A PILOT SCALE STUDY OF LOW DISSOLVED OXYGEN OXIC-ANOXIC PROCESS FOR BIOLOGICAL NITROGEN REMOVAL FROM TROPICAL SEWAGE

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FACULTY OF ENGINEERING
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ABSTRACT

Complying with nitrogen effluent discharge limits in domestic sewage treatment comes with high energy and chemical costs. Conventional biological nitrogen removal (BNR) process, which maintains high dissolved oxygen (DO) concentrations (>2 mg/L), often consumes half of the total plant's energy for aeration. Stakeholders in the sewage industry are seeking ways to improve energy sustainability. Low-DO oxic-anoxic (low-DO OA) meets the criteria as a simpler, cost-effective and efficient biological treatment process due to its operation at low DO level (<1 mg/L). The lab-scale low-DO OA reactor completely removed ammoniacal-nitrogen (NH_4-N) and produced low effluent nitrate-nitrogen (NO_3-N) when treating local sewage. However, the feasibility of the low-DO OA process has yet to be validated at a scaled-up level for nitrogen removal. This project aimed to evaluate the feasibility of an on-site pilot-scale low-DO OA process in treating domestic sewage with low chemical oxygen demand-to-nitrogen ratio (COD/N) in tropical climate. Despite the low COD/N influent sewage, further denitrification experiments revealed that slowly biodegradable COD (sbCOD), predominantly present in particulate settleable solids (PSS) in domestic wastewater, can effectively support nitrogen removal efficiency. Nitrification batch tests demonstrated that operating the BNR at low DO concentrations is feasible. Nitrifiers from low DO sludge samples have a higher oxygen affinity with a lower half-saturation constant (K_o) of 0.18 mg/L compared to high DO sludge samples of 0.4 mg/L. The low-DO condition may create a favourable condition for the growth of K-strategist nitrifiers such as comammox *Nitrospira*. The pilot-scale sequencing batch reactor (pilot SBR) with a working volume of 150 L was operated in three phases: Phase 1 (low-DO OA), Phase 2 (high-DO OA) and Phase 3 (low-DO OA with intermittent aeration). Operating at low-DO OA achieved higher NH_4-N (97 ± 3%) and total nitrogen (TN) (80 ± 6%) removal efficiencies compared to high-DO OA at NH_4-N (99%) and TN (70 ± 5%). Integrating low-DO OA with intermittent aeration improved the TN removal efficiency to 85 ± 6% with 99% NH_4-N removals. The low-DO OA process could reduce energy consumption by 18% while low-DO OA with intermittent aeration could potentially save up to 25% energy compared to the conventional BNR process. This pilot study provided a better understanding of the feasibility and energy efficiency of removing nitrogen from tropical sewage with a low COD/N ratio in Malaysia. The low-DO OA process could provide a simpler, cost-effective and sustainable alternative that can be retrofitted into conventional BNR systems, thereby reducing energy and chemical consumption in STPs.

Keywords: Activated sludge; domestic sewage treatment; energy saving; intermittent aeration; low dissolved oxygen.

KAJIAN SKALA PILOT PROSES OKSIK-ANOKSIK PADA OKSIGEN TERLARUT RENDAH UNTUK PENYINGKIRIAN NITROGEN SECARA BIOLOGI BAGI AIR KUMBAHAN TROPIKA

ABSTRAK

Mematuhi had pelepasan efluen nitrogen dalam rawatan air kumbahan domestik memerlukan kos tenaga dan kimia yang tinggi. Proses penyingkiran nitrogen (BNR) konvensional yang mengekalkan kepekatan oksigen terlarut (DO) tinggi (>2 mg/L) kerap menggunakan separuh daripada tenaga keseluruhan loji untuk sistem pengudaraan. Syarikat pengurusan kumbahan sedang mencari kaedah untuk meningkatkan kelestarian tenaga rawatan air kumbahan. Proses rawatan oksik-anoksik dengan DO rendah (low-DO OA) memenuhi kriteria sebagai proses rawatan biologi yang lebih mudah, kos berkesan dan cekap disebabkan operasinya pada tahap DO yang rendah (<1 mg/L). Walau bagaimanapun, kebolehlaksanaan proses low-DO OA untuk penyingkiran nitrogen masih belum disahkan pada tahap skala tinggi. Projek ini bertujuan untuk menilai kebolehlaksanaan proses low-DO OA pada tahap skala pilot dalam merawat kumbahan domestik dengan nisbah permintaan oksigen kimia kepada nitrogen (COD/N) yang rendah dalam iklim tropika. Walaupun kumbahan mengandungi COD/N yang rendah, eksperimen denitrifikasi selanjutnya mendedahkan bahawa COD yang terbiodegradasi dengan perlahan (sbCOD) di mana kebanyakannya terdapat dalam pepejal boleh larut zarah (PSS) dalam air kumbahan domestik, boleh menyokong kecekapan penyingkiran nitrogen dengan berkesan. Eksperimen nitrifikasi menunjukkan bahawa mengoperasikan BNR pada DO yang rendah adalah boleh dilaksanakan. Organisma nitrifikasi dari sampel enap cemar DO rendah mempunyai afiniti oksigen yang lebih tinggi dengan nilai setengah jenuh tetap yang lebih rendah (K_o) iaitu 0.18 mg/L berbanding sampel enap cemar DO tinggi sebanyak 0.4 mg/L. Keadaan low DO mungkin memberi kelebihan untuk pertumbuhan organisma nitrifikasi jenis K-strategist seperti comammox *Nitrospira*.

Reaktor kelompok penjujukan pilot (pilot SBR) dengan isi padu kerja 150 L dikendalikan dalam tiga fasa: Fasa 1 (low-DO OA), Fasa 2 (high-DO OA) dan Fasa 3 (low-DO OA DO dengan pengudaraan berselang-seli). Proses low-DO OA mencapai kecekapan penyingkiran NH_4-N (97 ± 3%) dan jumlah nitrogen (TN) (80 ± 6%) yang lebih tinggi berbanding high-DO OA tinggi pada NH_4-N (99%) dan TN (70 ± 5%). Mengintegrasikan low-DO OA dengan pengudaraan berselang-seli dapat meningkatkan kecekapan penyingkiran TN kepada 85 ± 6% dengan prestasi penyingkiran NH_4-N 99%. Proses low-DO OA boleh mengurangkan penggunaan tenaga sebanyak 18% manakala low-DO OA dengan pengudaraan berselang-seli berpotensi menjimatkan sehingga 25% tenaga berbanding proses BNR konvensional. Kajian skala pilot ini memberikan pemahaman yang terperinci mengenai kebolehlaksanaan dan kecekapan tenaga untuk menyingkirkan nitrogen daripada air kumbahan tropika dengan nisbah COD/N yang rendah di Malaysia. Proses low-DO OA boleh menyediakan alternatif yang lebih mudah, kos efektif dan lestari yang boleh diubahsuai ke dalam sistem BNR konvensional, sekali gus mengurangkan penggunaan tenaga dan kimia di STPs.

Kata Kunci: Enapcemar aktif; air kumbahan domestic; tenaga penjimatan; pengudaraan selang-selang; oksigen terlarut rendah.

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Symbol / Abbreviations		Definition
AO	:	Anoxic-oxic
AAO	:	Anaerobic-anoxic-oxic
AOR	:	Ammonium oxidation rate
BNR	:	Biological nitrogen removal
BOD	:	Biochemical oxygen demand
bCOD	:	Biodegradable chemical oxygen demand
C/N	:	Carbon-to-nitrogen ratio
COD	:	Chemical oxygen demand
DO	:	Dissolved oxygen
DOC	:	Dissolved organic carbon
HRT	:	Hydraulic retention time
K_o	:	Oxygen half saturation
MLR	:	Mixed liquor recycle
MLSS	:	Mixed liquor suspended solids
MLVSS	:	Mixed liquor volatile suspended solids
Na_2CO_3	:	Sodium carbonate
NO	:	Nitric oxide
N_2O	:	Nitrous oxide
NH_4^+	:	Ammonium
NO_2^-	:	Nitrite
NO_3^-	:	Nitrate
NH_4-N	:	Ammoniacal nitrogen
NH_4Cl	:	Ammonium chloride

NO_2-N	:	Nitrite nitrogen
NO_3-N	:	Nitrate nitrogen
PE	:	Population equivalent
PSS	:	Particulate settleable solids
PO_4-P	:	Phosphate phosphorus
RAS	:	Return activated sludge
rbCOD	:	Readily biodegradable chemical oxygen demand
$SAOR$	:	Specific ammonium oxidation rate
SBR	:	Sequential batch reactor
SND	:	Simultaneous nitrification and denitrification
SRT	:	Sludge retention time
STPs	:	Sewage treatment plants
sCOD	:	Soluble chemical oxygen demand
sbCOD	:	Slowly biodegradable chemical oxygen demand
TN	:	Total nitrogen
TP	:	Total phosphorus
TSS	:	Total suspended solids
TCOD	:	Total chemical oxygen demand
UDT	:	Unexpected downtime
VSS	:	Volatile suspended solids

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CHAPTER 1: INTRODUCTION

1.1 Research Background

The growing population is contributing to an increase in nitrogen pollution-related effects, notably eutrophication, in many parts of the world (Bell et al., 2021; Frank, 2022; Guo et al., 2023; Jipanin et al., 2019; Prasad & Prasad, 2019; Stuti, 2023). As nitrogen pollution can lead to water quality degradation, pose risks to public health and harm aquatic biodiversity, it also has a significant impact on the economy and tourism. In addressing this challenge, many sewage treatment plants (STPs) often integrate the biological nitrogen removal (BNR) process into their facilities to remove nitrogen pollutants efficiently from sewage before being discharged to groundwater, surface water or wetlands.

The conventional BNR activated sludge process is well-known to be energy intensive. The aeration system alone, which provides oxygen to sewage treatment, can consume over 45 to 75% of the total STP's energy (Ramli & Hamid, 2017). In Malaysia, the total electricity costs for sewage treatment operations rose from RM22.5 million in 2000 to RM256.3 million in 2020, marking a tenfold increase in two decades (IWK, 2021). The electricity cost is expected to increase annually by 6.7% due to population growth, inflation and stricter effluent standards (Aliman, 2019; The Malaysian Reserve, 2021). Given the high operating expenditure, the Malaysian sewerage industry targets to reduce 15% of its energy consumption by 2030 (KeTTHA, 2017).

Another challenge with the conventional BNR process is the unsatisfactory nitrogen removal performance when treating sewage with limited organic carbon sources (Gao et al., 2020; How, Sin, et al., 2020; Zou et al., 2022). The soluble chemical oxygen demand-to-nitrogen ratio (COD/N) of 3 to 6 g COD/g N in most domestic sewage is insufficient to achieve complete biological nitrogen removal, which typically requires 6 to 11 g

COD/g N (Gao et al., 2020; How et al., 2018; Zhao et al., 2018). Although dosing carbon sources (methanol, acetate and ethanol) has been a common practice to improve nitrogen removal performance, it may not be sustainable and economical due to the high chemical costs and potential environmental impact (Dasgupta et al., 2017).

Several energy- and carbon-efficient BNR technologies have been developed for treating low COD/N domestic sewage including anaerobic ammonia oxidation (anammox), partial nitritation combined with anammox (PN/A) and low dissolved oxygen (DO) nitrification (Dasgupta et al., 2017; How et al., 2018; Kuenen, 2008). While anammox and PN/anammox processes have gained momentum in recent decades, they are more complex and have high costs for plant retrofitting and process control (Ge et al., 2015; Wang et al., 2022). The low-DO nitrification process has emerged as a promising solution with low retrofitting costs and high ammonium removal performance (How et al., 2018; Keene et al., 2017). Many studies have demonstrated efficient ammonium removal (>95%) is possible under low DO conditions (<1 mg/L) (How et al., 2021; Wang et al., 2022; Zheng et al., 2019). Besides intermittent aeration conditions, the low DO condition can also provide an optimal condition for simultaneous nitrification and denitrification (SND), further enhancing the nitrogen removal performance (How et al., 2019; Miao et al., 2022; Srb et al., 2022).

Many BNR processes in Malaysia operate on a pre-anoxic configuration, also referred to as anoxic-oxic (AO), despite the high operating costs of mixed liquor recycles (MLR) stream (Shen et al., 2019; Winkler et al., 2011). On the contrary, post-anoxic configuration, also known as oxic-anoxic (OA), eliminates the need for MLR pumping. Several studies have reported remarkable denitrification performance (90 to 98%) without carbon dosage when treating low COD/N sewage (Gao et al., 2020; Winkler et al., 2011; Zhao et al., 2018). How et al. (2020) reported that the low-DO OA process achieved complete nitrification with low effluent nitrate nitrogen (NO_3-N) concentrations of 0.3 mg/L without carbon dosage when treating low COD/N tropical sewage (How, Nittami, et al., 2020). At the lab-scale, How et al. (2019) reported that the tropical sewage (around 30ºC) accelerates the particulate settleable solids (PSS) hydrolysis rate, providing sufficient readily biodegradable COD (rbCOD) for denitrification. The low-DO OA process is simple and can be easily retrofitted to the existing BNR facilities in STP, reducing both capital and operating costs. However, the feasibility of the low-DO OA process has not been assessed in a pilot-scale study as a precursor to its full-scale BNR application in Malaysia.

1.2 Research Questions

a) Does the hydrolysis rate of PSS provide sufficient rbCOD for denitrification in low COD/N tropical sewage when scaled up to a larger treatment setting?
b) What is the correlation between DO concentrations and the maximum nitrification potential in sludge samples from different sewage treatment conditions?
c) Is the low-DO OA process feasible to be implemented at a larger-scale application of BNR sewage treatment in Malaysia?
d) Does intermittent aeration in the low-DO OA process further contribute to energy savings and enhanced nitrogen removal performance?

1.3 Research Objectives

Following the promising lab-scale results in achieving effective nitrogen removal with low energy demand, a pilot-scale reactor of a cost-effective oxic-anoxic sewage treatment system for biological nitrogen removal was set up. The main aim of this research is to evaluate the feasibility of an on-site pilot-scale low-DO OA process as an efficient and cost-effective BNR sewage treatment in treating low COD/N tropical sewage in Malaysia. To achieve the main goal, four specific objectives are outlined:

a) To investigate the biodegradability of PSS in tropical sewage as carbon source for denitrification.
b) To analyse the effect of DO concentrations on the maximum nitrification potential using sludge samples from low and high DO STPs.
c) To evaluate the long-term nitrogen removal performance of the low-DO OA process compared to the high-DO OA process.
d) To determine the nitrogen removal performance of intermittent aeration in the low-DO OA process.

1.4 Dissertation Outline

The dissertation comprises five chapters. The current chapter (***Chapter 1***) briefly discusses the research background related to nitrogen pollution or eutrophication in water bodies. The limitations of the conventional BNR process in STPs were highlighted in the problem statement section. The research objectives are also outlined in this chapter.

Chapter 2 details relevant literature information. The chapter begins with a more detailed background of nitrogen pollution, including its causes and effects. The fundamentals and drawbacks of the conventional BNR process are also presented. To address the issue, several low-energy BNR processes are listed. Among the listed processes, the low-DO nitrification process is highlighted, and recent studies of low-DO nitrification are included. Different BNR process configuration is also discussed in this chapter, ultimately leading to the discussions of the low-DO OA process.

Chapter 3 provides a clear overview of materials and methods used throughout the experimental work including reactor description, batch experiments, energy-saving calculations and analytical procedures.

Chapter 4 presents the characteristics of tropical sewage characteristics. The denitrification batch activity test using different COD fractions as carbon source for denitrification was also included in this chapter.

Chapter 5 begins with the nitrification performance at different DO concentrations using sludge samples from STPs operating on low and high DO. In the following section, the feasibility of the low-DO OA process for tropical sewage was reported on the nitrogen removal performance, comparison with high-DO OA, SND occurrence and incorporation of intermittent oxic-anoxic under low DO conditions. The last section reveals the energy savings calculation.

Chapter 6 concludes the findings of the study, highlighting the project's significance and offering recommendations for future work.

CHAPTER 2: LITERATURE REVIEW

2.1 Nitrogen Pollution in Water Bodies

Issues concerning nitrogen-rich sewage include eutrophication, toxicity, methemoglobinemia and water quality degradation (Suwal, 2019; The Star, 2023; United Nations, 2023). High levels of NH_4, nitrite (NO_2) and nitrate (NO_3) in receiving water can cause excessive growth of aquatic plants, leading to dissolved oxygen depletion and biodiversity decline (Qadir et al., 2020). A study has found that 32% of infants suffered from methemoglobinemia condition, often referred to as blue baby syndrome, in Beit Lahia in Gaza Strip, Palestine, with 97% of groundwater exceeding World Health Organisation drinking water guidelines for nitrate (>50 mg/L) (Shaban et al., 2023). The nitrogen loads in the Yangtze River in China have increased dramatically in the past five decades, with over 66% of the nitrogen load in the area contributing to the frequent occurrence of harmful algal blooms (Guo et al., 2023; Wang et al., 2020; Xu, 2022). In 2023, nitrogen-rich sewage runoff caused plankton blooms in Bang Saen Beach in Thailand, turning seawater green and harming tourism and marine life (The Star, 2023). In Vietnam, over 80% of the nation's diseases such as malaria, typhoid and cholera are linked to polluted water containing nitrogen contaminants (Suwal, 2019). In Malaysia, inadequately treated sewage accounted for 70% of nitrogen pollution in the receiving water bodies (Department of Environment of Malaysia, 2022). Such global challenge of mitigating nitrogen-related environmental pollution has become increasingly pressing, given the negative impacts experienced by various regions worldwide. Therefore, many countries implemented nitrogen removal processes in their sewage treatment systems to meet effluent discharge limits, safeguarding water quality and mitigating environmental impacts of nitrogen pollution.

Since sewage effluents are the main contributor to nitrogen load in water environments, stringent regulations are imposed on the discharges of sewage treatment plants (STPs) (Edokpayi et al., 2017; Wang et al., 2023). Stringent sewage discharge limits were implemented on nitrogenous compounds (TN, NH_4-N and NO_3-N) in STPs worldwide to maintain the quality of discharged sewage at acceptable levels (Table 2.1). The sewage effluent discharge regulations differ from one country to another. In Malaysia, a new standard discharge regulation, Environmental Quality (Sewage) Regulations 2009, was introduced in December 2009 to replace Environment Quality (Sewage and Industrial Effluents) 1979. The new Malaysian regulations enforced stricter restrictions on the release of nitrogen compounds (NH_4-N and NO_3-N) in sewage effluent (Ariffin & Sulaiman, 2015). Similarly, Indonesia limits NH_4-N levels in the effluent to below 10 mg/L whereas in Thailand, there are limits on the discharge of total nitrogen (TN = organic nitrogen + NH_4-N + NO_2-N + NO_3-N) and should not exceed 20 mg/L. China, Europe and Japan have also mandated stringent limitations on nitrogen compounds in domestic sewage. With the tightening of sewage discharge limits, integrating the biological nitrogen removal (BNR) processes into sewage treatment has become more prevalent in many STPs to comply with the regulation effluent discharge limits (US EPA, 1993).

Table 2.1: Comparison of treated domestic sewage discharge standards from different countries.

Parameter	China		Europe			Indonesia	Japan	Malaysia		Thailand
	Grade 1-A	Grade 1-B	PE 2–10k	PE 10–100k	PE >100k			Standard A	Standard B	
pH	6–9	6–9	–	–	–	6–9	5.8–8.6 (non-coastal); 5–9 (coastal)	6-9	5.5–9	5.5–9
BOD$_5$ (mg/L)	10	20	25	25	25	30	160 (daily average of 120)	20	50	20
COD (mg/L)	50	60	125	125	125	100	160 (daily average of 120)	120	200	–
TSS (mg/L)	10	20	60	35	35	30	200 (daily average of 150)	50	100	30
TN (mg/L)	15	20	–	15	10	–	120 (daily average of 60)	–	–	20
NH_4-N (mg/L)	5	8	–	–	–	10	–	5 (enclosed water body); 10 (river)	5 (enclosed water body); 20 (river)	–
NO_3-N (mg/L)	–	–	–	–	–	–	–	5 (enclosed water body); 10 (river)	10 (enclosed water body and river)	–
TP (mg/L)	0.5	1	–	2	1	–	16 (daily average of 8)	5	10	2
References	Ministry of Environmental Protection of China (2014)		Council Directive (1991)			Ministry of Environment and Forestry of Indonesia (2016)	Ministry of Environment of Japan (2015)	Department of Environment of Malaysia (2018)		Ministry of Natural Resources and Environment of Thailand (2010)

NH_4-N: ammoniacal nitrogen; NO_3-N: nitrate nitrogen; PE: population equivalent; TN: total nitrogen; China Grade 1-A standard: treated sewage discharges for reuse; China Grade 1-B standard: treated sewage discharges into surface water; Malaysia standard A: sewage effluent released upstream of water supply intake; Malaysia standard B: sewage effluent released downstream of water supply intake.

2.2 Biological Nitrogen Removal Processes

Nitrogen contaminants are commonly removed from sewage using conventional biological (nitrification-denitrification) or physicochemical (membrane filtration, ion exchange, coagulation and flocculation) treatment methods (Larios-Martínez et al., 2022; Patel et al., 2021). Since biological treatment has been relatively inexpensive, effective and widely applied globally over the past few decades, it has often been preferred over physicochemical (Guven et al., 2023; Zhang, Shao, Wang, et al., 2021).

2.2.1 Conventional Nitrification and Denitrification

The conventional biological nitrogen removal (BNR) process involves aerobic nitrification and anoxic denitrification to convert ammonium to gaseous nitrogen. However, extensive aeration and organic carbon supplementation in the conventional BNR process to enhance nitrogen removal performance also result in increased energy and operating costs (Sections 2.2.1.1 and 2.2.1.2).

2.2.1.1 Nitrification

Nitrification is a two-step BNR process performed by distinct groups of autotrophic bacteria called nitrifiers or nitrifying bacteria. The nitrifiers oxidize dissolved ammonia to nitrite and nitrate under aerobic conditions. In the first step, ammonium is oxidized to nitrite by ammonia oxidizing bacteria (AOB), such as *Nitrosomonas, Nitrosospira, and Nitrosococcus* (Equation 2.1). Nitrite accumulation rarely happens because of its instant conversion to nitrate. In the second step, nitrite is oxidized to nitrate by nitrite oxidizing bacteria (NOB), such as *Nitrospira, Nitrobacter and Nitrospina* (Equation 2.2). A total of 4.57g of oxygen is required for complete nitrification (Equation 2.3), with 3.43 g utilized in the conversion of ammonium to nitrite and the remaining 1.14 g used in the oxidation of nitrite to nitrate (Tchobanoglous et al., 2014).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \quad \textbf{(2.1)}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad \textbf{(2.2)}$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \quad \textbf{(2.3)}$$

During the nitrification process, oxygen is supplied to sewage, serving as an electron acceptor for the substrate oxidation. To guarantee efficient ammonium removal and compliance with legal discharge limits for the effluent, conventional STPs operating on extended aeration or activated sludge systems often operate at DO concentrations above 2 mg/L (Bellucci et al., 2011; Wilén et al., 2008). In Malaysia, STPs are mandated to maintain their aeration zones at DO of 2.5 to 3.5 mg/L to prevent nitrification failure (National Water Services Commission, 2009). Operating at high DO (2 to 5 mg/L) prevents the formation of porous flocs (Wilen & Balmer, 1999). In addition, oxygen-limited environments during nitrification can promote excessive growth of filamentous organisms, negatively impacting sludge settling and increasing effluent turbidity.

Despite the need to ensure high DO levels to avoid problems mentioned earlier, such an operation is the direct reason for the high energy costs. High DO nitrification requires intensive aeration, where the air is continuously pumped into sewage (Drewnowski et al., 2019). The aeration system alone often makes up for 45 to 75% of the entire STP's electricity costs (Rosso et al., 2008). It has also been reported that up to 3% of the world's energy production is being used by STPs (IWA, 2021). In STPs, electricity costs are the largest contributor to the overall expenses, followed by operation and maintenance costs (Maniam, 2021). Indah Water Konsortium Sdn Bhd (IWK), Malaysia's national sewerage company, spent RM 22.52 million on electricity costs for sewage treatment operations in 2000 and RM 256.3 million in 2020, indicating a tenfold increase in electricity costs over two decades (IWK, 2021). Hence, the challenge of the conventional BNR process lies in ensuring sustainable and energy-positive treatment operation.

2.2.1.2 Denitrification

The BNR process is followed by denitrification, a reduction step that transforms nitrate into harmless nitrogen gas (N_2) via intermediates nitric oxide (NO) and nitrous oxide (N_2O). The denitrification step was accomplished by denitrifiers or denitrifying bacteria in an oxygen-free environment (Tchobanoglous et al., 2014). Denitrifiers utilize nitrate (or nitrite) instead of oxygen as an electron acceptor to support microbial growth (Fu et al., 2022). It is common for the alkalinity level in sewage to increase because hydroxide ions (OH^-) are released as by-products during denitrification. Equation 2.4 presents the stoichiometric equation for denitrification, with $C_{10}H_{19}O_3N$ denoting biodegradable organic matter in sewage.

$$C_{10}H_{19}O_3N + 10NO_3^- \rightarrow 5N_2 + 10CO_2 + 3H_2O + NH_3 + 10OH^- \quad \textbf{(2.4)}$$

Compared to autotrophic denitrification, heterotrophic denitrification is more common and effective way of nitrogen removal in sewage treatment (van Rijn et al., 2006). Most heterotrophic denitrifiers rely on organic carbon as electron donors to convert nitrate to nitrogen gas and subsequently release it into the atmosphere (Wang & Chu, 2016). An adequate amount of carbon source is important as it has a direct effect on the rate of denitrification for achieving efficient nitrogen removal (Xu et al., 2018). However, complying with regulatory effluent discharge limits for nitrate has been challenging with carbon-limited sewage (Fu et al., 2022).

Most organic matter in sewage is measured by total COD (TCOD), a parameter that can be characterized into biodegradable COD (bCOD) and non-biodegradable COD (nbCOD). The bCOD is further classified into readily biodegradable COD (rbCOD) and slowly biodegradable (sbCOD). The rbCOD, considered soluble organic matter, comprises simpler molecules with particle sizes smaller than 0.45 μm and can be rapidly consumed by heterotrophic microorganisms. On the other hand, the sbCOD fraction is more complex organics molecules with particle sizes larger than 1.2 μm. For the heterotrophic bacteria to use sbCOD, the particulate settleable solids (PSS) must be broken down into rbCOD by extracellular enzymes (Pásztor et al., 2009; Zhang et al., 2021). Domestic sewage typically comprises 26 to 70% of sbCOD and 6 to 25% of rbCOD (Choi et al., 2017; Guellil et al., 2001; Sophonsiri & Morgenroth, 2004). The available data on sewage characteristics in Malaysia indicate that the soluble COD in domestic sewage is insufficient for efficient denitrification (How et al., 2020). How et al. (2019) found that PSS breaks down 2.5 times faster into rbCOD for domestic sewage in tropical climates (30°C) than in colder climates (20°C). The acceleration of PSS hydrolysis rate provides sufficient sbCOD composition to enhance nitrogen removal activity for domestic sewage in tropical climates.

Limited availability of organic carbon in sewage with a low chemical oxygen demand-to-nitrogen (COD/N) ratio (<4) restricts nitrogen removal performance (Gao et al., 2020; How et al., 2019; Zou et al., 2022). As a result, external carbon sources, such as methanol, acetate and ethanol, are commonly added to sewage with limited biodegradable organic matter (Lu et al., 2014). A significant limitation of adding synthetic carbon sources in the BNR process is the increased operating costs (Badia et al., 2021). While acetate and ethanol can rapidly enhance nitrogen removal, they come at a higher cost with acetate priced at $1.2/kg (RM 5.70/kg) and ethanol at $0.96/kg (RM 4.50/kg) (Kim et al., 2017; Mike, 2023b, 2023a). Although methanol is usually preferred and more affordable at $0.3/kg (RM 1.40/kg), it responds relatively slower than acetate and ethanol during the initial feeding stage (Mike, 2023c; Sun et al., 2010). While adding carbon sources effectively reduces nitrogen levels in the effluent, it contributes to higher operating costs. Out of concern for sustainability, there is a need to explore potential BNR processes that can improve energy effectiveness and limit the reliance on organic matter without compromising nitrogen removal efficiency.

2.2.2 Energy and Chemical Efficient Biological Nitrogen Removal Methods

Over the past few decades, many innovative BNR technologies or methods that use less oxygen and organics have been successfully implemented in STPs, such as nitritation/denitritation, partial nitritation/anammox (PN/A), intermittent aeration in BNR and low-DO BNR.

2.2.2.1 Nitritation/Denitritation

The nitritation/denitritation process is an unconventional nitrogen removal approach that uses lower 25% oxygen consumption and 40% carbon requirement compared to conventional nitrification and denitrification (Noutsopoulos et al., 2018). The lower aeration energy is possible with the enrichment of AOB and suppression of NOB to oxidize NH_4-N to NO_2-N in nitritation (Figure 2.1) (Hellinga et al., 1999; Van Kempen et al., 2001). The accumulating NO_2-N from the nitritation serves as an electron acceptor for the heterotrophic denitrifiers to reduce NO_2-N directly to N_2 gas in denitritation, reducing reliance on organic carbon requirements (Dobbeleers et al., 2020). The critical elements to establishing nitritation/denitritation are high temperature (30 to 40°C), low DO condition (<0.5 mg/L), optimal pH (6.5-8), short sludge retention time (SRT) (~1 d) and high free ammonia (Gao et al., 2009; Lemaire et al., 2008; Zeng et al., 2009).

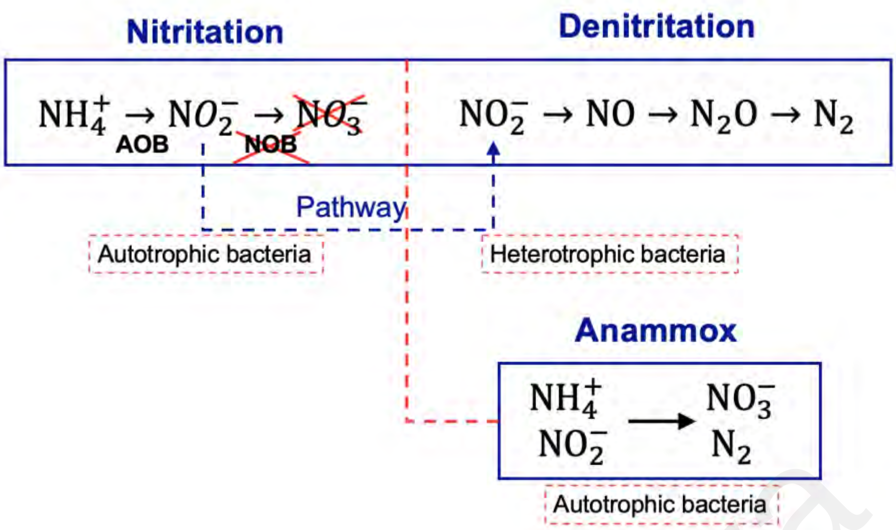


Figure 2.1: Schematic diagram of nitritation/denitritation and partial nitritation/Anammox processes (Malamis et al., 2013)**.**

A challenge lies in maintaining a balance between nitritation/denitritation steps as environmental conditions or sewage composition fluctuations can impact the process stability and nitrogen removal efficiency. Fluctuating temperatures in domestic sewage may lead to significant nitrite accumulation as the growth rate of NOB is higher than AOB (Kampschreur et al., 2007). The major hindrances in this BNR process are the toxicity associated with highly potent nitrite and nitrous oxide production (Oleszkiewicz et al., 2015). In addition, free ammonia in typical domestic sewage is insufficient to reach concentrations inhibiting NOB (Hellinga et al., 1999). As a result, the only viable parameters to control are DO concentrations and HRT (Liu et al., 2019; Oleszkiewicz et al., 2015). A few studies have linked low DO conditions to nitrification failure which could be caused by the adaptation of certain NOB groups to oxygen-limited environments (Zhou et al., 2018). While nitritation/denitritation represent a promising approach with energy- and carbon-efficient advantages, the process requires careful control for optimal performance.

2.2.2.2 Partial Nitritation/Anammox

With the discovery of anammox bacteria in 1990, the partial nitritation/anammox (PN/A) process emerged as a revolutionary nitrogen removal technology (Kuenen, 2008; Third et al., 2005). In the PN/A process, AOB and anammox bacteria directly transform NH_4-N to N_2 gas, saving 63% oxygen demand and 100% carbon requirement (Figure 2.1) (Kuai & Verstraete, 1998; Lackner et al., 2014). The PN/A nitrogen removal technology has been successfully applied in many full-scale STPs treating high-strength ammonia and low-organic carbon sewage (Cao et al., 2018; Han et al., 2020; Xu et al., 2022; Yang et al., 2023).

Despite the remarkable progress of the PN/A technology, there are still downsides impeding larger applications such as NOB inhibition, frequent plant failure, sensitivity to operational conditions, foaming, slow-growth rate bacteria, etc (Rong et al., 2022). Fluctuation in sewage composition or conditions (such as pH, temperature, solids concentration and NH_4-N levels) can disrupt the anammox population and nitrogen removal efficiency (Harb et al., 2021). Domestic sewage with low NH_4-N (<50 mg/L) and temperature (<25°C) can cause difficulty in inhibiting NOB and enriching anammox bacteria, causing NO_3-N cannot be removed by anammox bacteria (Cao et al., 2017; Wang et al., 2022). In addition, the anammox bacteria have a slow growth rate (0.003/h with a doubling time in 14 days) (Jetten et al., 1998). This can cause lengthy start-up and recovery times, requiring weeks or even months to stabilize the operation (Dasgupta et al., 2017; Wen et al., 2020; Weralupitiya et al., 2021). Therefore, adopting the PN/A process into full-scale STPs, especially in developing countries, may be unsuitable due to the operational complexity, specialized control equipment, high capital costs, research and development costs, and in-depth training for engineers and operators.

2.2.2.3 Intermittent Aeration in BNR

Another commonly employed enhanced nitrogen removal process with a simpler energy-efficient strategy in STPs is intermittent aeration in BNR (Habermeyer & Sá Nchez, 2005; Hanhan et al., 2011; Kimochi et al., 1998). Intermittent aeration can be applied in continuous mode (multi-stage anoxic-oxic and oxidation ditch) and batch (sequencing batch reactor) mode (Cao et al., 2013; Li et al., 2014; Miao et al., 2017, 2018; Rodríguez et al., 2011). The intermittent aeration in BNR operates in a cyclical order between the aeration phases to remove ammonium and non-aeration phases to enhance denitrification (Fulazzaky et al., 2015; Zhao et al., 2018). The advantages of intermittent aeration include efficient nitrogen removal, improved sludge settleability, reduced harmful N_2O emissions and decreased energy consumption (Ma et al., 2021; Srb et al., 2022; Sun et al., 2017).

Furthermore, it was well-known that the alternate aeration and non-aeration condition in the intermittent aeration creates a favourable condition for simultaneous nitrification and denitrification (SND) occurrence, further improving nitrogen removal efficiency (Ge et al., 2014; Izadi et al., 2021; Sun et al., 2017). The increased SND in intermittent aeration conditions enhances organic carbon utilization for denitrification instead of being oxidized by oxygen in aerated phases (Miao et al., 2022). The non-aeration phases in the intermittent aeration stored internal organic carbon as an effective substrate for SND, making for endogenous denitrification in the subsequent phases. The reduced duration of aeration lowers the aeration energy and cost of the BNR operation (Capodici et al., 2015). Hence, intermittent aeration is a suitable option for retrofitting into the existing BNR process, improving nitrogen removal efficiency while simulataneously reducing energy consumption.

2.2.2.4 Low DO BNR

The process optimization of low-DO BNR in the existing activated sludge BNR process has been recognised as another cost-effective retrofit option. Low-DO BNR minimizes oxygen requirement and achieves desirable nitrogen removal performance (Bellucci et al., 2011; Stewart et al., 2022; Zheng et al., 2022). Although early works suggest maintaining DO at a high level (i.e. 2 to 4 mg/L) in the aeration tank to prevent nitrification inhibition (Arnaldos et al., 2013; Blackburne et al., 2008; Park & Noguera, 2004), many recent studies have shown that nitrification performance is still favourable despite operating at low DO (<1 mg/L) concentration (How et al., 2021; Wang et al., 2022; Zheng et al., 2019). Considering the energy-intensive aeration in STPs, low DO operation is a promising BNR alternative that ensures long-term sustainability and contributes to energy footprint reduction in STPs.

A study that compared the aeration energy consumption between conventional high-DO and low-DO nitrification deduced that operating the nitrification at low DO (0.5 mg/L) decreased aeration energy by 23% compared to high DO (2 mg/L) (How et al., 2018). Similarly, Keene et al. (2017) reported a comparable range with an estimated energy reduction of 25% when operating the BNR process at low DO (0.33 mg/L) in comparison to high DO concentration (4.3 mg/L). The low-DO BNR process represents a promising retrofit BNR alternative in contributing toward a sustainable sewerage industry in the remediation of high operating costs. While nitritation/denitritation and PN/A processes are regarded as advanced BNR technologies with significant advantages, low-DO BNR stands out for its operational simplicity, lower capital costs, adaptability to existing BNR facilities and robust in handling fluctuation in sewage characteristics and loading. Hence, these strengths make low-DO BNR a more suitable energy-efficient alternative, particularly for STPs in developing countries.

2.3 Application of Low-DO in BNR Sewage Treatment

2.3.1 BNR Treatment Performance under Low DO

The low DO has been implemented in BNR, enhanced biological phosphorus removal and SND processes using reactors such as modified University of Cape Town (UCT)-type, flow-through biofilm reactor, moving bed biofilm reactor, continuous baffled reactor and sequencing batch reactors (SBR) (Awad et al., 2022; How et al., 2019; Keene et al., 2017; Luan et al., 2022; Wang et al., 2022). Table 2.2, shows an overview of low-DO BNR studies in treating domestic sewage. Contrary to early studies that mentioned operating at low DO inhibits the ammonium removal performance, many studies have reported complete or near-complete ammonium removal when operating the BNR system at low DO concentrations (<1 mg/L) (Blackburne et al., 2008; Park & Noguera, 2004).

Table 2.2: Comparative performance of different nitrogen removal processes operating under low DO treating municipal sewage.

Process	Reactor	Scale	Influent Concentrations			Operating Conditions					Effluent		Efficiency	Reference
			NH_4-N (mg/L)	TN (mg/L)	COD (mg/L)	T (ºC)	pH	DO (mg/L)	HRT (h)	SRT (d)	NH_4-N (mg/L)	NO_3-N (mg/L)	NH_4-N Removal (%)	
PN/SSAD	SBR	Lab	50	–	–	–	8.0	0.4–0.6	30	60	<2	8.14	99.4	(Hu et al., 2023)
SNDPR	Biofilm	Pilot	10–34	15–45	150–450	200	–	0.2–0.6	6–9	20	3–4.2	4.8–3.5	93	(Wang et al., 2022)
Nitrification	Membrane reactor	Lab	48	–	180	23	7–7.5	0.41	–	40	[a]0.1	[a]40	100	(Liu et al., 2021)
SND	AAO Batch	Lab	19.8–28.6	30.4–39.6	192–358	–	7.5	0.5	14	15	0.47	<10	97.9	(Wang et al., 2021)
Nitrification	SBR	Lab	19–25	25–33	–	30	7.5–8	0.2–0.6	16	20	0	<0.3	100	(How, Nittami, et al., 2020)
Nitrification	SBR with PFR	Lab	61–95	37–61	98–183	19.2–21.4	–	0.2–1	9	55–143	1–6.2	<5	93.5	(Roots et al., 2019)
SNAD	Biofilm SBR	Lab	70–80	–	200–300	30	7.3–8	0.7–3.5	–	–	–	<3	[a]95.6	(Zheng et al., 2019)
Nitrification	SBR	Lab	14–20	20–26	256–314	30	7.5–8	0.5	15	20	<2	<0.5	90	(How et al., 2018)
SND	MBR	Lab	6–32	–	–	21–23	–	<0.2	–	–	<1	19–25	96.9	(Palomo et al., 2018)
SND	Modified UCT	Pilot	27.7–37.7	22–43	–	11–25	–	[a]0.33	17	7.4–10.4	<1	–	99	(Keene et al., 2017)
SNDPR	SBR	Full	30–35	–	80–150	29–32	–	1-2	0.77–1.65	5	0.5–3	0–1	91.4–98.3	(Yang et al., 2016)
Nitrification	UCT	Lab	19–30	–	–	21–23	–	<0.3	11	10	<1	20–22	96.7	(Fitzgerald et al., 2015)
Nitrification	Complete mix	Lab	48	–	180	20	7.25	0.4	–	40	<1	–	99.98	(Liu & Wang, 2015)

–: Not mentioned; [a]: Average values; AAO: Anaerobic-anoxic-oxic; HRT: Hydraulic retention time; MBR: Membrane bioreactor; PN/SSAD: Partial nitrification and short-cut sulfur autotrophic denitrification; SBR: Sequential batch reactor; SND: Simultaneous nitrification and denitrification; SRT: Sludge retention time; SNAD: Simultaneous partial nitrification, anammox and denitrification; SNDPR: Simultaneous nitrification, denitrification and phosphorus removal; UCT: University of Cape Town

Numerous studies reported an increase in oxygen affinity in both ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) with a high nitrification rate in low DO environments (Arnaldos et al., 2013; Keene et al., 2017; Wang et al., 2021). A batch study by How et al. (2018) also reveals that nitrifiers in sludge samples have a higher affinity for oxygen, making the nitrification rate unimpacted at reduced low-DO levels. Although Fitzgerald et al. (2015) observed nitrification retardation when sludge samples was transferred from a conventional STP to a low-DO environment of 0.2 mg/L, prolonged exposure (100 days) to low-DO conditions resulted in an efficient conversion of ammonia to nitrate.

Operating nitrification at DO levels of 0.2 mg/L was still feasible for high ammonium removal efficiency (>93%) (Palomo et al., 2018; Roots et al., 2019; Wang et al., 2022). Wang et al. (2021) observed that decreasing DO concentrations from 2 to 0.5 mg/L did not affect the removal efficiency of ammonium or COD in an anaerobic-anoxic-oxic reactor treating domestic sewage. A comparative study by How et al. (2019) found that high NH_4-N removal performance of 95% and 93% were attained when DO levels reduced from 1.7 ± 0.2 to 0.9 ± 0.2 mg/L in an anoxic-oxic BNR reactor, respectively. In another major study, How et al. (2018) achieved 90% ammonium removal over a span of 42 days using a lab-scale sequencing batch reactor (SBR) despite low-DO concentrations (0.5 mg/L) operation in treating tropical sewage.

2.3.2 SND under Low DO BNR

The simultaneous nitrification and denitrification (SND) phenomenon was discovered in a full-scale SBR plant in Iowa, United States where it was observed that 80% of inorganic nitrogen was lost from the system (Irvine et al., 1987). The occurrence of SND in the same aerated vessel can reduce treatment time, oxygen, carbon and alkalinity consumption (Helmer & Kunst, 1998). There are various process variables that affect the effectiveness of SND including the DO concentration, COD/N ratio, configuration of the bioreactor and floc size characteristics (Jimenez et al., 2010).

It was widely reported that operating the activated sludge process at low DO (<1 mg/L) levels favours the occurrence of SND (Bueno et al., 2018; Dai et al., 2017; Ma et al., 2017; Wang et al., 2021). Under low DO conditions, the limited oxygen transfer rate in the sludge mixture leads to the formation of flocs with core-shell structure (Sun et al., 2010). The autotrophic nitrifying bacteria formed the outer aerobic shell, facilitating nitrification, while the denitrifying heterotrophic bacteria formed the inner anoxic core, responsible for denitrifying NO_2-N or NO_3-N into N_2 gas. Nitrification products that accumulate at the interphase between the core and shell create a concentration gradient for diffusion into the inner anoxic core (Miao et al., 2022). Maintaining low DO conditions during the BNR process enhances the SND, promoting an overall efficiency of nitrogen removal and reducing the need for energy-intensive aeration.

2.3.3 Microbial Activity under Low DO

How et al. (2020), Cao et al. (2018) and Yang et al. (2016) found that the genus *Nitrospira*-related NOB, associated with complete ammonia oxidizers (comammox), dominated the low-DO BNR nitrifying systems treating tropical sewage. Comammox *Nitrospira* exhibits higher yield growth and substrate affinity than canonical nitrifiers, enabling them to compete for oxygen under low DO conditions (How et al., 2021; Kits et al., 2017). Comammox is a critical functional group in energy-efficient nitrification for aeration due to its ability to perform two-step nitrification alone (Beach & Noguera, 2019; Daims et al., 2015; Palomo et al., 2018; Roots et al., 2019). Notably, two studies found that comammox *Nitrospira* thrives in a low DO environment and produces a lower amount of nitrous oxide (N_2O) than AOB due to the absence of nitric oxide reductase (Han et al., 2021; Kits et al., 2019). How et al. (2019) added that low DO environment and tropical temperate sewage may have promoted the growth of comammox *Nitrospira*. The presence of comammox *Nitrospira* could play an important role within the nitrifying community in enhancing treatment performance and reducing the emission of potent N_2O gas.

2.3.4 N_2O Emissions under Low DO Conditions

Operating at limited-oxygen conditions (DO<1mg/L) in BNR sewage treatment has faced considerable criticism because incomplete denitrification may result in nitric oxide (NO) and nitrous oxide (N_2O) emissions to the atmosphere (Ge et al., 2014; Kampschreur et al., 2007; Law et al., 2012). Such release of greenhouse gases (NO and N_2O) from sewage treatment are problematic as they have a negative impact on air quality, climates and health of personnel at STPs (Duan et al., 2021; Tallec et al., 2006; Thakur & Medhi, 2019). Although N_2O is generated from the STPs, the production of N_2O from the low-DO nitrifying systems was not observed in numerous studies (Li et al., 2021; Liu & Wang, 2013; Park & Noguera, 2004). Nitrite, an indicator of potential nitric oxide and nitrous oxide gas production, was not observed in many low DO reactors (How et al., 2018; Keene et al., 2017; Wang et al., 2021; Zheng et al., 2022).

Liu et al. (2021) demonstrated that operating a lab-scale membrane reactor at low DO levels (0.4 mg/L) for 141 days led to a decreased N_2O emission of 0.11% compared to high DO (2 mg/L) operation which showed an N_2O emission of 0.24% when fed with synthetic municipal sewage. Li et al. (2021) inferred that the presence of comammox might contribute to a 20% reduction in N_2O gas emissions during prolonged exposure to low DO levels in a weakly acidic (pH of 6.3 to 6.8) bioreactor. Interestingly, intermittently aerated SBR has a higher TN removal efficiency (93.5%), lower N_2O-emission factor (0.01 to 0.53%) and 25 times higher relative abundance of comammox than the continuous aerated SBR (Liu et al., 2021). Overall, the abundance of comammox bacteria in low-DO reactors may contribute to reduced energy consumption and N_2O emissions.

2.4 BNR Process Configurations

Two common types of BNR configurations in STPs are pre-anoxic and post-anoxic denitrifications. The main differences between these BNR configurations are the aerobic (oxic) and anoxic zone order (Tchobanoglous et al., 2014). Both pre-anoxic and post-anoxic denitrification can be combined with various design, operating conditions and reactor systems to provide an optimum sewage treatment system.

2.4.1 Pre-anoxic Denitrification

The pre-anoxic denitrification, also called anoxic-oxic (AO), is often considered and more common in practice for many conventional STPs (Winkler et al., 2011). As the name AO suggests, the anoxic phase comes before the aerobic phase, allowing denitrification before the nitrification (Figure 2.2). Pre-anoxic denitrification is a substrate-driven process as the biological oxygen demand in the influent serves as an electron donor to reduce nitrate. Hence, this AO configuration helps to reduce organic loading in the aerobic tank by utilizing nitrate as an electron acceptor instead of oxygen (Tchobanoglous et al., 2014).

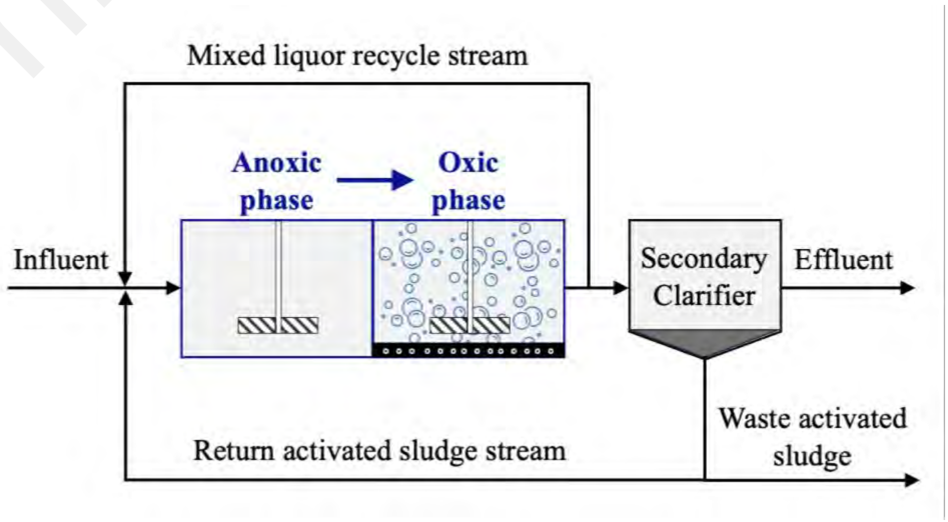


Figure 2.2: Illustration of pre-anoxic configuration.

There have been several studies on intermittent aeration to create multiple AO configurations where multiple nitrification and denitrification happened in the same reaction cycle (Hajsardar et al., 2016; Sheng et al., 2017; Song et al., 2017; Wang et al., 2015). In a study of the treatment of digested piggery sewage, higher NH_4-N (93 ± 6%) and TN (78 ± 1%) removal rates were achieved in intermittently aerated AO process compared to the conventional AO operation (Sheng et al., 2017). Although some studies suggested that multiple AO configurations may emit higher N_2O gas (Kampschreur et al., 2009; Rodríguez-Caballero et al., 2015), Rodríguez-Caballero et al. (2015) demonstrated that shortening the anoxic and aerobic reaction duration can facilitate the direct conversion of N_2O to N_2, thereby mitigating this potent gas release. With shorter aeration time, the intermittent aeration in the AO process reduces aeration energy consumption by 33 to 45% compared to the conventional AO process, making it an attractive way to decrease aeration operating expenses and carbon footprint (Rodríguez-Caballero et al., 2015).

However, since an incomplete NO_3-N removal typically happens for AO configuration where about 3 to 5 mg/L of effluent TN remains in the effluent, the produced NO_3-N in the aerobic zone is often recirculated back to the anoxic zone with a high flow rate of mixed liquor recycle (MLR) stream (Liu & Wang, 2015). The main issue associated with the high MLR flow rate for pumping is the high energy consumption (Coats et al., 2023). The high MLR flow rate can cause potential carryover of DO in the anoxic zone and dilution of carbon from the influent, reducing the organic carbon available for denitrification (Raboni et al., 2013; Winkler et al., 2011). How et al. (2019) observed that during low-DO (0.9 ± 0.1 mg/L) operation, the AO exhibited higher effluent NO_3-N concentrations at 13 mg/L, compared to the anaerobic oxic-anoxic reactor with effluent NO_3-N levels of 4 mg/L. While pre-anoxic denitrification is a commonly employed method, its inefficiency in denitrification and the elevated operating costs linked to the high MLR flow rate render this configuration unsuitable for a cost-effective BNR process.

2.4.2 Post-anoxic Denitrification

Post-anoxic denitrification, also known as the oxic-anoxic (OA) configuration, does not require the MLR recycling stream and produces effluent with lower NO_3-N (TN<3 mg/L) since the anoxic phase happens after the aerobic phase, as shown in Figure 2.3 (Xu et al., 2013). In the OA configuration, the denitrification rate is expected to be low because most biodegradable organics are oxidized in the aerobic (oxic) phase. The remaining organic matter may not be sufficient for the subsequent anoxic phase (Henze et al., 1997). As a result, additional carbon sources may be supplied in the post-anoxic setup, which can increase the operating costs.

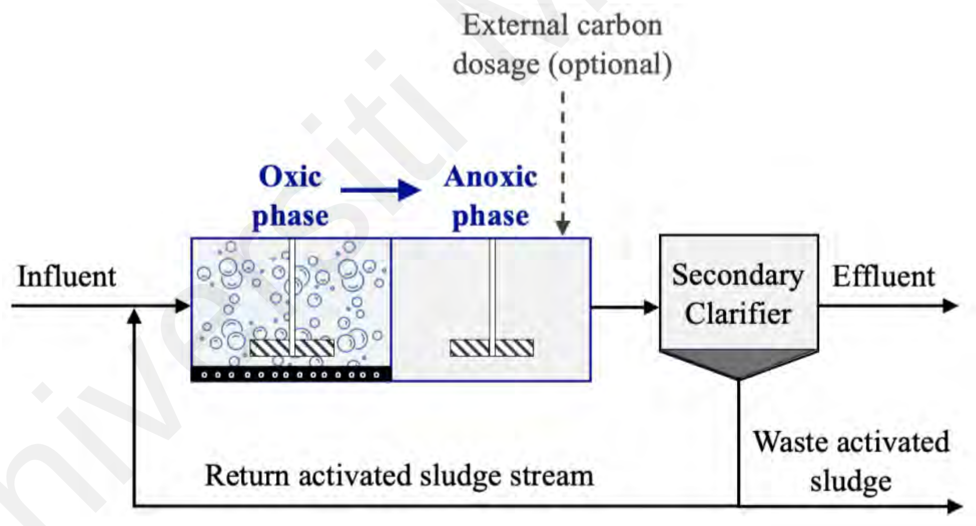


Figure 2.3: Illustration of post-anoxic configuration.

In response to the growing concerns about stricter nitrogen discharge limits, several studies have reported on post-anoxic denitrification for nitrogen removal (Gao et al., 2020; Gong et al., 2023; Rajpal et al., 2022; Winkler et al., 2011; Zhao et al., 2018). Vocks et al. (2005) attained a high nitrogen removal of 80% without an external carbon source in a post-anoxic membrane bioreactor, as post-anoxic denitrification could be driven by glycogen or polyhydroxyalkanoates. Several studies also achieved excellent

TN removal efficiency, i.e., ranging from 90 to 98%, with post-anoxic denitrification treating low COD/N ratio (1.3 to 4.4) sewage without any carbon supplement (Gao et al., 2020; How et al., 2019, 2020; Zhao et al., 2018). How et al. (2019) observed that effluent NO_3-N concentration was further reduced from 13 mg/L to 5 mg/L when changing the BNR configuration from anoxic-oxic (AO) to anoxic-oxic-anoxic (AOA) reactor under low-DO concentrations (0.9 ± 0.1 mg/L) treating tropical sewage. By simplifying the low-DO AOA to low-DO OA, How et al. (2020) achieved more desirable nitrogen removal performance with complete NH_4-N removal and low effluent NO_3-N (<0.3 mg/L) without carbon dosage. Such a simplified low-DO OA process could present a cost-effective retrofit alternative for the conventional BNR process to decrease aeration energy usage and eliminate carbon dosing in treating tropical sewage, particularly in developing countries with limited resources. Therefore, further investigation on low-DO OA system is anticipated, aiming at a larger scale as the small and lab-scale study has proved its potentialities to successfully remove nitrogen from the tropical sewage.

2.5 Summary

Chapter 2 provides a comprehensive literature review of the biological nitrogen removal process and emphasizes the need to eliminate harmful nitrogen from sewage. It starts by discussing the fundamentals of nitrification and denitrification, while also detailing the main constraints of the conventional BNR process. The chapter then explores energy and chemical-efficient BNR methods including nitritation/denitritation, partial nitritation/anammox, intermittent aeration in BNR, and low DO BNR. The discussion dives deeper into the studies on the low-DO BNR process for domestic sewage treatment. Under the low-DO BNR process, reviews on nitrogen treatment performance, SND occurrence, microbial activity and N_2O emissions were covered. The following chapter reviews the BNR process configurations, including pre-anoxic and post-anoxic denitrification, ultimately steering the research focus toward a simpler and more cost-effective BNR process, low-DO OA. While the lab-scale low-DO OA study has demonstrated positive results in treating tropical sewage, a comprehensive feasibility study at the pilot scale is important before adopting the process into a real-world application. The pilot study on low-DO OA will offer valuable insights into its practicality for nitrogen removal from domestic sewage in Malaysia.

CHAPTER 3: MATERIALS AND METHODS

3.1 Summary of Research Methodology

Chapter 3 consists of six sections with the first section providing a flowchart overview of the research methodology based on the main aim followed by four specific objectives (Figure 3.1). Section 3.2 outlines the procedure of a denitrification batch activity test using different fractions of COD, in soluble or particulate form, as carbon source. In the subsequent section, a description of the nitrification batch test using sludge samples from STPs operating on low and high dissolved oxygen levels was presented.

Section 3.4 provides details on the reactor, including reactor setup, sources of sewage and reactor operations. The pilot SBR was operated in three phases: Phase 1 on low-DO OA, Phase 2 on high-DO OA, and Phase 3 on intermittent oxic-anoxic under low DO condition. The three phases were evaluated based on the nitrogen removal performance and energy-saving calculation, along with the sewage characterization. In the subsequent section, energy-saving calculations for operating the BNR process under low DO conditions and with intermittent aeration were also included.

Section 3.6 explains the analytical techniques used for COD, cation (NH_4-N), anion (NO_2-N and NO_3-N), TN, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volume index (SVI).

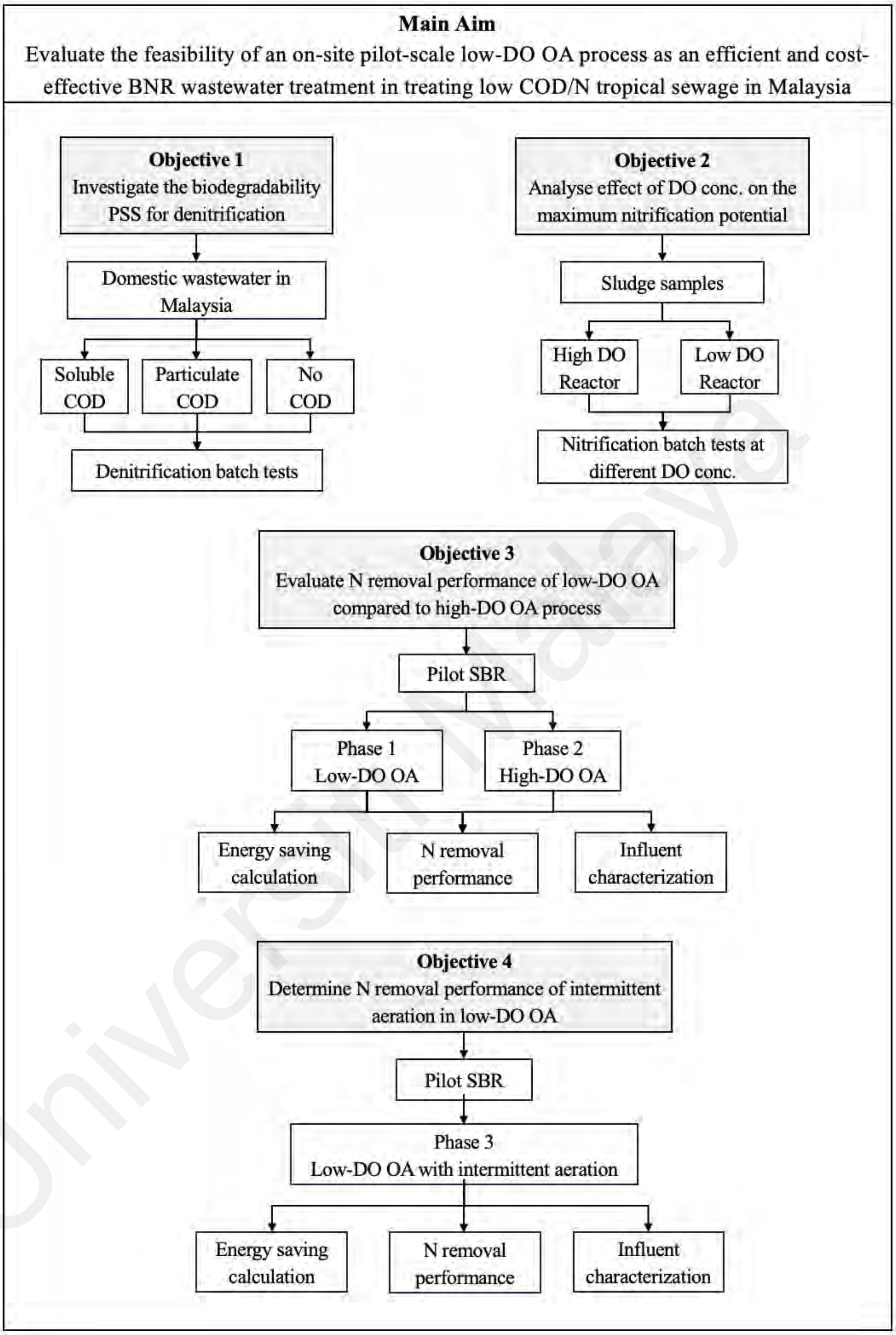


Figure 3.1: Flowchart of research methodology.

3.2 Denitrification Batch Activity Test using Different Fractions of COD

The denitrification batch experiments were carried out in accordance with the method described by van Loosdrecht et al. (2016) to investigate the biodegradability of particulate settleable solids (PSS) in tropical sewage as carbon source for denitrification. Three sets of batch tests (Set A, B and C) were conducted in 2-L beakers. The raw sewage from STP X in Kuala Lumpur, Malaysia, was collected and divided into soluble and particulate fractions. The filtered sewage through 0.2-μm pore-size filter papers was used in Set A to provide readily biodegradable COD (rbCOD) for denitrification. In Set B, PSS obtained from the settled portion of sewage, which underwent three consecutive washing cycles, was added to the nutrient solution to provide slowly biodegradable COD (sbCOD) as carbon source. Set C contains nutrient solution only. The concentration of nutrient solution was as follows, per litre: 1460 mg KH_2PO_4, 20 mg N-Allylthiourea, 1070 mg NH_4Cl, 660 mg $MgSO_4.7H_2O$ and 3.33 mL of trace element solution formulated by Ong et al. (2013).

Before conducting the experiment, the carbon source solutions underwent a 10-minute nitrogen gas sparging to create an oxygen-free environment in the beakers. Subsequently, all three sets were sealed with parafilm and subjected to mixing. Pre-washed sludge samples from a stabilized pilot reactor were added to attain 2,500 mg/L MLVSS concentration. The initial nitrate-nitrogen (NO_3-N) concentration in all three sets of solution was maintained between 50 to 60 mg/L. The pH and DO levels of the three sets were measured using a 405-DPAS-SC K851200 pH probe (Mettler-Toledo, USA) and an InPro6850i DO probe (Mettler-Toledo, USA) (Appendix 3). Sampling was performed every 15 to 30 minutes for nitrite-nitrogen (NO_2-N), NO_3-N and soluble COD (sCOD) analyses in Set A, B and C. The denitrification batch activity test setup is shown in Appendix 1.

3.3 Effect of DO on Ammonium Oxidation Rate with Sludge Samples from Low and High DO STPs

3.3.1 Nitrification Batch Activity Experiment

The nitrification batch activity test was carried out to evaluate the maximum nitrification potential of sludge samples from full-scale SBR operating on low and high DO concentrations. The Michaelis-Menten mathematical model was used to simulate the relationship between DO concentrations and specific ammonium uptake rate ($SAOR$). The batch test followed the procedure outlined in van Loosdrecht et al. (2016).

Prior to the execution of the experiment, sludge samples, 0.0187 M of ammonium chloride (NH_4Cl) stock solution, 0.8 M of sodium carbonate (Na_2CO_3) as alkaline solution and synthetic medium were prepared. The sludge samples were obtained from the full-scale SBR operating on the OA configuration at STP Y and Z in Selangor, Malaysia, which operated under low DO (0.5 mg/L) and high DO (2.8 mg/L), respectively. The sludge samples were washed with tap water three times to eliminate any inhibitory or toxic compounds. After washing, the sludge samples were resuspended in the synthetic medium. The synthetic medium adapted from Kampschreur et al. (2007) consisted of 134 mg/L Na_3PO_4, 35 mg/L $MgSO_4{\cdot}7H_2O$, 5 mg/L $CaCl_2{\cdot}2H_2O$, 180 mg/L NaCl, 30 mg/L KCl, 1 mg/L of yeast extract and 0.3 mL/L of trace element solution.

A 1-L batch reactor with a working volume of 0.8 L was used for the experiment. The initial MLVSS of each experiment set was around 3,500 mg/L. Before adding the substrate, the sludge mixture was controlled at a recommended pH range of 7.5 and 8.4 by manually adding an alkaline solution (van Loosdrecht et al., 2016). An air pump supplied air into the reaction vessel through an air bubble stone. Compressed air was regulated by a solenoid valve connected to the DO sensor. An M300 Process 2-channel 1/3 DIN transmitter (Mettler Toledo, Switzerland) was programmed with a specific DO setpoint to maintain desired DO conditions in the reaction mixture. Different sets of experiments were conducted with DO setpoints at 0, 0.3, 0.6, 1, 2, and 4 mg/L and each experiment was performed in duplicate. The pH and DO were recorded via the transmitter connected to a 405-DPAS-SC K851200 pH probe (Mettler-Toledo, USA) and an InPro6850i DO probe (Mettler-Toledo, USA). Once the pH and DO concentrations were stabilized at the desired conditions, the batch experiment started by introducing the NH_4Cl solution. The volume of the NH_4Cl dosage was determined to achieve an initial NH_4-N concentration of approximately 30 mg/L. Aliquots of 20 mL of mixed liquor samples were collected at 0, 0.25, 0.5, 1, 1.5, 2, 2.5 and 3 h for cation and anion analyses to measure the substrate uptake rate. The initial and final MLVSS concentrations were assessed. The nitrification batch activity test setup is shown in Appendix 4.

3.3.2 Oxygen Half Saturation (K_o) and Specific Ammonium Oxidation Rate ($SAOR$)

The NH_4-N time profile was generated for each set of batch experiments to determine the ammonium oxidation rate (AOR) based on the gradient. The $SAOR$ was calculated by dividing AOR (mg NH_4-N/L·h) by the respective average MLVSS (mg/L) concentration. Michaelis-Menten mathematical model was used to simulate the relationship between DO concentrations and $SAOR$, as shown in Equation 3.1.

$$SAOR = \frac{SAOR_{max} \times DO}{K_o + DO} \tag{3.1}$$

Where $SAOR_{max}$ is the maximum $SAOR$ (mg NH_4-N/g VSS·h) and K_o is the half-saturation constant of dissolved oxygen (mg/L). The $SAOR_{max}$ and K_o values were generated using MATLAB (The Mathworks Inc, USA) software version R2023a.

3.4 Pilot-scale Sequencing Batch Reactor

3.4.1 Reactor Setup

The pilot-scale sequencing batch reactor, hereinafter referred to as the pilot SBR, was set up at Indah Water Research Centre, Kuala Lumpur, Malaysia. The pilot SBR had a working volume of 150 L, a height of 94 cm, and an inner diameter of 56 cm. The vessel was constructed using inert and opaque fiber-reinforced plastic. An overhead agitator, with double impeller blades positioned at the middle and bottom of the shaft, was installed and operated at 300 rpm during reaction phases to ensure uniform mixing. In Figure 3.2, the pilot SBR system mainly consisted of an air compressor (Eurox, Italy), an air-operated diaphragm pump (Graco, USA), and an aeration pump (Itoshi Hailea, China). The air compressor was automatically activated during the filling phase to supply air to the diaphragm pump. Compressed air supplied by the aeration pump was delivered through the fine-bubble disc diffusers placed at the base of the reactor. The solenoid valve connected to the programmable logic controller (OMRON, Japan) helps to automatically activate and deactivate the aeration pump to maintain pre-selected minimum and maximum DO concentration in the reactor. The DO and pH were measured online with DO and pH probes (LEADTEC, Malaysia) connected to a dual-channel analyzer. The pilot SBR setup, control panel and P&ID are shown in Appendix 8 and Appendix 9.

3.4.2 Sources of Seed sludge and Domestic Sewage

Seed sludge collected from the return activated sludge line of STP X was inoculated into the pilot SBR to obtain an initial MLSS concentration of around 2,800 mg/L. The STP X has a current capacity of 430,000 population equivalent (PE) with a designed capacity of 750,000 PE. The treated sewage from STP X was utilized downstream of the water supply intake. After preliminary treatment, consisting of bar screening and grit removal, at STP X, the raw sewage was then directed to a 400-L buffer tank, where it was stored (Figure 3.2). The raw sewage in the buffer tank serves as the influent to the pilot

SBR. An intermittent slow mixing process is set up in the buffer tank to ensure homogeneity in the influent sewage. The mixing is activated just before the influent was pumped into the pilot SBR during filling phase. The influent sewage was sampled twice a week on the selected days, aligning with the sampling of the mixed liquor in the pilot SBR.

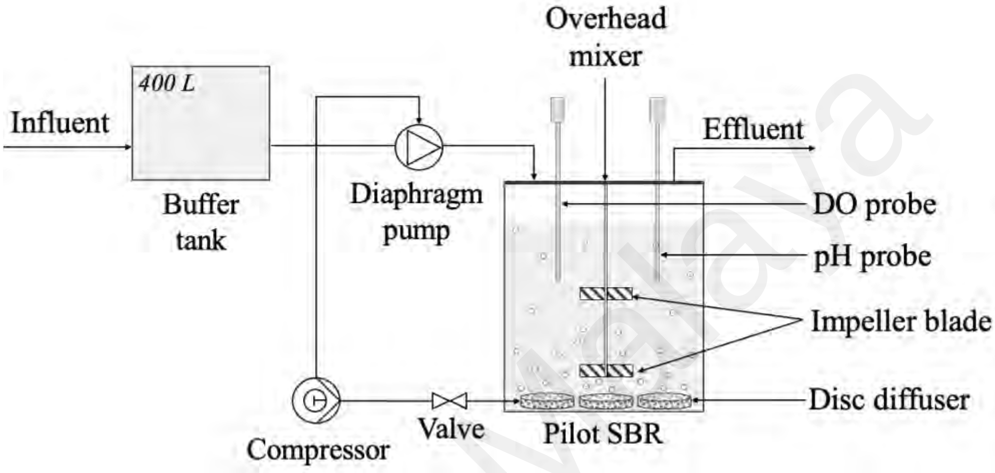


Figure 3.2: Schematic diagram of the pilot SBR system.

3.4.3 Reactor Operations

The reactor was operated under three phases, as indicated in Table 3.1. In Phase 1 and 2, the pilot SBR was operated with a 6-h cycle time under an oxic-anoxic (OA) configuration from March to November 2022. The 6-h OA cycle consisted of 10 min filling, 120 min oxic (aerobic), 180 min of anoxic (non-aerated), 30 min settling, 10 min withdrawing and 10 min idling phases (Figure 3.3).

Table 3.1: The pilot SBR operating conditions in Phase 1, 2 and 3.

Operating phase	Phase 1	Phase 2	Phase 3
Operation duration	Day 1 – 218	Day 219 – 260	Day 1 – 69
Process	Low-DO OA	High-DO OA	Low-DO OA with intermittent aeration
DO level in oxic phase (mg/L)	0.6 ± 0.2	2.0 ± 0.2	0.6 ± 0.2
Total Cycle time (h)	6	6	4
Hydraulic retention time (h)	18	18	12
Sludge retention time (d)	20	20	20
Volume exchange ratio	1/3	1/3	1/3

The DO concentration in the oxic phase was maintained at low (0.6 ± 0.2 mg/L) in Phase 1 and high (2.0 ± 0.2 mg/L) in Phase 2. In Phase 1 and 2, the hydraulic retention time (HRT), sludge retention time (SRT) and volume exchange ratio were maintained at 18 h, 20 d and 1/3. The HRT was shortened from 18 to 12 h in Phase 3. In Phase 3, intermittent aeration consisting of 30 minutes of oxic conditions followed by 30 minutes of anoxic conditions in the 3-h reaction time was operated in the 4-hour cycle time (Figure 3.3).

(a) Phase 1

Filling Phase	Low-DO Oxic	Anoxic	Settling Phase	Decanting Phase	*Idling*
10 min	120 min	180 min	30 min	10 min	10 min

(b) Phase 2

Filling Phase	High-DO Oxic	Anoxic	Settling Phase	Decanting Phase	*Idling*
10 min	120 min	180 min	30 min	10 min	10 min

(c) Phase 3

Filling Phase	Low-DO Oxic	Anoxic	Low-DO Oxic	Anoxic	Low-DO Oxic	Anoxic	Settling Phase	Decanting Phase	*Idling*
10 min	30 min	30 min	30 min	30 min	30 min	30 min	30 min	10 min	10 min

Figure 3.3: Summary of pilot-SBR operating sequences and cycle time for (a) Phase 1, (b) Phase 2 and (c) Phase 3.

The mixture of sludge and sewage (hereby referred to as mixed liquor) in the pilot SBR was sampled at regular intervals on selected cycles to monitor the evolution of nitrogen compounds. The sampling was performed twice a week on selected days, such as Tuesday and Friday to maintain a sufficient time gap between each sampling day and minimize any potential bias introduced by the weekend loading effect. All the samples were stored at 4°C for further analysis.

3.5 Energy Savings Calculations

3.5.1 Estimation of Energy Reduction for Low-DO BNR

The energy saving was estimated by considering the oxygen transfer rate (OTR) during the nitrification and DO concentration in the mixed liquor in tropical sewage (How et al., 2018; Keene et al., 2017; Tchobanoglous et al., 2014). The energy savings calculations was summarised in the flowchart in Figure 3.4.

Energy Savings Calculations for low DO (0.6 mg/L) and high DO (2 mg/L) BNR

Step 1: Total Biological Oxygen Requirement, R_o

$$R_O = Q(S_O - S) - 1.42P_{X,bio} + 4.33Q(TKN)$$

where

$$P_{X,bio} = \frac{QY(S_o - S)}{1 + (k_d)SRT} + \frac{f_d k_d QY(S_o - S)SRT}{1 + (k_d)SRT} + \frac{QY_n(TKN)}{1 + (k_{dn})SRT}$$

↓

Step 2: Total Oxygen Transfer Rate, OTR_{TOTAL}

$$OTR_{total} = OTR_{liquid} + R_O$$

where

$$OTR_{liquid} = \left[\left((Q - Q_{RAS})DO\right) + \left(Q(DO - DO_o)\right)\right]$$

↓

Step 3: Standard Oxygen Transfer Rate, SOTR

$$SOTR = \left(\frac{OTR_{total}}{\alpha F}\right)\left\{\frac{C^*_{\infty,20}}{\left[\beta \frac{C_{st}}{C^*_{s20}}\left(\frac{P_b}{P_s}\right)C^*_{\infty 20} - DO\right]}\right\}\left[(1.024)^{20-T}\right]$$

where

$$\frac{P_b}{P_s} = exp\left[-\frac{gM(z_b - z_a)}{RT}\right]$$

Step 4: Aeration Energy Requirement

$$Aeration\ energy\ requirement = \frac{SOTR}{1.3}$$

where

Aeration System	Transfer Rate (kg O_2/kW.h)
Low speed surface aerator	1.5 – 2.1
Low speed surface aerator with draft tube	1.2 – 2.8
High speed surface aerator	1.1 – 1.4

Figure 3.4: Summary of aeration energy savings calculations.

The aeration energy required for the low DO (0.6 mg/L) and high DO (2 mg/L) nitrification processes was calculated using data from the pilot SBR study. The oxygen required to biodegrade carbonaceous materials and oxygen required to oxidize ammonia were calculated based on Equation 3.2 and 3.3, respectively.

$$R_O = Q(S_O - S) - 1.42P_{X,bio} + 4.33Q(TKN) \tag{3.2}$$

Where R_o is the total biological oxygen required (g/d), Q is the influent flowrate (m^3/d), S_o is the influent substrate concentration (mg/L), S is the effluent substrate concentration (mg/L), $P_{X,bio}$ is the biomass as VSS wasted (g/d) and TKN is oxidized influent nitrogen (mg/L).

$$P_{X,bio} = \frac{QY(S_o - S)}{1 + (k_d)SRT} + \frac{f_d k_d QY(S_o - S)SRT}{1 + (k_d)SRT} + \frac{QY_n(TKN)}{1 + (k_{dn})SRT} \tag{3.3}$$

Where Y is the biomass yield from bCOD (VSS/g BOD), k_d is the endogenous decay coefficient for heterotrophic bacteria (1/d), SRT is the sludge retention time (d), f_d is the fraction of cell mass remaining as cell debris, Y_n is the biomass yield from ammonium (VSS/g NH_4-N) and k_{dn} is the endogenous decay coefficient for nitrifying bacteria (1/d).

Equation 3.4 determines the total oxygen transfer rate (OTR_{total}) in the aerobic tank. The OTR_{total} (kg O_2/d) is the summation of R_o and oxygen transfer rate to maintain DO in the tank (OTR_{liquid}). The OTR_{liquid} (kg O_2/d) was determined using Equation 3.5.

$$OTR_{total} = OTR_{liquid} + R_O \tag{3.4}$$

$$OTR_{liquid} = \left[\left((Q - Q_{RAS})DO\right) + \left(Q(DO - DO_o)\right)\right] \tag{3.5}$$

Where Q_{RAS} is the flowrate of return activated sludge (MGD), DO is the dissolved oxygen concentration in the tank (mg/L) and DO_o is the dissolved oxygen concentration in the previous tank (mg/L).

Equation 3.6 converts the OTR_{total} to the standard oxygen transfer rate ($SOTR$) based on fine bubble aeration design. The relative pressure at site elevation to standard pressure at site ($\frac{P_b}{P_s}$) was obtained from Equation 3.7.

$$SOTR = (\frac{OTR_{total}}{\alpha F})\left\{\frac{C^*_{\infty,20}}{\left[\beta \frac{C_{st}}{C^*_{s20}}\left(\frac{P_b}{P_s}\right) C^*_{\infty 20} - DO\right]}\right\}[(1.024)^{20-T}] \quad \textbf{(3.6)}$$

$$\frac{P_b}{P_s} = exp\left[-\frac{gM(z_b - z_a)}{RT}\right] \quad \textbf{(3.7)}$$

Where α is the relative transfer rate between clean water and sewage, β is the relative DO saturation between clean water and sewage, F is the diffuser fouling coefficient, $C^*_{\infty,20}$ is the saturated DO by diffused aeration at a standard temperature of 20ºC and sea level (mg/L), C_{st} is the saturated DO at operating temperature of 30ºC and sea level (mg/L), C^*_{s20} is the saturated DO at a standard temperature of 20ºC and sea level (mg/L), P_s is the standard pressure at sea level, P_b is the elevation-based pressure at the site (m), T is the temperature of the aeration tank (ºC), g is the gravitational constant (m/s^2), M is the gas constant (kg/kg-mole·K), z_a is the elevation at sea level (m) and z_b is the elevation of the site (m). For surface aeration design, $C^*_{s20} = C^*_{\infty,20}$.

Equation 3.8 shows the calculation for aeration energy requirement to operate at a particular DO concentration for high-speed surface aerator design.

$$Aeration\ energy\ requirement = \frac{SOTR}{1.3} \quad \textbf{(3.8)}$$

3.5.2 Estimation of Energy Reduction for Low-DO BNR with Intermittent Aeration

The percentage of energy reduction for low-DO BNR with intermittent aeration in Phase 3 was calculated based on the total energy consumption of STP Z in Selangor, Malaysia reported by Muzaffar et al. (2022). STP Z was operated in the SBR with a population equivalent and design capacity of 150,000 and 16,875 m^3/d, respectively. STP Z was operated in the conventional operational design mandated by Malaysia's National Water Services Commission (2009). To determine the energy reduction of the BNR process with intermittent aeration, it was assumed that the STP Z operated in the same DO levels. To determine the percentage of energy reduction, the calculated value of the BNR with intermittent aeration was compared.

3.6 Analytical Methods

3.6.1 Chemical Oxygen Demand (COD)

The COD test is used to measure the amount of oxygen required to chemically oxidize organic material in sewage sample. The sCOD and total COD (TCOD) were measured using reactor digestion method following the procedure (Hach, 2023). The sCOD of sewage samples was analysed using a low range COD test vials (Hach, USA) after filtration through a 0.45-µm pore size cellulose acetate syringe filter (Phenomenex, USA). The TCOD of the sewage samples was analysed using the high range COD test vials (Hach, USA) without filtration. The COD test vials were subjected to 150ºC digestion for 2 h in a DRB 200 digester (Hach, USA). The sCOD and TCOD concentrations of the samples were measured using a DR/890 colorimeter (Hach, USA).

3.6.2 Nitrogen Ion Concentration

The collected sewage samples were centrifuged (Sigma 3-16P, Sartorius, Germany) for 5 minutes at 3,500 rpm. The supernatant of sewage samples was filtered through a regenerated cellulose syringe filter of 0.2-µm pore size (Phenomenex, USA) into the ion chromatography (IC) vials. The samples were then analyzed for anion (NO_2^- and NO_3^-) and cation (NH_4^+) concentrations using an 861 Advanced Compact Ion Chromatography equipped with 863 compact IC autosampler (Metrohm, Switzerland). The column used for anion analysis was Metrosep A supp 5 150/4.0 mm column (Metrohm, Switzerland) while the cation analysis used Metrosep C 4 150/4.0 mm column (Metrohm, Switzerland). For anion analysis, the reagents include deionized water, regeneration solution of 0.01 M sulphuric acid, eluent solution containing 3.2 mM sodium carbonate and 1 mM sodium bicarbonate. An eluent solution consisting of 1.7 mM nitric acid and 0.7 mM dipicolinic acid was used for cation analysis. The anion and cation concentrations were determined from the conductivity peak area with standard solutions as a reference.

3.6.3 Total Nitrogen (TN)

For TN analysis, sewage samples were filtered through a 0.45-μm pore-size cellulose acetate syringe filter (Sartorius, Germany) and injected into glass vials. The TN concentration of the samples was measured using a TOC-V CSN analyzer (Shimadzu, Japan) through oxidative combustion chemiluminescence.

3.6.4 MLSS and MLVSS

$MLSS$ is a measurement the concentration of suspended solids, both organic and inorganic, in sewage samples, indicating the biomass content in the mixed liquor. MLVSS is a specifically measures the concentration of the volatile solids, which are organic materials in the mixed liquor. Prior to $MLSS$ and $MLVSS$ analyses, 1.2-μm glass microfiber filter discs (Whatman, United Kingdom) were pre-treated based on the procedure described in Standard Methods for the Examination of Water and Sewage (APHA/AWWA/WEF, 1998). For MLSS analysis, the sewage samples were filtered through the pre-treated filter discs and the solids retained on the filter discs were dried in a Jouan EU115 oven (Thermo Fisher Scientific, USA) at 104 ± 1ºC for more than 1 h. For MLVSS analysis, the dried solids were ignited in a KL 15/11 muffle furnace (Thermoconcept, Germany) at 550 ± 50ºC for more than 15 min. The $MLSS$ and $MLVSS$ were calculated using Equations 3.9 and 3.10.

$$MLSS, \frac{\mathrm{mg}}{\mathrm{L}} = \frac{(\mathrm{A} - \mathrm{B}), \mathrm{g} \times 1000, \frac{\mathrm{mg}}{\mathrm{g}}}{\mathrm{V, L}} \quad \textbf{(3.9)}$$

$$MLVSS, \frac{\mathrm{mg}}{\mathrm{L}} = \frac{(\mathrm{A} - \mathrm{C}), \mathrm{g} \times 1000, \frac{\mathrm{mg}}{\mathrm{g}}}{\mathrm{V, L}} \quad \textbf{(3.10)}$$

Where A is weight of the filter disc and the weight of dried solids after drying at 104 ± 1ºC for 1 h (g), B is the weight of the pre-treated filter disc (g), C is the weight of the filter disc and the weight of dried solids after igniting at 550 ± 50ºC for 15 min (g) and V is the volume of the sample (L).

3.6.5 Sludge Volume Index (SVI)

SVI is an indicative parameter used to characterize the settling properties or quality of activated sludge samples. The volume of settled sludge of mixed liquor samples was obtained after 30 min of settling in a 1-L graduated cylinder. The *SVI* was calculated from settled sludge volume ($V_{settled}$) and *MLSS*, concentration using Equation 3.11.

$$SVI, \frac{\mathrm{mL}}{\mathrm{g}} = \frac{V_{settled}, \frac{\mathrm{mL}}{\mathrm{L}} \times 1000, \frac{\mathrm{mg}}{\mathrm{g}}}{MLSS, \frac{\mathrm{mg}}{\mathrm{L}}} \quad \textbf{(3.11)}$$

3.6.6 Removal Efficiency Calculations

The COD, NH_4-N, TSS and TN removal efficiencies were defined by Equation 3.12.

$$Removal\ Efficiency, \% = \frac{\mathrm{A}, \frac{\mathrm{mg}}{\mathrm{L}} - \mathrm{B}, \frac{\mathrm{mg}}{\mathrm{L}}}{\mathrm{A}, \frac{\mathrm{mg}}{\mathrm{L}}} \times 100\% \quad \textbf{(3.12)}$$

Where A is the influent concentration and B is the effluent concentration.

CHAPTER 4: CHARACTERISATION OF TROPICAL SEWAGE

4.1 Influent Characteristics of Domestic Sewage in Tropical Climates

The tropical sewage, representing the influent sewage for Phase 1, 2 and 3 of the pilot SBR, was characterised from March 2022 to June 2023 (Table 4.1). The average temperature of sewage (28 to 32°C) aligns with the optimum temperature (25 to 35°C) for bacteria activity (Tchobanoglous et al., 2014). How et al. (2018) mentioned that warm tropical sewage (30°C) helps to accelerate microbial metabolism rate, thereby leading to an increase in biological reaction rate. The pH of sewage during Phase 1, 2 and 3 ranged from 6.4 to 7.1, which falls within the favourable pH conditions (6 to 9) for most biological organisms.

Table 4.1: Average composition of physical and chemical characteristics of raw sewage during Phase 1, 2 and 3.

Parameter	Unit	Phase 1	Phase 2	Phase 3
Temperature	°C	29 ± 1.1	30 ± 1.0	30 ± 0.7
pH	-	6.8 ± 0.3	6.8 ± 0.2	6.7 ± 0.3
TSS	mg/L	164 ± 50	169 ± 54	319 ± 100
VSS	mg/L	147 ± 43	135 ± 51	279 ± 87
TN	mg/L	28 ± 3	27 ± 2	31 ± 4
NH_4-N	mg/L	23 ± 3	24 ± 4	26 ± 4
NO_2-N	mg/L	0	0	0
NO_3-N	mg/L	0	0	0
TCOD	mg/L	265 ± 67	250 ± 54	364 ± 99
DOC	mg/L	26 ± 7	27 ± 5	39 ± 6
sCOD	mg/L	77 ± 20	65 ± 21	85 ± 33
PO_4-P	mg/L	1.9 ± 0.5	2.2 ± 0.4	3.6 ± 1.9
TP	mg/L	9 ± 4	13 ± 5	28 ± 6
sCOD/N	-	3.2 ± 1.3	2.6 ± 0.4	2.8 ± 1.1
TCOD/N	-	9 ± 3	9.1 ± 1.7	12 ± 3.2
VSS/TSS	-	0.87 ± 0.1	0.79 ± 0.1	0.87 ± 0.04

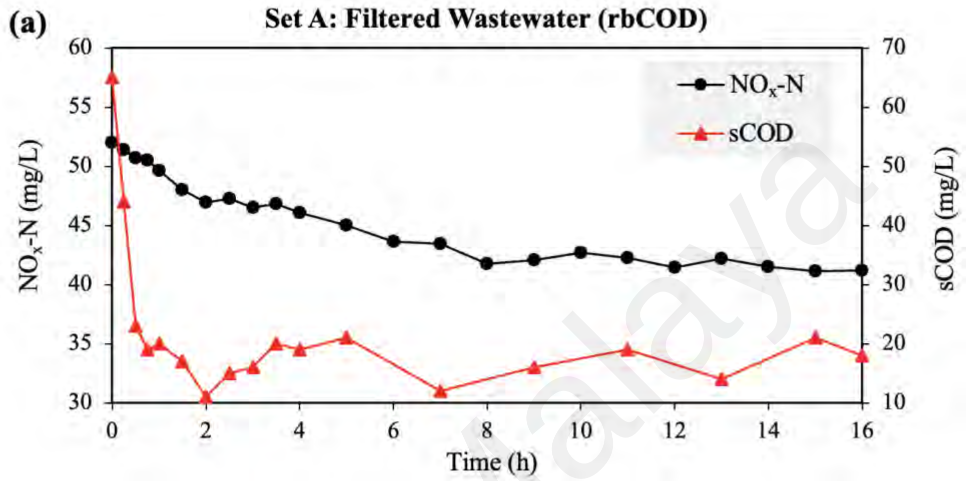
During Phase 1 to 3, the TSS in sewage ranged from 114 to 419 mg/L, while the VSS ranged from 84 to 366 mg/L. The VSS-to-TSS ratio (VSS/TSS) ranged from 0.78 to 0.91, higher than the typical ratio of 0.7 to 0.75 (Henze et al., 1997). The high VSS/TSS ratio could indicate a large portion of biodegradable particulate settleable solids (PSS) present in tropical sewage. The TN (25 to 35 mg/L) and NH_4-N (20 to 30 mg/L) concentrations were considered as low-to-medium strength (Tchobanoglous et al., 2014). The NO_2-N and NO_3-N levels in influent sewage were negligible, implying that there was no significant nitrogen conversion in the influent sewage.

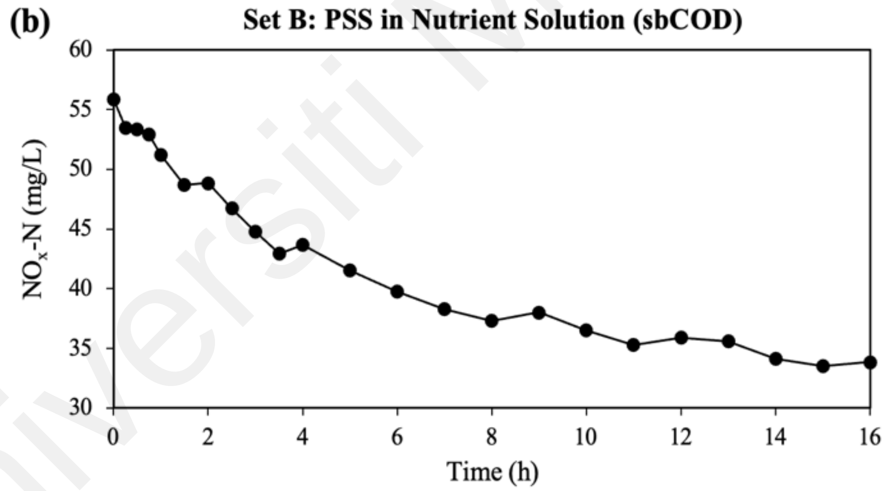
In warm climate regions, the soluble chemical oxygen demand (sCOD) concentration typically falls within the range of 66 to 170 mg/L, which was notably lower than the sCOD levels in cold regions (15–21°C) at 182 to 232 mg/L (Pfluger et al., 2018; Shen et al., 2019; Shi et al., 2008). The sewage in Singapore, a region with tropical climate like Malaysia, has been observed to demonstrate a similar range of sCOD concentrations, typically around 70 mg/L (Wu et al., 2017). The warm sewage in Malaysia and Singapore may be responsible for the accelerated microbial metabolisms in the sewer system, thereby increasing the sCOD biodegradation rate (Shi et al., 2008).

The sCOD/N ratio in raw sewage of all three operating phases (2 to 4) was generally lower than the recommended ratio (6 to 11), thereby limiting the heterotrophic denitrification. On the other hand, the TCOD/N ratio (8 to 11) is relatively higher than the recommended value for efficient denitrification (Gao et al., 2020; Wang, et al., 2020; Wang et al., 2015; Zhao et al., 2018). As indicated by the COD, TSS and VSS data, the large portion of biodegradable organic matter present in PSS as slowly biodegradable COD (sbCOD) may have facilitated denitrification activity in the low soluble COD/N ratio tropical sewage. To validate this hypothesis, denitrification batch activity experiments were carried out in Section 4.2.

4.2 Biodegradability of Particulate Settleable Solids for Denitrification

The denitrification batch activity test was conducted to evaluate the feasibility of utilizing sbCOD from PSS for denitrification following the procedure outlined in Section 3.2. Figure 4.1a–c illustrates the concentration profiles of sCOD and NO_x-N in Set A (filtered raw sewage), Set B (PSS in nutrient solution) and Set C (nutrient solution only), respectively. The activated sludge collected from the pilot SBR operating on Phase 1 (low-DO OA) was used in all three sets. The raw data for the denitrification batch tests are attached in Appendix 2.





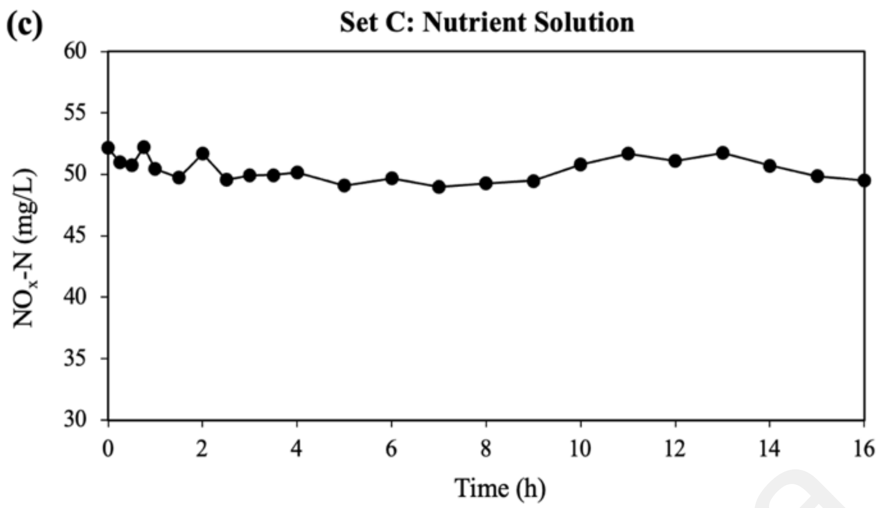


Figure 4.1: sCOD and NO_x-N concentrations for denitrification batch activity tests in (a) Set A with filtered sewage (rbCOD), (b) Set B with PSS in nutrient solution (sbCOD) and (c) Set C with nutrient solution only. The sCOD profile was only available in Set A due to its soluble biodegradability carbon source.

In Set A, there was a decrease of 9 mg/L of NO_x-N concentration during the initial 8 h of the experiment and it remained stable at around 42 mg/L in the following hours (Figure 4.1a). The sCOD concentration in Set A reduced from 65 to 15 mg/L within the first 2 h and remained consistently low, staying below 25 mg/L until the experiment over. The likely explanation is that denitrifying bacteria were actively utilizing readily biodegradable chemical oxygen demand (rbCOD) present in the filtered sewage for denitrification during the first 8 h. Subsequently, denitrification activity degraded as the available rbCOD was depleted.

In Set B, sbCOD from the PSS was used as the biodegradable organics. Compared to Set A, 19 mg/L of NO_3-N concentration was reduced within the first 8 hours in Set B. The significant reduction in the first 8 hours was likely due to active PSS hydrolysis. In the following hours, the NO_3-N concentration further decreased by 4 mg/L. The decrease in NO_3-N levels was linked to the rbCOD, which became available because of PSS hydrolysis. In Set C, no biodegradable organic sources were present in the nutrient solution. No reduction in NO_3-N was observed, indicating no denitrification activity occurred. No sCOD data were displayed for Set A and B because the carbon source used was not soluble.

The findings from the denitrification batch experiments suggested that sbCOD as PSS in tropical sewage has higher fractions than those sCOD from filtered tropical sewage. These aligned with previous studies that indicated sbCOD from PSS in sewage significantly contributes to biodegradable COD (bCOD) for denitrification (Choi et al., 2017; Sophonsiri & Morgenroth, 2004). Sophonsiri & Morgenroth (2004) and How et al. (2019) demonstrated that approximately 45 to 50% of the TCOD was present as sbCOD in PSS. The warm sewage temperature in tropical regions elevates the PSS hydrolysis rate. An increase in the PSS hydrolysis rate enhances the utilization of sbCOD for denitrification, thereby improving BNR performance. In a study by How et al. (2019), PSS hydrolysis was 2.5 times higher in warm sewage conditions (30°C) than the reported sewage values at 20°C. The higher PSS hydrolysis rate in warm sewage has the potential to provide a sufficient rbCOD for denitrification, particularly in low soluble COD/N sewage. Utilizing the sbCOD in PSS as a biodegradable organic source makes it possible to eliminate the need for an external carbon source, leading to a reduction in operating costs for external carbon dosage.

CHAPTER 5: FEASIBILITY OF LOW-DO OA PROCESS FOR TROPICAL SEWAGE

5.1 Nitrification Performance of Sludge under Low and High DO Conditions

The effect of DO concentrations on nitrification performance was investigated through batch experiments. To compare the performance, nitrifying activated sludge samples were taken from the oxic tanks of two full-scale SBRs, each operating at low DO (0.5 mg/L) and high DO concentrations (2.8 mg/L). The relationship between DO and specific ammonium oxidation rate ($SAOR$) of low and high DO SBR samples was represented by saturation kinetics using Michaelis-Menten model (Figure 5.1). The raw data of the nitrification batch activity tests and DO and pH profiles can be obtained from Appendix 5 and Appendix 6.

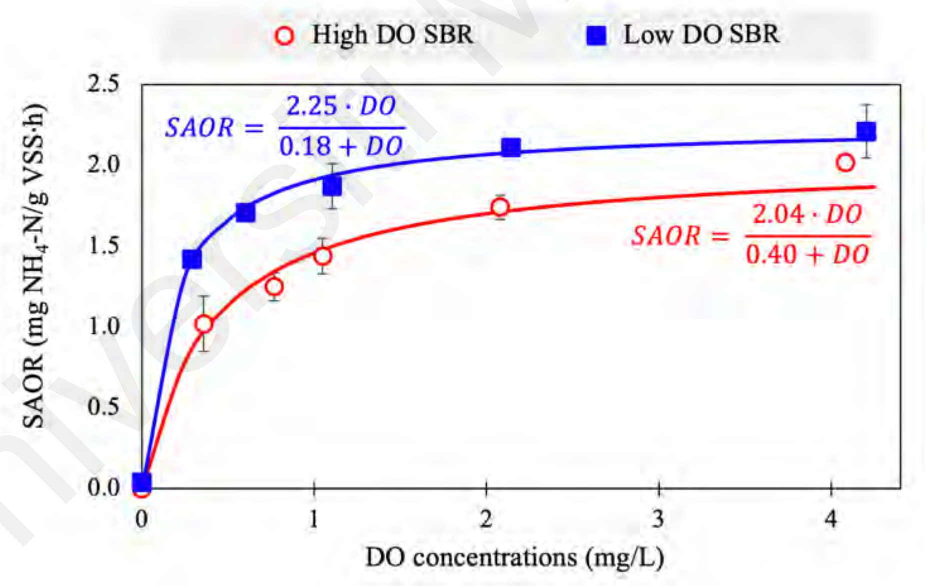


Figure 5.1: The $SAOR$ rate as a function of DO during the nitrification batch experiments using nitrifying activated sludge from low and high DO SBR.

The $SAOR$ rates of low and high DO SBR samples were 2.25 and 2.04 mg NH_4-N/g VSS·h, respectively (Table 5.1). Interestingly, the low-DO SBR samples did not reduce the $SAOR$ rate. The results achieved in this study align with the literature, where $SAOR$ values ranged from 0.8 to 3.6 mg N/g VSS·h (Fan et al., 2017; How et al., 2018; Keene et al., 2017). How et al. (2018) also proved that the $SAOR$ rate remained largely unaffected by the limited oxygen conditions, with the $SAOR$ rate at 70% of the maximum under low DO (0.5 mg/L). Fan et al. (2017) found no significant difference in $SAOR$ rates when using sludge from a high DO (1 mg/L) and low DO (0.5 mg/L) pilot-scale anoxic-oxic reactor, with $SAOR$ values of 4.28 and 4.59 mg NH_4-N/g VSS·h, respectively. The $SAOR$ results show that the nitrification performance under low DO conditions was comparable to that achieved under high DO conditions, suggesting that the ammonium oxidation was still efficient when oxygen availability was limited.

Table 5.1: Best-fit Michaelis-Menten kinetic parameters for ammonium oxidation of activated sludge from full-scale low and high DO concentrations.

Parameters	Units	Full-scale low-DO SBR	Full-scale high-DO SBR
Average MLVSS	mg/L	3396 ± 334	3259 ± 243
$SAOR$	mg NH_4-N/g VSS·h	2.25	2.04
K_o	mg/L	0.18	0.40
R^2	–	0.9936	0.9997

Besides the $SAOR$, it is essential to determine the affinity of nitrifiers to oxygen using the half-saturation constant (K_o). The high DO SBR samples exhibited half-saturation constants twice as high at 0.40 mg/L compared to the low DO SBR samples at 0.18 mg/L (Table 5.1). The K_o values in this study were within the range (0.14 to 1.38 mg/L) of the reported literature (How et al., 2018; Keene et al., 2017; Wang et al., 2021). The trend was consistent with Wang et al. (2021) study, which found higher K_o (0.39 mg/L) under high DO (2 mg/L) conditions than low DO (0.5 mg/L) at lower K_o (0.29 mg/L) in an anaerobic-anoxic-oxic reactor. Our results implied that nitrifiers in the low DO SBR sludge samples had a higher affinity for oxygen than those in the high DO SBR. This could be due to the adaptation of nitrifiers to low DO environments. Although nitrifiers in the high DO SBR can still efficiently use oxygen, they experience lower selective pressure to adapt to lower DO concentrations. The sludge samples from low DO SBR have higher oxygen affinity, making operating at low DO levels feasible in treating tropical sewage.

The nitrifying bacteria with higher oxygen affinity from low-DO SBR sludge samples implies the domination of slow-growing and low-growth rate K-strategists. The K-strategist nitrifiers, such as *Nitrospira*, *Nitrobacter* and *Nitrosospira*, were found to be the most abundant in the low-DO reactors (How et al., 2018; Keene et al., 2017; Wang et al., 2021; Yang et al., 2016). Beach & Noguera (2019) and Cao et al. (2018) reported that low-DO conditions favoured the growth of *Nitrospira*. *Nitrospira* species exhibit all the necessary enzymes required to perform complete nitrification (Daims et al., 2015). Besides low-DO conditions, *Nitrospira*-related comammox also thrives in the optimal environment at 37ºC and is adaptable in a highly oligotrophic environment (Kits et al., 2017). *Nitrospira* comammox could potentially exist in abundance in low-DO and warm-temperature sewage in contributing to stable nitrification performance.

5.2 Pilot-scale SBR Performance

5.2.1 Treatment Performance of Low-DO OA and High-DO OA

The pilot SBR was operated for 218 days under Phase 1 (Low-DO OA) and continued with Phase 2 (High-DO OA) until Day 260. The raw data of Phase 1 and 2 are shown in Appendix 10. The DO concentration during the oxic phase was maintained at 0.6 ± 0.2 mg/L and at 2.0 ± 0.2 mg/L in Phase 1 and 2, respectively. The seed sludge from return activated sludge stream was inoculated into the pilot SBR to achieve the initial MLSS of 2,800 mg/L. The sCOD, NH_4-N and TN treatment performance of both phases were illustrated in Figure 5.2a–c.

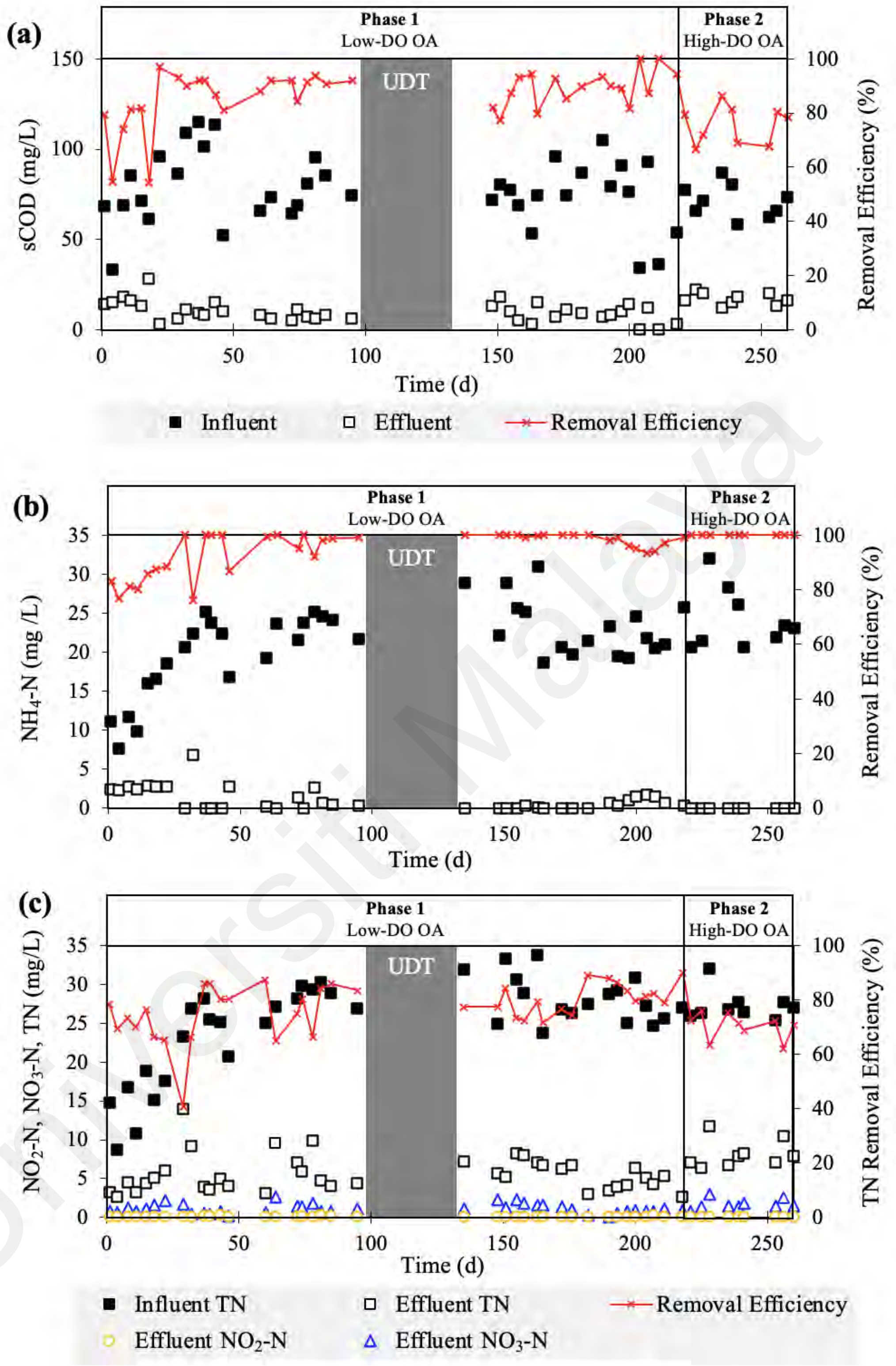


Figure 5.2: Pilot SBR treatment performance during Phase 1 (Low-DO OA) and Phase 2 (High-DO OA) of (a) sCOD, (b) NH_4-N and (c) NO_2-N, NO_3-N, TN profiles. UDT refers to unexpected downtime caused by the pilot SBR compressor issue.

The pilot SBR operating on low-DO OA showed effective treatment performance after 37 days of operation. In Phase 1 (Day 37 to 98), high removal efficiencies for sCOD, NH_4-N and TN were attained with average removals of 90 ± 4%, 97 ± 4% and 80 ± 8%, respectively (Figure 5.2a–c). Low effluent NH_4-N (0.7 ± 1.0 mg/L) and NO_3-N (3.1 ± 1.8 mg/L) concentrations were observed, even under low DO conditions, in compliance with Malaysia's discharge standard A limits for NH_4-N (5 mg/L) and NO_3-N (10 mg/L). Nitrite concentration in the effluent was less than 0.5 mg/L. The findings of this study align with the lab-scale low-DO OA study by How et al. (2020) where they achieved >99% NH_4-N removal and maintained low effluent NO_3-N concentration of 0.3 mg/L under low DO concentrations (0.4 ± 0.2 mg/L). The influent and effluent mixed liquor from Phase 1 treatment were shown in Appendix 15.

Between Day 99 to 130, the pilot SBR operation was halted due to an unexpected downtime (UDT) resulting from a compressor malfunction. During the UDT period, the activated sludge was temporarily stored at 4°C in the fridge until operations resumed. Despite the UDT lasting for 31 days, the sCOD, NH_4-N and TN removal efficiencies remained satisfactory at 89 ± 7%, 98 ± 2%, and 80 ± 6%, respectively as the operations resumed, suggesting that the low-DO OA sludge has good resilience.

The biomass concentration was also monitored in the study (Figure 5.3a). In Phase 1 (Day 37 to 95), the MLSS and MLVSS were consistently maintained around 2,914 ± 445 mg/L and 2,231 ± 350 mg/L, respectively (Figure 5.3a). In the first 22 days following the UDT, the MLSS and MLVSS values fluctuated sharply. Specifically, the MLSS fluctuated from around 1,410 to 3,528 mg/L. On Day 156 onwards, the MLSS and MLVSS remained relatively stable at around 3,039 ± 318 mg/L and 2,389 ± 257 mg/L. The stoppage of the pilot SBR operation could have impacted the sludge settleability and may need a period of adjustment to adapt and regain its settleability.

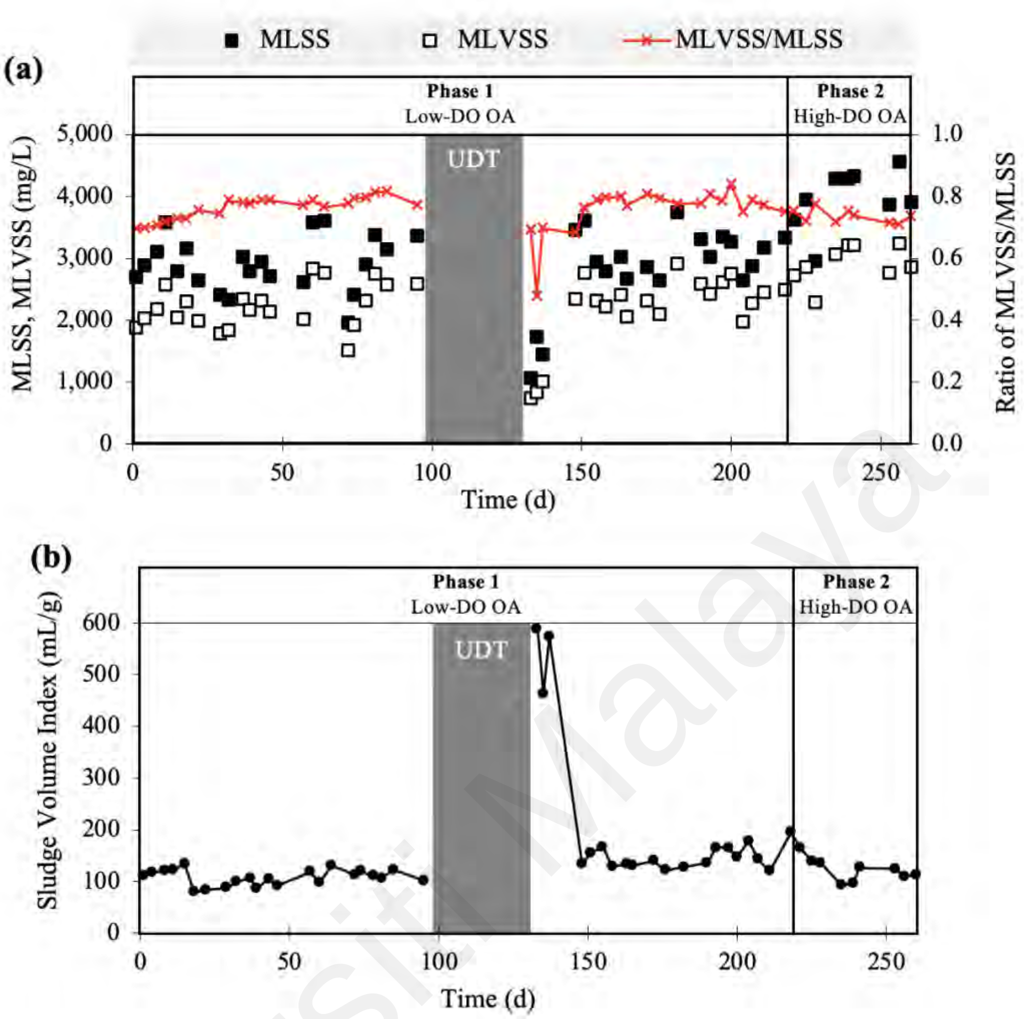


Figure 5.3: Pilot SBR biomass concentration profile during Phase 1 (Low-DO OA) and Phase 2 (High-DO OA) for (a) MLSS, MLVSS and (b) SVI profiles.

Early studies have documented that operating nitrification at low DO can cause sludge bulking, which hampers settling and reduces treatment efficiency (Hashemi et al., 2005; Weon et al., 2004; Wilen & Balmer, 1999). Sludge bulking is linked to the overgrowth of excessive filamentous bacteria and the formation of porous flocs (Martins et al., 2004). Hence, the sludge volume index (SVI) parameter is used to assess the settleability of activated sludge in sewage treatment. SVI values below 120 mL/g are favourable for good settling characteristics, while SVI values above 150 mL/g indicate sludge bulking and poor settling (Tchobanoglous et al., 2014). In Phase I (Day 37 to 98), the SVI remained low, with an average of 109 mL/g, despite operating at low DO conditions (Figure 5.3b). Although the SVI value fluctuated significantly after the UDT, it eventually stabilized at an average of 145 mL/g from Day 156 onwards. In Phase 1, the activated sludge demonstrated good settling characteristics, as evidenced by low SVI values and no sludge bulking. The photos of the volume of sludge settleability measurement for Phase 1 are available in Appendix 16.

The DO concentration was raised from 0.6 ± 0.2 mg/L to 2.0 ± 0.2 mg/L on Day 221 to compare the treatment performance with high-DO OA system (Phase 2). As anticipated, complete nitrification was achieved when operating at high DO concentration. Therefore, operating at such high DO may be unnecessary, given that nearly similar nitrification efficiency (97 ± 3%) was attained even at low DO concentration. In terms of TN removal performance, Phase 1 (80 ± 6%) achieved higher TN removal efficiency than Phase 2 (70 ± 5%). The result was consistent with the findings by Wang et al. (2021), who observed a decrease in TN removal efficiency from 79% to 69% when increasing the DO level from 0.6 to 2.0 mg/L in an anaerobic-anoxic-oxic reactor. Fan et al. (2017) also observed a slight decline in TN removal efficiency from 88% to 86% when increasing the DO concentrations from 0.5 to 1.0 mg/L in the oxic reactor of an anoxic-oxic activated sludge reactor. Operating at low DO conditions had a positive impact on the TN removal performance, which was likely attributed to the simultaneous nitrification-denitrification (SND) occurrence. The limited oxygen diffusion within the sludge floc during the oxic phase may have contributed to the enhancement of SND. Detailed analysis of the SND occurrence was discussed in Section 5.2.4.

5.2.2 Treatment Performance of Low-DO OA with Intermittent Aeration

Figure 5.4a–b shows the treatment performance of sCOD, NH_4-N and TN over 79 days in Phase 3 with intermittent 30 min aeration and non-aeration (Appendix 11). The DO and pH profiles are shown in Appendix 14. The low-DO OA with intermittent aeration attained complete NH_4-N oxidation after 12 days of operation. Between Day 12 to 33, the average sCOD and TN removal efficiencies were 82 ± 2% and 85 ± 6%, respectively. The low-DO OA with intermittent aeration worked fairly well in complying with the NO_3-N discharge limit with low effluent NO_3-N (2.6 ± 0.2 mg/L). From Day 34 to 51, the pilot SBR operation was stopped for UDT due to an inlet pump problem. The pilot SBR operation was resumed on Day 62 with new activated sludge. Remarkably, the sCOD, NH_4-N and TN removal performance remained highly effective, with removal efficiencies of 85 ± 7%, >99% and 85 ± 3%, respectively.

The sCOD and NH_4-N removal efficiencies in Phase 3 were comparable to those achieved in Phase 1. Interestingly, the TN removal efficiency in Phase 3 (85 ± 3%) was slightly higher than in Phase 1 (80 ± 8%). Therefore, incorporating intermittent aeration into the low-DO OA process represents a feasible strategy for reducing energy consumption while maintaining treatment effectiveness.

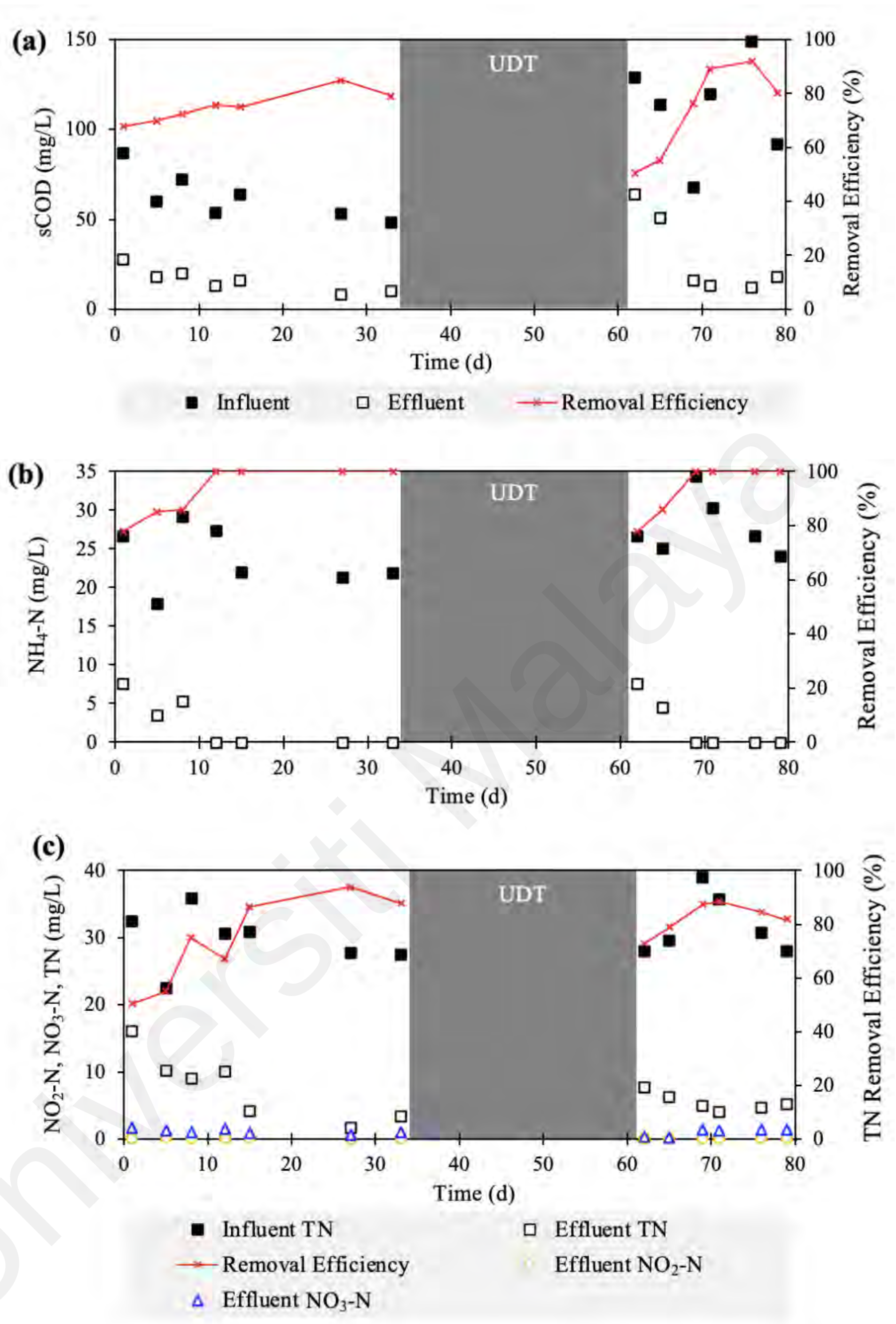


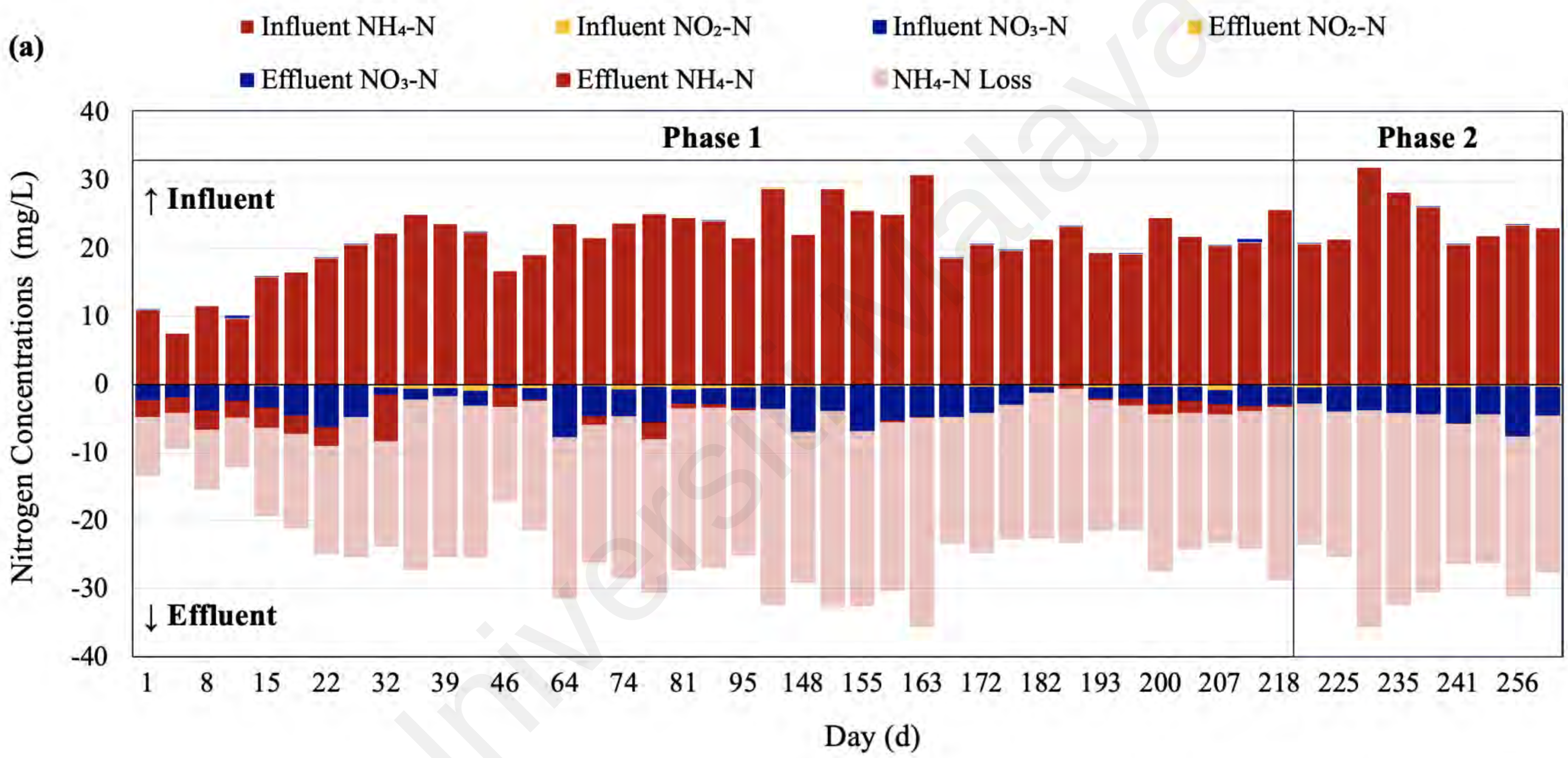
Figure 5.4: Pilot SBR treatment performance during Phase 3 (Low-DO OA with intermittent aeration) of (a) sCOD, (b) NH_4-N and (c) NO_2-N, NO_3-N, TN profiles.

5.2.3 Nitrogen Mass Balance

Figure 5.5a was plotted to analyse the nitrogen mass balance for influent and effluent in the OA systems during Phase 1 and 2. During Phase 1, the low-DO OA performance results clearly showed good nitrification performance under low-DO concentrations (0.6

± 0.2 mg/L) with low effluent NH_4-N (<3 mg/L) most of the time. After Day 37 of stabilization in Phase 1, a significant portion of NH_4-N was eliminated from the sewage despite operating at low DO levels. Despite the low COD/N tropical sewage and no external carbon sources being added, NO_3-N concentrations in the effluent remained low (<8 mg/L) during Phase 1 and 2 due to good denitrification activity. The most probable reason could be sufficient rbCOD was produced from the high PSS hydrolysis rate in tropical sewage to convert nitrate to nitrogen gas efficiently (Section 4.2). In Phase 3, the NH_4-N was completely removed on Day 12. Although there was some NH_4-N in effluent on Day 62 due to the resumption of the operation after the UDT, it was completely removed on Day 69. The NO_2-N accumulation was also not observed in Phase 3. The NO_3-N concentration in the effluent was also lower (Figure 5.5b) compared to Phase 1 and 2. The intermittent aeration may have further enhanced denitrification activity by utilizing biodegradable organics in raw sewage.

In a BNR system, nitrogen loss primarily occurs through denitrification, where NO_3-N is converted to nitrogen gas or potentially nitrous oxide (N_2O) emissions. Previous studies concluded that the low-DO BNR operation in activated sludge systems could elevate N_2O emissions (Kampschreur et al., 2008; Peng et al., 2015; Tallec et al., 2006). It is important to note that the biomass used in the previous studies was cultivated in a high DO treatment tank. The cultivation likely resulted in the enrichment of nitrifiers adapted to high DO conditions. Additionally, the earlier studies on low DO BNR were typically conducted over short-term periods. However, many recent studies have proven that operating at low DO or intermittent aeration did not contribute to an increase in N_2O release (Liu et al., 2021; Ma et al., 2021; Wang et al., 2022). A long-term low DO (0.5 mg/L) operation with 40 d of SRT decreased the release of N_2O by 54% (Liu et al., 2021). Under a longer period, low DO emerged as a selection factor that could change the nitrifier and abundance and communities accordingly. In this low-DO OA study, the efficient NH_4-N oxidation under low DO or intermittent aeration might not promote N_2O production.



(a)
Influent NH4-N
Influent NO2-N
Influent NO3-N
Effluent NO2-N
Effluent NO3-N
Effluent NH4-N
NH4-N Loss
Phase 1
Phase 2
↑ Influent
↓ Effluent
Nitrogen Concentrations (mg/L)
40
30
20
10
0
-10
-20
-30
-40
1
8
15
22
32
39
46
64
74
81
95
148
155
163
172
182
193
200
207
218
225
235
241
256
Day (d)

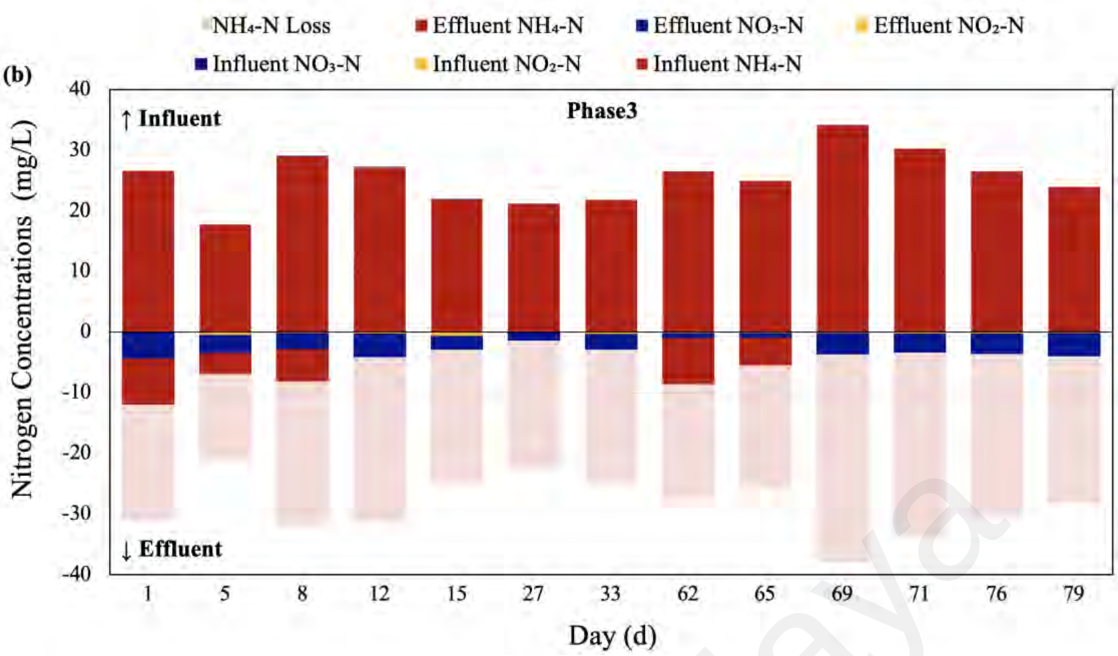
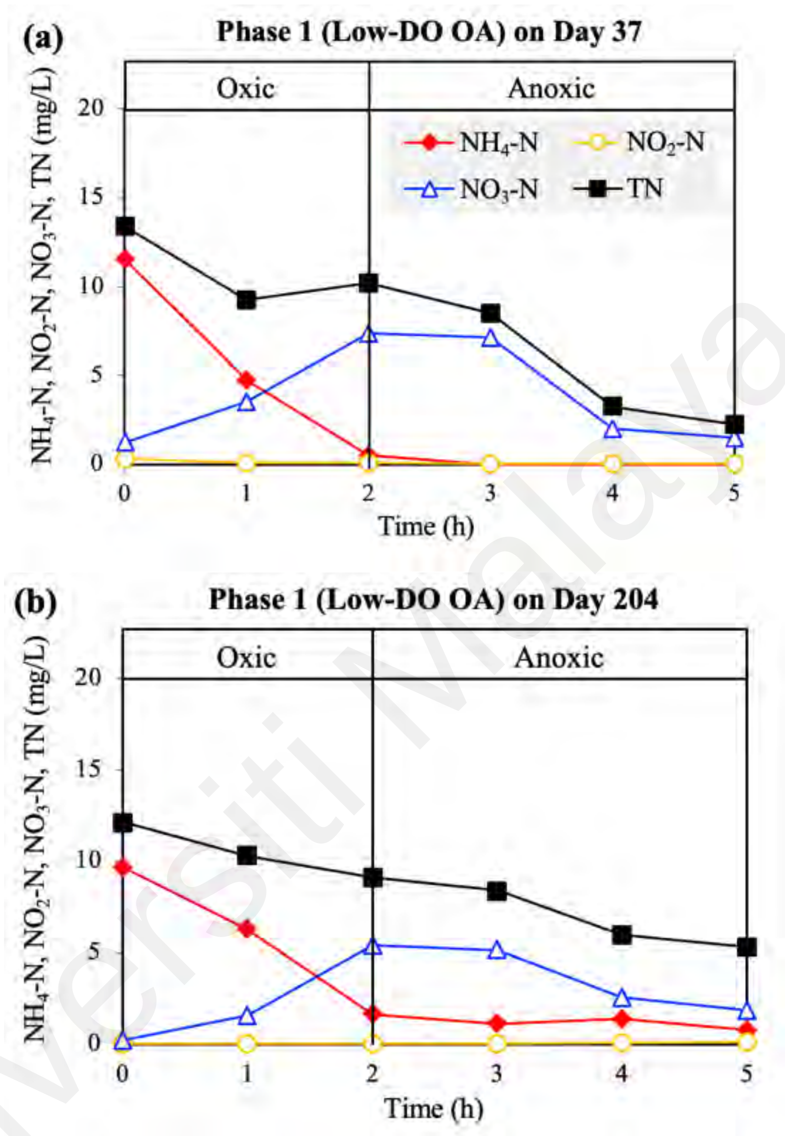


Figure 5.5: Time profile of nitrogen mass balance of influent (top) and effluent (below) during (a) Phase 1 and 2 and (b) Phase 3.

5.2.4 Variation of Nitrogen in Cyclic Studies

The detailed graphical representations of NH_4-N, NO_2-N, NO_3-N and TN concentrations from Phase 1, Phase 1 (after UDT) and Phase 2 were presented in Figure 5.6a–c. During the low-DO OA operation, the SND occurrence was identified when ΔNH_4-N being oxidized was greater than ΔNO_3-N being produced in the oxic phase. From Figure 5.6a, 12 mg/L of ΔNH_4-N was oxidized while only 6 mg/L of ΔNO_3-N produced, suggesting the SND happened in the oxic phase. Similarly, 10 mg/L of ΔNH_4-N was oxidized while only 5 mg/L of ΔNO_3-N produced in the oxic phase (Figure 5.6b). A similar pattern indicating SND was also observed in low-DO (0.9 mg/L) anaerobic-oxic-anoxic, where the amount of ΔNH_4-N oxidized during the oxic phase exceeded the production of ΔNO_3-N by 4 mg/L (How et al., 2019). It is well known that operating at low DO concentrations (<1 mg/L) during the nitrification in the activated sludge system likely contributed to the SND occurrence (How et al., 2019; Keene et al., 2017; Miao et al., 2017; Wang et al., 2021). The representative pH and DO profiles from Phase 1 and 2 were included in Appendix 12 and Appendix 13.



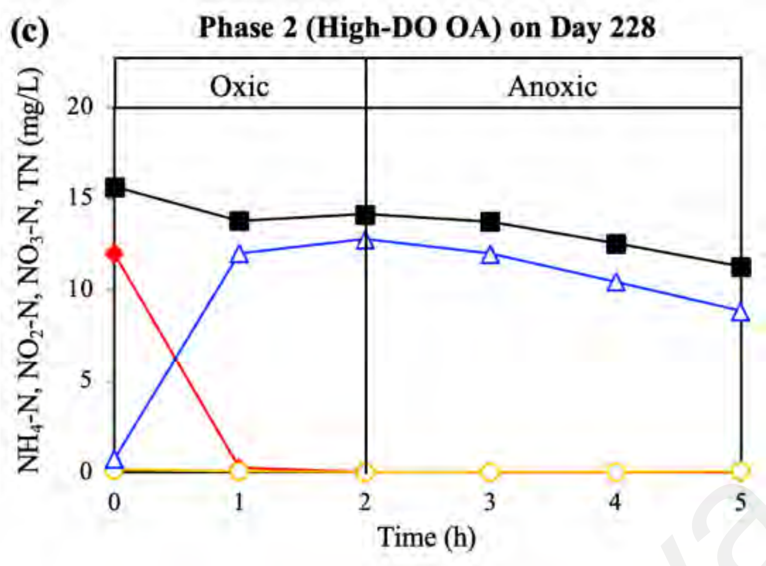


Figure 5.6: Detailed cyclic profiles of NH_4-N, NO_2-N, NO_3-N and TN during (a) Phase 1 (Low-DO OA) on Day 37, (b) Phase 1 (Low-DO OA) on Day 204 and (c) Phase 2 (High-DO OA) on Day 228.

The SND occurrence is influenced by specific hydraulic and oxygen environments that impact the formation of large sludge flocs with a modified internal structure. These flocs' size and structure lead to DO concentration gradients, creating anoxic micro-zones in the centre of the flocs for heterotrophic denitrification, while the outer layers of the flocs create aerobic micro-zones for nitrification (Gogina & Gulshin, 2016). By operating the oxic phase in an environment with limited oxygen availability, it is possible to maintain the coexistence of nitrifiers and denitrifiers, thereby enhancing nitrogen removal performance through SND (Zhou et al., 2012). Consequently, SND reduces aeration energy consumption, lower chemical usage and eliminates the need for additional carbon sources.

No SND was observed under high DO conditions in Phase 2. The amount of ΔNO_3-N produced (12.8 mg/L) during the oxic phase closely matched the oxidized ΔNH_4-N (12 mg/L), as depicted in Figure 5.6c. Ma et al. (2017) demonstrated that SND efficiency increased from 39% to 81% as the DO level decreased from 4.6 to 0.4 mg/L in a sequencing batch biofilm reactor treating coal gasification sewage. Similarly, higher SND efficiency (74%) was reported under low DO concentration (0.7 mg/L) compared to a high DO concentration (1.2 mg/L), where the SND efficiency was 66% in an SBR reactor (Yan et al., 2019). Operating the BNR system under high DO concentration (≥ 2 mg/L) suppresses denitrification and restricts SND from occurring, resulting in a decrease in TN removal efficiency.

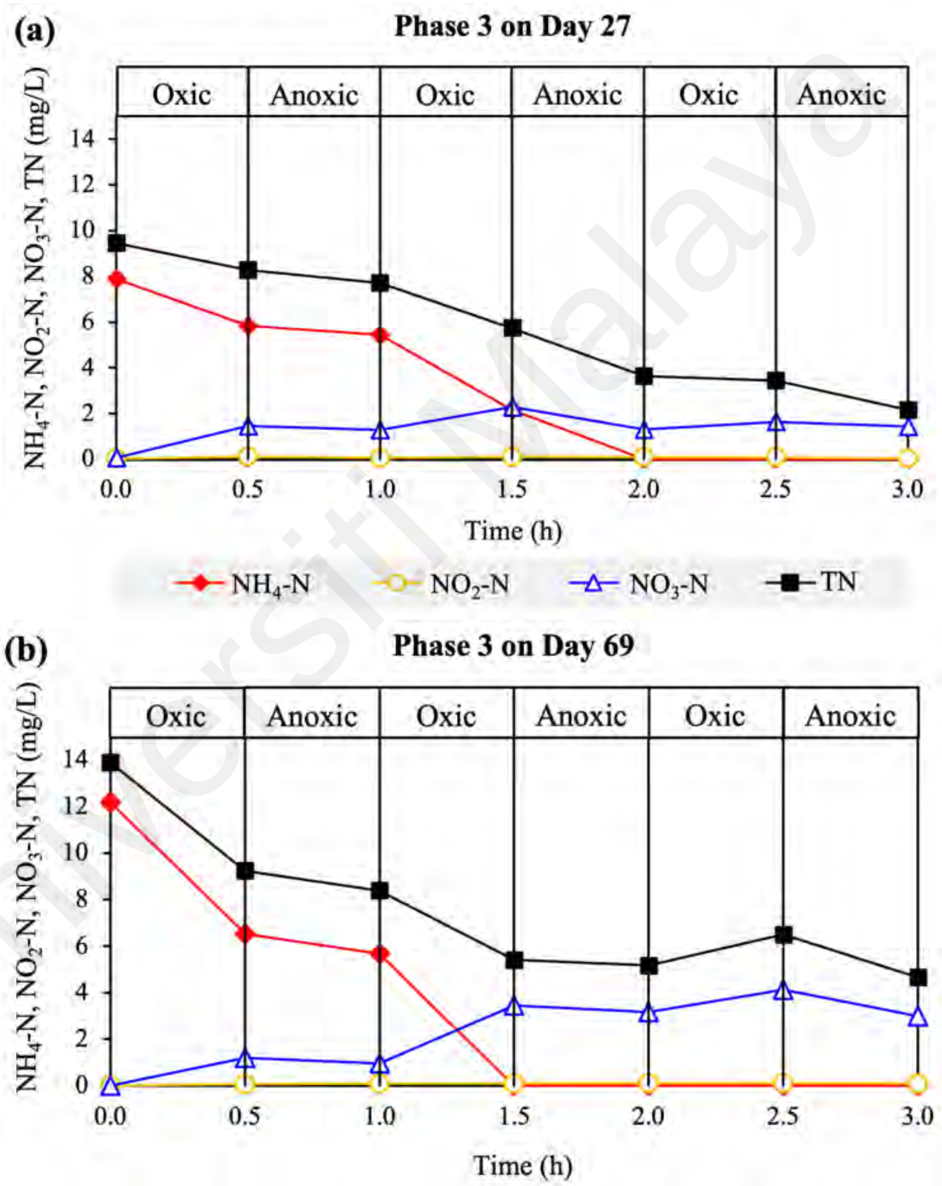


Figure 5.7: Detailed cyclic profiles of NH_4-N, NO_2-N, NO_3-N and TN during (a) Phase 3 on Day 27 and (b) Phase 3 on Day 69.

The detailed NH_4-N, NO_2-N, NO_3-N and TN concentration profiles for a selected cycle from Day 27 (before UDT) and 69 (after UDT) of Phase 3 were depicted in Figure 5.7.

The NH_4-N was completely consumed within 2 h on Day 27 and 1.5 h on Day 69, respectively, indicating that the DO concentration was sufficient for effective NH_4-N removal, even under low DO intermittent aeration. The ΔNO_3-N remained lower than the ΔNH_4-N during oxic phase, suggesting a clear indication of SND occurrence. The fact that the overall produced ΔNO_3-N is lower than the consumed ΔNH_4-N suggests that a portion of the produced NO_3-N is being utilized by the denitrifying bacteria during the oxygen-limited aerated phase. By implementing intermittent aeration on the low-DO OA process, energy consumption is reduced compared to continuous aeration in low-DO OA systems. The intermittent aeration approach allows for more efficient use of oxygen and reduces the overall energy requirements for aeration, resulting in energy savings in the sewage treatment process. Therefore, operating the low-DO OA in intermittent aeration can lead to reduced operating costs and improved treatment performance, making it an attractive option for energy-efficient biological nitrogen removal in sewage treatment plants.

5.3 Energy Savings

5.3.1 Energy Reduction of Low-DO BNR

The low-DO OA process has demonstrated its efficient performance in removing nitrogen when treating tropical sewage (30°C) with a low COD/N ratio. An energy-saving analysis was performed to compare the aeration energy consumption between low-DO (0.6 mg/L) and high-DO (2 mg/L) nitrification processes. The calculation was estimated from the dissolved oxygen transfer rate for nitrification and bulk DO concentration in sewage, assuming the surface aerator as the aeration system. Table 5.2 presents the estimated energy savings of operating the nitrification process at low DO compared with the high DO levels.

Table 5.2: Energy saving of low DO nitrification process.

Parameters	Low DO (0.6 mg/L)	High DO (2 mg/L)
Oxygen transfer rate required, OTR_{LIQUID} (kg O_2/day)	4.4	14.7
Total oxygen transfer rate required, OTR_{TOTAL} (kg O_2/day)	644.4	654.7
Total oxygen transfer rate at standard condition, SOTR (kg O_2/day)	766	931
Energy required (kWh/day)	589	716
Energy saving of low DO nitrification (%)	$\frac{716 - 589\ kWh/day}{716\ kWh/day} \times 100\% = 18\%$	

Based on the calculations, operating the nitrification at DO level of 0.6 mg/L can result in an 18% reduction in aeration energy usage when compared to nitrification at DO level of 2 mg/L. Comparable energy reduction values at 23% and 25% were observed by How et al. (2018) and Keene et al. (2017) when operating at low DO concentrations of 0.5 mg/L and 0.33 mg/L compared to the conventional high DO conditions, respectively. Hence, operating the BNR process with low DO levels does improve the energy efficiency of STPs.

5.3.2 Energy Reduction of Low-DO BNR with Intermittent Aeration

The low-DO BNR with intermittent aeration in Phase 3 has demonstrated satisfactory nitrogen removal performance in treating tropical sewage (30°C) with a low COD/N ratio. An energy-saving analysis to obtain the estimated energy reduction of BNR with intermittent aeration was performed in Table 5.3. The calculation was estimated assuming that the DO concentrations in nitrifying systems were operated at the same concentrations. The energy reduction of BNR with intermittent aeration obtained from Table 5.3 was then used to estimate the energy saving when operating in low-DO BNR with intermittent aeration.

Table 5.3: Energy saving calculation of the BNR with intermittent aeration.

Parameters	Conventional BNR	BNR with intermittent aeration
Total energy consumption (kWh/year)	1,916,385	−
Power consumption (kW)	222	−
Total aeration operation in one day (h)	12	9
Energy required (kWh/day)	2662	1996
Energy reduction of BNR with intermittent aeration (%)	$\frac{2662 - 1996\ kWh/day}{2662\ kWh/day} \times 100\% = 25\%$	

Based on the calculation on Table 5.3, integrating BNR with intermittent aeration can reduce energy consumption by 25% compared to the conventional BNR process. Using the estimated energy savings of low-DO nitrification from Section 5.3.1, it was deduced that low-DO BNR with intermittent aeration results in an additional 7% energy savings. The low-DO BNR with intermittent aeration reduced the energy intensity and operation of the aerator system, further enhancing the energy efficiency of STPs.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

In this dissertation, a pilot-scale reactor of a cost-effective oxic-anoxic biological nitrogen removal treating tropical sewage was developed. The studies have demonstrated that the low-DO OA process is feasible as an efficient and cost-effective BNR treatment for treating tropical sewage with a low COD/N ratio. The research conclusions for all four objectives are summarised as follows:

a) Biodegradability of PSS in tropical sewage

The denitrification batch activity experiment results indicate that sbCOD from PSS can effectively serve as biodegradable organics to support denitrification. The high rate of PSS hydrolysis in warm sewage supplies adequate organic matter for nitrogen removal in the post-anoxic stage of the low-DO OA process. The findings affirm the feasibility of utilising tropical sewage with higher PSS biodegradability for enhanced nitrogen removal efficiency, particularly in carbon-limited sewage.

b) Maximum nitrification potential

The nitrification batch activity experiment aimed to determine the maximum nitrification performance of nitrifiers in sludge samples from a full-scale low-DO SBR and full-scale high-DO SBR. The sludge samples from the low-DO SBR exhibited a lower half-saturation constant (K_o) of 0.18 mg/L, in contrast to the high-DO SBR with a K_o of 0.4 mg/L, indicating a higher oxygen affinity in the low-DO SBR. The nitrifying bacteria from low-DO SBR samples with higher oxygen affinity may suggest the potential K-strategist survival, a slow-growing and low-growth rate nitrifier such as comammox *Nitrospira*.

c) Treatment performance of low-DO OA

The 150-L pilot-scale SBR was successfully established for nitrogen removal in low COD/N tropical sewage. In the OA system, decreasing DO levels from 2 ± 0.2 to 0.6 ± 0.2 mg/L did not adversely impact NH_4-N and COD removal performance. Both the low-DO and high-DO OA process exhibited excellent NH_4-N removal, with the low-DO OA achieving 97 ± 3%, comparable to the high-DO OA at 99%. Applying low-DO operation to the OA system also encourages SND occurrence, resulting in a 10% increase in TN removal efficiency compared to one with high-DO without any carbon dosage. Despite the 31-day operational breakdown, the sludge exhibited strong resilience and recovered the BNR performance. The low-DO OA process is a simple and cost-effective retrofit alternative for the conventional high-DO BNR process, resulting in savings of over 18% in aeration energy.

d) Treatment performance of low-DO OA with intermittent aeration

The study of intermittent aeration, 30 min aeration and 30 min non-aeration sequentially for a total period of 4 hours in the low-DO OA operation was carried out in the pilot SBR system for further energy-saving considerations. The low-DO OA with intermittent aeration has complete NH_4-N removals and higher TN removals (85 ± 6%) compared to the Phase 1 low-DO OA only. Besides low DO conditions, the intermittent aeration environment further enhanced the SND, improving the nitrogen removal performance. Low-DO BNR with intermittent aeration can reduce energy consumption by 25%. Integrating intermittent aeration in the low-DO OA process offers a viable approach to reduce energy consumption further while preserving treatment efficiency.

6.2 Significance of the Study

Removing nitrogen pollutants from sewage using the conventional BNR method to comply with stringent regulatory regulations poses a significant financial burden for many STPs. Results of the pilot study on the low-DO OA process have so far been encouraging as an energy-efficient and cost-effective BNR sewage treatment. The outcomes of the pilot-scale study will benefit the following aspects:

a) Improved knowledge of tropical sewage characteristics

This research could help to improve knowledge of the sewage characteristics in Malaysia. Although tropical sewage in this study contains low soluble biodegradable organics with sCOD/N of 2 to 4 carbon, further denitrification batch test reveals that the high portion of particulate carbon can support denitrification. The warm sewage (about 29°C) in Malaysia can benefit from the higher PSS hydrolysis rate which can provide sufficient carbon for denitrification and eliminate carbon dosage, reducing chemical costs. Beyond economic benefits, eliminating carbon dosage minimizes the carbon footprint and enhances operational simplicity.

b) Energy-efficient and cost-effective BNR process

The low-DO OA process treating tropical sewage with low COD/N results in efficient nitrogen removal performance. Contrary to the conventional BNR process, low-DO OA and low-DO OA with intermittent aeration can reduce energy consumption at STPs without compromising the effluent quality. The simple and low-cost low-DO OA can contribute to Sustainable Development Goal (SDG) 6 in improving water quality and reducing pollution to ensure safe and clean water for all by 2030. At the same time, the present study on low-DO OA might help to solve energy usage reduction in STPs, aligning with SDG 7 in improving the energy efficiency of sewage treatment.

6.3 Recommendations for Future Research

Based on the outcomes of this project, future work should concentrate on the following recommendations:

a) Full-scale Application

The low-DO OA process and its combination with intermittent aeration in BNR sewage treatment presents a significant energy and carbon savings opportunity without compromising treatment performance. The positive outcomes from the pilot-scale study suggest scaling up the low-DO OA reactor to full-scale testing. Full-scale low-DO OA reactor utilizing direct on-site sewage influent can provide more precise results. Factors such as operational efficiency, compliance with regulations, maintenance and energy consumption can assess the feasibility of implementing the low-DO OA process at full scale before its real-world application.

b) Analysis of Microbial Community Structure

Microbial community analysis would provide value-added knowledge on the responsible microorganisms involved in the simultaneous nitrification and denitrification during the oxic phase (Section 5.2.4). Analysing the microbial community helps to understand and validate the composition, abundance and diversity of functional microorganisms involved in the low-DO BNR process.

c) Measurement of N_2O Emissions

Numerous experts have expressed concerns regarding the potential increase of N_2O gas emissions from the low-DO operation in this BNR system. It is unknown whether the low-DO OA operation would contribute to N_2O emissions. Therefore, it is essential to detail the measurement of N_2O emissions in the low-DO OA process in future research.

LIST OF PUBLICATIONS AND PAPERS PRESENTED

List of Publication:

1) **Leong, C. L.**, How, S. W., Rabuni, M. F., Mohd Aris, A., Khor, B. C., Curtis, T. P., & Chua, A. S. M. (2023). Pilot Study of Oxic–Anoxic Process under Low Dissolved Oxygen for Nitrogen Removal from Low COD/N Tropical Sewage. *Water*, *15*(11), 2070.

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List of Papers Presented in Conferences:

1) **Leong, C. L.**, How, S. W., Rabuni, M. F., Mohd Aris, A., Khor, B. C., Curtis, T. P., & Chua, A. S. M. (2022). Pilot-scale Evaluation of Oxic-Anoxic Process under Low Dissolved Oxygen Condition for Nitrogen Removal from Tropical Sewage. *The 13th International Symposium on Southeast Asian Water Environment (SEAWE2022),* 13-15 December 2022, Bangkok, Thailand.

2) **Leong, C. L.**, How, S. W., Rabuni, M. F., Mohd Aris, A., Khor, B. C., Curtis, T. P., & Chua, A. S. M. (2022). Pilot Study of Low-dissolved-oxygen Oxic-anoxic Process Treating Low COD/N Tropical Sewage for Nitrogen Removal. *The 9th IWA-ASPIRE Conference & Exhibition 2023*, 22-26 October 2023, Kaohsiung, Taiwan.

REFERENCES

Aliman, K. H. (2019, February 28). Tariff review may relieve Indah Water's structural deficit. *The Edge Malaysia*.

APHA/AWWA/WEF. (1998). Standard Methods for the Examination of Water and Wastewater. In *American Public Health Association/American Water Works Association/Water Environment Federation* (20th ed.). https://doi.org/10.1016/B978-0-12-382165-2.00237-3

Ariffin, M., & Sulaiman, S. N. M. (2015). Regulating sewage pollution of Malaysian rivers and its challenges. *Procedia Environmental Sciences*, *30*, 168–173.

Arnaldos, M., Kunkel, S. A., Stark, B. C., & Pagilla, K. R. (2013). Enhanced heme protein expression by ammonia-oxidizing communities acclimated to low dissolved oxygen conditions. *Applied Microbiology and Biotechnology*, *97*(23), 10211–10221. https://doi.org/10.1007/s00253-013-4755-7

Awad, H., El-Mewafi, M., Negm, M. S., & Gar Alalm, M. (2022). A divided flow aerobic-anoxic baffled reactor for simultaneous nitrification-denitrification of domestic wastewater. *Science of the Total Environment*, *833*. https://doi.org/10.1016/j.scitotenv.2022.155247

Badia, A., Kim, M., & Dagnew, M. (2021). Nitrite denitrification using biomass acclimatized with methanol as complementary carbon source: long-term performance and kinetics study. *Environmental Science: Water Research & Technology*, *7*(1), 93–106.

Beach, N. K., & Noguera, D. R. (2019). Design and assessment of species-level qPCR primers targeting comammox. *Frontiers in Microbiology*, *10*(JAN), 1–15. https://doi.org/10.3389/fmicb.2019.00036

Bell, V. A., Naden, P. S., Tipping, E., Davies, H. N., Carnell, E., Davies, J. A. C., Dore, A. J., Dragosits, U., Lapworth, D. J., Muhammed, S. E., Quinton, J. N., Stuart, M., Tomlinson, S., Wang, L., Whitmore, A. P., & Wu, L. (2021). Long term simulations of macronutrients (C, N and P) in UK freshwaters. *Science of The Total Environment*, *776*, 145813. https://doi.org/10.1016/J.SCITOTENV.2021.145813

Bellucci, M., Ofit¸eru, I. D. O., Graham, D. W., Head, I. M., & Curtis, T. P. (2011). Low-Dissolved-Oxygen Nitrifying Systems Exploit Ammonia-Oxidizing Bacteria with Unusually High Yields. *APPLIED AND ENVIRONMENTAL MICROBIOLOGY*, *77*(21), 7787–7796. https://doi.org/10.1128/AEM.00330-11

Blackburne, R., Yuan, Z., & Keller, J. (2008). Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor. *Biodegradation*, *19*(2), 303–312. https://doi.org/10.1007/s10532-007-9136-4

Bueno, R. F., Piveli, R. P., Campos, F., & Sobrinho, P. A. (2018). Simultaneous nitrification and denitrification in the activated sludge systems of continuous flow. *Environmental Technology*, *39*(20), 2641–2652. https://doi.org/10.1080/09593330.2017.1363820

Cao, G., Wang, S., Peng, Y., & Miao, Z. (2013). Biological nutrient removal by applying modified four step-feed technology to treat weak wastewater. *Bioresource Technology*, *128*, 604–611. https://doi.org/10.1016/J.BIORTECH.2012.09.078

Cao, Y., Kwok, B. H., Van Loosdrecht, M. C. M., Daigger, G., Png, H. Y., Long, W. Y., & Eng, O. K. (2018). The influence of dissolved oxygen on partial nitritation/ anammox performance and microbial community of the 200,000 m3/d activated sludge process at the Changi water reclamation plant (2011 to 2016). *Water Science and Technology*, *78*(3), 634–643. https://doi.org/10.2166/wst.2018.333

Cao, Y., van Loosdrecht, M. C. M., & Daigger, G. T. (2017). Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies. *Applied Microbiology and Biotechnology*, *101*(4), 1365–1383.

Capodici, M., Di Bella, G., Di Trapani, D., & Torregrossa, M. (2015). Pilot scale experiment with MBR operated in intermittent aeration condition: analysis of biological performance. *Bioresource Technology*, *177*, 398–405.

Choi, Y.-Y., Baek, S.-R., Kim, J.-I., Choi, J.-W., Hur, J., Lee, T.-U., Park, C.-J., & Lee, B. J. (2017). Characteristics and biodegradability of wastewater organic matter in municipal wastewater treatment plants collecting domestic wastewater and industrial discharge. *Water, 9*(6), 409.

Coats, E. R., Appel, F. J., Guho, N., Brinkman, C. K., & Mellin, J. (2023). Interrogating the performance and microbial ecology of an enhanced biological phosphorus removal/post-anoxic denitrification process at bench and pilot scales. *Water Environment Research, 95*(4), e10852.

Council Directive. (1991). *Council Directive 91/271/EEC of 21 May 1991 concerning urban wastewater treatment*.

Dai, H., Lu, X., Peng, L., Li, X., & Dai, Z. (2017). Enrichment culture of denitrifying phosphorus removal sludge and its microbial community analysis. *Environmental Technology*, *38*(22), 2800–2810.

Daims, H., Lebedeva, E. V., Pjevac, P., Han, P., Herbold, C., Albertsen, M., Jehmlich, N., Palatinszky, M., Vierheilig, J., Bulaev, A., Kirkegaard, R. H., Von Bergen, M., Rattei, T., Bendinger, B., Nielsen, P. H., & Wagner, M. (2015). Complete nitrification by Nitrospira bacteria. *Nature*, *528*(7583), 504–509. https://doi.org/10.1038/nature16461

Dasgupta, S., Wu, S., & Goel, R. (2017). Coupling autotrophic denitrification with partial nitritation-anammox (PNA) for efficient total inorganic nitrogen removal. *Bioresource Technology*, *243*, 700–707. https://doi.org/10.1016/j.biortech.2017.06.130

Department of Environment of Malaysia. (2018). Environmental Quality Report 2018. *Jabatan Alam Sekitar*, 36–105. https://www.doe.gov.my/portalv1/wp-content/uploads/formidable/5/Kualiti-Air-Sungai.pdf

Department of Environment of Malaysia. (2022). *Environmental Quality Report*. https://enviro2.doe.gov.my/ekmc/digital-content/laporan-kualiti-alam-sekeliling-eqr-2022/

Dobbeleers, T., Caluwé, M., Dockx, L., Daens, D., D'aes, J., & Dries, J. (2020). Biological nutrient removal from slaughterhouse wastewater via nitritation/denitritation using granular sludge: an onsite pilot demonstration. *Journal of Chemical Technology & Biotechnology*, *95*(1), 111–122.

Drewnowski, J., Remiszewska-Skwarek, A., Duda, S., & Łagód, G. (2019). Aeration process in bioreactors as the main energy consumer in a wastewater treatment plant. Review of solutions and methods of process optimization. *Processes*, *7*(5). https://doi.org/10.3390/pr7050311

Duan, H., Zhao, Y., Koch, K., Wells, G. F., Zheng, M., Yuan, Z., & Ye, L. (2021). Insights into Nitrous Oxide Mitigation Strategies in Wastewater Treatment and Challenges for Wider Implementation. *Environmental Science & Technology*, *55*(11), 7208–7224. https://doi.org/10.1021/acs.est.1c00840

Edokpayi, J. N., Odiyo, J. O., & Durowoju, O. S. (2017). Impact of Wastewater on Surface Water Quality in Developing Countries: A Case Study of South Africa. In H. Tutu (Ed.), *Water Quality* (p. Ch. 18). IntechOpen. https://doi.org/10.5772/66561

Fan, H., Qi, L., Liu, G., Zhang, Y., Fan, Q., & Wang, H. (2017). Aeration optimization through operation at low dissolved oxygen concentrations: Evaluation of oxygen mass transfer dynamics in different activated sludge systems. *Journal of Environmental Sciences (China)*, *55*, 224–235. https://doi.org/10.1016/j.jes.2016.08.008

Fitzgerald, C. M., Camejo, P., Oshlag, J. Z., & Noguera, D. R. (2015). Ammonia-oxidizing microbial communities in reactors with efficient nitrification at low-dissolved oxygen. *Water Research*, *70*, 38–51. https://doi.org/10.1016/j.watres.2014.11.041

Frank, O. (2022, July 8). *Toxic red tide along Massachusetts coast prompts shellfishing ban*. Boston 25 News. https://www.boston25news.com/news/local/toxic-red-tide-along-massachusetts-coast-prompts-shellfishing-ban/FFEOTFN62FBBZMHA5E7UWY7SPY/

Fu, X., Hou, R., Yang, P., Qian, S., Feng, Z., Chen, Z., Wang, F., Yuan, R., Chen, H., & Zhou, B. (2022). Application of external carbon source in heterotrophic denitrification of domestic sewage: A review. In *Science of the Total Environment* (Vol. 817). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2022.153061

Fulazzaky, M. A., Abdullah, N. H., Yusoff, A. R. M., & Paul, E. (2015). Conditioning the alternating aerobic–anoxic process to enhance the removal of inorganic nitrogen pollution from a municipal wastewater in France. *Journal of Cleaner Production*, *100*, 195–201.

Gao, D., Peng, Y., Li, B., & Liang, H. (2009). Shortcut nitrification–denitrification by real-time control strategies. *Bioresource Technology*, *100*(7), 2298–2300.

Gao, X., Zhang, T., Wang, B., Xu, Z., Zhang, L., & Peng, Y. (2020). Advanced nitrogen removal of low C/N ratio sewage in an anaerobic/aerobic/anoxic process through enhanced post-endogenous denitrification. *Chemosphere*, *252*. https://doi.org/10.1016/j.chemosphere.2020.126624

Ge, S., Peng, Y., Qiu, S., Zhu, A., & Ren, N. (2014). Complete nitrogen removal from municipal wastewater via partial nitrification by appropriately alternating anoxic/aerobic conditions in a continuous plug-flow step feed process. *Water Research*, *55*, 95–105. https://doi.org/10.1016/j.watres.2014.01.058

Gogina, E., & Gulshin, I. (2016). Simultaneous nitrification and denitrification with low dissolved oxygen level and C/N ratio. *Procedia Engineering*, *153*, 189–194.

Gong, B., Duan, K., Chen, S., & Wang, Y. (2023). Enhanced nutrients removal and microbial mechanisms in a pilot-scale anaerobic-oxic-anoxic (A/O/A) system: Synergistic roles of denitrifying polyphosphate accumulating organisms and endogenous denitrifiers. *Process Safety and Environmental Protection*, *179*, 47–56. https://doi.org/https://doi.org/10.1016/j.psep.2023.08.085

Guellil, A., Thomas, F., Block, J.-C., Bersillon, J.-L., & Ginestet, P. (2001). Transfer of organic matter between wastewater and activated sludge flocs. *Water Research*, *35*(1), 143–150.

Guo, H., Cai, R., & Tan, H. (2023). Projected harmful algal bloom frequency in the Yangtze River Estuary and adjacent waters. *Marine Environmental Research*, *183*, 105832. https://doi.org/https://doi.org/10.1016/j.marenvres.2022.105832

Guven, H., Ersahin, M. E., Ozgun, H., Ozturk, I., & Koyuncu, I. (2023). Energy and material refineries of future: Wastewater treatment plants. *Journal of Environmental Management*, *329*, 117130. https://doi.org/10.1016/J.JENVMAN.2022.117130

Habermeyer, P., & Sá Nchez, A. (2005). *Optimization of the Intermittent Aeration in a Full-Scale Wastewater Treatment Plant Biological Reactor for Nitrogen Removal*.

Hach. (2023). *COD Digestion Vials, Low Range*. Hach. https://www.hach.com/p-cod-tnt-vial-test/2125825

Hajsardar, M., Borghei, S. M., Hassani, A. H., & Takdastan, A. (2016). Simultaneous ammonium and nitrate removal by a modified intermittently aerated sequencing batch reactor (SBR) with multiple filling events. *Polish Journal of Chemical Technology*, *18*(3), 72–80. https://doi.org/doi:10.1515/pjct-2016-0051

Han, P., Wu, D., Sun, D., Zhao, M., Wang, M., Wen, T., Zhang, J., Hou, L., Liu, M., Klümper, U., Zheng, Y., Dong, H. P., Liang, X., & Yin, G. (2021). N2O and NOy production by the comammox bacterium Nitrospira inopinata in comparison with canonical ammonia oxidizers. *Water Research*, *190*, 116728. https://doi.org/10.1016/J.WATRES.2020.116728

Han, X., Zhang, S., Yang, S., Zhang, L., & Peng, Y. (2020). Full-scale partial nitritation/anammox (PN/A) process for treating sludge dewatering liquor from anaerobic digestion after thermal hydrolysis. *Bioresource Technology*, *297*, 122380. https://doi.org/10.1016/J.BIORTECH.2019.122380

Hanhan, O., Insel, G., Yagci, N. O., Artan, N., & Orhon, D. (2011). Mechanism and design of intermittent aeration activated sludge process for nitrogen removal. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, *46*(1), 9–16. https://doi.org/10.1080/10934529.2011.526073

Harb, R., Laçin, D., Subaşı, I., & Erguder, T. H. (2021). Denitrifying anaerobic methane oxidation (DAMO) cultures: Factors affecting their enrichment, performance and integration with anammox bacteria. *Journal of Environmental Management, 295*, 113070. https://doi.org/10.1016/J.JENVMAN.2021.113070

Hashemi, S. H., Azimi, A. A., Torabian, A., Nabi Bidhendi, G., & Mahmoodkhani, R. (2005). Low dissolved oxygen sludge bulking in sequencing batch reactors. *International Journal of Environmental Studies*, *62*(4), 415–420. https://doi.org/10.1080/00207230500118785

Hellinga, C., Van Loosdrecht, M. C. M., & Heijnen, J. J. (1999). Model based design of a novel process for nitrogen removal from concentrated flows. *Mathematical and Computer Modelling of Dynamical Systems*, *5*(4), 351–371.

Helmer, C., & Kunst, S. (1998). Simultaneous nitrification/denitrification in an aerobic biofilm system. *Water Science and Technology*, *37*(4–5), 183–187.

Henze, M., Harremoës, P., Jansen, J. la, Arvin, E., & Treatment, W. (1997). Biological and chemical processes. *Wastewater Treatment*.

How, S. W., Chua, A. S. M., Ngoh, G. C., Nittami, T., & Curtis, T. P. ; (2019). Enhanced nitrogen removal in an anoxic-oxic-anoxic process treating low COD/N tropical wastewater: Low-dissolved oxygen nitrification and utilization of slowly-biodegradable COD for denitrification. *Science of the Total Environment*, *693*, 133526. https://doi.org/10.1016/j.scitotenv.2019.07.332

How, S. W., Lim, S. Y., Lim, P. B. ;, Aris, Mukhammad. ;, Ngoh, G. C., Curtis, T. P. ;, & Chua, A. S. M. (2018). Low-dissolved-oxygen nitrification in tropical sewage: An investigation on potential, performance and functional microbial community. *Water Science and Technology*, *77*(9), 2274–2283. https://doi.org/10.2166/wst.2018.143

How, S. W., Nittami, T., Ngoh, G. C., Curtis, T. P. ;, & Chua, A. S. M. (2020). An efficient oxic-anoxic process for treating low COD/N tropical wastewater: Startup, optimization and nitrifying community structure. *Chemosphere*, *259*, 127444. https://doi.org/10.1016/j.chemosphere.2020.127444

How, S. W., Shoji, T., Tan, C. K., Curtis, T. P. ;, & Chua, A. S. M. (2021). Kinetic characterization of a low-dissolved-oxygen oxic-anoxic process treating low COD/N tropical wastewater revealed selection of nitrifiers with high substrate affinity. *Journal of Water Process Engineering*, *43*(July), 1–9. https://doi.org/10.1016/j.jwpe.2021.102235

How, S. W., Sin, J. H., Wong, S. Y. Y., Lim, P. B., Aris, A. M., Ngoh, G. C., Shoji, T., Curtis, T. P. ;, & Chua, A. S. M. (2020). Characterization of slowly-biodegradable organic compounds and hydrolysis kinetics in tropical wastewater for biological nitrogen removal. *Water Science and Technology*, *81*(1), 71–80. https://doi.org/10.2166/wst.2020.077

Hu, M., Zhang, H., & Tian, Y. (2023). Achieving nitrogen removal with low material and energy consumption through partial nitrification coupled with short-cut sulfur autotrophic denitrification in a single-stage SBR. *Bioresource Technology*, *380*, 128999. https://doi.org/10.1016/J.BIORTECH.2023.128999

Huang, W., Gong, B., Wang, Y., Lin, Z., He, L., Zhou, J., & He, Q. (2020). Metagenomic analysis reveals enhanced nutrients removal from low C/N municipal wastewater in a pilot-scale modified AAO system coupling electrolysis. *Water Research*, *173*. https://doi.org/10.1016/j.watres.2020.115530

IWA. (2021, February 23). *Circular Economy: Tapping the Power of Wastewater - International Water Association*. International Waster Association. https://iwa-network.org/learn/circular-economy-tapping-the-power-of-wastewater/

IWK. (2021, August 30). *IWK committedly treats wastewater, ensures environmental sustainability*. The Malaysian Reserve. https://themalaysianreserve.com/2021/08/30/iwk-committedly-treats-wastewater-ensures-environmental-sustainability/

Izadi, P., Izadi, P., & Eldyasti, A. (2021). Enhancement of simultaneous nitrogen and phosphorus removal using intermittent aeration mechanism. *Journal of Environmental Sciences (China)*, *109*, 1–14. https://doi.org/10.1016/j.jes.2021.02.026

Jetten, M. S. M., Strous, M., Van De Pas-Schoonen, K. T., Schalk, J., Van Dongen, U. G. J. M., Van De Graaf, A. A., Logemann, S., Muyzer, G., Van Loosdrecht, M. C. M., & Kuenen, J. G. (1998). The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews*, *22*(5), 421–437.

Jimenez, J., Dursun, D., Dold, P., Bratby, J., Keller, J., & Parker, D. (2010). Simultaneous nitrification-denitrification to meet low effluent nitrogen limits: Modeling, performance and reliability. *WEFTEC 2010*, 2404–2421.

Jipanin, S. J., Shaleh, S. R. M., Lim, P. T., Leaw, C. P., & Mustapha, S. (2019). The Monitoring of Harmful Algae Blooms in Sabah, Malaysia. *Journal of Physics: Conference Series*, *1358*(1), 012014.

Kampschreur, M. J., Picioreanu, C., Tan, N., Kleerebezem, R., Jetten, M. S. M., & van Loosdrecht, M. C. M. (2007). Unraveling the Source of Nitric Oxide Emission During Nitrification. *Water Environment Research*, *79*(13), 2499–2509. https://doi.org/10.2175/106143007x220815

Kampschreur, M. J., Tan, N. C. G., Kleerebezem, R., Picioreanu, C., Jetten, M. S. M., & Loosdrecht, M. C. M. van. (2008). Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. *Environmental Science & Technology*, *42*(2), 429–435.

Kampschreur, M. J., Temmink, H., Kleerebezem, R., Jetten, M. S. M., & van Loosdrecht, M. C. M. (2009). Nitrous oxide emission during wastewater treatment. *Water Research*, *43*(17), 4093–4103.

Keene, N. A., Reusser, S. R., Scarborough, M. J., Grooms, A. L., Seib, M., Santo Domingo, J., & Noguera, D. R. (2017). Pilot plant demonstration of stable and efficient high rate biological nutrient removal with low dissolved oxygen conditions. *Water Research*, *121*, 72–85. https://doi.org/10.1016/j.watres.2017.05.029

KeTTHA. (2017). *Green Technology Master Plant Malaysia 2017-2030*. Ministry of Energy, Green Technology and Water. https://www.pmo.gov.my/wp-content/uploads/2019/07/Green-Technology-Master-Plan-Malaysia-2017-2030.pdf

Kim, E., Shin, S. G., Jannat, M. A. H., Tongco, J. V., & Hwang, S. (2017). Use of food waste-recycling wastewater as an alternative carbon source for denitrification process: A full-scale study. *Bioresource Technology*, *245*, 1016–1021. https://doi.org/10.1016/J.BIORTECH.2017.08.168

Kimochi,’, Y., Inamori, Y., Mizuochi, M., Xu, K.-Q., & Matsumura4, M. (1998). Nitrogen Removal and N20 Emission in a Full-Scale Domestic Wastewater Treatment Plant with Intermittent Aeration. In *JOURNAL OF FERMENTATION AND BIOENGINEEREW* (Vol. 86, Issue 2).

Kits, K. D., Jung, M. Y., Vierheilig, J., Pjevac, P., Sedlacek, C. J., Liu, S., Herbold, C., Stein, L. Y., Richter, A., & Wissel, H. (2019). *Low yield and abiotic origin of N2O formed by the complete nitrifier Nitrospira inopinata. Nat Commun 10: 1836*.

Kits, K. D., Sedlacek, C. J., Lebedeva, E. V, Han, P., Bulaev, A., Pjevac, P., Daebeler, A., Romano, S., Albertsen, M., Stein, L. Y., Daims, H., & Wagner, M. (2017). Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle. *Nature*, *549*(7671), 269–272. https://doi.org/10.1038/nature23679

Kuai, L., & Verstraete, W. (1998). Ammonium removal by the oxygen-limited autotrophic nitrification-denitrification system. *Applied and Environmental Microbiology*, *64*(11), 4500–4506.

Kuenen, J. G. (2008). Anammox bacteria: From discovery to application. *Nature Reviews Microbiology*, *6*(4), 320–326. https://doi.org/10.1038/nrmicro1857

Lackner, S., Gilbert, E. M., Vlaeminck, S. E., Joss, A., Horn, H., & van Loosdrecht, M. C. M. (2014). Full-scale partial nitritation/anammox experiences–an application survey. *Water Research*, *55*, 292–303.

Larios-Martínez, A. D., Barrales-Fernández, C., Alvarez-Chavez, P. E., Méndez-Carreto, C., Sandoval-Salas, F., Ruiz-Colorado, N., Godbout, S., Fournel, S., & Avalos-Ramírez, A. (2022). Chapter 4 - Modular treatment approach for drinking water and wastewater: introduction to a sustainable approach to decentralized treatment systems. In S. Kaur Brar, P. Kumar, & A. Cuprys (Eds.), *Modular Treatment Approach for Drinking Water and Wastewater* (pp. 55–66). Elsevier. https://doi.org/https://doi.org/10.1016/B978-0-323-85421-4.00003-6

Law, Y., Ye, L., Pan, Y., & Yuan, Z. (2012). Nitrous oxide emissions from wastewater treatment processes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1593), 1265–1277.

Lemaire, R., Marcelino, M., & Yuan, Z. (2008). Achieving the nitrite pathway using aeration phase length control and step-feed in an SBR removing nutrients from abattoir wastewater. *Biotechnology and Bioengineering*, *100*(6), 1228–1236.

Li, B.-L., Zhang, Z., Li, Y., Song, M.-T., & Zhang, C. (2014). Enhanced nitrogen removal and energy saving of intermittent aeration-modified oxidation ditch process. *Desalination and Water Treatment*, *52*(25–27), 4895–4903.

Li, D., Fang, F., & Liu, G. (2021). Efficient Nitrification and Low-Level N2O Emission in a Weakly Acidic Bioreactor at Low Dissolved-Oxygen Levels Are Due to

Comammox. *Applied and Environmental Microbiology*, *87*(11), 1–13. https://doi.org/10.1128/AEM.00154-21

Liu, G., & Wang, J. (2013). Long-Term Low DO Enriches and Shifts Nitrifier Community in Activated Sludge. *Environmental Science and Technology*, *47*(10), 5109–5117.

Liu, G., & Wang, J. (2015). Quantifying the chronic effect of low DO on the nitrification process. *Chemosphere*, *141*, 19–25. https://doi.org/10.1016/j.chemosphere.2015.05.088

Liu, G., Wu, X., Li, D., Jiang, L., Huang, J., & Zhuang, L. (2021). Long-Term Low Dissolved Oxygen Operation Decreases N2O Emissions in the Activated Sludge Process. *Environmental Science and Technology*, *55*(10), 6975–6983. https://doi.org/10.1021/acs.est.0c07279

Liu, T., Liu, S., He, S., Tian, Z., & Zheng, M. (2021). Minimization of N2O emission through intermittent aeration in a sequencing batch reactor (SBR): Main behavior and mechanism. *Water (Switzerland)*, *13*(2). https://doi.org/10.3390/w13020210

Liu, W., Chen, W., Yang, D., & Shen, Y. (2019). Functional and compositional characteristics of nitrifiers reveal the failure of achieving mainstream nitritation under limited oxygen or ammonia conditions. *Bioresource Technology*, *275*, 272–279.

Lu, H., Chandran, K., & Stensel, D. (2014). Microbial ecology of denitrification in biological wastewater treatment. In *Water Research* (Vol. 64, pp. 237–254). Elsevier Ltd. https://doi.org/10.1016/j.watres.2014.06.042

Luan, Y. N., Yin, Y., An, Y., Zhang, F., Wang, X., Zhao, F., Xiao, Y., & Liu, C. (2022). Investigation of an intermittently-aerated moving bed biofilm reactor in rural wastewater treatment under low dissolved oxygen and C/N condition. *Bioresource Technology*, *358*. https://doi.org/10.1016/j.biortech.2022.127405

Ma, W., Han, Y., Ma, W., Han, H., Zhu, H., Xu, C., Li, K., & Wang, D. (2017). Enhanced nitrogen removal from coal gasification wastewater by simultaneous nitrification and denitrification (SND) in an oxygen-limited aeration sequencing batch biofilm reactor. *Bioresource Technology*, *244*, 84–91.

Ma, Y., Piscedda, A., Veras, A. D. L. C., Domingo-Félez, C., & Smets, B. F. (2021). Intermittent aeration to regulate microbial activities in membrane-aerated biofilm reactors: Energy-efficient nitrogen removal and low nitrous oxide emission. *Chemical Engineering Journal*, 133630. https://doi.org/10.1016/j.cej.2021.133630

Malamis, S., Katsou, E., Di Fabio, S., Bolzonella, D., & Fatone, F. (2013). Biological nutrient removal from the supernatant originating from the anaerobic digestion of the organic fraction of municipal solid waste. *Critical Reviews in Biotechnology*, *34*. https://doi.org/10.3109/07388551.2013.791246

Maniam, N. (2021, September 10). Our services never stop. *The Star*.

Martins, A. M. P., Pagilla, K., Heijnen, J. J., & Van Loosdrecht, M. C. M. (2004). Filamentous bulking sludge - A critical review. In *Water Research* (Vol. 38, Issue 4, pp. 793–817). Elsevier Ltd. https://doi.org/10.1016/j.watres.2003.11.005

Miao, Y., Peng, Y., Zhang, L., Li, B., Li, X., Wu, L., & Wang, S. (2018). Partial nitrification-anammox (PNA) treating sewage with intermittent aeration mode:

Effect of influent C/N ratios. *Chemical Engineering Journal*, *334*, 664–672. https://doi.org/10.1016/J.CEJ.2017.10.072

Miao, Y., Zhang, L., Li, B., Zhang, Q., Wang, S., & Peng, Y. (2017). Enhancing ammonium oxidizing bacteria activity was key to single-stage partial nitrification-anammox system treating low-strength sewage under intermittent aeration condition. *Bioresource Technology*, *231*, 36–44. https://doi.org/10.1016/J.BIORTECH.2017.01.045

Miao, Y., Zhang, L., Yu, D., Zhang, J., Zhang, W., Ma, G., Zhao, X., & Peng, Y. (2022). Application of intermittent aeration in nitrogen removal process: development, advantages and mechanisms. *Chemical Engineering Journal*, *430*. https://doi.org/10.1016/j.cej.2021.133184

Mike. (2023a). *Acetate price index*. Business Analytiq. https://businessanalytiq.com/procurementanalytics/index/ethyl-acetate-price-index/

Mike. (2023b). *Ethanol price index*. Business Analytiq. https://businessanalytiq.com/procurementanalytics/index/ethanol-price-index/

Mike. (2023c). *Methanol price index*. Business Analytiq. https://businessanalytiq.com/procurementanalytics/index/methanol-price-index/

Ministry of Environment and Forestry of Indonesia. (2016). *Water Quality Standard of Domestic Wastewater*.

Ministry of Environment of Japan. (2015). *National Effluent Standards of Japan*. https://www.env.go.jp/en/water/wq/nes.html

Ministry of Natural Resources and Environment of Thailand. (2010). *Enhancement and Conservation of National Environmental Quality Act*.

Muzaffar, W. M. B. W., Aznah, A., & Halim, H. (2022). Energy efficiency and nutrient removal performance: Comparison between several types of activated sludge process. *IOP Conference Series: Earth and Environmental Science*, *1091*(1). https://doi.org/10.1088/1755-1315/1091/1/012056

National Water Services Commission. (2009). *Malaysia Sewerage Industry Guidelines* (4th ed., Vol. 3). National Water Services Commission.

Noutsopoulos, C., Mamais, D., Statiris, E., Lerias, E., Malamis, S., & Andreadakis, A. (2018). Reject water characterization and treatment through short-cut nitrification/denitrification: assessing the effect of temperature and type of substrate. *Journal of Chemical Technology & Biotechnology*, *93*(12), 3638–3647.

Oleszkiewicz, J., Kruk, D. J., Devlin, T., Lashkarizadeh, M., & Yuan, Q. (2015). Options for improved nutrient removal and recovery from municipal wastewater in the Canadian context. *Environ. Technol*, *20*, 681–695.

Ong, Y. H., Chua, A. S. M., Lee, B. P., & Ngoh, G. C. (2013). Long-term performance evaluation of EBPR process in tropical climate: Start-up, process stability, and the effect of operational pH and influent C:P ratio. *Water Science and Technology*, *67*(2), 340–346. https://doi.org/10.2166/wst.2012.552

Palomo, A., Pedersen, A. G., Fowler, S. J., Dechesne, A., Sicheritz-Pontén, T., & Smets, B. F. (2018). Comparative genomics sheds light on niche differentiation and the evolutionary history of comammox Nitrospira. *ISME Journal*, *12*(7), 1779–1793. https://doi.org/10.1038/s41396-018-0083-3

Park, H. D., & Noguera, D. R. (2004). Evaluating the effect of dissolved oxygen on ammonia-oxidizing bacterial communities in activated sludge. *Water Research*, *38*(14–15), 3275–3286. https://doi.org/10.1016/j.watres.2004.04.047

Pásztor, I., Thury, P., & Pulai, J. (2009). Chemical oxygen demand fractions of municipal wastewater for modeling of wastewater treatment. *International Journal of Environmental Science & Technology*, *6*, 51–56.

Patel, A., Arkatkar, A., Singh, S., Rabbani, A., Solorza Medina, J. D., Ong, E. S., Habashy, M. M., Jadhav, D. A., Rene, E. R., Mungray, A. A., & Mungray, A. K. (2021). Physico-chemical and biological treatment strategies for converting municipal wastewater and its residue to resources. *Chemosphere*, *282*, 130881. https://doi.org/10.1016/J.CHEMOSPHERE.2021.130881

Peng, L., Ni, B.-J., Ye, L., & Yuan, Z. (2015). The combined effect of dissolved oxygen and nitrite on N2O production by ammonia oxidizing bacteria in an enriched nitrifying sludge. *Water Research*, *73*, 29–36.

Pfluger, A., Vanzin, G., Munakata-Marr, J., & Figueroa, L. (2018). An anaerobic hybrid bioreactor for biologically enhanced primary treatment of domestic wastewater under low temperatures. *Environmental Science: Water Research & Technology*, *4*(11), 1851–1866.

Prasad, R., & Prasad, S. (2019). Algal Blooms and Phosphate Eutrophication of Inland Water Ecosystems with Special Reference to India. *INTERNATIONAL JOURNAL OF PLANT AND ENVIRONMENT*, *5*(01), 01–08. https://doi.org/10.18811/ijpen.v5i01.1

Qadir, M., Drechsel, P., Jiménez Cisneros, B., Kim, Y., Pramanik, A., Mehta, P., & Olaniyan, O. (2020). Global and regional potential of wastewater as a water, nutrient and energy source. *Natural Resources Forum*, *44*(1), 40–51. https://doi.org/10.1111/1477-8947.12187

Raboni, M., Torretta, V., Viotti, P., & Urbini, G. (2013). Pilot experimentation with complete mixing anoxic reactors to improve sewage denitrification in treatment plants in small communities. *Sustainability*, *6*(1), 112–122.

Rajpal, A., Bhatia, A., Tomar, N., Kazmi, A. A., Ojha, C. S. P., & Tyagi, V. K. (2022). Insight into a novel post-anoxic integrated biofilm process for wastewater treatment and reclamation. *Journal of Water Process Engineering*, *49*, 102957. https://doi.org/https://doi.org/10.1016/j.jwpe.2022.102957

Ramli, N. A., & Hamid, M. F. A. (2017). Analysis of energy efficiency and energy consumption costs: A case study for regional wastewater treatment plant in Malaysia. *Journal of Water Reuse and Desalination*, *7*(1), 103–110. https://doi.org/10.2166/wrd.2016.196

Rodríguez, D. C., Pino, N., & Peñuela, G. (2011). Monitoring the removal of nitrogen by applying a nitrification–denitrification process in a Sequencing Batch Reactor (SBR). *Bioresource Technology*, *102*(3), 2316–2321. https://doi.org/10.1016/J.BIORTECH.2010.10.082

Rodríguez-Caballero, A., Aymerich, I., Marques, R., Poch, M., & Pijuan, M. (2015). Minimizing N2O emissions and carbon footprint on a full-scale activated sludge sequencing batch reactor. *Water Research*, *71*, 1–10.

Rong, C., Wang, T., Luo, Z., Guo, Y., Kong, Z., Wu, J., Qin, Y., Hanaoka, T., Sakemi, S., Ito, M., Kobayashi, S., Kobayashi, M., & Li, Y. Y. (2022). Seasonal temperatures impact on the mass flows in the innovative integrated process of anaerobic membrane bioreactor and one-stage partial nitritation-anammox for the treatment of municipal wastewater. *Bioresource Technology*, *349*, 126864. https://doi.org/10.1016/J.BIORTECH.2022.126864

Roots, P., Wang, Y., Rosenthal, A. F., Griffin, J. S., Sabba, F., Petrovich, M., Yang, F., Kozak, J. A., Zhang, H., & Wells, G. F. (2019). Comammox Nitrospira are the dominant ammonia oxidizers in a mainstream low dissolved oxygen nitrification reactor. *Water Research*, *157*, 396–405. https://doi.org/10.1016/j.watres.2019.03.060

Rosso, D., Larson, L. E., & Stenstrom, M. K. (2008). Aeration of large-scale municipal wastewater treatment plants: State of the art. *Water Science and Technology*, *57*(7), 973–978. https://doi.org/10.2166/wst.2008.218

Shaban, J., Al-Najar, H., Kocadal, K., Almghari, K., & Saygi, S. (2023). The Effect of Nitrate-Contaminated Drinking Water and Vegetables on the Prevalence of Acquired Methemoglobinemia in Beit Lahia City in Palestine. *Water (Switzerland)*, *15*(11). https://doi.org/10.3390/w15111989

Shen, Y., Yang, D., Wu, Y., Zhang, H., & Zhang, X. (2019). Operation mode of a step-feed anoxic/oxic process with distribution of carbon source from anaerobic zone on nutrient removal and microbial properties. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-018-37841-8

Sheng, X., Liu, R., Song, X., Chen, L., & Tomoki, K. (2017). Comparative study on microbial community in intermittently aerated sequencing batch reactors (SBR) and a traditional SBR treating digested piggery wastewater. *Frontiers of Environmental Science & Engineering*, *11*(3), 8. https://doi.org/10.1007/s11783-017-0929-3

Shi, C. Y., Long, W. Y., Meng, A. C., & Raajeevan, K. S. (2008). *Biological nitrogen removal activated sludge process in warm climates*. IWA Publishing.

Song, X., Liu, R., Chen, L., Dong, B., & Kawagishi, T. (2017). Advantages of intermittently aerated SBR over conventional SBR on nitrogen removal for the treatment of digested piggery wastewater. *Frontiers of Environmental Science & Engineering*, *11*(3), 13. https://doi.org/10.1007/s11783-017-0941-7

Sophonsiri, C., & Morgenroth, E. (2004). Chemical composition associated with different particle size fractions in municipal, industrial, and agricultural wastewaters. *Chemosphere*, *55*(5), 691–703. https://doi.org/10.1016/j.chemosphere.2003.11.032

Srb, M., Lánský, M., Charvátová, L., Koubová, J., Pecl, R., Sýkora, P., & Rosický, J. (2022). Improved nitrogen removal efficiency by implementation of intermittent aeration. *Water Science and Technology*, *86*(9), 2248–2259. https://doi.org/10.2166/wst.2022.317

Stewart, R. D., Bashar, R., Amstadt, C., Uribe-Santos, G. A., McMahon, K. D., Seib, M., & Noguera, D. R. (2022). Pilot-scale comparison of biological nutrient removal (BNR) using intermittent and continuous ammonia-based low dissolved oxygen aeration control systems. *Water Science and Technology*, *85*(2), 579–590. https://doi.org/10.2166/wst.2021.630

Stuti, M. (2023, March 13). *Toxic red tide algae bloom causes closure of Florida beaches and cancellation of festival*. The Independent.

https://www.independent.co.uk/climate-change/news/florida-red-seaweed-invades-b2299554.html

Sun, S.-P., Nàcher, C. P. i, Merkey, B., Zhou, Q., Xia, S.-Q., Yang, D.-H., Sun, J.-H., & Smets, B. F. (2010). Effective Biological Nitrogen Removal Treatment Processes for Domestic Wastewaters with Low C/N Ratios: A Review. *Environmental Engineering Science*, *27*(2), 111–126. https://doi.org/10.1089/ees.2009.0100

Sun, Y., Guan, Y., Pan, M., Zhan, X., Hu, Z., & Wu, G. (2017). Enhanced biological nitrogen removal and N 2 O emission characteristics of the intermittent aeration activated sludge process. *Reviews in Environmental Science and Bio/Technology*, *16*, 761–780.

Suwal, S. (2019). *Water in Crisis - Vietnam*. The Water Project. https://thewaterproject.org/water-crisis/water-in-crisis-vietnam

Tallec, G., Garnier, J., Billen, G., & Gousailles, M. (2006). Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: Effect of oxygenation level. *Water Research*, *40*(15), 2972–2980. https://doi.org/10.1016/J.WATRES.2006.05.037

Tchobanoglous, G., Stensel, D., Tsuchihashi, R., & Burton, F. (2014). *Wastewater Engineering Treatment and Resource Recovery* (Fifth). McGraw-Hill Education.

Thakur, I. S., & Medhi, K. (2019). Nitrification and denitrification processes for mitigation of nitrous oxide from waste water treatment plants for biovalorization: challenges and opportunities. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2019.03.069

The Malaysian Reserve. (2021, August 30). *IWK committedly treats wastewater, ensures environmental sustainability*. https://themalaysianreserve.com/2021/08/30/iwk-committedly-treats-wastewater-ensures-environmental-sustainability/

The Star. (2023, October 3). Plankton blooms in the Gulf of Thailand signal bad pollution, warn experts. *The Star*.

Third, K. A., Paxman, J., Schmid, M., Strous, M., Jetten, M. S. M., & Cord-Ruwisch, R. (2005). Treatment of nitrogen-rich wastewater using partial nitrification and Anammox in the CANON process. *Water Science and Technology*, *52*(4), 47–54. https://doi.org/10.2166/wst.2005.0086

United Nations. (2023, January 16). *Four reasons why the world needs to limit nitrogen pollution*. UN Environment Programme. https://www.unep.org/news-and-stories/story/four-reasons-why-world-needs-limit-nitrogen-pollution

US EPA. (1993). Process design manual of nitrogen control. *EP A.[Links]*.

van Loosdrecht, M., H. Nielsen, P., M. Lopez-Vazquez, C., & Brdjanovic, D. (2016). *Experimental Methods in Wastewater Treatment*.

van Rijn, J., Tal, Y., & Schreier, H. J. (2006). Denitrification in recirculating systems: Theory and applications. *Aquacultural Engineering*, *34*(3), 364–376. https://doi.org/10.1016/J.AQUAENG.2005.04.004

Vocks, M., Adam, C., Lesjean, B., Gnirss, R., & Kraume, M. (2005). Enhanced post-denitrification without addition of an external carbon source in membrane bioreactors. *Water Research*, *39*(14), 3360–3368.

Wang, C., Lin, Q., Yao, Y., Xu, R., Wu, X., & Meng, F. (2022). Achieving simultaneous nitrification, denitrification, and phosphorus removal in pilot-scale flow-through biofilm reactor with low dissolved oxygen concentrations: Performance and mechanisms. *Bioresource Technology*, *358*. https://doi.org/10.1016/j.biortech.2022.127373

Wang, H., Guan, Y., Li, L., & Wu, G. (2015). Characteristics of biological nitrogen removal in a multiple anoxic and aerobic biological nutrient removal process. *BioMed Research International*, *2015*.

Wang, H., Sun, Y., Zhang, L., Wang, W., & Guan, Y. (2021). Enhanced nitrogen removal and mitigation of nitrous oxide emission potential in a lab-scale rain garden with internal water storage. *Journal of Water Process Engineering*, *42*. https://doi.org/10.1016/j.jwpe.2021.102147

Wang, H., Yan, H., Zhou, F., Li, B., Zhuang, W., & Shen, Y. (2020). Changes in nutrient transport from the Yangtze River to the East China Sea linked to the Three-Gorges Dam and water transfer project. *Environmental Pollution*, *256*. https://doi.org/10.1016/j.envpol.2019.113376

Wang, J., & Chu, L. (2016). Biological nitrate removal from water and wastewater by solid-phase denitrification process. *Biotechnology Advances*, *34*(6), 1103–1112. https://doi.org/10.1016/J.BIOTECHADV.2016.07.001

Wang, L., Li, B., Li, Y., & Wang, J. (2021). Enhanced biological nitrogen removal under low dissolved oxygen in an anaerobic-anoxic-oxic system: Kinetics, stoichiometry and microbial community. *Chemosphere*, *263*, 128184. https://doi.org/10.1016/j.chemosphere.2020.128184

Wang, X., Wang, S., Xue, T., Li, B., Dai, X., & Peng, Y. (2015). Treating low carbon/nitrogen (C/N) wastewater in simultaneous nitrification-endogenous denitrification and phosphorous removal (SNDPR) systems by strengthening anaerobic intracellular carbon storage. In *Water Research* (Vol. 77, pp. 191–200). Elsevier Ltd. https://doi.org/10.1016/j.watres.2015.03.019

Wang, Y., Ding, X., Chen, Y., Zeng, W., & Zhao, Y. (2023). Pollution source identification and abatement for water quality sections in Huangshui River basin, China. *Journal of Environmental Management*, *344*, 118326. https://doi.org/10.1016/J.JENVMAN.2023.118326

Wang, Z., Zheng, M., Duan, H., Yuan, Z., & Hu, S. (2022). A 20-year journey of partial nitritation and anammox (PN/A): from sidestream toward mainstream. *Environmental Science & Technology*, *56*(12), 7522–7531.

Wen, R., Jin, Y., & Zhang, W. (2020). Application of the anammox in China—A review. In *International Journal of Environmental Research and Public Health* (Vol. 17, Issue 3). MDPI. https://doi.org/10.3390/ijerph17031090

Weon, S. Y., Lee, S. I., & Koopman, B. (2004). Effect of temperature and dissolved oxygen on biological nitrification at high ammonia concentrations. *Environmental Technology*, *25*(11), 1211–1219. https://doi.org/10.1080/09593332508618369

Weralupitiya, C., Wanigatunge, R., Joseph, S., Athapattu, B. C. L., Lee, T. H., Kumar Biswas, J., Ginige, M. P., Shiung Lam, S., Senthil Kumar, P., & Vithanage, M. (2021). Anammox bacteria in treating ammonium rich wastewater: Recent perspective and appraisal. In *Bioresource Technology* (Vol. 334). Elsevier Ltd. https://doi.org/10.1016/j.biortech.2021.125240

Wilen, B., & Balmer, P. (1999). The Effect of Dissolved Oxygen Concentration on the Activated Sludge Process. *War. Res*, *33*(2), 391–400.

Wilén, B., Markiewicz, A., & Nilsson, Å. (2008). Variation in dissolved oxygen concentration and its effect on the activated sludge properties studied at a full scale wastewater treatment plant. *Proceedings of IWA World Water Congress & Exhibition, 19-24 September 2010 in Montreal*, *1*, 4.

Winkler, M., Coats, E. R., & Brinkman, C. K. (2011). Advancing post-anoxic denitrification for biological nutrient removal. *Water Research*, *45*(18), 6119–6130. https://doi.org/10.1016/j.watres.2011.09.006

Wu, B., Li, Y., Lim, W., Lee, S. L., Guo, Q., Fane, A. G., & Liu, Y. (2017). Single-stage versus two-stage anaerobic fluidized bed bioreactors in treating municipal wastewater: Performance, foulant characteristics, and microbial community. *Chemosphere*, *171*, 158–167.

Xu, D., Chen, H., Li, X., Yang, Q., Zeng, T., Luo, K., & Zeng, G. (2013). Enhanced biological nutrient removal in sequencing batch reactors operated as static/oxic/anoxic (SOA) process. *Bioresource Technology*, *143*, 204–211. https://doi.org/https://doi.org/10.1016/j.biortech.2013.05.092

Xu, J., Cui, Q., Bu, C., Ismail, S., & Ni, S. Q. (2022). Partition of Anammox and Nitrifiers Through Bio-Carriers for Full-Scale Sidestream Partial Nitrification–Anammox Plant. *Frontiers in Bioengineering and Biotechnology*, *10*. https://doi.org/10.3389/fbioe.2022.819937

Xu, W. (2022, September 28). New move aims to protect Yangtze. *China Daily Global*.

Xu, Z., Dai, X., & Chai, X. (2018). Effect of different carbon sources on denitrification performance, microbial community structure and denitrification genes. *Science of The Total Environment*, *634*, 195–204. https://doi.org/10.1016/J.SCITOTENV.2018.03.348

Yan, L., Liu, S., Liu, Q., Zhang, M., Liu, Y., Wen, Y., Chen, Z., Zhang, Y., & Yang, Q. (2019). Improved performance of simultaneous nitrification and denitrification via nitrite in an oxygen-limited SBR by alternating the DO. *Bioresource Technology*, *275*, 153–162.

Yang, L., Yao, H., Jia, F., Han, B., Chen, Y., Jiang, J., Liu, T., & Guo, J. (2023). Effects of ammonia nitrogen and organic carbon availability on microbial community structure and ecological interactions in a full-scale partial nitritation and anammox (PN/A) system. *Water Research*, *244*, 120524. https://doi.org/10.1016/J.WATRES.2023.120524

Yang, Q., Shen, N., Lee, Z. M. P., Xu, G., Cao, Y., Kwok, B., Lay, W., Liu, Y., & Zhou, Y. (2016). Simultaneous nitrification, denitrification and phosphorus removal (SNDPR) in a full-scale water reclamation plant located in warm climate. *Water Science and Technology*, *74*(2), 448–456. https://doi.org/10.2166/wst.2016.214

Zeng, W., Zhang, Y., Li, L., Peng, Y., & Wang, S. (2009). Control and optimization of nitrifying communities for nitritation from domestic wastewater at room temperatures. *Enzyme and Microbial Technology*, *45*(3), 226–232.

Zhang, J., Shao, Y., Liu, G., Qi, L., Wang, H., Xu, X., & Liu, S. (2021). Wastewater COD characterization: RBCOD and SBCOD characterization analysis methods. *Scientific Reports*, *11*(1), 691. https://doi.org/10.1038/s41598-020-80700-8

Zhang, J., Shao, Y., Wang, H., Liu, G., Qi, L., Xu, X., & Liu, S. (2021). Current operation state of wastewater treatment plants in urban China. *Environmental Research*, *195*, 110843. https://doi.org/10.1016/J.ENVRES.2021.110843

Zhao, J., Wang, X., Li, X., Jia, S., & Peng, Y. (2018). Combining partial nitrification and post endogenous denitrification in an EBPR system for deep-level nutrient removal from low carbon/nitrogen (C/N) domestic wastewater. *Chemosphere*, *210*(2), 19–28. https://doi.org/10.1016/j.chemosphere.2018.06.135

Zhao, W., Huang, Y., Wang, M., Pan, C., Li, X., Peng, Y., & Li, B. (2018). Post-endogenous denitrification and phosphorus removal in an alternating anaerobic/oxic/anoxic (AOA) system treating low carbon/nitrogen (C/N) domestic wastewater. *Chemical Engineering Journal*, *339*, 450–458.

Zheng, M., Mu, G., Zhang, A., Wang, J., Chang, F., Niu, J., Wang, X., Gao, T., & Zhao, Z. (2022). Predominance of comammox bacteria among ammonia oxidizers under low dissolved oxygen condition. *Chemosphere*, *308*. https://doi.org/10.1016/j.chemosphere.2022.136436

Zheng, Z., Huang, S., Bian, W., Liang, D., Wang, X., Zhang, K., Ma, X., & Li, J. (2019). Enhanced nitrogen removal of the simultaneous partial nitrification, anammox and denitrification (SNAD) biofilm reactor for treating mainstream wastewater under low dissolved oxygen (DO) concentration. *Bioresource Technology*, *283*(January), 213–220. https://doi.org/10.1016/j.biortech.2019.01.148

Zhou, X., Guo, X., Han, Y., Liu, J., Ren, J., Wang, Y., & Guo, Y. (2012). Enhancing nitrogen removal in an Orbal oxidation ditch by optimization of oxygen supply: practice in a full-scale municipal wastewater treatment plant. *Bioprocess and Biosystems Engineering*, *35*, 1097–1105.

Zhou, X., Liu, X., Huang, S., Cui, B., Liu, Z., & Yang, Q. (2018). Total inorganic nitrogen removal during the partial/complete nitrification for treating domestic wastewater: Removal pathways and main influencing factors. *Bioresource Technology*, *256*, 285–294.

Zou, L., Zhou, M., Luo, Z., Zhang, H., Yang, Z., Cheng, H., Li, R., He, Q., & Ai, H. (2022). Selection and synthesization of multi–carbon source composites to enhance simultaneous nitrification–denitrification in treating low C/N wastewater. *Chemosphere*, *288*. https://doi.org/10.1016/j.chemosphere.2021.132567