have a stable ability to cause NI.

As mentioned in Section 3.2, the landfill leachate originates from a construction and demolition waste landfill. This leachate typically has a high pH and contains elevated levels of heavy metals probably but variation ranges are large, as shown in Table 2.3.2. Several studies have indicated that a high pH and high concentrations of certain heavy metals can negatively affect the bio-treatment process through a series of NI effects. Therefore, it is assumed that this batch of landfill leachate contains relatively low levels of alkalinity and heavy metals.

Section 2.5 indicates that nitrification rates can accelerate following inhibition due to microbial resistance. This phenomenon is often attributed to environmental stressors such as high free ammonia, high free nitrous acid, high salinity, low dissolved oxygen or extreme temperature, as detailed in Table 2.5.1. Ammonia conversion rates of experimental group were consistently high, sometimes exceeding controlled group at 90-120 minutes, as shown in Fig.B1. It is assumed that the nitrifiers are resistant to highly toxic environments.

One limitation of this study is the lack of analysis of landfill leachate composition, particularly the measurement of ammonia concentration. Additionally, the highest tested landfill leachate content (30%) may not be sufficient to fully verify the NI effect. It is recommended that future trials include landfill leachate content of up to 50% to better assess its impact. Another limitation is that the experiment was conducted with a single batch of landfill leachate lacking diversity. A potential improvement would be to analyze multiple batches of landfill leachate and identify the specific components responsible for high NI degrees. However, this would require a large-scale study over an extended period. Furthermore, investigating nitrifier resistance over time in response to varying landfill leachate concentrations could be a valuable area of research.

Numerous studies have linked landfill leachate causing NI because of its high COD in WWTPs [Paśmionka et al., 2022; Y. M. Kim et al., 2008; L. Wang et al., 2014; Aktaş et al., 2001]. However, landfill leachate inevitably becomes part of raw wastewater, posing a significant risk if it leads to large-scale nitrifier loss. To mitigate this risk, it is essential to monitor the quality of both raw wastewater and effluent, measuring key parameters such as COD, BOD, ammonia concentration and pH value. Modern WWTPs are increasingly equipped with advanced detection systems, but the most effective strategy is to prevent and control landfill leachate contamination at its source. For example, landfills should implement precisely monitoring systems for leachate composition.

Landfill leachate is only one of the major contributors to NI, and its complex composition suggests that NI is likely caused by the interaction of multiple compounds. Additionally, operational factors such as the ratio of carbon and nitrogen, temperature, aeration intensity, and sludge retention time must also be carefully managed to minimize NI risks.

The study of landfill leachate causing NI is an extensive and highly practical area of research within the engineering field. Its findings contribute to improving operational efficiency in both WWTPs and landfills. Investigating the specific compounds within landfill leachate requires a more scientific approach, involving a huge number of experiments. However, due to variations in microbial communities, data from previous and valuable studies should be used primarily as a reference of Section 2.2). Given these complexities, it can be concluded that research on landfill leachate causing NI is a vast and challenging subject, requiring further studies to fully understand its mechanisms and mitigation strategies.

CHAPTER

FIVE

CONCLUSION

As society develops, the discharge of wastewater with increasingly complex compositions continues to grow. Consequently, environmental standards are becoming stricter, necessitating upgrades in WWTPs to enhance their ability to treat various impurities and toxic substances. Among all wastewater treatment processes, biological treatment plays a crucial role, especially nitrification. However, sensitive microorganisms cannot withstand powerful and continuous toxic shocks, making it essential to analyze the degree of NI caused by toxicants.

Through literature review, several substances and extreme conditions have been identified as key factors contributing to NI. The heavy metals include Ag, Hg, Cd, Cu, Ni and Zn. The typical chemical compounds include cyanide, phenol, TCMP, ATU, TAA etc. According to statistics, the highest toxic substances are Ag (EC_{50}=0.008 mg/L) and NPE (EC_{50}=0.0003 mg/L). The extreme environmental conditions, such as high FA, high salinity, low pH or extreme temperature, would cause NI significantly.

To verify the impact of toxicants on NI, landfill leachate was tested in controlled experiments and ATU proved effectivity of assay method - ammonia conversion. Controlled experiment I showed ATU caused NI obviously and stratified. ATU is a biological nitrification inhibitor. The results showed that EC_{50} of ATU was 0.41±0.03 mg/L and total inhibition was at 2.18±0.03 mg/L. Since EC_{50} was less than 1 mg/L, the results met expectations, further validating ATU as a stable nitrification inhibitor in laboratory conditions. It meant that this assay method could indicate the degree of NI. In Controlled experiment I, this batch of landfill leachate didn't show a significant inhibition effect overall. However, 20% and 30% landfill leachate caused NI at 110 minutes, but nitrification rates unexpectedly increased between 110-120 minutes. The results suggested that this batch of landfill leachate lacked stable NI effects, potentially due to nitrifiers' resistance.

The potential solution of nitrifiers protection is classification and limitation of wastewater sources in WWTP. Implementing strict pre-treatment process and source control at catchment can help minimize toxic. Enhancing nitrifier variability is an effective method to improve adaptability of nitrifiers. The other method, building emergency tank for accident is applying in most large scale WWTPs.

The study of NI sources is complex and requires long term verification. It spans across biological, chemical, and engineering fields. The study of NI sources requires interdisciplinary collaboration. Future research should focus on larger datasets, advanced detection techniques, and a more detailed breakdown of nitrification mechanisms.

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APPENDICES

APPENDIX

A

CONTROLLED EXPERIMENT I

A1 Data collection

Table A1: Data collection for controlled experiment I

Time (min)	Nitrate concentration for all groups (mg N•L^{-1})					R_{CN} (mg N•L^{-1}•min^{-1})			NI%
	A	B	C	D	M_E	R_{CNC}	R_{CNE}	ΔR_{CN}	
0.125 mg/L ATU									
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
30	1.06	0.71	0.90	0.74	0.78	0.11	0.08	0.03	26%
40	2.39	1.78	2.06	1.96	1.93	0.13	0.11	0.02	19%
50	3.93	2.95	3.28	3.35	3.19	0.15	0.13	0.03	19%
60	5.44	4.10	4.65	4.68	4.48	0.15	0.13	0.02	18%
70	7.24	5.35	6.22	6.29	5.95	0.18	0.15	0.03	18%
80	9.06	6.71	7.63	7.78	7.37	0.18	0.14	0.04	19%
90	9.75	7.89	9.11	9.26	8.75	0.07	0.14	-0.07	10%
100	10.10	9.22	10.22	10.60	10.02	0.04	0.13	-0.09	1%
110	12.20	10.63	10.86	10.59	10.69	0.21	0.07	0.14	12%
120	13.82	11.44	11.13	10.95	11.17	0.16	0.05	0.11	19%
0.25 mg/L ATU									
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
30	0.64	0.32	0.37	0.41	0.37	0.06	0.04	0.03	42%
40	2.07	1.31	1.53	1.35	1.40	0.14	0.10	0.04	33%
50	3.41	2.44	2.68	2.30	2.47	0.13	0.11	0.03	28%
60	4.99	3.58	3.91	3.38	3.62	0.16	0.11	0.04	27%
70	6.61	4.70	5.13	4.37	4.73	0.16	0.11	0.05	28%

Time (min)	Nitrate concentration for all groups (mg N•L^{-1})					R_{CN} (mg N•L^{-1}•min^{-1})			NI%
	A	B	C	D	M_E	R_{CNC}	R_{CNE}	ΔR_{CN}	
80	8.47	5.92	6.47	5.55	5.98	0.19	0.12	0.06	29%
90	9.36	7.11	7.85	6.63	7.20	0.09	0.12	-0.03	23%
100	10.74	8.39	8.95	7.66	8.33	0.14	0.11	0.02	22%
110	11.95	9.00	8.91	10.11	9.34	0.12	0.10	0.02	22%
120	13.63	9.18	9.89	10.43	9.83	0.17	0.05	0.12	28%
				0.5 mg/L ATU					
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
30	0.45	0.38	0.00	0.14	0.18	0.45	0.18	0.28	61%
40	1.87	1.01	0.69	0.82	0.84	1.41	0.67	0.75	55%
50	3.39	1.57	1.47	1.42	1.49	1.52	0.65	0.87	56%
60	4.98	1.98	2.23	2.04	2.09	1.59	0.60	0.99	58%
70	6.64	2.47	3.00	2.47	2.65	1.67	0.56	1.11	60%
80	8.05	2.83	3.60	3.20	3.21	1.41	0.56	0.85	60%
90	10.98	3.24	4.18	3.63	3.68	2.93	0.47	2.45	66%
100	12.89	3.62	4.71	4.06	4.13	1.91	0.45	1.46	68%
110	13.43	4.00	5.30	4.56	4.62	0.54	0.48	0.05	66%
120	14.37	4.32	5.76	4.85	4.98	0.94	0.36	0.58	65%
				1.0 mg/L ATU					
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	1.50	1.00	0.85	0.99	0.95	1.50	0.95	0.56	37%
20	3.24	1.66	1.57	1.59	1.61	1.74	0.66	1.08	50%
30	4.96	1.91	1.92	1.91	1.91	1.72	0.30	1.42	61%
40	6.80	2.17	2.06	2.05	2.10	1.84	0.18	1.65	69%
50	8.63	2.36	2.14	2.26	2.25	1.83	0.16	1.67	74%
60	9.46	2.32	2.27	2.30	2.29	0.83	0.04	0.79	76%
70	10.25	2.44	2.34	2.25	2.34	0.79	0.05	0.74	77%
80	12.50	2.53	2.36	2.33	2.40	2.25	0.06	2.19	81%
90	14.06	2.58	2.40	2.38	2.45	1.56	0.05	1.51	83%
100	15.45	2.43	2.50	2.36	2.43	1.40	-0.03	1.42	84%
110	17.64	2.82	2.52	2.53	2.62	2.18	0.19	1.99	85%
120	18.05	2.73	2.53	2.44	2.56	0.41	-0.06	0.47	86%
				2.0 mg/L ATU					
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
20	0.12	0.08	0.07	0.00	0.05	0.12	0.05	0.06	55%
30	1.39	0.18	0.12	0.00	0.10	1.28	0.05	1.23	93%
40	2.95	0.34	0.13	0.00	0.16	1.55	0.06	1.50	95%
50	4.65	0.35	0.14	0.00	0.16	1.70	0.01	1.69	97%
60	6.38	0.20	0.18	0.00	0.13	1.73	-0.04	1.77	98%

Time (min)	Nitrate concentration for all groups (mg N•L^{-1})					R_{CN} (mg N•L^{-1}•min^{-1})			NI%
	A	B	C	D	M_E	R_{CNC}	R_{CNE}	ΔR_{CN}	
70	7.03	0.23	0.26	0.00	0.16	0.65	0.04	0.61	98%
80	8.05	0.36	0.27	0.00	0.21	1.02	0.05	0.97	97%
90	10.02	0.42	0.33	0.00	0.25	1.97	0.04	1.94	98%
100	11.83	0.38	0.28	0.00	0.22	1.81	-0.03	1.84	98%
110	12.47	0.59	0.28	0.00	0.29	0.64	0.07	0.57	98%
120	13.53	0.52	1.59	0.00	0.70	1.05	0.41	0.64	95%

1. Explanations for abbreviations: M_E: the average nitrate concentration of experimental groups (B, C and D);
2. A group is controlled group, B, C and D are experimental groups;
3. R_{CN} is average rate of nitrate production by bacteria in 10min, R_{CNC} is for controlled group, R_{CNP} is for experimental groups;
4. ΔR_{CN} is the difference of nitrate production rate between controlled group and experimental groups;
5. The equations of R_{CN} and NI% refer to Section 3.5.

A2 Figures

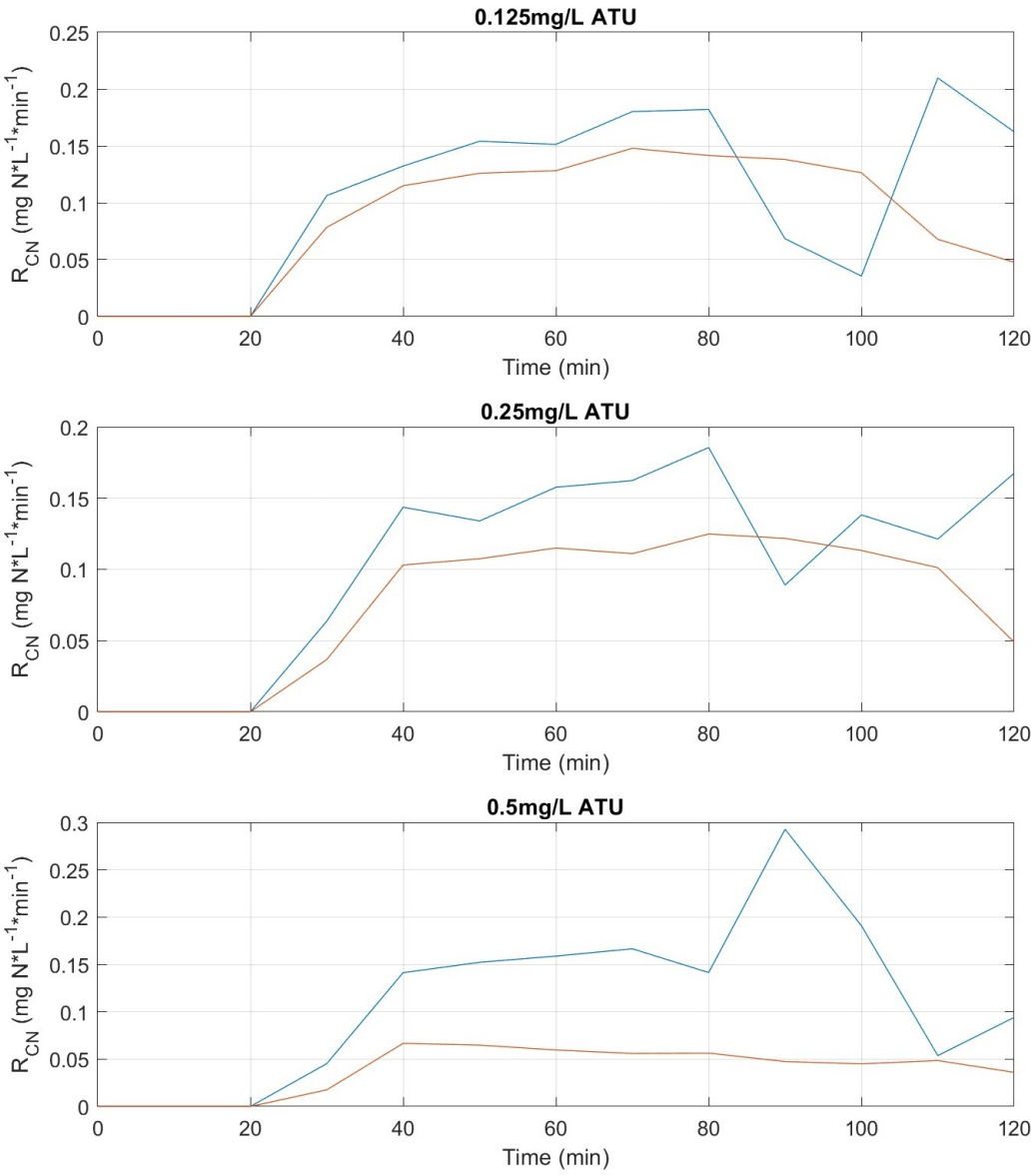

Fig. A1. Comparisons of R_{CN} for controlled and experimental groups, figures are for 0.125, 0.25 and 0.5 mg/L ATU respectively (blue line: controlled group; red line: experimental group).

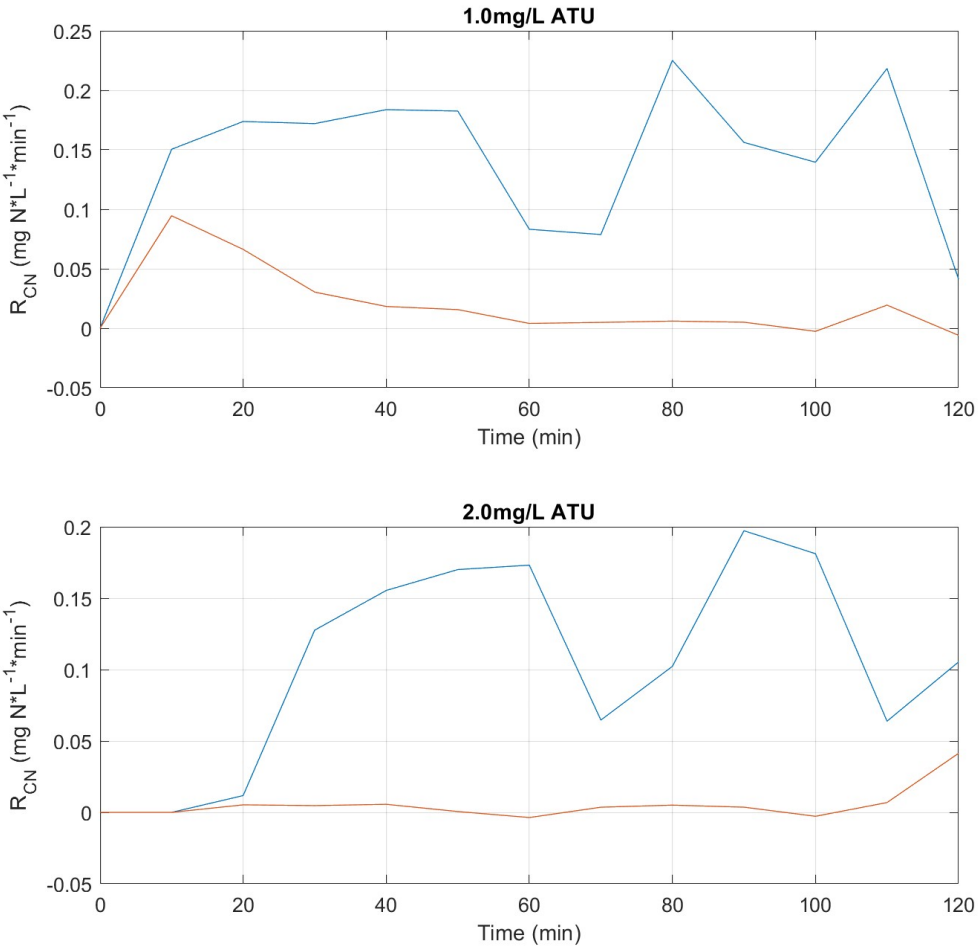

Fig. A2. Comparisons of R_{CN} for controlled and experimental groups, figures are for 1.0 and 2.0 mg/L ATU respectively (blue line: controlled group; red line: experimental group).

APPENDIX

B

CONTROLLED EXPERIMENT II

B1 Data collection

Table B1: Data collection for controlled experiment I

Time (min)	Nitrate concentration for all groups (mg N•L^{-1})					R_{CN} (mg N•L^{-1}•min^{-1})			NI%
	A	B	C	D	M_E	R_{CNC}	R_{CNE}	ΔR_{CN}	
					10% LFL				
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	2.47	2.21	2.67	2.62	2.50	0.25	0.25	0.00	-1%
20	4.73	4.57	5.07	5.18	4.94	0.23	0.24	-0.02	-4%
30	6.99	6.14	6.74	6.66	6.51	0.23	0.16	0.07	7%
40	8.85	8.29	9.06	9.02	8.79	0.19	0.23	-0.04	1%
50	11.13	10.46	11.40	11.16	11.01	0.23	0.22	0.01	1%
60	13.28	12.44	13.89	13.40	13.24	0.21	0.22	-0.01	0%
70	15.48	14.66	16.25	16.04	15.65	0.22	0.24	-0.02	-1%
80	16.44	17.57	19.44	18.67	18.56	0.10	0.29	-0.19	-13%
90	19.33	19.12	21.13	20.52	20.26	0.29	0.17	0.12	-5%
100	21.43	21.25	23.47	22.79	22.50	0.21	0.22	-0.01	-5%
110	23.53	23.38	25.82	25.05	24.75	0.21	0.22	-0.01	-5%
120	25.63	25.51	28.17	27.32	27.00	0.21	0.22	-0.01	-5%
					20% LFL				
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	1.67	-0.82	0.16	1.23	0.19	0.17	0.02	0.15	89%
20	3.96	0.58	2.31	2.21	1.70	0.23	0.15	0.08	57%
30	5.96	1.74	4.05	3.58	3.12	0.20	0.14	0.06	48%
40	7.79	3.53	5.89	4.40	4.61	0.18	0.15	0.03	41%
50	9.79	4.99	7.96	5.65	6.20	0.20	0.16	0.04	37%
60	12.27	6.34	10.63	6.87	7.95	0.25	0.17	0.07	35%
70	14.22	8.93	12.31	7.92	9.72	0.20	0.18	0.02	32%

Time (min)	Nitrate concentration for all groups (mg N•L^{-1})					R_{CN} (mg N•L^{-1}•min^{-1})			NI%
	A	B	C	D	M_E	R_{CNC}	R_{CNE}	ΔR_{CN}	
80	15.89	10.51	14.60	9.01	11.37	0.17	0.17	0.00	28%
90	17.65	12.23	16.65	10.07	12.98	0.18	0.16	0.02	26%
100	17.57	14.23	19.07	11.26	14.85	-0.01	0.19	-0.20	15%
110	17.48	15.85	21.25	12.63	16.57	-0.01	0.17	-0.18	5%
120	17.05	17.43	23.40	13.36	18.06	-0.04	0.15	-0.19	-6%
				30% LFL					
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0%
10	1.82	1.85	1.48	0.74	1.35	0.18	0.14	0.05	26%
20	4.64	3.67	3.13	2.22	3.01	0.28	0.17	0.12	35%
30	7.16	5.55	4.76	2.84	4.38	0.25	0.14	0.11	39%
40	9.43	7.52	6.65	3.88	6.02	0.23	0.16	0.06	36%
50	11.66	9.14	8.42	4.84	7.47	0.22	0.14	0.08	36%
60	13.99	11.44	10.39	5.89	9.24	0.23	0.18	0.06	34%
70	16.48	12.87	11.98	7.15	10.66	0.25	0.14	0.11	35%
80	18.77	14.87	13.75	8.12	12.25	0.23	0.16	0.07	35%
90	18.51	17.41	16.07	9.40	14.29	-0.03	0.20	-0.23	23%
100	18.42	18.12	18.77	10.55	15.81	-0.01	0.15	-0.16	14%
110	19.25	21.88	21.09	11.32	18.10	0.08	0.23	-0.15	6%
120	18.95	23.82	22.08	12.44	19.45	-0.03	0.14	-0.17	-3%

1. Explanations for abbreviations: M_E: the average nitrate concentration of experimental groups (B, C and D);
2. A group is controlled group, B, C and D are experimental groups;
3. R_{CN} is average rate of nitrate production by bacteria in 10min, R_{CNC} is for controlled group, R_{CNP} is for experimental groups;
4. ΔR_{CN} is the difference of nitrate production rate between controlled group and experimental groups;
5. The equations of R_{CN} and NI% refer to Section 3.5.

B2 Figures

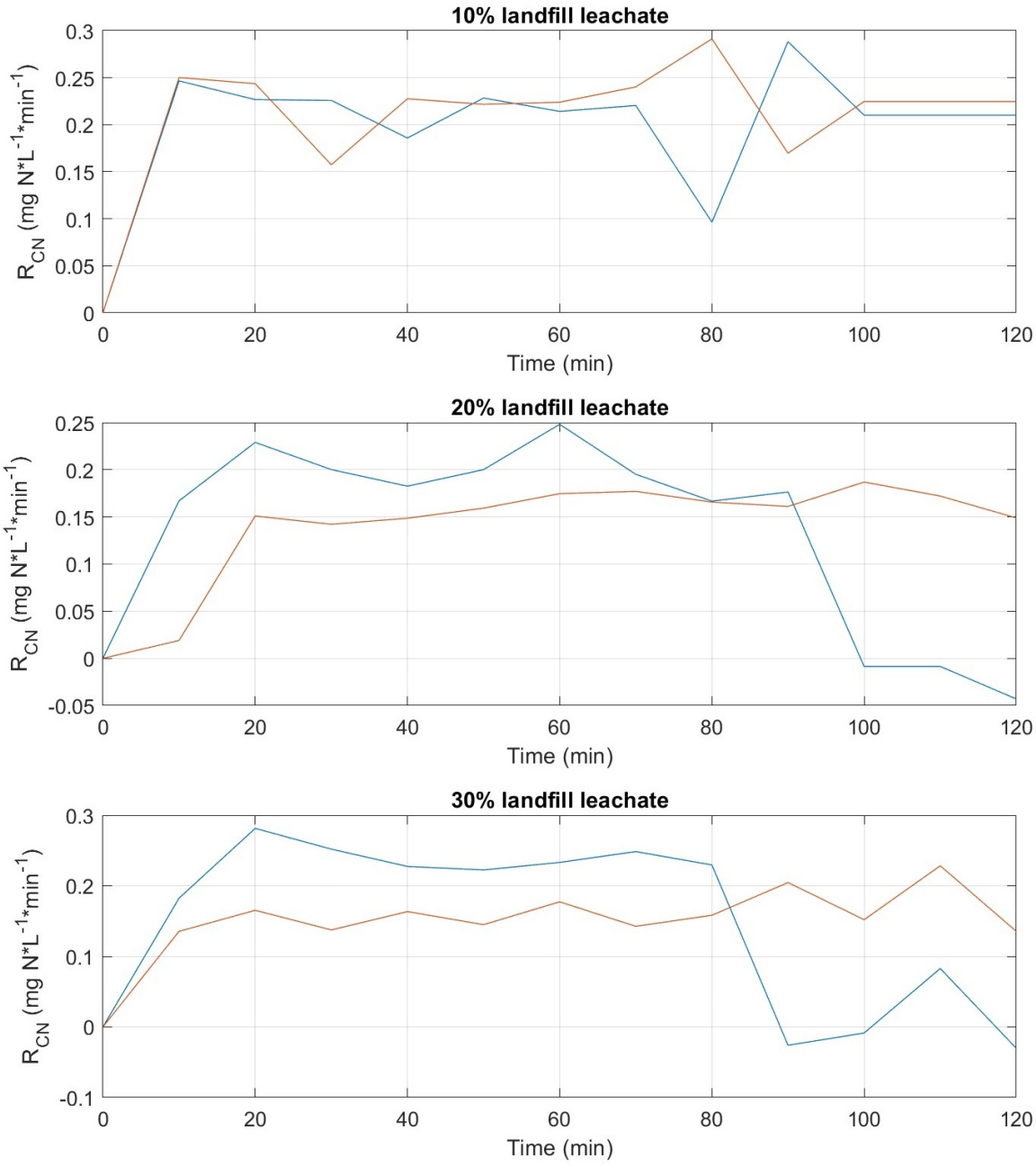

Fig. B1. Comparisons of R_{CN} for controlled and experimental groups, figures are for 10%, 20% and 30% LFL respectively (blue line: controlled group; red line: experimental group).