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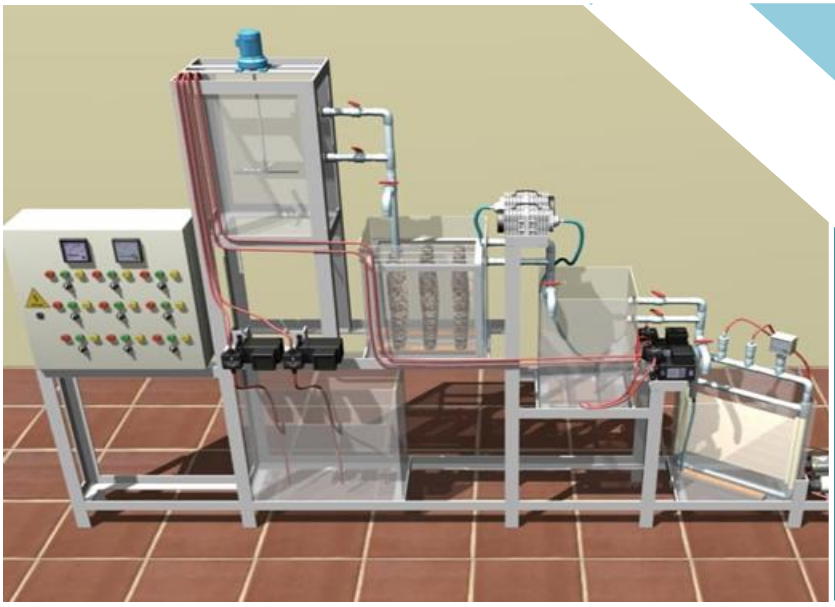
DEPARTMENT OF ENVIRONMENTAL SCIENCES AND MANAGEMENT

Thesis presented for obtaining the degree of DOCTOR OF SCIENCES

Science and Sanitary of the Environment

COMBINATION OF PARTIAL NITRIFICATION AND INERT COD REMOVAL PROCESSES IN A MEMBRANE BIOREACTOR TO TREAT LEACHATE IN VIETNAM



By TRAN THI NGOC DIEU

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FOREWORD

The rapid industrialization and urbanization in Vietnam have brought significant environmental challenges, one of which is the management of landfill leachate. Landfill leachate contains high concentrations of nitrogen, refractory organic compounds, heavy metals, and other pollutants. Landfill leachate causes severe risks to both the environment and public health if it is not treated.

This thesis, entitled "Combination of Partial Nitrification and Inert COD Removal Processes in a Membrane Bioreactor to Treat Leachate in Vietnam," aims to tackle these pressing issues by integrating advanced biological treatment technologies.

This research's key is applying partial nitrification to reduce nitrogen concentrations efficiently while minimizing the oxygen demand required for complete nitrification. By limiting the process of the conversion of ammonium to nitrate, partial nitrification creates favorable conditions for subsequent nitrogen removal through denitrification. This method not only enhances treatment efficiency but also significantly reduces operational costs.

Another critical focus of this thesis is the treatment of inert COD, which comprises refractory organic compounds that are difficult to degrade biologically. By utilizing the advanced filtration capabilities of the MBR system, this research effectively separates and removes these compounds, ensuring compliance with stringent discharge standards. The MBR technology also facilitates the retention of slow-growing nitrifying bacteria, enhancing system stability and performance.

The study employs Activated Sludge Models ASM1 and ASM3 to optimize these processes, which are widely recognized for their ability to simulate complex biological treatment systems. These models serve as essential tools for simulating biological treatment processes, providing insights into system dynamics, and facilitating the optimization of operational parameters.

This research is significant not only for its scientific contributions but also for its practical applications. By combining partial nitrification, inert COD removal, MBR technology, and advanced modeling techniques (ASM1 and ASM3), the study presents a comprehensive framework for improving landfill leachate treatment in Vietnam.

This serves as a foundation for further advancements in leachate treatment and inspires researchers and practitioners to explore innovative approaches for sustainable waste management.

SUMMARY OF THE DOCTORAL DISSERTATION

Chapter 1: INTRODUCTION

The introductory chapter established the global and national context of landfill leachate management, highlighting the pressing challenges faced by developing countries such as Vietnam. Landfill leachate is characterized by extremely high pollutant concentrations, including ammonium levels often exceeding 800 – 1,200 mgN.l^{-1} and chemical oxygen demand (COD) values ranging between 2,500 – 5,000 mg.l^{-1}, with a substantial fraction composed of refractory organics. The persistence of these pollutants results in significant environmental risks, particularly when untreated or insufficiently treated leachate infiltrates surface and groundwater.

The objective of the research was to explore advanced biological treatment strategies capable of achieving reliable nitrogen and COD removal from landfill leachate, with a focus on combining Membrane Bioreactor (MBR) technology and Activated Sludge Models (ASM1 and ASM3). This integrated approach was designed to optimize biological nitrogen removal, characterize refractory COD behavior, and provide a robust modeling framework for system design and operation.

The methodology outlined the rationale for adopting MBR systems, which offer stable biomass retention, high effluent quality, and flexibility under variable leachate conditions. Furthermore, the incorporation of ASM models allowed for systematic parameter estimation and simulation, essential for predicting treatment performance.

The introduction concluded by identifying the knowledge gap: limited data existed on applying ASM1 and ASM3 to landfill leachate in tropical regions, and little was known about the dynamics of partial nitrification and refractory COD removal in MBRs under Vietnamese conditions. This research therefore sought to fill this gap through combined experimental and modeling approaches, providing both scientific and practical contributions to sustainable leachate management.

Chapter 2: LITERATURE REVIEW

The literature review systematically analyzed previous studies on landfill leachate characteristics, treatment technologies, and modeling approaches. It emphasized the distinction between young and old leachates: young leachates with high BOD/COD ratios and rapidly biodegradable organic matter, and old

leachates with BOD/COD ratios below 0.1, dominated by refractory humic substances and inert COD. In Vietnam, most leachates correspond to intermediate to old stages, making treatment particularly challenging.

The review highlighted conventional biological processes such as activated sludge, sequencing batch reactors, and constructed wetlands. These systems often failed to consistently meet effluent standards due to inhibitory compounds and high ammonium loads. Advanced options such as MBRs have shown superior performance, with reported nitrogen removal efficiencies of 70 – 90% and COD removal of 60 – 80%, but their application to tropical landfill leachate remains underexplored.

In terms of nitrogen removal, the review underscored the importance of partial nitrification–denitrification pathways as more energy-efficient alternatives to full nitrification. Factors such as dissolved oxygen, pH, and free ammonia concentrations were recognized as critical control parameters. For COD, the persistence of soluble inert fractions (S_I), often comprising 20 – 30% of total COD, was consistently reported as a limiting factor.

The review also analyzed activated sludge models, particularly ASM1 and ASM3, as tools for simulating biological treatment. ASM1 is effective in representing nitrogen transformations, whereas ASM3 offers improved description of COD fractions and storage processes. However, few studies have attempted parameter calibration for landfill leachate.

The chapter concluded that while MBR technology combined with ASM modelling presents a promising approach, research is needed to calibrate models for leachate conditions, optimize partial nitrification, and quantify refractory COD behavior—objectives directly addressed in the present thesis.

Chapter 3: LANDFILL LEACHATE CHARACTERIZATION FOR SIMULATION OF BIOLOGICAL TREATMENT WITH ACTIVATED SLUDGE MODEL No.1 (ASM1) AND No.3 (ASM3)

Chapter 3 provided a detailed analysis of the composition and seasonal variability of landfill leachate in Vietnam, forming the experimental basis for subsequent modelling and treatment studies. Composite samples were collected over both dry and rainy seasons. Influent COD concentrations averaged 2,800 – 3,200 $mg.l^{-1}$, with slowly biodegradable COD (X_S) accounting for 45 – 50% and inert soluble COD (S_I) contributing 20 – 25%. Biodegradable soluble COD (S_S) rarely exceeded 30%, confirming the refractory nature of the leachate.

Nitrogen species were dominated by ammonium, with concentrations consistently between 850 – 1,100 mgN.l^{-1}, while nitrate and nitrite remained negligible (< 5 mgN.l^{-1}), indicating the absence of significant nitrification in the landfill environment.

Alkalinity levels of 2,000 – 3,000 mg.l^{-1}, $CaCO_3$ were sufficient to buffer nitrification, though the presence of possible inhibitory compounds was noted. Seasonal variations revealed slightly higher COD and ammonium during the rainy season due to increased leaching of fresh waste layers.

Respirometric assays confirmed slow biodegradation rates. Oxygen uptake tests showed that only 50 – 55% of COD was biodegradable within 5 days, with endogenous respiration dominating thereafter. Biodegradation kinetics indicated reduced maximum heterotrophic growth rates ($\mu_H \approx 2.5 – 2.8\ d^{-1}$) compared to municipal wastewater values, highlighting stress conditions for biomass.

Chapter 4: CHARACTERIZATION OF SLUDGE FOR STOICHIOMETRIC AND KINETIC PARAMETERS FOR MODEL No.1 AND No.3 FOR LEACHATE TREATMENT

This chapter focused on the estimation and calibration of kinetic and stoichiometric parameters for Activated Sludge Models ASM1 and ASM3 to enable their application to landfill leachate treatment. Since leachate differs significantly from municipal wastewater, with high ammonium and refractory COD fractions, the default model parameters were unsuitable. The aim was therefore to determine accurate values for parameters governing ammonium oxidation, heterotrophic COD degradation, and decay processes, and to compare the predictive capacity of ASM1 and ASM3 under leachate conditions.

Respirometric batch tests were carried out using acclimated activated sludge exposed to different substrates, measuring oxygen uptake rates (OUR) under controlled conditions. Data analysis showed that the maximum autotrophic growth rate (μ_A) was approximately 0.25 d^{-1}, considerably lower than the typical value for municipal wastewater (~0.8 d^{-1}). The half-saturation constant for ammonium (K_{NH_4}) averaged 2.3 – 2.6 mgN.l^{-1}, nearly double the ASM default, reflecting the reduced affinity of nitrifiers in the inhibitory leachate environment. For heterotrophic biomass, the maximum growth rate (μ_H) was around 2.8 d^{-1}, while the yield coefficient was reduced to 0.55 gCOD/gCOD compared with the standard 0.67, indicating stress conditions. The fraction of inert COD was confirmed at 20 – 25% of total COD, with slowly biodegradable COD making up almost half of the COD load.

Model calibration demonstrated that ASM1 could adequately represent nitrogen transformations but underestimated COD removal, as it lacks explicit storage and hydrolysis mechanisms. In contrast, ASM3 achieved a better fit, particularly in describing slowly biodegradable COD fractions and endogenous storage, with correlation coefficients exceeding 0.9 for OUR curves. Sensitivity analysis.

highlighted the strong influence of μ_A, K_{NH_4}, and heterotrophic yield on model predictions.

Chapter 5: MODELLING OF PARTIAL NITRIFICATION AND DENITRIFICATION

This chapter investigated the feasibility of achieving stable partial nitrification in landfill leachate treatment, with the dual aim of suppressing nitrite oxidation and promoting ammonium conversion to nitrite as a precursor for subsequent denitrification. The background highlighted that conventional nitrification in leachate often fails due to high ammonium concentrations, inhibitory compounds, and the accumulation of inert COD, making controlled partial nitrification a more energy-efficient and sustainable option.

The experimental approach employed controlled batch and sequencing batch reactor (SBR) runs under varying dissolved oxygen (DO), pH, and temperature conditions. Respirometric assays were also used to quantify nitrification rates and inhibition levels. The principal objective was to define the operating window that suppresses nitrite oxidizing bacteria (NOB) while maintaining the activity of ammonium oxidizing bacteria (AOB).

Results demonstrated that ammonium concentrations in the raw leachate averaged 800 – 1,200 $mgN.l^{-1}$, with alkalinity consistently above 2,500 $mg.l^{-1}$, $CaCO_3$, favoring nitrification potential. Under controlled DO at 0.5 – 1.0 $mg.l^{-1}$, nitrite accumulation ratios exceeded 60 – 70%, while higher DO (> 2 $mg.l^{-1}$) led to rapid nitrite oxidation and nitrate formation. Temperature was another key factor: at 30 – 32°C, AOB exhibited maximum activity ($\mu_A \approx 0.28\ d^{-1}$), whereas NOB growth was strongly inhibited, resulting in stable nitrite accumulation. pH between 7.5 – 8.0 supported sustained partial nitrification, while lower pH caused inhibition of both AOB and NOB. Batch tests showed nitrite accumulation up to 450 $mgN.l^{-1}$, confirming effective NOB suppression.

Model simulations using calibrated ASM1 and ASM3 parameters aligned well with experimental data, with ASM3 providing more accurate predictions of nitrite accumulation dynamics. Sensitivity analyses confirmed that DO and free ammonia concentrations were the most influential factors governing the balance between AOB and NOB activity.

In conclusion, Chapter 5 demonstrated that partial nitrification of landfill leachate is achievable and stable under optimized operating conditions of low DO (0.5 – 1.0 $mg.l^{-1}$, moderate pH (7.5 – 8.0), and mesophilic temperature (~30°C). This strategy not only reduces oxygen demand but also establishes a suitable feed for subsequent anoxic denitrification, thereby improving the overall nitrogen removal efficiency.

Chapter 6: NITROGEN REMOVAL IN LANDFILL LEACHATE TREATMENT WITH MEMBRANE BIOREACTOR IN VIETNAM

This chapter examined the performance of a pilot-scale Membrane Bioreactor (MBR) treating landfill leachate, with a particular focus on nitrogen removal efficiency under tropical operational conditions. The motivation stemmed from the limitations of conventional activated sludge systems, which struggle with high ammonium and refractory COD levels in leachate. The pilot MBR was designed to integrate controlled partial nitrification with subsequent denitrification, ensuring stable effluent quality.

The pilot system had a treatment capacity of 5 $m^3.d^{-1}$, operated under continuous flow, with submerged hollow-fiber membranes providing solid–liquid separation. Influent leachate contained COD ranging from 2,500 – 3,200 $mg.l^{-1}$, and ammonium 800 – 1,100 $mgN.l^{-1}$. Operating conditions maintained a hydraulic retention time (HRT) of 24 – 36 hours, sludge retention time (SRT) > 40 days, and controlled dissolved oxygen at 0.5 – 1.0 $mg.l^{-1}$ in the aerobic zone to facilitate partial nitrification.

Results indicated excellent nitrogen removal. Ammonium removal consistently exceeded 90 – 95%, with effluent ammonium reduced to < 50 $mgN.l^{-1}$. Total nitrogen removal reached 75 – 82%, depending on influent load and temperature, with effluent TN concentrations typically between 120 – 180 $mgN.l^{-1}$. Nitrite accumulation was observed in intermediate stages, confirming the effectiveness of partial nitrification–denitrification. COD removal ranged from 70 – 78%, limited by the presence of slowly biodegradable and inert COD fractions identified earlier. Transmembrane pressure (TMP) increased gradually but remained manageable, with membrane fouling controlled through periodic backwashing and air scouring.

Model validation using ASM1 and ASM3 showed that ASM3 provided closer agreement with measured data, particularly in simulating COD fractions and nitrogen transformations. The calibrated model predicted effluent concentrations with an R^2 above 0.9, confirming its applicability for leachate MBR design.

In conclusion, the pilot-scale MBR demonstrated robust performance for nitrogen removal from landfill leachate, achieving > 90% ammonium removal and > 75% total nitrogen reduction under optimized low-DO conditions. While COD removal was moderate due to the persistence of refractory organics, the system consistently produced stable effluent suitable for discharge or further polishing. These findings confirm that MBR technology, supported by calibrated ASM modeling, is a viable solution for leachate treatment in tropical developing countries such as Vietnam.

Chapter 7: REFRACTORY COD REMOVAL IN LANDFILL LEACHATE TREATMENT WITH MEMBRANE BIOREACTOR IN VIETNAM

This chapter focused on the capacity of the pilot Membrane Bioreactor (MBR) to remove refractory organic matter, a major challenge in landfill leachate treatment. While biological processes are effective for nitrogen removal, the persistence of inert or slowly biodegradable COD typically limits effluent quality. The objective of this chapter was to quantify COD removal efficiency, identify the refractory fraction, and evaluate strategies to enhance performance.

Influent COD concentrations during pilot operation averaged 2,500 – 3,200 $mg.l^{-1}$, with fractionation showing that 45 – 50% of the COD was slowly biodegradable (X_S), while 20 – 25% was inert soluble COD (S_I) resistant to biological degradation. Standard operation at a hydraulic retention time of 24 – 36 hours and sludge retention time > 40 days achieved overall COD removal of 72 – 78%, with effluent COD typically 550 – 700 $mg.l^{-1}$. Despite the relatively high efficiency, effluent COD remained above discharge standards, primarily due to the persistence of S_I.

Batch assays confirmed that only 5 – 10% of S_I could be further degraded even under extended aeration, reinforcing its refractory nature. Adsorption onto biomass contributed minimally, with less than 7% COD reduction attributable to biosorption. Model simulations with ASM1 and ASM3, using the calibrated parameters from Chapter 4, accurately reproduced COD removal trends, and sensitivity analyses confirmed that effluent COD was strongly controlled by the proportion of inert fractions rather than kinetic parameters.

In conclusion, the MBR demonstrated consistent removal of biodegradable COD but could not completely eliminate refractory COD, which persisted in effluents at levels of several hundred $mg.l^{-1}$. The findings emphasize the need for complementary polishing steps — such as advanced oxidation processes (ozonation, Fenton, or activated carbon adsorption) — to achieve regulatory

standards. Importantly, the study provided quantitative evidence that inert COD accounts for up to one-quarter of total COD in Vietnamese landfill leachate, limiting the achievable performance of biological treatment alone. This insight is critical for future leachate treatment design and highlights the role of integrated treatment trains combining MBR with advanced physicochemical processes.

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LIST OF ABBREVIATIONS

A

AD: Anaerobic Digestion

AE: Aerobic

AF: Anaerobic Filter

AMO: Ammonia Monooxygenase

AN1: Anoxic Reactor 1

AN2: Anoxic Reactor 2

ANAMMOX: Anaerobic Ammonium Oxidation

AOB: Ammonia Oxidizing Bacteria

AOP: Advanced Oxidation Process

ASM1: Activated Sludge Model No. 1

ASM3: Activated Sludge Model No. 3

ASP: Activated Sludge Process

ATU: Allylthiourea

AUR: Ammonia Uptake Rate

B

BET: Brunauer–Emmett–Teller (surface area analysis)

BNR: Biological Nitrogen Removal

BOD: Biochemical Oxygen Demand

BOD_5: 5-day Biochemical Oxygen Demand

C

CBOD: Carbonaceous Biochemical Oxygen Demand

CFV: Cross Flow Velocity

COD: Chemical Oxygen Demand

D

DO: Dissolved Oxygen

DS: Dissolved Solids

DSS: Dissolved Suspended Solids

E

EC: Electrical Conductivity

ED: Electrodialysis

EPS: Extracellular Polymeric Substances

F

F/M: Food/Microorganism

FA: Free Ammonia

FNA: Free Nitrous Acid

FS: Flat Sheet

G

GAC: Granular Activated Carbon

H

HAO: Hydroxylamine Oxidoreductase

HF: Hollow Fiber

HPLC: High-Performance Liquid Chromatography

HRT: Hydraulic Retention Time

I

IWA : International Water Association

L

LR: Loading Rate

M

MBBR: Moving Bed Bioreactor

MBR: Membrane Bioreactor

MCRT: Mean Cell Residence Time

MF: Microfiltration

MLSS: Mixed Liquor Suspended Solids

MLVSS: Mixed Liquor Volatile Suspended Solids

MW: Mass Weight

N

NAR: Nitrite Accumulation Ratio

Nar: Nitrate reductase

NH_4^+–N: Ammonium Nitrogen

Nir: Nitrite reductase

NIT: Nitrification

NOB: Nitrite Oxidizing Bacteria

Nor: Nitric oxide reductase

NPR1: Nitrite Production Rate

NPR2: Nitrate Production Rate

NUR1: Nitrite Uptake Rate

NUR2: Nitrate Uptake Rate

O

ORP: Oxidation-Reduction Potential

ORL: Organic Loading Rate

OUR: Oxygen Uptake Rate

OURex: Exogenous Oxygen Uptake Rate

P

PCBs: Polychlorinated Biphenyls

PES: Polyethersulfone

PF: Plate and Frame

PN: Partial Nitrification

ppm: Parts Per Million

PTFE: Polytetrafluoroethylene

PVDF: Polyvinylidene Fluoride

Q

Q: Flow rate

qAUR: Specific Ammonia Uptake Rate

qNUR: Specific Nitrite Uptake Rate

qNPR1: Specific nitrite production rate

qNPR2: Specific nitrate production rate

qNUR1: Specific Nitrite Uptake Rate

qNUR2: Specific Nitrate Uptake Rate

R

RBC: Rotating Biological Contactor

Ref: Reference

RO: Reverse Osmosis

S

SBR: Sequencing Batch Reactor

SDNR: Specific DeNitrification Rate

SOUR: Specific Oxygen Uptake Rate

SRT: Sludge Retention Time

STOWA:` Foundation for Applied Water Research

SW: Spiral wound

T

TAN: Total Ammoniacal Nitrogen

TB: Tubular

TCOD: Total Chemical Oxygen Demand

TDS: Total Dissolved Solids

TF: Tricking Filter

ThOD: Theoretical Oxygen Demand

TKN: Total Kjeldahl Nitrogen

TMP: Transmembrane Pressure

TN: Total Nitrogen

TOC: Total Organic Carbon

TP: Total Phosphorus

TS: Total Solids

TSS: Total Suspended Solids

U

UASB: Upflow Anaerobic Sludge Blanket

UF: Ultrafiltration

UV: Ultraviolet light

V

VFA: Volatile Fatty Acids

VOC: Volatile Organic Compounds

VS: Volatile Solids

VSS: Volatile Suspended Solids

W:

WWT: Waste Water Treatment

WWTP: Wastewater Treatment Plant

X

XOCs: Xenobiotic Organic Compounds

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CHAPTER 1: INTRODUCTION

1.1. COMPOSITION OF LANDFILL LEACHATE

Landfill leachate is created from landfill sites. The composition of landfill leachate is affected by many factors including composition of solid waste, hydrogeological conditions and operating methods. Besides, the biochemical activity, moisture, temperature [1] as well as landfill age [2, 3] also affect leachate composition. Leachate often contains high concentrations of easily biodegradable and non-biodegradable organic matter as well as inorganic ions [4]. Forty-five toxic and organic constituents were consistently detected in the leachate from all the landfills [5]. According to Kjeldsen *et al.(2002),* the pollutants found in landfill leachate can be divided into four groups [6]:

- *Dissolved organic matter*

Total organic carbon, volatile fatty acids and more inert compounds such as fulvic and humic compounds are found in landfill leachate and can be quantified via the measurement of Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) [7].

- *Inorganic macro components*

Many inorganic macro components are found in landfill leachate such as calcium, magnesium, sodium, potassium, ammonium, iron, manganese, chloride, sulfate and hydrogen carbonate [7]. These ions are not particularly toxic but can cause environmental problems when they are discharged in large amounts in the environment [8-10].

- *Inorganic trace elements*

Inorganic trace elements which include metals like cadmium (Cd^{2+}), chromium (Cr^{3+}), copper (Cu^{2+}), lead (Pb^{2+}), nickel (Ni^{2+}), zinc (Zn^{2+}), iron (Fe^{2+}), manganese (Mn^{2+}), mercury (Hg^{2+}) and also some metalloids, may be found in landfill leachate [6, 8, 9, 11]. The concentrations of inorganic trace elements in leachate span over a large range. The inorganic trace elements at low concentration are mercury (0.00005 - 0.16 $mg.l^{-1}$, and cadmium (0.0001 - 0.4 $mg.l^{-1}$). The inorganic trace elements found at higher concentration include nickel (0.015 – 13 $mg.l^{-1}$) and zinc (0.03 - 1000 $mg.l^{-1}$) [12]. Besides, inorganic trace elements vary according to depth of landfill site, composition of waste matters and landfill age [13]. The average metal concentrations, however, are fairly low [14]. Moreover, the concentrations of inorganic trace elements in leachate are higher at earlier stages of landfill due to solubility of heavy metals

at low pH. The pH at later stages increases and a decline in metal solubility occurs resulting in considerable reduction in concentrations of heavy metals [10]. Besides, metals can be precipitated at high pH. The main cause of metal precipitation in landfills is not hydroxides precipitation at high pH though (pH is usually not high enough. The group of metals including Zn, Ni, Pb, and Cu exhibit hydroxide precipitation in the pH range of 8–11, particularly Cd requires around pH 11) but metallic sulfur precipitation associated to the sulfate reduction process.

- *Xenobiotic organic compounds (XOCs)*

Xenobiotic organic compounds found in landfill leachates are known to include aromatic hydrocarbons, halogenated hydrocarbons (including pesticides), and plasticizers [10]. However, the concentration of the XOCs in a landfill leachate decreases with time [12]. Anaerobic processes are known to facilitate the biodegradation of some xenobiotics. Other inorganic compounds like borates, arsenates and other compounds like organometallics are also found in landfill leachate but their concentrations are generally low and so these compounds are considered of secondary importance [6].

1.2. CHARACTERISTICS OF LANDFILL LEACHATE

The two main factors characterizing landfill leachate are the rate of volumetric flow and the composition [13]. Leachate characteristics vary with time and from site to site [15] because they depend on the type of wastes disposed, rainfall, age of the landfill, but also on its design and operating conditions [13, 16]. Moreover leachate characteristics change through time and a strong relationship exists between the state of waste decomposition and leachate characteristics [5, 13, 17]. The basic parameters usually measured to characterize landfill leachate include pH, alkalinity, COD, BOD, the BOD/COD ratio, forms of nitrogen (mainly ammonium-N and TKN), oxidation–reduction potential, color, the concentrations of toxic metals, as well as total dissolved solids (TDS) and total suspended solids (TSS) [6, 18, 19]. Before a landfill site reaches a stable state, several parameters change enormously; general trends in gas and leachate emissions from landfills through time are shown in Figure 1.01 [20].

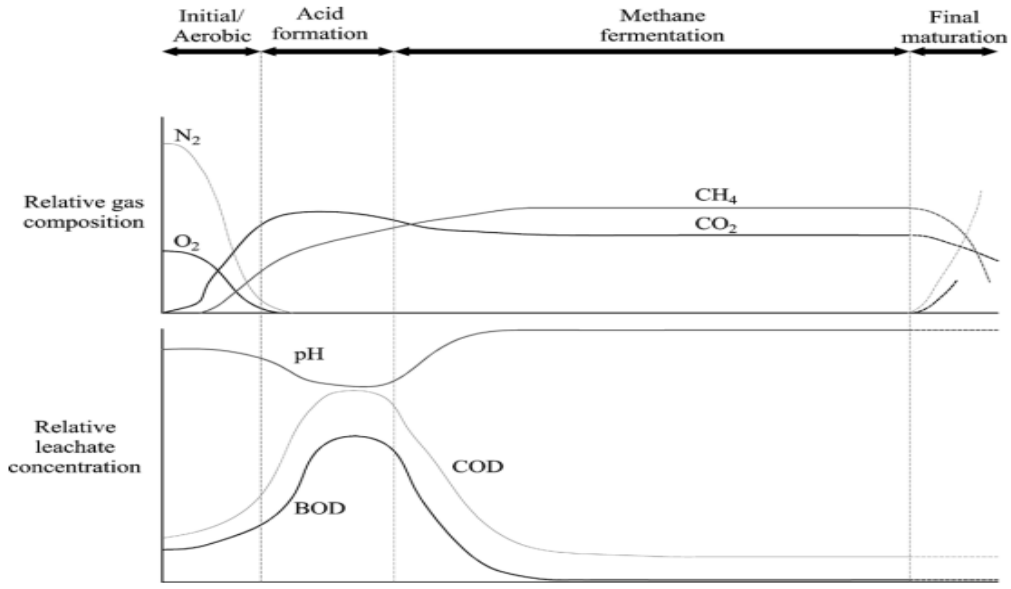

Figure 1.01. Waste stabilization phases

During the initial aerobic phase, oxygen is rapidly consumed, resulting in the production of carbon dioxide; this phase generally lasts only a few days as wastes are quickly covered by newly dumped wastes. As oxygen becomes depleted, waste decomposition enters an anaerobic phase during which fermentation reactions take place leading to the accumulation of carboxylic acids (among others), and a pH decrease which is why it is called the acid phase. During this phase leachate contains many compounds at high concentrations; in particular, they contain easily degradable organic compounds at high concentrations such as volatile fatty acids.

The onset of the initial methanogenic phase likely occurs when the pH of the landfill is sufficiently neutralized to allow at least limited growth of methanogenic bacteria. As acids that accumulated in the acid phase are converted to methane and carbon dioxide by methanogenic bacteria, the methane production rate increases [6]. Consequently, COD and BOD concentrations begin to decrease, and the pH increases as acids are consumed. The BOD to COD ratios also decrease as the degradability of the organic matter during the methanogenic phase decreases [12].

As most landfills are around 30 years old, data on phases occurring after the stable methanogenic phase are scarce and what happens in landfills at later phases is somewhat hypothetical.

- *Color*

The color of leachate samples ranges from orange brown to dark brown [19] because the leachate is formed by the percolation of rainwater through open landfill or through the cap of the completed site in landfill site [21]. The landfill

leachate has a dark color due to the presence of high molecular weight organic substances. Generally, the leachate from stabilized landfills contains high concentrations in organic substances such as humic and fulvic compounds, associated with this leachate color.

- *Odor*

More than 100 odorous compounds were found in landfill leachate such as ammoniac (NH_3), methyl mercaptan (CH_3SH), hydrogen sulfide (H_2S) and dimethyl sulfide ($(CH_3)_2S$), etc. [22-26]. According to Jae *et al*, the hydrogen sulfide was formed from waste containing organic wastes including food wastes, paper and sludge of wastewater treatment plants decomposed by anaerobic bacteria [26] or other sulfate sources. Solid wastes containing many organic components during their decomposition, may be a source of bad smells due to the presence of fatty organic acids.Yamamoto *et al.* (2004), also found formic acid, acetic acid, propionic acid, butyric acid in leachate [27]. Those compounds are formed during the first steps of the methanogenesis process.

- *pH*

pH is the most important parameter that influencesexisting forms of pollutants in leachate. The pH varies according to age of landfills [28]. After the initial acid phase, pH of leachate increases with time due to the decrease of the concentration of the partially ionized free volatile fatty acids [29]. The increase in pH indicates that a steady state has been reached between acid producing processes (cellulose and lignin degradation) and acid consuming processes (methane formation) at the landfill. Chen (1996) [30], found the value of pH increases with landfill age up to a stabilized state also because of biological decomposition of organic nitrogen into ammoniacal nitrogen. Generally, the value of pH of old leachate is higher than young leachate.

- *Electrical conductivity*

Electrical conductivity (EC) is a means to measure the ionic concentration within a solution [17, 29]. According to Vadillo *et al*. (1999) [31], the EC of the leachate can reach 41 $mS.cm^{-1}$. The value of EC changes due to variations in moisture content, temperature and biochemical processes.

- *Alkalinity*

The pH increases during methanogenic phase, and as a result supports the growth of methanogenic bacteria because of the bicarbonate buffering capacity system. Leachate from old landfills give rise to alkaline leachates [1], and the level of alkalinity ranging from 1000 to 5000 mg $CaCO_3.l^{-1}$ was recommended [29] to facilitate anaerobic processes.

- *Total dissolved solids and total suspended solids*

Total Dissolved Solids (TDS) comprise mainly inorganic salts and dissolved organics. According to Muhammad Umar *et al.*, the TDS parameter is used to license discharge of landfill leachate in many countries such as the U.K [32]. A value of TDS concentration ranging from 6042 to 9780 $mg.l^{-1}$ is recommended [19]. Total Suspended Solids (TSS) consist in organic matter, inorganic matter, clay, microorganisms, etc. They may also contain fine particles of soil from the daily capping [33, 34]. Leachate from landfills normally contains only small amounts of suspended solids because leachate is filtrated through the solid wastes. Compared to other components in leachate treatment, suspended solids are easy to remove.

- *Biochemical Oxygen Demand*

The BOD of leachate indicates the age of the landfill and this parameter decreases with time. Many authors found that BOD value varies according to age of landfills. The value of BOD can get maximum when the landfill site is processing for 6 months to 2 years (during acidogenesis and acetogenesis steps), then it will reduce until the landfill is at steady state through from 6 to 15 years [28, 35].

- *Chemical oxygen demand*

Total organic compounds content represented by COD in leachate is measured by establishing the amount of oxygen consumed to totally oxidize organic matter into inorganic products by chemical oxidation. Generally, as landfill age increases, organic concentration in leachate decreases [18]. According to Longe. *et al.*, the highest COD value is 85 $g.l^{-1}$ in the youngest leachate. This value declines asf landfill ages and reaches 7 $g.l^{-1-}$ [36]. As mentioned previously it depends on many factors, but it will decrease with time.

- *BOD_5/COD ratio*

It is commonly known that organics in leachate are characterized by different biodegradability. Generally, the BOD_5/COD ratio describes the degree of biodegradation and gives information on the age of the landfill. The BOD_5/COD in leachate is known to decrease with increasing age of landfill [35]. For a young leachate, a BOD_5/COD ratio higher than 0.4 is typical in the acidogenic phase [10]. Indeed, a low BOD_5/COD ratio indicates a high proportion of inert organic compounds difficult to be biologically degraded in leachate . An older leachate in the methanogenic phase is not as easily biodegraded as a young leachate. In this phase, high concentrations of refractory organic compounds are found because some substances have been biodegraded during the acidogenic

phase. The ratio of BOD_5/COD is an important indicator of the age and stability of a landfill and its leachate [35]. Medium age leachates typically have a BOD_5/COD ratio between 0.1 and 0.3, otherwise, the ratio is less than 0.1 for an old leachate .

- *TOC*

Organic matter is one of major pollutants present in landfill leachate. The BOD/TOC could also reflect the oxidation of organic carbon in leachate and this value is high in the initial stages of landfills. When the landfill reaches steady state then the value of BOD/TOC is reduced.

- *Nitrogen*

The high ammonia concentration typically observed in landfill leachates is primarily attributed to the hydrolysis and fermentation of nitrogen-containing biodegradable substrate [18]. Organic nitrogen and ammonia are two main componentsfound in landfill leachate. Old leachate is rich in ammoniacal nitrogen derived from the hydrolysis and fermentation of nitrogenous fractions of biodegradable substrates. NH_4^+-N is high in young leachates and decreases with time like all the other compounds, but this decrease is not as rapid as for organic compounds, thus the N/COD ratio increases with time. The average concentrations of TKN and ammonia in leachate range from 1000 to 2100 $mg.l^{-1}$ and from 689 to 1822 $mg.l^{-1}$, respectively [37]. The ammoniacal nitrogen concentration in leachate depends on the amount of easily biodegradable compound such as proteins in the solid wastes. Therefore, this value of ammoniacal nitrogen is different between countries and are found at high value in Asia.

- *Total phosphorus*

Phosphorus is a main component within the inorganic pollutants from the leachate. The levels of total phosphorous in leachates are low, with the highest total phosphorus content of only 30.3 $mg.l^{-1}$ [3, 10]. Thus phosphorus could be a limiting factor for the growth of bacteria to treat leachates.

- *Sulphate, chloride*

The sulphate concentration in leachate mainly depend on the organic matter present decomposed in the solid wastes. It is expected to decrease as landfill ages due to the reduction of sulphates to sulphides concomitant with the initiation of anaerobic conditions in the landfill. Therefore, the concentration of sulphate in leachate can also be considered as an indicator of the stabilization of waste within a landfill site. The chloride parameter in leachate is interesting to assess the variation of leachate dilution [29]. As chlorides are not

degraded/transformed in the landfill and are considered as conservative elements, this property can be used to facilitate mass balances on the landfill, including water balance [12, 13].

1.2.1. Characteristics of landfill in the world

The principal pollutants in leachate are organic and ammoniacal nitrogen. The parameters change with time, composition of solid waste, weather, depth of landfill, etc. For example, the composition of landfill leachates in Hong Kong sites is expressed in Table 1.01 [38].

Table 1.01. Composition of landfill leachates in Hong Kong sites

Parameters	**Closed landfill sites**						**Active landfill sites**		
($mg.l^{-1}$)	MYTC[1]	STW[2]	MTL[3]	NCW[4]	GDB[5]	JV[6]	PP[7]	SW[8]	JB[9]
pH (no unit)	7.1 – 8.3	7.0 – 8.3	7.8-9.0	7.8-7.9	7.9-8.1	5.6-7.3	6.2-8.9	6.8-9.1	7.5-8.5
SS	1000	> 5000	2000	480	1100	1000	> 2500	2000	-
COD	1660	17000	50000	750	1700	6610	30000	13000	-
BOD_5	100	7300	22000	81	160	1600	5000	5000	-
Ammoniacal -nitrogen	2000	3000	13000	760	2300	1500	3000	11000	1900
Kjeldahl-N	2200	3200	13000	960	2500	2000	3800	11000	-
Phosphate	20	10	90	2.5	22	10	270	30	-
Sulphide	0.4	3.4	4.4	-	-	10	7.5	3.6	4
Sulphate	7	1000	-	< 1	19	39	150	440	150
Chloride	630	12000	30000	770	2300	3400	3600	30000	1600
Chromium	0.4	1	5.3	0.35	0.22	0.12	4	0.3	0.3
Nickel	0.1	0.5	0.1	0.06	0.45	0.1	0.25	0.15	0.1
Copper	< 0.01	0.5	0.1	0.08	0.04	0.02	0.3	1	0.05
Zinc	0.51	1	0.3	0.29	0.21	1	1	2.2	1.5
Cadmium	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Lead	0.3	1	0.06	0.1	0.6	0.04	0.3	0.1	0.05

[1]. MYTC: Ma Yau Tong Central;
[2]. STW: Sai Tso Wan
[3]. MTL: Ma Tso Lung
[4]. NCW: Ngau Chi Wan
[5]. GDB: Gin Drinkers Bay
[6]. JV: Jordan Valley
[7]. PP: Pillar Point
[8]. SW: Shuen Wan
[9]. JB: Junk Bay

As shown in Table 1.01, the landfill leachate contains high concentrations of organic compounds and varies in wide range from 750 to 50.000 $mg.l^{-1}$, whereas biodegradable organics range from 100 – to 22.000 $mg.l^{-1}$. The leachate also includes significant nitrogen compounds such as TNK and ammonia. It is also important to notice that the majority of TNK is ammonium accounting from 760 to 13.000 $mg.l^{-1}$. According to Lo (1996) [38], the ammonia concentrations in leachate are usually found to be high following hydrolysis and fermentation of protein fraction of biodegradable substrates. Table 1.01 also shows that the average concentrations of hazardous substances, in this case heavy metals, were fairly low.

The leachate composition from different sanitary landfills as reported in the literature is subject to wide variations. Characteristics of leachate change with age are summarized in Table 1.02. The presented data in Table 1.02 indicate the landfill age has a significant effect on its composition, especially on organic and ammonia concentrations.

Table 1.02. Effect of landfill age on leachate quality

Country	**Age** years	**pH** -	**COD;** $mgO_2.l^{-1}$	**BOD;** $mgO_2.l^{-1}$	**NH_4^+-N;** $mg.l^{-1}$	**Ref.**
India	< 5	6.5	> 10000	> 0.3*	-	[28]
	5-10	6.5-7.5	4000-10000	0.1-0.3*	-	
	>10	> 7.5	< 4000	< 0.1*	-	
Malaysia	>10	7.59-8.05	117-855	29-158	210-857	[39]
Bangladesh	>10	6.93	1630	-	1252	[40]
Toronto	< 5	6.52	3641.2	2031.62	288.6	[7]
	5-10	5.72	875.44	195.83	260.03	
Belgium	5-10	7.56 ± 0.16	3040 ± 243	223 ± 21	1274±60	[41]

* BOD_5/COD ratio

The data show that the degree of landfill stabilization has significant effect on leachate characteristics. The concentrations of organic compounds in leachate from young landfills are high, whereas leachate from stabilized landfills often contains lower levels of organic matter. Values of COD vary in wide range between countries due to differences in solid waste compounds. The BOD/COD ratio decreases rapidly with the aging of the landfill, ranging from less 0.3 to than 0.1 due to the degradation of biodegradable organic compounds in leachate after several years.

1.2.2. Characteristics of landfill leachate in Vietnam

In Vietnam, with the rapid economic growth and seemingly uncontrolled urbanization, the amount of generated solid wastes has been increasing dramatically, especially in big cities such as Ho Chi Minh city and Hanoï. Generally, leachate in Vietnam contains high organic, nitrogen compounds. Besides, landfill sites in Vietnam generate large amounts of leachate containing highly toxic and non-biodegradable organic chemicals caused by the continuous decomposition of solid waste [42]. Characteristic of leachate in Vietnam are presented in Table 1.03 [43-47].

Table 1.03. Characteristics of leachate in Vietnam

Parameters		Phuoc Hiep		Nam Son
		dry season	rainy season	
pH	-	5.8	6.6	8.31
EC	µS/cm	4575	4275	10 - 22
COD	$mgO_2.l^{-1}$	9000	5670	2152 – 22780
BOD	$mgO_2.l^{-1}$	-	-	780 – 12300
TSS	$mg.l^{-1}$	240	1520	425 – 2240
TDS	$mg.l^{-1}$	2400	7.500	-
TN	$mg.l^{-1}$	-	-	485 – 2150
NH_4^+-N	$mg.l^{-1}$	736	1062	150 – 1050
NO_3^--N	$mg.l^{-1}$	37.2	3.8	-
TP	$mg.l^{-1}$	-	-	7 - 25
Hardness	$mgCaCO_3.l^{-1}$	795	525	-
Alkalinity	$mgCaCO_3.l^{-1}$	-	-	1000 – 10000
Ca^{2+}	$mg.l^{-1}$	1825	824	135 - 650
Mg^{2+}	$mg.l^{-1}$	567	378	50 – 1500
Cl^-	$mg.l^{-1}$	1550	1449	850 – 1850
SO_4^{2-}	$mg.l^{-1}$	1500	863	100 – 1500
Fe	$mg.l^{-1}$	60.1	35.1	10.4
Na^+	$mg.l^{-1}$	2600	1517	-
K^+	$mg.l^{-1}$	1400	263	-
Cd	$mg.l^{-1}$	0.003	0.01	0.01 – 0.02
Pb	$mg.l^{-1}$	0.07	0.05	0.05 – 0.07

It can be noted that the principal pollutants in leachate are organics and ammoniacal nitrogen. The variation of these parameters with time and weather may have important implications on leachate.

1.3. HAZARDS RELATED TO LANDFILL LEACHATE

The serious pollution problems from the municipal solid waste landfill sites area are an issue because contaminated matter will continue to be produced after closure of the landfill site and this process will last for 30 – 50 years [48], even up to 200 years [49].

Leachate from a landfill can continue to pose a groundwater contamination problem for many years after the closure of the landfill [50]. There are many causes that lead to potential environmental impacts of a landfill on groundwater quality such as leachate characteristics [51], the percolation of fluid through the solid waste, the mass of contaminant in the facility, the material of the leachate containment system and the site hydrogeology [51, 52].

Depending on the type of landfill, leachate with high concentration of pollutants may enter underground, surface water and soil [3]. These pollutants are negatively impacting water resources and the environment [53, 54]. Toxic compounds inside leachate affect fish, and cause other animals and human being, which feed on fish, to be subjected to the same toxic effect [17].

Ammoniacal nitrogen in landfill leachate is the contaminant with the greatest potential to adversely impact surface waters and ground waters [10].

Leachate containing ammonia and organic matter at high levels can decrease dissolved oxygen if discharged without treatment; it can also stimulate development of algae due to nutrient enrichment, which in turn die and are decomposed leading to further decrease of oxygen content of surface water[40]. Generally, leachate consisting of mixtures of many chemicals is a potential risk to human health. The health effects of organic toxicants are listed in Table 1.04. [48].

Table 1.04. Health effects of organic toxicants in landfill leachate

Organic compounds	Health effects
Dichloromethane	short-term exposure – headaches, fatigue, and feeling of drunkenness. exposure to high concentration - unconsciousness and death. long-term exposure – damage to liver and brain.

Organic compounds	Health effects
	cancer – lungs, liver and pancreas
1,2-dichloroethane (12-DCE_a)	short-term exposure may cause eye problems, headache, feeling of drunkenness, fatigue, central nervous system depression, convulsions, unconsciousness and death. long-term exposure – damage to liver, kidneys, lungs and adrenal glands
Trichloroethene (TCE_e)	short-term exposure – beginning with headache, dizziness, and confusion and progressing with increasing exposure to unconsciousness and death. long – term exposure – damage liver and kidney to leukemia Skin – rushes and liver cancer.
Tetrachloroethene (T_eCE_e)	inhalation – dizziness, headache, sleepiness, confusion, nausea, difficulty in speaking and walking, unconsciousness, and death Skin – irritation
Toluene	inhalation – fatigue, confusion, headache, dizziness and drowsiness. ingestion – abdominal spasms Skin – irritation. long-term exposure – liver and kidney damage.
Hexachlorobenzene	ingestion – liver disease with associated skin lesions called porphyria cutanea tarda children – abnormal physical development.
Pentachlorophenol (PeCP)	short-term exposure – increases in temperature, profuse sweating, and difficulty breathing even death. long-term exposure - damage to liver and immune system, damage to thyroid and reproductive system.
Aldrin/Dieldrin	ingestion – convulsions and some died. long-term exposure – headaches, dizziness, irritability, vomiting, and uncontrolled muscle movements
Lindane	short-term exposure – central nervous system stimulation (usually developing within 1 hour), mental/motor impairment, excitation, clonic (intermittent) and tonic (continuous) convulsions, increased respiratory rate and/or failure, pulmonary edema and dermatitis.

Organic compounds	Health effects
	long-term exposure – kidney, pancreas, testes, and nasal mucous membrane damage.
Polychlorinated biphenyl PCBs	long-term exposure – anemia, damage to liver, stomach, thyroid gland and immune system skin – chloracne and rashes cancer – liver and biliary tract.

1.4. OVERVIEW OF LANDFILL LEACHATE TREATMENT

1.4.1. Introduction

Leachates contain different contaminants at high concentrations which have to be removed before they enter the water systems. That is the best way to control the environmental pollution from landfill leachate. In general, the treatment methods are selected based on the characteristics of organic matter in leachate, expressed as the ratio of BOD/COD [55], nitrogen concentration, etc. Some methods for removing contaminants have been applied such as:

- *Filtration*

Filtration involves passing flow through a filtering medium or a combination of two or three media to remove suspended or colloidal matter. Filtration is usually applied as pre-treatment for adsorption processes, membrane separation processes and ion exchange processes to prevent rapid fouling or plugging due to high loadings of suspended solids. Filtration may also be used after precipitation/flocculation or biological processes for removal of residual suspended solids in the clarified effluent.

- *Membrane Filtration*

Microfiltration and ultrafiltration have been proved to be effective in the removal of large organic particles from aqueous leachate streams [56]. The greatest potential for application of these membrane technologies probably involves sites where leachate contains only one primary contaminant. As membranes exhibiting greater efficiency and chemical resistance are developed, microfiltration and ultrafiltration have become more viable treatment alternatives.

- *Adsorption*

The adsorption process is used as a stage of integrated chemical-physical-biological process for landfill leachate treatment [57]. Adsorption efficiency

depends on pretreatment to remove suspended solids, oil, and grease, etc. According to YalılıKılıç *et al*. (2007) [58] adsorption process followed by chemical coagulation provides an effluent suitable for direct discharging into receiving media in Turkish. On the other hand, adsorption by activated carbon has been used along with biological treatment for effective treatment of landfill leachate [57, 59, 60]. The adsorption by activated carbon has become a well-developed process widely applied and has become recognized as standard technology for the treatment of most landfill leachate, especially, removal of mixed organic and inorganic toxicants. However, activated carbon columns need to be regenerated frequently with costly granular activated carbon, which led to limit its application for the treatment of landfill leachate in developing countries [61].

- *Precipitation*

During the leachate treatment process, metals can be precipitated from wastewater as hydroxides, sulphides, or carbonates by adding an appropriate chemical precipitant and adjusting the pH to favour insolubility. Although better removal efficiencies are possible with sulphide precipitation because of the low solubility of metal sulphides, hydroxide precipitation with lime or caustic as the precipitant is practiced more widely because of its materials-handling and cost advantages.

- *Oxidation/Reduction*

To significantly remove most COD and amount of color of landfill leachate under appropriate conditions, the oxidation method is used [62]. The important advantage of this method is that it oxidizes organic pollutants into carbon dioxide and water [63]. Wet air oxidation is one of available technologies for the treatment of landfill leachate. It involves the aqueous-phase oxidation of concentrated organic and inorganic wastes in the presence of oxygen at elevated temperature and pressure. Oxidation/reduction technology is well developed and has many applications. In treatment of young leachate that contains relatively high COD, Fenton process can be used as a pretreatment for improving BOD_5/COD ratio stage [21]. The ozone processes are attractive methods for the treatment of landfill leachates due to their high oxidative power [64]. Leachate contains various pollutants and inert contamination can be removed by oxidation-reduction at high efficiency. However, the cost is expensive and yields a chemical sludge with toxic components.

- *Stripping*

Ammonia stripping has also the advantage of precipitating organic and heavy metals present in the leachate. The concurrent COD and phosphorus removal via lime precipitation is independent of airflow rate. The change in color of the raw leachate from dark brown to pale yellow after recirculation indicates the removal of the organic fractions that contribute to the color [65].

- *Activated sludge*

Biological treatment has become one of the most often used treatment processes; it is the most common method for the removal of organic, nitrogen, or phosphorus components from wastewaters [66]. The aerobic process is often applied to remove biodegradable organic compounds in leachate. A combination of aerobic and anoxic environment is necessary for the achievement of organic and nitrogen removal from wastewater. The connection of anaerobic and aerobic environment is required to biologically remove phosphates. On the other hand, association of different regimes (anaerobic, anoxic, aerobic) will achieve the optimal treatment efficiency.

- *Sequencing batch reactor*

The sequencing batch reactor (SBR), like the conventional activated-sludge process can be used in order to biodegrade organic contaminants. SBRs can achieve good BOD and nutrient removal [67]. With a SBR, the leachate can be accumulated in a holding tank for intermittent treatment. The SBR also has greater operational flexibility to accommodate changing feed characteristics (flow and/or organic loading) and can achieve more complete treatment through adjustment of reaction parameters than the conventional activated-sludge system [62, 68].

- *Membrane bioreactor*

The combination of membrane separation technology and bioreactors has led to a new focus on wastewater treatment. Membrane bioreactors (MBR) have been widely applied on industrial wastewater treatment and leachate treatment [69]. In MBR, biodegradable organic matter and the nitrogen compounds will be reduced based on oxidizing process by microorganisms [62, 68, 70]. The sludge flow is channeled through an ultrafiltration unit where the mixed liquor and water are separated from each other. The filtrate is drained off as effluent and the concentrate is recirculated to the aeration chamber [10]. In the activated sludge process the water is separated from the biomass by the settling process. In membrane bioreactor the separation is done by membrane, which is more efficient but yields a higher energy consumption, membrane have also a

positive effect to keep slowly growing species in the bioreactor, such as nitrifiers.

- *Aerated lagoon*

An aerated lagoon depends on the biodegradation of biologically degradable organic compounds in landfill leachate by stimulating the growth and activity of bacteria in natural ponds. It is an extensive aeration, activated sludge process without sludge recycling. This system usually requires relatively deeper stabilization pond. Oxygen can be input into the process either by pumping it into the base of the pond, or by lifting the liquids into the air. There are, however, many other critical aspects to the use of this technology, such as sludge removal rates, that will require to be addressed in the operational design of such systems. The aerated lagoon is efficient only for young leachates when the BOD/COD ratio is high [62].

- *Rotating biological contactor*

The rotating biological contactor (RBC) is a biofilm process. An RBC consists of a series of closely spaced plastic disks on a horizontal shaft, rotating and partially submerged in wastewater. The readily biodegradable organics in leachate can be treated by rotating biological contactors [62, 68, 71]. Like other biological processes, RBC's are easily inhibited or ineffective at high concentrations of metals, refractory organics, or other toxic conditions [71]. [71]. The usual design of RBS makes that denitrification is not very efficient, if needed, in RBC.

- *Trickling filter*

The attached growth process in trickling filters is an aerobic biological treatment process based on the growth of micro-organisms that form a biofilm over a bed of rocks or plastic medium. This method has been investigated for the biological nitrogen lowering from municipal landfill leachate [13]. The wastewater trickles through the filter bed, contacts the slime layer formed on the medium, and is collected by an under-drain system. The micro-organisms assimilate and oxidize substances in the leachate; as the micro-organisms grow, the slime layer increases. Periodic sloughing of the slime layer into the under-rain system results from organic and hydraulic loadings on the filter, and a new slime layer begins to grow. Sloughed solids are separated from the treated effluent by settling. It seems that treatment of leachate with trickling filters is difficult. With leachate a biomass with clogging capacity seems to develop in trickling filter [62, 71, 72].

- *Moving bed bioreactor*

The suspended biological reactor is filled with specially designed biofilm carrier elements which are free floating and moving around in the reactor with a mixer or air mixer. This is an aerobic biological process in which the air is supplied from a side channel air blower and through a coarse bubble air distribution system at the bottom of the tank. The main advantage is that the biofilm attached to the carriers provides a higher and more stable biomass concentration compared to suspended growth in conventional activated sludge systems. This leads to better resistance to shock loads, higher treatment efficiency, and reduced sludge production. On the other hand, the systemhas less vulnerability and sludge loss compared to other aeration biological process. As soon as biofilm mass has been accumulated, the reactor volume can be favorably exploited [62, 71]. A combination of a cross-flow MBR and MBBR for the treatment of stabilized leachate was done by Canciani *et al*. [73].

- *Anaerobic process*

Anaerobic treatment is the biological treatment without use of air or elemental oxygen. Many applications are directed towards the removal of organic pollution in leachate (containing high concentrations of organic acids), slurries and sludge. Organic pollutants are converted by anaerobic micro-organisms to a gas containing methane and carbon dioxide, known as “biogas”. There are several disadvantages for the anaerobic treatment. They can be best summarized as the following points such as relatively long periods are required to start-up process; sensitivity to variable loads and possible toxicity problems; anaerobic processes have been traditionally limited to pre-treatment applications; additional treatment could be required to meet discharge standard; and high temperature required to accelerate the process [74]. Moreover, in the case of landfill leachate, the landfill is already an anaerobic bioreactor, with a long residence time. An additional fermenter should not have a deep effect, as far as methanogenesis is active in the landfill.

- *Up-flow anaerobic sludge bed*

The Up-flow anaerobic sludge bed (UASB) is applied to remove organic compounds with high concentration in leachate by anaerobic technology [62]. In addition, the UASB lends itself to a design where liquid, gas and solid phases can be separated within one vessel. The UASB reactor because of easy operation, minimal sludge production, and energy efficiency is taken into consideration in some situations for leachate treatment. Additionally, during this process, methane as the by-product could be used as fuel and suspended

solids removal might be needed before using UASB. A lower quality effluent will be produced by UASB and it is necessary to combine it with a post-treatment .

- *Biological nitrogen reduction*

Nitrogen is the dominant nutrient in landfill leachate. The nitrogen occurs mainly as ammonium nitrogen and as organic nitrogen. The contents of oxidized nitrogen (nitrites and nitrates) are generally negligible. Therefore, nitrification and denitrification are essential processes of leachate treatment. The biological method of nitrification/denitrification is probably the most efficient and cheapest process to eliminate nitrogen from leachate [75]. Nitrification is the conversion process of ammonium into nitrites by biological process in aerobic conditions which is followed by the oxidation of these nitrites into nitrates. This process is carried out by the cooperation of two kinds of bacteria. The first step is done by *Nitrosomonas* type bacteria and the oxidation from nitrites to nitrates is done by *Nitrobacter* type bacteria [62, 71].

$$2NH_4^+ + 3O_2 \xrightarrow{\text{Nitrosomonas}} 2NO_2^- + 2H_2O + 4H^+ \quad (1.1)$$

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

Because, denitrification is an anoxic process, it does not require molecular oxygen to be present in the water, although oxygen atoms are present within the nitrates [71]. The denitrification processes is applied widely in order to remove nitrogen nutrients before discharging wastewater into environment [62, 71].

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2 \quad (1.2)$$

So nitrification combined to denitrification are the main processes used to remove ammonium from leachates and transform it into gaseous nitrogen (N_2).

In old leachate, the biodegradable organic concentration is too low and insufficient to supply for the heterotrophic bacteria performing denitrification process, thus external organic carbon will be needed such as methanol or sugar.

- *Natural treatment systems*
  + *Irrigation*

The irrigation system consists of parts for collection, storage, pumping and distribution of the leachate over the treatment area. The location of the treatment area could be a completed part of the landfill or on ground situated outside the landfill, such as forests or meadowland. With the help of irrigation, the leachate quantity could be reduced through increased evaporation.

Meanwhile, the nutrient content in leachate will also be decreased, partly because of the incorporation of nutrients in vegetation and partly because of other processes in the soil. Nitrogen can be converted by nitrification/denitrification, and a major part of the phosphorous can be adsorbed in the soil. Organic materials in low doses are degraded by microorganisms. Metals can be oxidized and precipitated or be adsorbed to the soil particles [62]. A good management of the risks is necessary if this system is supplied. Also the infiltration of contaminated waters has to be avoided.

+ *Overland flow*

Suspended and colloidal organic materials in the leachate are removed by sedimentation and filtration through surface grass and organic layers. Removal of total nitrogen and ammonia is inversely related to application rate, slope length and soil temperature. Phosphorus and trace elements removal is achieved by sorption on soil clay colloids and precipitation as insoluble complexes of calcium, iron and aluminum. Overland flow systems also remove pathogens from effluent at levels comparable with conventional secondary treatment systems, without chlorination. A monitoring program should always be incorporated into the design of overland flow projects both for leachate water and effluent quality and for application rates [62]. The considerable ammonium concentration of leachates can also inhibit the growth of the grass.

+ *Aquatic systems*

There are three primary mechanisms when floating aquatic plant systems are used to treat wastewater:

- ☑ Metabolism through a mixture of facultative microorganisms on plant roots, suspended in the water column and in the detritus at the bottom of the pond.
- ☑ Sedimentation of wastewater solids and internally produced biomass (dead plants and microorganisms).
- ☑ Incorporation of nutrients in living plants and subsequent harvest. Floating aquatic plant systems are typically effective in reducing concentrations of BOD and suspended solids.

In addition to these primary mechanisms, nitrate nitrogen may be removed by denitrification, and total nitrogen and phosphorus removal can be consistently achieved if the plants are harvested routinely. Floating aquatic plant systems are typically effective in reducing concentrations of BOD and suspended solids.

Actually, there are many selections of treatment methods from among the potentially applicable technologies. However, with single stage treatment it is difficult to respect the stringent discharge limits because of complicated and hazardous leachate characteristics. The main reason is the very peculiar property of leachate: it changes with time in quantity and quality; thus it is very difficult to design a single system that will adapt to these changes during the lifetime of the landfill site. Therefore, combinations of physical, chemical and biological methods are used for effective treatment of landfill leachate.

The following sections describe the most commonly used treatment methods with associated advantages and disadvantages and different techniques in more details are summarized from [18, 61, 76-80] and shown in Table 1.05.

Table 1.05. Advantages and disadvantages of different leachate treatment techniques

Treatment techniques	Advantages/Target of removal	Disadvantages/Remark
Primary treatment (precipitation/ clarification)	- reduces metal concentrations and color - removes compounds with MW (mass weight) higher than 5000	- produces important amounts of sludge
Ammonium stripping	- removes/decreases ammoniacal nitrogen	- requires other equipment for air pollution control
Coagulation–flocculation	- removes/decreases heavy metals and suspended and flocculated solids	- high sludge production and subsequent disposal may be a problem
Chemical precipitation	- removes/decreases heavy metals	- requires further disposal due to sludge generation
Ultrafiltration	- removes SS which are not easily settled - renders further settling unnecessary - removes high molecular weight compounds	- fouling - necessitates efficient pretreatment - costly and limited applicability due to membrane fouling
Microfiltration	- removes/decreases suspended solids	- used after metal precipitation
Nano-filtration	- removes/decreases sulphate salts and	- costly but requires lower pressure than reverse osmosis

Treatment techniques	Advantages/Target of removal	Disadvantages/Remark
	hardness ions, like Ca^{2+} and Mg^{2+}	
Reverse osmosis	- removes/decreases organic and inorganic compounds	- costly and extensive pre-treatment is required prior to RO - management of concentrate is needed
Ion exchange	- dissolved compounds, cations/anions	- used as a polishing step after biological treatments and treatment cost is high
Activated carbon adsorption	- removes organics which are difficult to degrade biologically - can efficiently be combined with another biological process - removes/decreases organic compounds	- necessitates regeneration of carbon or it is waste with the sludge - large carbon? footprint - relatively expensive
Activated sludge	- effective removal of low MW compounds - relatively cheap in comparison with other biological processes - demonstrated full-scale performance in Ontario - Removes nitrogen	- not efficient on compounds with MW higher than 5000 - sensitive to shock loads - nutrients additions may be required - sensitive to seasonal volume variations - frequent settling problems
Sequencing batch reactors	- equalization, primary clarification (in most cases), biological treatment, and secondary clarification can be achieved in a single reactor vessel. - operating flexibility and control. - minimal footprint.	- a higher level of sophistication is required (compared to conventional systems), especially for larger systems, of timing units and controls. - higher level of maintenance (compared to conventional systems)

Treatment techniques	Advantages/Target of removal	Disadvantages/Remark
	- potential capital cost savings by eliminating clarifiers and other equipment. - useful for efficient N removal	associated with more sophisticated controls, automated switches, and automated valves. - potential of discharging floating or settled sludge during the draw or decant phase with some SBR configurations. - potential plugging of aeration devices during selected operating cycles, depending on the aeration system used by the manufacturer. - potential requirement for equalization after the SBR, depending on the downstream processes.
Tricking filters	- long residence time produce a more highly mineralized sludge - no blower necessary - synthetic media allows for much higher loading rate	- requires a clarifier for sloughed off solids - oxygen transfer is a limiting factor for BOD > 450 $mg.l^{-1}$ - clogging (biomass developed)
Rotating biological contractors	- quite reliable because of large amount of biomass - withstands hydraulic and organic surges - low power requirements and can operate at higher BOD/Phosphorus ratio	- requires clarifier for sloughed off solids - potential for scaling caused by iron and calcium precipitate - rather limited Nitrogen removal capacities
Recirculation and Land (spray) irrigation	- volume reduced by evaporation - organics reduced by natural biological activity and sunlight	- limited by climatic conditions - metals and refractory organic do not degrade - strong odor - liquid volume increases in landfill

Therefore, the biological method has been applied to remove COD and nitrogen in leachate as well as wastewater which contain nitrogen and biodegradable compounds at high concentrations. Combining with biological processes to remove nitrogen and organic will have many advantages compared to other methods because of their lower cost and the fact that they are more environmental friendly.

1.4.2. Examples of landfill leachate treatment in the world

Landfill leachate is characterized by high concentrations of organic matter and ammonium nitrogen. The composition of organic, inorganic and heavy metal components in the leachate always fluctuates remarkably, making more difficult to be dealt with [55]. To treat landfill leachate, many kinds of methods have been widely combined. It is mainly based on biological processes for the purpose of removing organic compounds and nitrogen. Physical-chemical methods are used to remove particulate or colloidal particles and precipitation for removing metal. In most countries, especially in China, about 80 percent of wastewater treatment plants use the pre-denitrification process (anoxic/oxic) process for biological nitrogen removal [81]. COD and color removal of leachate can be treated by electro-coagulation approach [82], However, high ammonia concentration and phosphorus deficiency in leachate may hamper the efficiency of biological treatment [33]. In Germany, a combination of oxidation agents as ozone or hydrogen peroxide and ultraviolet light (UV) is often used to treat leachate [83]. Leachate treatment systems in Korea and Germany are shown in Figure 1.02.

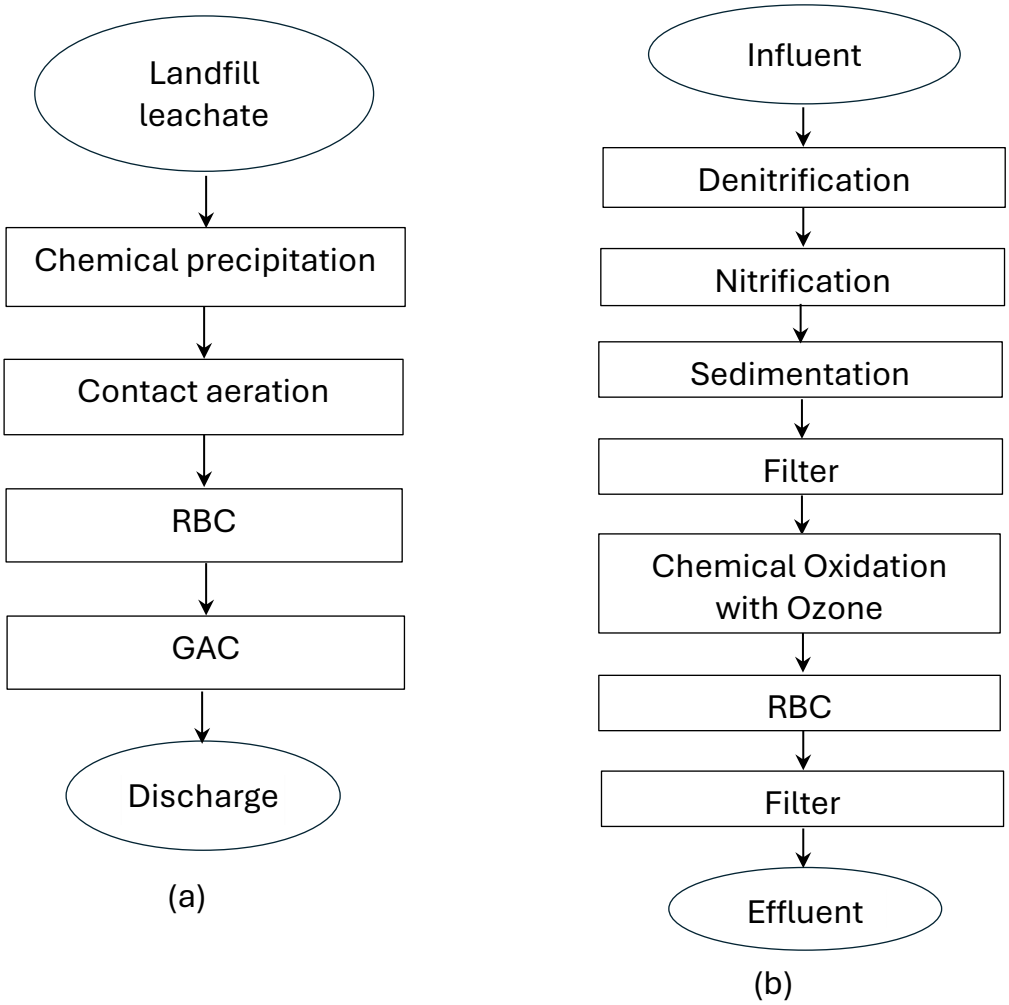

Figure 1.02. Some examples for leachate treatment, Korea (a) and Germany (b)

In the example from Korea [84] the process was composed of contact aeration, rotating biological contactor and GAC adsorption process. According to the authors, it was difficult to remove COD and nitrogen by these processes because the microbial activity in leachate was low and caused biomass washout and frequent replacements of activated carbon.

In the example from Germany [83], biological treatment processes were used firstly to remove nitrogen through nitrification and denitrification processes and part of biodegradable organics were also reduced. Thus, the cost for treatment is cheaper due to oxidation of easy biodegradable components implemented in these processes. Then, organics were oxidized only partly by ozone to biological degradable intermediate products and removed by biological treatment in RBC.

1.4.3. Examples of landfill leachate treatment in Vietnam

The most significant factor influencing the effectiveness of leachates treatment is the age of the landfill. Leachate from young landfills contain high concentrations of readily biodegradable organic matter. Thus, biological treatment is selected to remove biodegradable organic and nitrogen. The

concentration of several parameters contained in young leachate can inhibit biological treatment such as suspended solids and heavy metals and free ammonia, therefore, physical/chemical process and very careful design are needed for pretreatment of young leachate. In Vietnam, there are some important parameters used to select technology for young leachate treatment, mainly COD, nitrogen and heavy metals. The leachate treatment technology has been applied at the operating landfills in Binh Duong and Ho Chi Minh City is shown in Figure 1.03. (provided by Binh Duong water environment joint stock company – Waste treatment plant and Ho Chi Minh City Urban Environment Company Limited). The treatment system can be divided in different stages:

- *Preliminary treatment*

In collection tank, leachate is pumped to fine screens to remove large particles, then lime is added to the mixing tank for reducing some heavy metal ions in the leachate. At the equalization tank, the aeration system is applied to increase mixing capacity and reduce the odor created by anaerobic process. After that, chemical sludge is settled before going to the next stage.

- *Removal of ammonia and calcium*

Ammonia is removed by stripping, sodium hydroxide solution is added to maintain the pH value range from 10 to 11. Calcium is precipitated by adding sulfuric acid and removed in the selector.

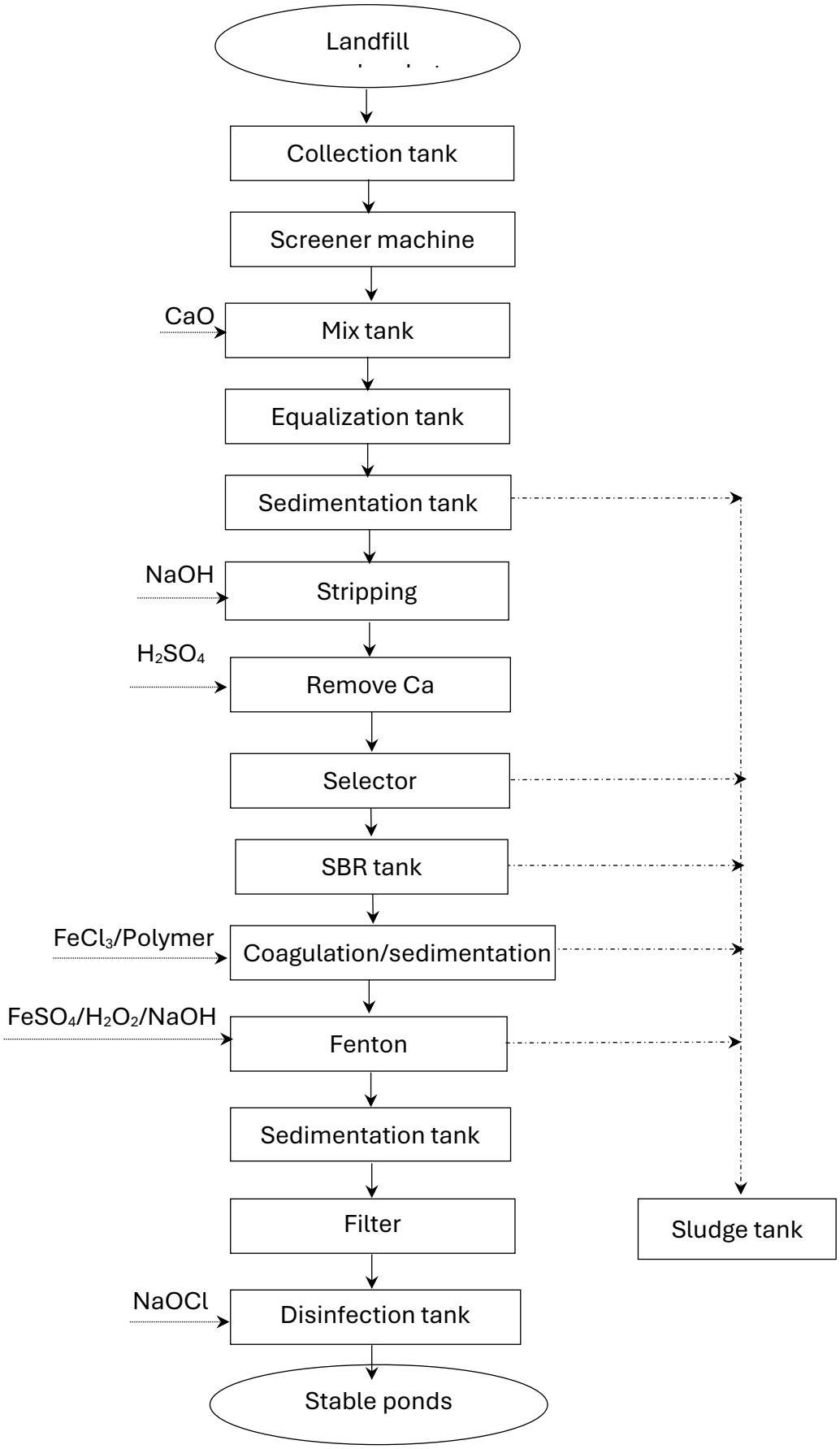

Figure 1.03. Leachate treatment systems in Binh Duong and Hiep Phuoc landfill site (Vietnam)

- *Biological treatment*

SBR technology is applied to treat leachate in Vietnam. The biodegradable organic compounds and nitrogen are removed by microbial populations in the activated sludge. Nitrogen is removed due to the nitrification process occurring during aeration periods, while denitrification process is done during sedimentation time.

- *Physico-chemical treatment*

Leachate after biological treatment is pumped to the physico-chemical treatment tank to remove suspended residues and color in leachate. Ferric chloride is commonly added as coagulant. Besides, polymers are also used to enhance the floc-settling rate

- *Advanced oxidation treatment*

Advanced oxidation processes based on the activation of hydrogen peroxide with catalysts to produce HO* radicals are known as a more efficient oxidative process and has the aim to enhance chemical oxidation efficiency of refractory organic matter.

- *Filtration and disinfection*

The sand filtration tank is used to remove suspended solids after settling. Besides, the leachate is disinfected before discharging into nature by sodium hypochlorite

In general, the leachate in Viet Nam contains high pollutants concentrations such as organic compounds, suspended solids, metals, especially significant contents of nitrogen. Besides the part of inert COD ratio accounts noticeably in leachate treatment in Vietnam. Recently, applying technology for leachate treatment in Vietnam has encountered disadvantage including effluent nitrogen concentrations higher than the Vietnamese discharge standard. On the other hand, the cost for treatment is high due to using external carbon for denitrification process in SBR, chemicals for coagulation and Fenton process as well as treating chemical sludge produced by the treatment system. In order to resolve the above issues, applying new technology to remove COD and nitrogen, decrease the treatment cost and achieve requests before discharge into the environment is necessary. It is the motivation to chose the topic **"COMBINATION OF PARTIAL NITRIFICATION AND INERT COD REMOVAL PROCESSES IN A MEMBRANE BIOREACTOR TO TREAT LEACHATE IN VIETNAM"** as the subject of this thesis.

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CHAPTER 2: LITERATURE REVIEW

2.1. BIOLOGICAL PROCESSES IN NITROGEN AND COD REMOVAL

2.1.1. Conversions in biological treatment plants

The most important method used widely in wastewater treatment plants is the biological treatment method. Biological nitrogen removal in wastewater treatment is mostly carried out by multi-step microbial processes. Wastewater treatment with high COD and nitrogen concentration, usually implements three stages: COD removal is achieved in a first stage, then the ammonia is converted into nitrite and nitrate in a second stage, the final stage being nitrate removal. In the second stage, the nitrification process takes place after COD has been removed in the wastewater.

Denitrification, in the third stage, is a heterotrophic process and needs an organic carbon source, thus making the process more complicated. Major biological treatment processes used for wastewater treatment are summarized in Table 2.01 [1-3].

Table 2.01. Major biological treatment processes used for wastewater treatment

Type	Common name	Use
Aerobic process		
Suspended growth	Activated sludge process	BOD removal, nitrification
	Aerated lagoons	BOD removal, nitrification
	Aerobic digestion	Stabilization, BOD removal
Attached growth	Trickling filters	BOD removal, nitrification
	Rotating biological contactors	BOD removal, nitrification
	Packed bed reactors	BOD removal, nitrification
Hybrid (combined) suspended and attached growth processes	Trickling filter/activated sludge	BOD removal, nitrification
Anoxic process		
Suspended growth	Suspended growth denitrification	Denitrification
Attached growth	Attached growth denitrification	Denitrification

Type	Common name	Use
Anaerobic processes		
Suspended growth	Anaerobic contact process	BOD removal
	Anaerobic digestion	Stabilization, solids and pathogen destruction,
Attached growth	Anaerobic packed and fluidized bed	BOD removal, waste stabilization, denitrification
Sludge blanket	Upflow anaerobic sludge blanket	BOD removal, especially high-strength wastes
Hybrid	Upflow sludge blanket/attached growth	BOD removal
Combined aerobic, anoxic, and anaerobic process		
Suspended growth	Single or multistage process, various specialized processes	BOD removal, nitrification, denitrification, and phosphorus removal
Hybrid	Single or multistage process with packing for attached growth	BOD removal, nitrification, denitrification, and phosphorus removal
Lagoon process		
Aerobic lagoons	Aerobic lagoons	BOD removal, nitrification
Maturation (tertiary) lagoons	Maturation (tertiary) lagoons	BOD removal
Facultative lagoons	Facultative lagoons	BOD removal
Anaerobic lagoons	Anaerobic lagoons	BOD removal, waste stabilization

2.1.2. COD removal

Bacterial biomass ($C_5H_7NO_2$) is synthesized from organic compounds with general formula $C_xH_yO_z$ found in wastewater by following biological oxidation [4, 5]:

$$n\left(C_xH_yO_z\right) + nNH_3 + n\left(x + \frac{y}{4} + \frac{z}{2}\text{-}5\right)O_2 \longrightarrow n(C_5H_7NO_2) + n(x\text{-}5)CO_2 + \frac{n}{2}(y\text{-}4)H_2O \quad (2.1)$$

While synthesizing cells it simultaneously oxidizes and decomposes part of the cell material in order to gain energy for synthesizing (endogenous respiration)[6], as shown in Eq. (2.2).

$$C_5H_7NO_2 + 5O_2 \rightarrow 5CO_2 + NH_3 + 2H_2O \quad (2.2)$$

2.1.2.1. The influence of the environmental factors

Both physical and chemical characteristics of the environment influence microbial growth. These factors can determine the types of organisms that can grow and influence the rate of growth under these conditions.

- *Temperature*

One of the most important physical factors affecting growth is temperature. Each microorganism is able to grow within a specific temperature range. While most species can grow only between 0°C and 40 °C, others can grow below 0 °C to above 90 °C. Based on optimum growth temperatures, microorganisms can be classified as: psychrophiles (less than 20 °C), mesophiles (20 – 45 °C), and thermophiles (greater than 45 °C).

The temperature dependency for the biological process can be described by the van't Hoff exponential expression [6]:

$$\mu_{max}(T) = \mu_{max}(20^oC).\exp\big(k(T-20)\big) \quad (2.3)$$

Where

T Temperature; °C

k Temperature constant

For aerobic processes, the expression applies in the temperature range 0 – 32 °C. For the temperature range 32 – 40 °C, the removal rate is constant after which it usually declines drastically to be zero around 45 °C. The anaerobic processes can also take place in the thermophilic range 50 – 60 °C. Here the process rate will be approximately 50 % higher than at 35 °C [5].

- *pH*

Another factor that influences growth rate is pH. Some general statements can be made about microorganisms' pH preferences [7]:

+ Bacteria have optimum pH near 7 (5 – 9 range).
+ Fungi prefer acidic environments (pH minimum of 1 to 3 pH, optimum near 5).
+ Blue-green algae thrive at pH higher than 7.
+ Most protozoa develop at a pH range 5 to 8.

The aerobic conversions are pH dependent. The rather unusual shape of the curve is due to a combination of the pH dependency of the micro-organisms and the selection of individual micro-organisms. The kinetic of pH can be shown in Equation (2.4) [6]:

$$\mu_{max}(pH) = \mu_{max}(opt.pH)\frac{K_{pH}}{K_{pH}+I} \quad (2.4)$$

Where

K_{pH} is the pH constant.

$I = 10^{|optimumpH-pH|}-1$

- *Oxygen*

If some bacteria can grow only in the absence of oxygen, many bacteria and fungi and protozoa are able capable of growth in either the presence or absence of oxygen. Oxygen is required for two purposes by aerobes. Mainly, for the electron transport system necessary for generation of energy and a small amount is used in enzymatic reactions. The oxygen dependency for aerobic process can be described by a Monod-like expression [6]:

$$\mu_{obs} = \mu_{max}.\frac{S_{O2}}{S_{O2}+K_{S,O2}} \quad (2.5)$$

Where

S_{O2} is the oxygen concentration in the reactor

$K_{S,O2}$ is the half saturation constant for oxygen

Combination with Monod equation for substrate:

$$\mu_{obs} = \mu_{max}.\frac{S_2}{S_2 + K_S}.\frac{S_{O2}}{S_{O2} + K_{S,O2}} \quad (2.6)$$

- *Nutrients*

The elements accounting for ratio of 90% of the dry weight of a cell, include C, O, N, and H, plus P and S elements, occupy the account almost in molecules of the cell. The remaining elements with lower ratio are K, Na, Ca, Mg, Cl, Fe, etc. There are four elements that occupy the bulk of the cell wich are C, O, N and H, in which C and N are the most abundant

Therefore, the major difference in nutritional requirements of micro-organisms is the different source of C and N that they can use for synthesis of cellular material [6].

Nitrogen and phosphorus effect on aerobic process can be described in a double Monod expression which conceals that the microbial growth is inhibited

when the concentrations of nitrogen and phosphorus are low. Some species can be inhibited by high nitrogen concentrations.

- *Toxic substances*

Non-competitive reversible inhibition influences the kinetics of growth by reducing the maximum specific growth rate [6].

$$\mu_{obs} = \mu_{max} \cdot \frac{K_{S,I}}{K_{S,I} + C_I} \quad (2.7)$$

Where

$\mu_{max'}$ is max specific growth rate with inhibition

μ_{max} is max specific growth rate without inhibition

$K_{S,I}$ half-saturation constant under inhibition

C_I concentration of inhibitory compound

μ_{obs} observed specific growth rate

2.1.2.2. Heterotrophic microorganisms

Genera of aerobic heterotrophic bacteria in standard activated sludge excluding zoogloeal strains are summarized in Table 2.02 [8, 9].

Table 2.02. Genera of aerobic heterotrophic bacteria in standard activated sludge excluding zoogloeal strains

Alcaligenes	*Sphaerotilus*	*Arthrobacter*
Acinetobacter	*Paracoccus*	*Microbacterium*
Caulobacter	*Aeromonas*	*Comamonas-Pseudomonas*
Cytophaga	*Bacillus*	*Pseudomonas* (fluorescent group)
Debaromyces	*Brevibacterium*	*Flavobacterium-Cytophaga*
Flavobacterium	*Comomonas*	*Unidentified* (gram-negative rods)
Micrococcus	*Pseudomonas*	*Aureobacterium-Microbacterium*
Hyphomicrobium	*Coryneform*	

2.1.3. Nitrogen removal

2.1.3.1. Nitrogen removal pathways

Conventional microbial nitrogen removal is based on autotrophic nitrification and heterotrophic denitrification.

In the first step of nitrification, AOB oxidize ammonium to nitrite via hydroxylamine. Membrane-bound ammonia monooxygenase is involved in these reactions [10].

$$NH_3 + O_2 + 2H^+ + 2e^- \xrightarrow{AMO} NH_2OH + H_2O \quad (2.8)$$

$$NH_3 + N_2O_4 + 2H^+ + 2e^- \xrightarrow{AMO} NH_2OH + 2NO + H_2O \quad (2.9)$$

The hydroxylamine resulting from ammonia oxidation is further oxidized to nitrite by the hydroxylamine oxidoreductase.

$$NH_2OH + 0.5O_2 \xrightarrow{HAO} NHO_2 + 2H^+ + 2e^- \quad (2.10)$$

$$\text{Or } NH_2OH + H_2O \xrightarrow{HAO} HNO_2 + 4H^+ + 4e^- \quad (2.11)$$

In the second step, NOB oxidize nitrite to nitrate with the involvement of membrane-bound nitrite oxidoreductase [11].

$$NO_2^- + 0.5O_2 \xrightarrow{NOR} NO_3^- \quad (2.12)$$

In anoxic denitrification, NO_3^- and NO_2^- are reduced to gaseous nitrogen with a variety of electron donors, such as methanol, acetate, and organic substances in wastewater [11].

$$2NO_3^- + 10H^+ + 10e^- \longrightarrow N_2 + 2OH^- + 4H_2O \quad (2.13)$$

$$2NO_2^- + 6H^+ + 6e^- \longrightarrow N_2 + 2OH^- + 2H_2O \quad (2.14)$$

Oxygen demand for nitrification and denitrification are of interest when calculating the oxygen consumption by nitrogenous matter. Figure 2.01 schematically shows the electron transfer in the nitrification and the denitrification process [12].

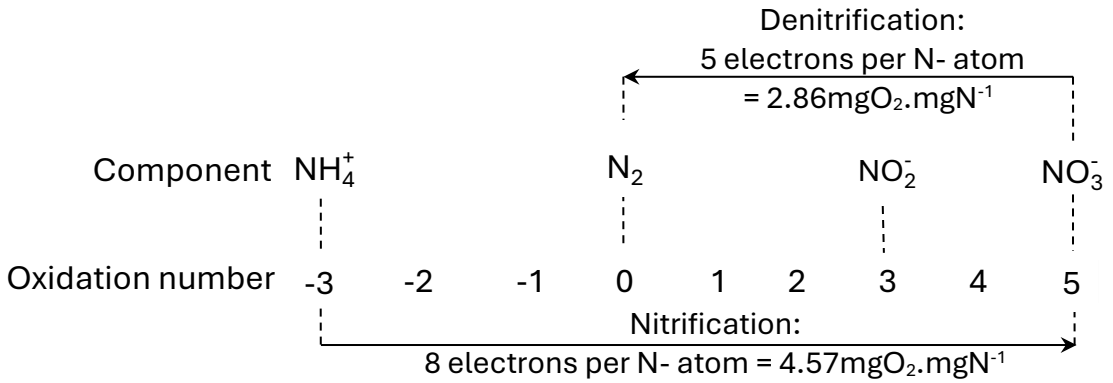


Figure 2.01. Variation of the nitrogen oxidation number in the process of nitrification and denitrification

2.1.3.2. Nitrification

- *Introduction*

Nitrification is a biological process, in which bacteria consume dissolved oxygen to transform ammonia into nitrite followed by the oxidation of nitrite to nitrate. It is carried out by two microbial groups: autotrophic ammonia oxidizers

and autotrophic nitrite oxidizers [13]. The process of ammonium oxidation is referred to as nitrification, and is carried out by two different groups of nitrifiers. The first group oxidizes ammonium to form nitrite. The most frequent genus is *Nitrosomonas* but there are other nitrifiers as well. The process for the ammonium oxidizing bacteria is [14, 15]:

$$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + H_2O + 2H^+ + \text{energy} \quad (2.15)$$

The oxidation of nitrite to nitrate is carried out by *Nitrobacteria*-like bacteria and by other minor species. The process for the nitrite oxidizing bacteria is:

$$NO_2^- + 0.5O_2 \longrightarrow NO_3^- \quad (2.16)$$

Most nitrifying bacteria are autotrophic and thus use carbon dioxide as the carbon source. The carbon dioxide should be reduced before the carbon can form part of the cell mass, and this reduction takes place through the oxidation of the nitrogen source of the organism concerned. For oxidation of ammonium, the expression for growth is [4, 16]:

$$15CO_2 + 13NH_4^+ \longrightarrow 10NO_2^- + 3C_5H_7NO_2 + 23H^+ + 4H_2O \quad (2.17)$$

For oxidation of nitrite, the corresponding growth expression is [17]:

$$5CO_2 + NH_4^+ + 10NO_2^- + 2H_2O \longrightarrow 10NO_3^- + C_5H_7O_2N + H^+ \quad (2.18)$$

Some of the ammonium ions are used as a nutrient source for nitrogen and are assimilated into new cellular material. The growth of new cells in the activated sludge process leads to an increase in the mixed liquor volatile suspended solids [6, 7]:

$$4CO_2 + HCO_3^- + NH_4^+ + 4H_2O \longrightarrow C_5H_7O_2N + 5O_2 + 3H_2O \quad (2.19)$$

The oxidation of ammonium takes place in several steps while the oxidation from nitrite to nitrate is a single step [18].

$$NH_4^+ \rightarrow NH_2OH \rightarrow \ldots\ldots\ldots \rightarrow NO_2^- \rightarrow NO_3^- \quad (2.20)$$

- *Alkalinity*

Alkalinity and pH effects on nitrification were studied by John W. Shanahan *et al.* (2015) [19]. According to Henze et al. (2000) [20], the equation of reaction for nitrification is expressed as follows:

$$22NH_4^+ + 37O_2 + 4CO_2 + HCO_3^- \longrightarrow C_5H_7NO_2 + 21NO_3^- + 20H_2O + 42H^+ \quad (2.21)$$

- *The influence of environmental factors*

+ *Substrate*

Substrate composition impacts the removal efficiency of NH_4^+-N. The removal efficiency of NH_4^+-N by the isolates was higher at a C/N ratio of 8 than that at C/N ratio of 4. At a C/N ratio of 8, there was not any effect of the substrate composition on the removal efficiency of NH_4^+-N but the effect of substrate composition was significant at the C/N ratio of 4. This indicates that the isolates required high concentrations of organic carbon [21].

+ *Temperature*

The temperature dependence of the maximum growth rate during nitrification is expressed as [22]:

$$\mu_{max,NS} = 0.042 \exp (0.0351\ T - 2.174) \quad (2.22)$$

$$\mu_{max,NB} = 0.042 \exp (0.0587\ T - 1.13) \quad (2.23)$$

The rate of nitrification is a temperature function between 8°C and 30°C. Low wastewater temperatures in winter negatively affect the nitrification [22]. On the other hand, no thermophilic species were identified for nitrification.

+ *Oxygen*

Nitrifying bacteria are strict aerobes. If oxygen is not supplied, the nitrification rate is limited entirely. This means that there is always a limiting effect of the oxygen concentration on the nitrification rate when aerating with air. Oxygen becomes a limiting factor for nitrification when its concentration is lower than 2 $mg.l^{-1}$ [23].

+ *Inhibiting substance*

The inhibition of NOB is critical for shortcut nitrification and denitrification because NOB oxidize nitrite to nitrate and convert partial nitrification to complete nitrification. Many parameters have been found to selectively inhibit NOB, including DO concentration, temperature, SRT, substrate concentration, aeration pattern, and chemical inhibitors [11].

- *Microorganisms of nitrification*

According to Gerardi (2002) [24] although there are many organisms that are capable of oxidizing ammonium ions and nitrite ions, the principal organisms responsible for most, if not all, nitrification in the activated sludge process are two genera of nitrifying bacteria, *Nitrosomonas* and *Nitrobacter*. These genera of bacteria possess special enzymes and cellular structures that permit them

to achieve significant nitrification. Besides the nitrifying bacteria, there are two protozoa (*Epistylis* and *Vorticella*) that are present in relatively large numbers during rapid nitrification [24].

There are several genera of nitrifying bacteria. The genera can be grouped according to those that oxidize ammonium ions and those that oxidize nitrite ions as summarized in Table 2.03 [24, 25].

Table 2.03. Genera of nitrifying bacteria

Energy substrate	Oxidized product	Genera of nitrifying bacteria
$\mathbf{NH_4^+}$	NO_2^-	*Nitrosococcus* *Nitrosocystis* *Nitrosolobus* *Nitrosomonas* *Nitrospira*
$\mathbf{NO_2^-}$	NO_3^-	*Nitrobacter* *Nitrococcus* *Nitrospira*

2.1.3.3. Denitrification

Biological denitrification is a process in which nitrate is reduced to nitrogen gas by microorganisms in the absence of dissolved oxygen. Denitrification can occur provided sufficient sources of nitrate and organic carbon are present [26]. Denitrification process is the use of nitrite ions or nitrate ions by facultative anaerobes (denitrifying bacteria) to degrade cBOD. Denitrification is often combined with aerobic nitrification to remove various forms of nitrogenous compounds from wastewater, denitrification occurs when an anoxic condition exists. Anaerobic respiration occurs when free molecular oxygen is not available and another molecule is used to degrade cBOD. Molecules other than free molecular oxygen that can be used for the degradation of cBOD are dependent on its availability, the presence of other molecules, and the enzymatic ability of the bacterial population.

During anoxic respiration, nitrite and nitrate ions are reduced (oxygen removed from the ions) through several biochemical steps or reaction. The main gaseous product of the biochemical reactions is nitrogen gas [16, 27].

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \qquad (2.24)$$

When nitrite and nitrate ions are reduced to ammonium ions inside the bacterial cell, the nitrogen in the in ammonium ions is incorporated into cellular material. This reduction of nitrogen is termed “assimilatory” nitrite or nitrate reduction.

The specific growth rate of bacteria is influenced by both the concentration of the organic substrate and the concentration of nitrite or nitrate. The kinetics of denitrification can be described by a double Monod kinetic model and an additional term to include the inhibiting effect of dissolved oxygen concentration on denitrification for NO_3^- [6, 22].

- *The influence of carbon on denitrification*

Denitrification is considered to be a heterotrophic process, conducted by microorganisms that require a reduced organic substrate for energy and cell synthesis. Denitrification is achieved with organic compounds in wastewater, also called the internal carbon source. Besides, easily degradable external carbon sources, such as methanol, ethanol, acetic acid and glucose have been used as the electron donor.

- *Microorganisms of denitrification*

Many genera of denitrifying bacteria are able to utilize nitrate or nitrite ions as electron acceptors for cBOD degradation. Some genera, such as Enterobacter and Escherichia, are restricted to nitrate, whereas others, such as Alcaligenes, can utilize only nitrite. When nitrate is reduced only to nitrite without further reduction, nitrite may accumulate in the system. The genera of bacteria that include denitrifying species are summarized in Table 2.04 [12, 24, 25].

Table 2.04. Genera of bacteria that include denitrifying species

Acetobacter	*Bacillus*	*Halobacterium*	*Propionicbacterium*
Achromobacter	*Chromobacterium*	*Hyphomicrobium*	*Pseudomonas*
Acinetobacter	*Corynebacterium*	*Kingella*	*Rhizobium*
Agrobacter	*Denitrobacillus*	*Methanonas*	*Rhodopseudomonas*
Alcaigenes	*Enterobacter*	*Moraxella*	*Spirillum*
Arthrobacter	*Escherichia*	*Neisseria*	*Thiobacillus*
Axotobacter	*Flavobacterium*	*Paracoccus*	*Xanthomonas*

2.1.3.4. Partial nitrification and denitrification

Partial nitrification is the oxidation of wastewater ammonia to nitrite, but not to nitrate. To achieve partial nitrification, the subsequent oxidation of nitrite to nitrate must be prevented. Partial nitrification can be combined with the ANAMMOX process, but even if it is combined with conventional denitrification, already a significant benefit is achieved in terms of use of resources.

- *Influence of DO on partial nitrification*

The oxygen concentration is the most important limiting factor and could be used as a tool for partial nitrification. Low DO concentrations can affect the specific growth rate of both AOB and NOB, depending on their oxygen saturation constants. At low oxygen concentrations the population structure changes, which could affect nitrite accumulation rates.

- *Influence of alkalinity on partial nitrification*

The effect of influent alkalinity on partial nitritation was evaluated by Samik Bagchi *et al.* (2010) [28]. According to the findings in Liang *et al.* (201) [29], the optimal influent alkalinity for achieving 50% partial nitritation in a fixed-bed biofilm system is half of the stoichiometric alkalinity.

2.1.3.5. ANaerobic AMMonium Oxidation

In the ANAMMOX process, ammonium is oxidized under anoxic, i.e. oxygen depleted, conditions with nitrite as electron acceptor. The ANAMMOX process should always be combined with a partial nitritation process, such as the SHARON process, where half of the ammonium is oxidized to nitrite.

Partial nitrification coupled with ANAMMOX has gained a lot of interest in recent years. This partial nitrification – ANAMMOX process stands for a totally autotrophic strategy for the removal of nitrogen, particularly for from wastewater with high nitrogen and low organic contents.

Some advantages of partial – ANAMMOX process are the reduction of oxygen requirements during nitrification, a decrease of the sludge production and dioxide carbon emissions with respect to heterotrophic denitrification, and the possibility of working with higher nitrogen loading rates. The partial nitrification of ammonia to nitrite reduces not only the oxygen requirements for the oxidation, but also the amount of added organic matter for denitrification [30, 31]. Compared to conventional nitrification and denitrification, the aeration and carbon-source demand is reduced by over 50 and 100 %, respectively [29, 32]. Table 2.05 shows difference reactions between conventional ammonium removal and partial nitrification - ANAMMOX process.

Table 2.05. Comparison of conventional ammonium removal and partial-ANAMMOX

Process	**Conventional ammonium removal** [7, 33]	**Partial – ANAMMOX** [33, 34]
Nitrification	$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O$	$NH_4^+ + 1.5O_2 \longrightarrow NO_2^- + 2H^+ + H_2O$

Process	Conventional ammonium removal [7, 33]	Partial – ANAMMOX [33, 34]
Denitrification /Anammox	$2NO_3^- + 8g\ COD + 2H^+ \longrightarrow N_2 + 3g\ sludge$	$NO_2^- + NH_4^+ \longrightarrow N_2 + 2H_2O$
Total overall equation	$2NH_3 + 4O_2 + 8g\ COD \longrightarrow N_2 + 3g\ sludge$	$2NH_4^+ + 1.5O_2 \longrightarrow N_2 + 2H^+$

2.2. MEMBRANE BIOREACTOR

2.2.1. Membrane fundamentals

2.2.1.1. Membrane and membrane separation process

The membrane can be considered essentially as a barrier, which separates two phases and transports in a selective manner of various chemicals. Transport through a membrane can be by convection or by diffusion of individual molecules, induced by an electric field or concentration, pressure or temperature gradient. The membrane thickness may vary from as small as 100 microns to several millimeters [35]. Membrane separation process overview[10] is shown in Figure 2.02.

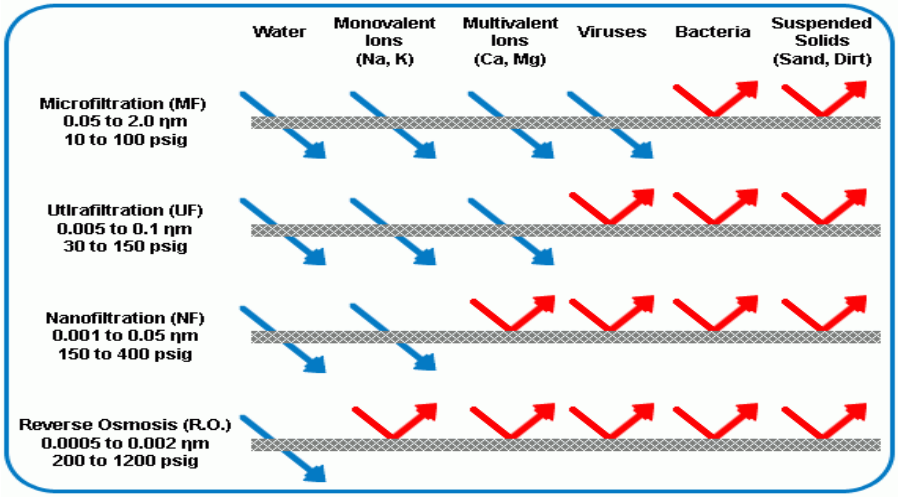


Figure 2.02. Membrane separation process overview

2.2.1.2. Membrane materials and characteristics

There are mainly two different types of membrane material, these being polymeric and ceramic. Metallic membrane filters also exist, but these have very specific applications which do not relate to MBR technology [35, 36]. Materials and characteristics are summarized in Table 2.06.

[10] http://www.aguayuda.org/index.php/solutions/water/

Table 2.06. Materials and characteristics

Anopore (ANP) (membrane used in Anotop filters)	Anopore is a hydrophilic membrane with excellent organic solvent compatibility. Suitable for use with both aqueous and organic samples. The membrane has very narrow pore size distribution. Not suitable for use with very acidic or very basic samples
Cellulose acetate (CA)	Hydrophilic membrane. Limited solvent resistance. Very low protein binding capacity, which makes it an excellent choice for protein recovery applications.
Cellulose nitrate (CN)	Hydrophilic membrane. Limited resistance to organic solvents. High liquid flow rate. High protein binding capacity, which makes it unsuitable for protein recovery applications.
Nylon/polyamide (NYL)	Hydrophilic membrane. Resistant to a range of organic solvents. Suitable for use with high pH samples. Binds proteins, which makes it unsuitable for protein recovery applications
Polycarbonate (PC)	Hydrophilic membrane. Manufactured from thin polycarbonate film, this membrane has a very narrow pore size distribution suitable for aqueous and some organic solvents
Polyethersulfone (PES)	Hydrophilic membrane. Broad solvent compatibility. Suitable for filtration of aqueous and compatible organic solvents. Higher liquid flow than either PTFE or PVDF. Low in extractables. Low protein binding
Polypropylene (PP)	Slightly hydrophobic membrane. Resistant to a wide range of organic solvents.
Polytetrafluoroethylene (PTFE)	Hydrophobic membrane. Resistant to organic solvents as well as strong acids and bases. Low protein binding. Low in extractables. Main applications are the filtration of non-aqueous samples. Prior to filtering of aqueous samples, the membrane must be pre-wetted with a water miscible organic solvent.

PVDF	Hydrophilic membrane. Resistant to a broad range of organic solvents. Low protein binding
Regenerated cellulose (RC)	Hydrophilic membrane. Resistant to a very wide range of solvents. Suitable for use with either aqueous solutions or organic solvents. Compatible with HPLC solvents. Very low protein binding capacity, which makes it an excellent choice for protein recovery applications

2.2.1.3. Membrane configuration

There are three different configurations in terms of the shape of membrane as: flat sheet, tubular, and hollow fiber [37]. Membrane configurations [11] are shown in Figure 2.03.

(a) Flat (sheet) (b) Tubular (c) Hollow fiber

Figure 2.03. Membrane configurations

Characteristics of tubular membranes, flat sheet membranes and hollow fiber membranes are summarized in Table 2.07 [38].

Table 2.07. Characteristics of tubular, flat sheet and hollow fiber membranes

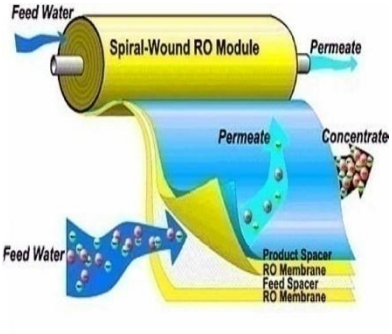
Characteristics	**Tubular membranes**	**Flat sheet membranes**	**Hollow fiber membranes**
Arrangement	External – recycling	External/submerged	External/submerged
Packing density	Low	Moderate	High
Energy demand	High (turbulent flow)	Low-moderate (laminar flow)	Low
Cleaning	Efficient + physical cleaning possible	Moderate	Back washing possible
Replacement	Tubes or element	Sheet	Element

[11] http://onlinembr.info/Membrane%20process/Membrane%20Process.html

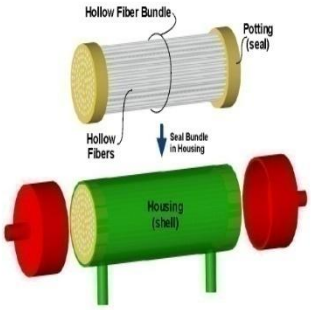
Membranes can be configured into membrane modules in different ways. Flat sheet membranes are used to construct spiral wound modules or they can be mounted on a frame, resulting in the plate and frame modules. Tubular membranes are usually anisotropic membranes with the separating layer on the inside. Hollow fiber membranes are often isotropic membranes that can be operated inside out or outside in. Submerged hollow fiber can be oriented horizontally or vertically; for application in MBR where air scouring is applied, vertical orientation seems favorable. Examples of membrane and module configuration [39] are shown in Figure 2.04.

(a) Plate and frame

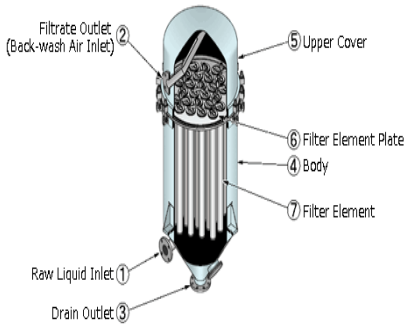
(b) Immersed or submerged



(c) Spiral-wound



(d) Hollow fiber



(e) Tubular

Figure 2.04. Examples of membrane and module configuration

For the treatment of suspensions, flat sheet, tubular and capillary membranes (hollow fibers) are preferred. In recent years, membrane processes have found wide application and nowadays membrane processes exist for most of the fluid separations encountered in industry. Membrane and module configurations is summarized in Table 2.08 [37].

Table 2.08. Summary of membrane and module configurations

Membrane configuration	Module configuration or operating method	Driving force	Pore size	Common Applications
Flat Sheet (FS)	Plate and frame (PF)	Pressure	MF/UF	WWT, ED[12]
	Immersed membranes	Vacuum	MF/UF	iMBR

[12] Electrodialysis

Membrane configuration	Module configuration or operating method	Driving force	Pore size	Common Applications
	Spiral wound (SW)	Pressure	UF/RO	DS[13], PR[14]
Hollow Fiber (HF)	Contained in pressure vessels	Pressure	MF/UF/RO	WT[15], PR, etc.
	Immersed module without pressure vessels	Vacuum	MF/UF	WT, iMBR
	Pressure filtration	Pressure	MF/UF	WWT[16], PR, sMBR
Tubular (TB)	Vacuum filtration with bubbling	Vacuum	MF/UF	sMBR

2.2.1.4. Fouling/cake layer mass transfer

Fouling rate in a membrane process is dependent on two factors, namely the permeate flux and the fouling potential of the feed water [40]. The flux can be expressed as [41]:

$$J = \frac{\Delta p}{\mu R_m} \quad (2.25)$$

Where

- J the flux; m/s
- Δp the transmembrane pressure; bar
- μ the fluid viscosity; $kg.m^{-1}.s^{-1}$
- R_m the resistance of the membrane; $m.kg^{-1}$

Membrane fouling occurs through one or more of the following mechanisms: accumulation of solute and gradual irreversible changes to the polarized layer (such as cake formation); surface adsorption/deposition of solutes; and adsorption/deposition of solute within the membrane.

Membrane fouling MBRs is attributed to the physicochemical interaction between the biofluid and membrane. As soon as the membrane surface comes into contact with the biological suspension, deposition of biosolids onto it take place leading to flux decline [42].

There are some types of membrane fouling:

[13] Desalination

[14] Process Recovery

[15] Water Treatment

[16] Wastewater Treatment

- Inorganic fouling is caused by the accumulation of inorganic precipitates, for example metal hydroxides on membrane surface or with pore structure. Precipitates are formed when the concentration of chemical species are exceeding their saturation concentrations [43, 44].
- Organic fouling is caused by the deposition of proteins, polysaccharides, humic acids and other organic substances (either soluble or colloidal) originating from feed water or microbial secretion. In particular, a gel layer can be formed in MBRs with sub-critical flux operation due to continuous organic fouling [45, 46].
- Fouling phenomena by natural organic matters such as algae, bacteria fall into the size range of particle and colloids [45, 47].
- Microbial fouling is a result of formation of biofilms on membrane surfaces. Once bacteria attach to the membrane, they start to multiple and produce extracellular polymeric substances to form a viscous, slimy, hydrated gel. Extracellular polymeric substances typically consist of heteropolysaccharides and have negative charge density [48, 49].

Classification of fouling is adapted from Kraume *et al.,* and factors affecting are described in Figure 2.05 [36, 42, 50].

Table 2.09. Classification of fouling

Definition (with preferred term)	Fouling rate (mbar/min)	Time interval	Cleaning method applied
Cake, reversible or removable	0.1 – 1	10 min	Physical cleaning (e.g. relaxation, backflush)
Residual fouling	0.01 – 0.1	1 – 2 weeks	Maintenance cleaning (e.g. chemical enhanced backflush)
Irreversible fouling	0.001 – 0.01	6 – 12 months	Chemical cleaning
Permanent, long-term or irreversible fouling	0.0001 – 0.001	Several years	Cannot be removed

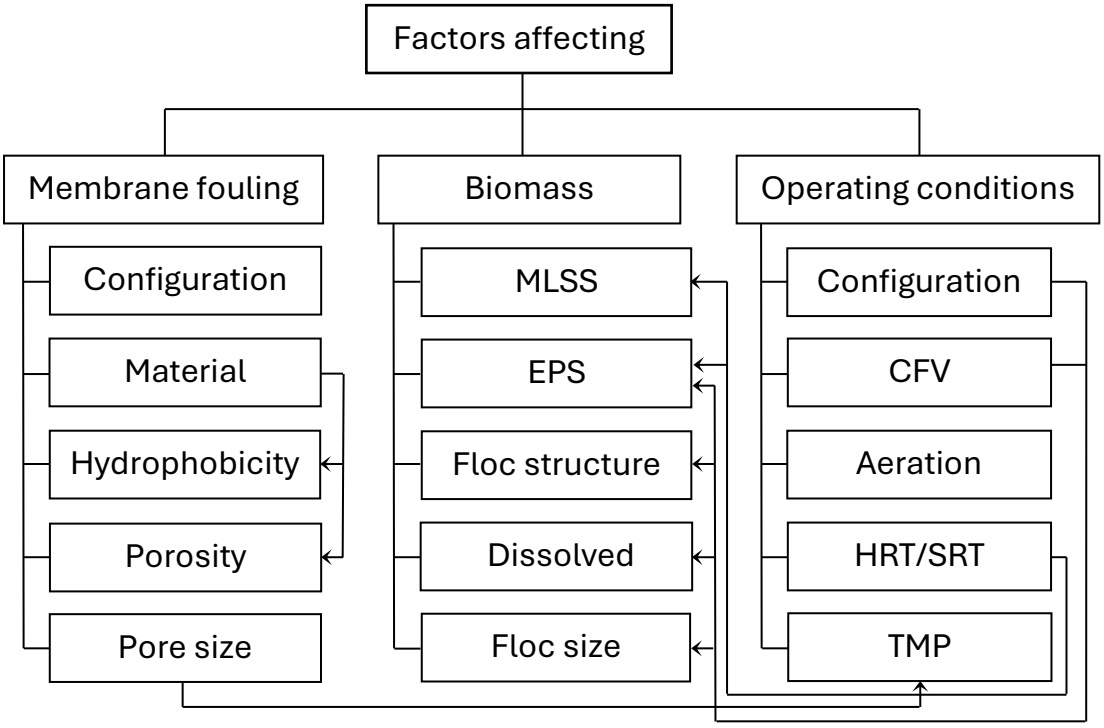


Figure 2.05. Schematic diagrams of factors influencing membrane fouling membrane bioreactor process

2.2.2. Biological treatment

2.2.2.1. Biological treatment rationale

Biological treatment processes remove suspended organic compounds through biodegradation, as well as suspended matter through physical separation [51, 52]. Membrane biological reactors consist in a biological reactor with suspended biomass and solids separation by microfiltration membranes with nominal pore sizes ranging from 0.1 to 0.4 µm. These are found in many applications in wastewater and leachate treatment. Membrane biological reactor systems may be used with aerobic or anaerobic suspended growth bioreactors to separate treatment wastewater from the active biomass [53]. The MBR process consists of a suspended growth biological reactor with a membrane unit either located external to the bioreactor or mounted directly within called submerged [42].

Table 2.10. Examples of biological processes and their characteristics

	Process configuration		Feeding regime		Redox conditions		
	Fixed film	Suspended growth	Continuous	Fed-batch	Aerobic	Anoxic	Anaerobic
AD[17]		x	(x)	(x)			x
AF[18]	x		x				x
ASP[19]		x	x		x	x	x
BAF[20]	x		x		x		
RBC[21]	x		x		x		
SBR[22]		x		x	x	x	
TF[23]	x				x		
UASB[24]		x	x				x
MBR[25]		x	x		x	x	

2.2.2.2. Microbiology

Biological treatment is the conversion of organic and inorganic matter into innocuous products by micro-organisms [51]. Microbial metabolism types in wastewater biotreatment are summarized in Table 2.11 [36].

Table 2.11. Microbial metabolism types in wastewater biotreatment

Component	Process	Electron acceptor	Type
Organic-carbon	Aerobic biodegradation	O_2	Aerobic
Ammonia	Nitrification	O_2	Aerobic
Nitrate	Denitrification	NO_3^-	Facultative
Sulphate	Sulphate reduction	SO_4^{2-}	Anaerobic
Organic-carbon	Methanogenesis	CO_2	Anaerobic

[17] AD: anaerobic digestion

[18] AF: anaerobic filter

[19] ASP: activated sludge process

[20] BAF: biological aerated filter

[21] RBC: rotating biological contactor

[22] SBR: sequencing batch reactor

[23] TF: trickling filter

[24] UASB: up-flow anaerobic sludge blanket

[25] MBR: membrane bioreactor

2.2.2.3. Nutrient removal

In the biochemical stage of wastewater treatment, nitrogen is removed from wastewater by microbes. These microbes live and growth enmeshed in EPS (Extracellular polymeric substances) that bind them into discrete micro-colonies forming three-dimensional aggregated microbial structures called flocs [54]. MBR technology allows obtaining similar or higher BNR rates than with conventional activated sludge systems even if working with a high loading rate, and always with a much better effluent quality in terms of suspended solids [55, 56].

2.2.2.4. Membrane system configurations

MBR system can be classified into two categories according to the location of the membrane component [57]. The first is a recirculated configuration with an external membrane unit. Sludge and water are separated by pressure outside. The concentrated sludge is then recycled back into the reactor. The second is submersed in the activated sludge to draw the water through the membrane, while the sludge is retained on the membrane surface [58]. Comparison of external and internal membrane based MBR system configurations is summarized in Table 2.12 [57].

Table 2.12. Comparison of external and internal membrane based MBR system configurations

Comparative Factor	External MBR Systems	Internal MBR Systems
Membrane Area Requirement	Characterized by higher flux and therefore lower membrane area requirement.	Lower flux but higher membrane packing density (i.e., membrane area per unit volume)
Space or Footprint Requirements	Higher flux membranes with bioreactor operating at higher VSS concentration and skidded assembly construction, results in compact system.	Higher membrane packing density and operation at bioreactor VSS concentration of 10 g/l or greater translates to compact system. yielding compact systems.
Bioreactor and Membrane Component Design and Operation Dependency	Bioreactor can be designed and operated under optimal conditions including those to achieve biological N and P removal, if required.	Design and operation of bioreactor and membrane compartment or tank are not independent. High membrane tank recycles required

Comparative Factor	External MBR Systems	Internal MBR Systems
Membrane Performance Consistency	Less susceptible to changes wastewater and biomass characteristics.	More susceptible to changes wastewater and biomass characteristics requiring alteration in membrane cleaning strategy and/or cleaning frequency.
Recovery of Membrane Performance	Off-line cleaning required every 1 to 2 months. Simple, automated procedure normally requiring less than 4 hours.	Off-line "recovery" cleaning required every 2 to 6 months. A more complex procedure requiring significantly more time and manual activity, at least on occasion may be required (i.e., physical membrane cleaning).
Membrane Life or Replacement	Requirements Results to-date imply an operating life of 7 years or more can be achieved with polymerics prior to irreversible fouling. Operating life of ceramics much longer.	Results to-date imply an operating life of 5 years may be possible prior to irreversible fouling and/or excessive membrane physical damage.
Full Scale Application Status	Conventional membrane based systems have a very long track record. Few nonconventional systems in operation in the U.S.	Full scale application widespread in the U.S.
Economics	Non-conventional designs translate to comparable power costs. Comparable capital cost at least at lower wastewater feed rates	Power and capital cost advantage at higher wastewater feed rates.

2.2.2.5. Membrane characteristics

The effects of pore size (and distribution of pore size) on membrane fouling are strongly related to the feed solution characteristics and in particular the particle size distribution. Depending on the pore size and the type of biomass filtered, results reported in the literature have shown opposite trends. Pore blocking typically occurs when particle size exceeds pore size, while smaller particles may still cause partial restriction if they accumulate within the pores [43]. Effect of pore size on MBR hydraulic performances is summarized in Table 2.13.

Table 2.13. Effect of pore size on MBR hydraulic performances

Membranes tested	Optimum	Test duration	Other	Ref.
0.1, 0.22, 0.45µm	0.22µm	20 h	-	[59]
20, 30, 50, 70 kDa	70 kDa 50 kDa	110 min 110 days	Concentrated feed, anaerobic	[60]
70 kDa, 0.3µm	70 kDa	8 h	-	[61]
30 kDa, 0.3µm	30 kDa 0.3 µm	2 h	CFV = 0.1 m/s CFV = 3.5 m/s	[62]
200 kDa, 0.1, 1µm	1 µm	3 h	Flux-step test	[63]
0.3, 1.5, 3, 5 µm	5 µm 0.3 µm	25 min 45 days	-	[64]
0.4, 5 µm	0.4 µm No effect	1 day From 50 days	-	[65]
0.01, 0.2, 1 µm	No effect	A few hours	Flux-step test	[66]
200 kDa, 0.1, 1 µm	0.1 µm	n/a	Anaerobic	[50]

2.2.2.6. Feed and biomass characteristics

Activated sludge is a complex and variable heterogeneous suspension containing both feed water components, substrates and metabolites produced during the biological reactions as well as the biomass itself [42]. EPS are the polymolecular substances produced by microorganisms under specific conditions. In an activated sludge system, EPS mainly contain two forms, soluble EPS and bound EPS according to their location around the cell [42, 67]. Production of EPS in MBR process can be influenced by several factors namely: SRT, temperature, aeration and pH [67, 68].

EPS can cause MBRs fouling because of surface attachment and pore intrusion. Therefore, cleaning of membrane with both physical and chemical methods is essential in order to improve MBR performance [67].

The MBR fouling rate increased with SRT, presumably due to large amount of foulants and high fluid viscosity. Thus higher air flow intensity was needed for fouling control at prolonged SRT [68].

2.2.3. Membrane advantages

- *High biomass concentration:* Complete separation between the HRT and SRT provides optimum control of biological reactions and greater reliability and flexibility in use than settling tanks [54]. The MBR allows the biomass

concentrations to be higher than for traditional treatment plants (from 20 to 30 $g.l^{-1}$).

- *Disinfectant properties:* these systems are capable of treating simultaneously biologically and disinfecting the effluent. In membrane filtration, the removal of bacteria and viruses can be achieved without any chemical addition. All the process equipment can also be tightly closed; no odor dispersion occurs.
- *Sludge age control:* Complete control of the sludge age is important to allow the development of slow growing microorganisms such as nitrifying bacteria. Excess sludge from MBR process is much lower than that of conventional activated sludge process about one fifth fold. Low F/M ratio and longer sludge age (from 50 to 100 days) in the reactor may be the reason for low sludge production rate. In addition, the microscopic observation of microorganism populations indicates that with increased sludge age, reduction in filamentous bacteria increased rotifers and nematodes that contribute to reduce the sludge production by predation. Ability to absorb variations and fluctuations in the hydraulic and organic load to the system. Process intensification is obtained through high biomass concentrations and reduced sludge production compared to other aerobic processes.
- *High rate decomposition:* MBR can prevent undecomposed polymer substances, so treatment efficiency is improved significantly. If these polymers are biodegradable, they can be broken down with a reduction in the accumulation of substances within the treatment process. The low molecular weights or dissolved organic substances can be eliminated by membrane separation alone and broken down and gasified by microorganisms or produced new bacteria cells. Most MBR studies indicate the effluent BOD_5 is below 5 $mg.l^{-1}$.
- *Treated water quality:* in conventional technology, treated water quality strongly depends on settling ability in sedimentation tank. MBR can separate solid and liquid by membrane filtration process. Therefore, the final effluent does not contain suspended matter, and almost no bacteria. This enables the direct discharge of the final effluent into surface water and the reuse of the effluent for cooling, toilet flushing, lawn watering or process water.
- *Flexibility in operation:* SRT can be controlled completely independent from HRT. So the system can be run at very long SRT providing favorable conditions for the growth of slow-growing microorganisms, which are able to degrade bio-refractory compounds.

- *Compact plant size:* Because the MBR process is independent upon sludge settling quality, high biomass concentration can be maintained up to 40 $g.l^{-1}$ in the reactor. Even if it has been demonstrated that in many cases such a high biomass concentration is not necessary. Therefore, the size of the bioreactor is reduced because of capacity of high volumetric loading rate. In addition, secondary settling tank, filter, sludge thickener or post treatment for further BOD, SS removal are not necessary in MBR process, thus the plant becomes more compact.
- *Advantages over immerse systems:*
  + No fine Bubble Diffuser Maintenance;
  + Easier to clean membranes chemically without upsetting the biological process in the tanks;
  + No unnecessary movement, hence less stress on hollow fibers during permeation process;
  + Better ability to control environment around the membrane modules;
  + Higher fluxes with smaller space requirement using less membrane surface area.

2.3. BIOREACTOR MODELISATION AND SIMULATION

2.3.1. Introduction

Bio-process modelling is increasingly used in design, modification and troubleshooting of WWTPs [69]. Process models for WWTPs find application in forecasting, fault detection, monitoring plant operations and research [70].

A Task Group on Mathematical Modelling for Design and Operation of Activated Sludge Processes was established by the International Association on Water Pollution Research and Control in 1982.

At that time, the research group headed by Prof. Marais at the University of Cape Town, South Africa researched and achieved significant success in modelling of activated sludge processes [71]. Today, they are known under many names: ASM1, ASM2, ASM2d, ASM3, etc. ASM models are summarized in Table 2.14 [18, 37, 71].

Table 2.14. ASM models

	ASM1	ASM2	ASM2d	ASM3
Published	1987	1995	1999	1999
Components	COD, TN	COD, TN, TP	COD, TN, TP	COD, TN, TP
Remark	New comprehen sive model	Biological phosphorus removal was added to ASM1	Role of PAO in denitrification was added to ASM2	New discoveries were added to ASM1

The ASM1 and ASM3 models enable the simulation of the processes of removal of organic compounds and nitrogen compounds from sludge taking into account unit processes occurring both in the wastewater and the active sludge [71, 72].

2.3.2. Activated sludge model N°1 (ASM1) and N°3 (ASM3)

In 1983, A working group destined to promote and facilitate the practical methods of designing and operating the biological wastewater treatment systems was formed by International Water Association (IWA) [73].

As a result, the ASM1 has been presented in 1987 [71, 74]. ASM1 describes an activated sludge system with carbon oxidation, nitrification, and denitrification.

The models used 13 state variables and described the elimination of organic carbon and nitrogen [75]. The structure of the ASM is based on ordinary differential equations. These equations describe the dynamic changes in COD and nitrogen through the process. To define the different components in the model, COD and nitrogen are divided into fractions that are represented by state variables. There are also kinetic and stoichiometric parameters to describe the dynamic changes in the model. The quantification of the total organic matter in wastewater and its characterization is of primary importance for the correct design, management and optimization of a WWTP [76]. In particular, interest in biodegradability characterization has been increased from the simulation models for the activated sludge process that do not use traditional parameters.

There are 13 components and 8 important reactions in ASM1 and 13 components and 12 processes in the ASM3 [77]. which are carried out by two types of microorganisms: heterotrophs and autotrophs both under aerobic and anoxic conditions and decay reaction. THREE other reactions take place: ammonification of soluble organic nitrogen, hydrolysis of entrapped organics and of entrapped organic nitrogen [78].

2.3.3. State variables in ASM1

2.3.3.1. Soluble components (S)

- *Inert soluble organic matter, S_I*

The inert organic matter is present in the influent but also produced during the activated sludge process. S_I made up of dissolved non-biodegradable molecules. It is calculated as the difference between S (Soluble COD) and S_S (Soluble readily biodegradable organic matter) [76]. It can also be determined as the soluble fraction of COD, COD_{sol}, remaining after long-term BOD test with the influent wastewater [77]. The inert organic matter in the influent is equal to the filtered COD in the effluent [79].

- *Readily biodegradable organic matter, S_S*

This fraction of the soluble COD is directly available for consumption by heterotrophic organisms [15]. It is consumed by growth of heterotrophic bacteria (both under aerobic and anoxic conditions); it is the hydrolysisresult of slowly biodegradable matter entrapped in the biofloc, besides being introduced through the influent wastewater. S_S is preferentially determined with the aid of a bioassay (respiration test) [80, 81].

- *Dissolved oxygen, S_O*

Oxygen concentration is consumed by aerobic growth of both heterotrophic and autotrophic bacteria; no addition of oxygen in the reactor is modeled [71] in the ASM models themselves.

- *Nitrate nitrogen fraction, nitrate (and nitrite), S_{NO}*

After oxygen, nitrates and nitrites are the second electron acceptors in the model. They are consumed for energy by growth of facultative heterotrophic bacteria and it is formed as a result of autotrophs growth under aerobic condition. In the original ASM1 and ASM3 models, for sake of simplicity, the model assumes that nitrification of ammonium nitrogen is one single step process and that nitrate nitrogen is the only oxidized form of nitrogen.

- *Soluble ammonium nitrogen, Ammonia, S_{NH}*

It is assumed to include both ionized and un-ionized forms of ammonium. It derives from ammonification of soluble organic nitrogen and it is used for energy by growth of autotrophic nitrifying bacteria. In addition, it is assimilated into new cells during both heterotrophic and autotrophic cell synthesis.

- *Alkalinity, S_{AKL}*

In the original ASM1 and ASM3 models, alkalinity was not indispensable in the description of substrate removal and neither affected other processes in the model, but it may help for checking the variation of pH as itimpacts processes of addition or removal of protons. The models (ASM1 and ASM2) consider the influence on alkalinity of these processes such as ammonification and conversion of ammonia to amino acids, denitrification that produces an increase of alkalinity and nitrification that has the significant impact with the net release of two protons per nitrogen, thus decreasing alkalinity.

- *Soluble biodegradable nitrogen fraction, S_{ND}*

It is converted to ammonia by ammonification and is the result of hydrolysis of particulate organic nitrogen.

- *Dinitrogen S_{N2}*

S_{N2} is assumed to be the only product of denitrification. S_{N2} may be subject to gas exchange, parallel with oxygen andnitrogen dioxide. It can then be used to predict problems due to supersaturation with nitrogen gas in secondary clarifiers. Alternatively, the N_2 contained in the influent and gas exchange can be neglected. S_{N2} may then be used to calculate the amount of nitrogen lost due to denitrification. S_{N2} has a negative ThOD (Theoretical Oxygen Demand).

2.3.3.2. Particulate components (X)

- *Inert suspended organic matter, X_I*

This material is not degraded in the activated sludge systems. It is flocculated onto the activated sludge. X_I maybe a fraction of the influent and is produced in the context of biomass decay [71, 77]. The X_I concentration could be determined as the particulate COD remaining after long-term BOD test [79, 82]. The assumption that no X_I is produced during the test has to be made to use this method. The assumption may be questionable since X_I will be produced due to decay during the BOD test and corrections for this must be considered [79]. In 1996 STOWA (Dutch Foundation for Applied Water Research) made guidelines for characterization of wastewater and defined X_I as the difference between particulate COD and X_S [77].

- *Slowly biodegradable substrate, X_S*

Slowly biodegradable substrates are high molecular weight, soluble, colloidal, and particulate organic substrates, which must undergo cell external hydrolysis before they are available for degradation. It is assumed that the products of

hydrolysis of X_S are either readily biodegradable (S_S) or inert (S_I) soluble organics [6, 71].

- *Heterotrophic biomass, X_{BH}*

These organisms are responsible for hydrolysis of particulate substrates X_S and can metabolize all degradable organic substrates. They can form organic storage products in the form of poly-hydroxy alkanoates or glycogen [15, 71].

- *Autotrophic biomass, X_{BA}*

It is assumed to grow under only aerobic condition and is destroyed by decay [71]. Ammoniacal nitrogen is oxidized to nitrate also resulting in production of autotrophic biomass in wastewater. The presence of autotrophic biomass may be of importance to prevent wash out of the nitrifiers in wastewater. The X_{BA} concentration can in principle be determined by experiments in lab-scale, but in practice the procedure may not be simple and X_{BA} may rather be adjusted during the model calibration.

- *Particular production from biomass decay, X_P*

It is an inert particulate result of biomass decay. As it will be explained in more detail in the next section, decay of both heterotrophic and autotrophic bacteria is assumed to generate two fractions: X_S and X_P. In ASM1 X_S re-enters the cycle of hydrolysis, conversely, X_P is not further transformed and accumulates in the system as inert particulate. This assumption may not reflect reality; however, it is introduced in the model to take into account the fact that not all biomass is active [71].

- *Particulate biodegradable nitrogen fraction, X_{ND}*

It is hydrolysed to S_{ND}, concomitantly together with the hydrolysis of slowly biodegradable COD. It increases in parallel with the decay of heterotrophic and autotrophic biomass [71].

- *Cell internal storage products of heterotrophic organisms, X_{STO} (ASM3)*

It includes poly-hydroxy-alkanoates, glycogen, etc. It occurs only associated with X_H (Heterotrophic active biomass); it is, however, not included in the mass f_{XH}. X_{STO} cannot be directly compared with analytically measured PHA or glycogen concentrations; X_{STO} is only a functional compound required for modeling but not directly identifiable chemically. X_{STO} may, however, be recovered in COD analysis and must satisfy ThOD conservation.

- *Suspended solids, X_{SS}*

To compute biokinetic concentration via stoichiometry, suspended solids are introduced in models. Treatment plant operators typically follow SS in day to

day analysis. In the influent, S_S ($X_{SS,0}$) include an inorganic fraction of SS and the 'soluble' fraction of $X_{S,0}$, which passes membrane filters. SS measured in the influent are therefore smaller than $X_{SS,0}$ used to describe the influent in the terms of the model compounds. Describing influent SS correctly should allow predicting MLSS as observed in the activated sludge reactors. in ASM3, if chemicals are added in order to precipitate phosphorus, the precipitates formed must be added to the concentration of Suspended Solids computed in the influent ($X_{SS,0}$). Alternatively, X_{SS} may be used to model Volatile Suspended Solids [18, 71, 72]. ASM state variables are summarized in Table 2.15.

Table 2.15. ASM state variables ASM1 or ASM3

State Variable	Symbol	Unit	Model	
			ASM1	ASM3
Soluble inert organic matter	S_I	$gCOD.m^{-3}$	x	x
Readily biodegradable substrate	S_S	$gCOD.m^{-3}$	x	x
Dissolved oxygen	S_O	$gO_2.m^{-3}$	x	x
Nitrite and nitrate nitrogen	S_{NO}	$gN.m^{-3}$	x	x
Free and ionized ammonia	S_{NH}	$gN.m^{-3}$	x	x
Soluble biodegradable organic nitrogen	S_{ND}	$gN.m^{-3}$	x	-
Dinitrogen, released by denitrification	S_{N2}	$gN.m^{-3}$	-	x
Alkalinity	S_{ALK}	Molar units	x	x
Particulate inert organic matter	X_I	$gCOD.m^{-3}$	x	x
Slowly biodegradable substrate	X_S	$gCOD.m^{-3}$	x	x
Active heterotrophic biomass	X_{BH}	$gCOD.m^{-3}$	x	x
Active autotrophic biomass	X_{BA}	$gCOD.m^{-3}$	x	x
Particular production from biomass decay	X_P	$gCOD.m^{-3}$	x	-
Particular biodegradable organic nitrogen	X_{ND}	$gN.m^{-3}$	x	
Organics stored by heterotrophs	X_{STO}	$gCOD.m^{-3}$	-	x
Total suspended solids	X_{SS}	$gSS.m^{-3}$	-	x

2.3.4. ASM processes

2.3.4.1. ASM1 processes

- *Aerobic growth of heterotroph*

The *aerobic growth of heterotrophs* occurs at the expense of soluble substrate utilizing oxygen and results in a production of heterotrophic biomass. The growth is modeled using Monod kinetics, which is assumed to be subject to double nutrient limitation their rate depending on the concentration of both S_S and S_O. In general, this process produces new biomass and removal of COD. Ammonia is used as nitrogen source for synthesis and is incorporated into the cell mass.

The degradation of soluble readily biodegradable substrate under consumption of oxygen leads to growth of heterotrophic biomass. This process is in general the main contributor to the production of biomass and removal of COD. The process is described by Monod function [71]:

$$\mu_H \cdot \frac{S_S}{K_S+S_S} \cdot \frac{S_O}{K_{O,H}+S_O} \cdot X_{BH} \qquad (2.26)$$

Where

μ_H maximum specific growth rate for heterotrophic biomass; $1.d^{-1}$

K_S substrate half saturation coefficient for heterotrophic biomass; $gCOD.l^{-1}$

K_{OH} oxygen half saturation coefficient for heterotrophic biomass; $gO_2.l^{-1}$

- *Anoxic growth of heterotrophs*

The *anoxic growth of heterotrophs* occurs in absence of dissolved oxygen with nitrate as the terminal electron acceptor, with S_S as substrate and resulting in heterotrophs biomass. The same Monod kinetics used in the aerobic growth are applied, except that the maximum rate of substrate is less under anoxic conditions. For this reason, the kinetic rate expression is multiplied by a factor $\eta_g < 1$. Ammonia serves as nitrogen source for cell synthesis.

$$\mu_H \cdot \frac{S_S}{K_S+S_S} \cdot \frac{K_{OH}}{K_{OH}+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}} \cdot \eta_g \cdot X_{BH} \qquad (2.27)$$

Where

η_g correction factor for μ_H under anoxic conditions

K_{NO} nitrate half-saturation coefficient for heterotrophic biomass; $gN.l^{-1}$

- *Aerobic growth of autotrophs*

In *aerobic growth of autotrophs*, S_{NH} serves as the energy source for growth of the nitrifiers, resulting in autotrophic cell mass and nitrate nitrogen as products.

This process is associated to the oxygen demand and once again the growth rate is modeled using Monod kinetics.

$$\mu_A \cdot \frac{S_{NH}}{K_{NH}+S_{NH}} \cdot \frac{S_O}{K_{OA}+S_O} \cdot X_{BA} \quad (2.28)$$

Where

μ_A maximum specific growth rate for autotrophic biomass; $1.d^{-1}$

K_{NH} ammonia half-saturation coefficient for autotrophic biomass; $gN.l^{-1}$

K_{OA} oxygen half saturation coefficient for autotrophic biomass; $gO_2.l^{-1}$

- *Decay of heterotrophs*

The *decay of heterotrophs* is modeled on the death – regeneration approach. The organisms die at a certain rate and a portion of the material is considered to be nonbiodegradable adding up to the X_P fraction. The remainder adds up to X_S. Organic nitrogen associated with X_S becomes available as particulate organic nitrogen.

This process rate considered is:

$$b_H . X_{BH} \quad (2.29)$$

Where

b_H decay coefficient for heterotrophic biomass; 1/d

- *Decay of autotrophs*

The *decay of autotrophs* takes exactly the same modeling approach than the decay of the heterotrophs.

This process rate considered is:

$$b_A . X_{BA} \quad (2.30)$$

Where

b_A decay coefficient for autotrophic biomass; 1/d

- *Ammonification of soluble organic nitrogen*

The *ammonification of soluble organic nitrogen* regards the conversion of S_{ND} into S_{NH} by a first order process mediated by active heterotrophs.

$$k_a . S_{ND} . X_{BH} \quad (2.31)$$

Where

k_a ammonification rate; $1/gCOD^{-1}.d^{-1}$

- *Hydrolysis of entrapped organics*

In *the hydrolysis of entrapped organics*, slowly biodegradable substrate trapped in the sludge mass is broken down, producing S_S for the organisms to growth. The process is modeled on the basis of reaction kinetics and occurs in aerobic and anoxic environments. The rate of hydrolysis is reduced under anoxic conditions compared to aerobic conditions by a factor $\eta_g < 1$.

$$k_h \cdot \frac{\frac{X_S}{X_{BH}}}{K_X + \left(\frac{X_S}{X_{BH}}\right)} \cdot \left(\frac{S_O}{K_{OH}+S_O} + \eta_b \cdot \frac{K_{OH}}{K_{OH}+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}}\right) \cdot X_{BH} \qquad (2.32)$$

Where

K_h maximum specific hydrolysis rate; gCOD.gCOD biomass^{-1}.d^{-1}

μ_b correction factor for hydrolysis under anoxic conditions

K_X hydrolysis half-saturation coefficient; gCOD.gCOD biomass^{-1}

- *Hydrolysis of entrapped organic nitrogen*

In *the hydrolysis of entrapped organic nitrogen*, X_{ND} is broken down to soluble organic nitrogen at a rate defined by the hydrolysis reaction for entrapped organics.

$$k_h \cdot \frac{\frac{X_S}{X_{BH}}}{K_X + \left(\frac{X_S}{X_{BH}}\right)} \cdot \frac{X_{ND}}{X_S} \cdot \left(\frac{S_O}{K_{OH}+S_O} + \eta_b \cdot \frac{K_{OH}}{K_{OH}+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}}\right) \cdot X_{BH} \qquad (2.33)$$

Table 2.16. ASM1 basic processes

Process	**Basic Reaction**
Aerobic growth of heterotrophs	$S_S + S_O + S_{NH} \longrightarrow X_{BH}$
Anoxic growth of heterotrophs	$S_S + S_{NO} + S_{NH} \longrightarrow X_{BH}$
Aerobic growth of autotrophs	$S_O + S_{NH} \longrightarrow X_{BA} + S_O$
Decay of heterotrophs	$X_{BH} \longrightarrow X_P + X_S + X_{ND}$
Decay of autotrophs	$X_{BA} \longrightarrow X_P + X_S + X_{ND}$
Ammonification of soluble organic nitrogen	$S_{ND} \longrightarrow S_{NH}$
Hydrolysis of entrapped organics	$X_S \longrightarrow S_S$
Hydrolysis of entrapped organic nitrogen	$X_{ND} \longrightarrow S_{ND}$

2.3.4.2. ASM3 processes

Heterotrophic organisms, aerobic and denitrifying activity

- *Hydrolysis*

This process makes available all slowly biodegradable substrates X_S contained in the influent to an activated sludge system. Hydrolysis is assumed to be active independently of the electron donor. This process is different from the hydrolysis process in ASM1; it is of less dominating importance for the rates of oxygen consumption and denitrification [72].

$$k_H \cdot \frac{\frac{X_S}{X_H}}{K_X + \frac{X_S}{X_H}} \cdot X_H \qquad (2.34)$$

- *Aerobic storage of readily biodegradable substrate*

This process describes the storage of readily biodegradable substrate S_S in the form of cell internal storage products X_{STO}. This process requires energy, which is obtained from aerobic respiration. It is assumed that all substrates first become stored material and later are assimilated to biomass. This is definitely not observed in reality; however, at this moment no reliable model is available which can predict the substrate flux into storage, assimilation and dissimilation respectively. Therefore, the Task Group suggest for the time being this simplest assumption. However, using a low yield coefficient for storage (Y_{STO}) and a higher one for subsequent growth (Y_H) allows to approximate the consequences of direct growth rather than storage followed by growth [71].

$$k_{STO} \cdot \frac{S_O}{K_O + S_O} \cdot \frac{S_S}{K_S + S_S} \cdot X_H \qquad (2.35)$$

- *Anoxic storage of readily biodegradable substrate*

This process is identical to aerobic storage, but denitrification rather than aerobic respiration provides the energy required. Only a fraction of the heterotrophic organisms X_H in activated sludge is capable of denitrification. ASM3 considers this by reducing the anoxic heterotrophic storage rate as compared to the aerobic rate [72, 83].

$$k_{STO} \cdot \eta_{NOX} \frac{S_O}{K_O + S_O} \cdot \frac{S_{NO}}{K_{NO} + S_{NO}} \cdot \frac{S_S}{K_S + S_S} X_H \qquad (2.36)$$

- *Aerobic growth of heterotrophs*

The substrate for the growth of heterotrophic organisms is assumed to consist entirely of stored organics X_{STO}. This assumption simplifies ASM3 considerably [71, 83].

$$\mu_H \cdot \frac{S_{O_2}}{K_{O_2}+S_{O_2}} \cdot \frac{S_{NH_4}}{K_{NH}+S_{NH}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot \frac{\frac{X_{STO}}{X_H}}{\frac{K_{STO}+X_{STO}}{X_H}} \cdot X_H \quad (2.37)$$

- *Anoxic growth of heterotrophs*

This process is similar to aerobic growth but respiration is based on denitrification. Only a fraction of the heterotrophic organisms X_H in activated sludge is capable of denitrification. ASM3 considers this by reducing the anoxic heterotrophic storage rate as compared to the aerobic rate [71].

$$\mu_H \cdot \eta_{NO} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}} \cdot \frac{S_{NH}}{K_{NH}+S_{NH}} \cdot \frac{S_{ALK}}{K_{ALK}+S_{ALK}} \cdot \frac{\frac{X_{STO}}{X_H}}{\frac{K_{STO}+X_{STO}}{X_H}} \cdot X_H \quad (2.38)$$

- *Aerobic endogenous respiration of heterotrophs*

This process describes all forms of biomass loss and energy requirements not associated with growth by considering related respiration under aerobic conditions: decay (maintenance), endogenous respiration, lysis, predation, motility, death, and so on. The model of this process is significantly different from the decay (lysis) process introduced in ASM1 [71].

$$b_{H,O} \cdot \frac{S_O}{K_O+S_O} \cdot X_H \quad (2.39)$$

- *Anoxic endogenous respiration of heterotrophs*

This process is similar to aerobic endogenous respiration but typically slower. Especially protozoa (predation) are considerably less active under denitrifying than under aerobic conditions [71].

$$b_{H,NO} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}} \cdot X_H \quad (2.40)$$

- *Aerobic respiration of storage products*

This process is analogous to endogenous respiration. It assures that storage products, X_{STO} decay together with biomass [71, 72].

$$b_{STO,O} \cdot \frac{S_O}{K_O+S_O} \cdot X_{STO} \quad (2.41)$$

- *Anoxic respiration of storage products*

This process is analogous to the aerobic process but under denitrifying conditions [71].

$$b_{STO,NO} \cdot \frac{K_O}{K_O+S_O} \cdot \frac{S_{NO}}{K_{NO}+S_{NO}} \cdot X_{STO} \quad (2.42)$$

Autotrophic organisms, nitrifying activity [71]

- *Aerobic growth of autotrophs (nitrification)*

$$\mu_A \cdot \frac{S_O}{K_{A,O}+S_O} \cdot \frac{S_{NH}}{K_{A,NH}+S_{NH}} \cdot \frac{S_{ALK}}{K_{A,ALK}+S_{ALK}} \cdot X_A \quad (2.43)$$

- *Aerobic endogenous respiration of autotrophs*

$$b_{A,O} \cdot \frac{S_O}{K_{A,O}+S_O} \cdot X_A \quad (2.44)$$

- *Anoxic endogenous respiration of autotrophs*

$$b_{A,NO} \cdot \frac{K_{A,O}}{K_{A,O}+S_O} \cdot \frac{S_{NO}}{K_{A,NO}+S_{NO}} \cdot X_A \quad (2.45)$$

2.3.5. Parameters, stoichiometry and kinetics in ASM

Parameters, stoichiometry and kinetics in ASM1 are described in Table 2.17 and ASM3 in Table 2.18 [71, 84].

Table 2.17. Model parameters and values at neutral pH for ASM1 (domestic wastewater)

Model parameters	Unit	Symbol	Default values	
			20°C	10°C
Stoichiometric parameters				
Heterotrophic yield	$gCOD.gCOD^{-1}$	Y_H	0.67	0.67
Autotrophic yield	$gCOD.gCOD^{-1}$	Y_A	0.24	0.24
Nitrogen fraction in biomass	$gN.gCOD^{-1}$	i_{XB}	0.086	0.086
Nitrogen fraction in endogenous mass	$gN.gCOD^{-1}$	i_{XP}	0.06	0.06
Fraction of biomass leading to particulate material	-	f_P	0.08	0.08
Kinetic parameters heterotrophs				
Maximum specific growth rate	day^{-1}	$\hat{\mu}_H$	6,0	3.0
Substrate saturation constant	$gCOD.m^{-3}$	K_S	20.0	20.0
Oxygen saturation constant	$gO_2.m^{-3}$	$K_{O,H}$	0.20	0.20
Nitrate decay rate	day^{-1}	b_H	0.62	0.20
Anoxic growth correction factor	-	η_g	0.8	0.8
Kinetic parameters autotrophs				
Maximum specific growth rate	day^{-1}	$\hat{\mu}_A$	1	0.35
Ammonium saturation constant	$g\ NH_4^+-N.m^{-3}$	K_{NH}	1.0	1.0
Oxygen saturation constant	$g\ O_2.m^{-3}$	K_{OA}	0.5	0.5

Model parameters	Unit	Symbol	Default values	
			20°C	10°C
Aerobic endogenous respiration rate of X_A	day^{-1}	b_{A,O_2}	0.15	0.05
Hydrolysis parameters				
Maximum specific hydrolysis rate	day^{-1}	k_h	3.0	1.0
Anoxic hydrolysis correction factor	-	η_h	0.4	0.4
Half-saturation coefficient for hydrolysis of X_S	-	K_X	0.03	0.01
Ammonification				
Ammonification rate constant	$m^3.gCOD^{-1}.day^{-1}$	k_a	0.08	0.04

Table 2.18. Typical stoichiometric and composition parameters for ASM3

Characterization	Symbol	Units	Value
Production of S_I in hydrolysis	f_{S_I}	$gCOD_{S_I}.gCOD_{X_S}^{-1}$	0
Aerobic yield of stored product per S_S	Y_{STO,O_2}	$gCOD_{X_{STO}}.gCOD_{S_S}^{-1}$	0.85
Anoxic yield of stored product per S_S	$Y_{STO,NO}$	$gCOD_{X_{STO}}.gCOD_{S_S}^{-1}$	0.8
Aerobic yield of heterotrophic biomass	Y_{H,O_2}	$gCOD_{X_H}.gCOD_{X_{STO}}^{-1}$	0.63
Anoxic yield of heterotrophic biomass	$Y_{H,NO}$	$gCOD_{X_H}.gCOD_{X_{STO}}^{-1}$	0.54
Yield of autotrophic biomass per NO_3^--N	Y_A	$gCOD_{X_A}.gN_{S_{NOX}}^{-1}$	0.24
N content of S_I	i_{N,S_I}	$gN.gCOD_{S_I}^{-1}$	0.01
N content of S_S	i_{N,S_S}	$gN.gCOD_{S_S}^{-1}$	0.03
N content of X_I	i_{N,X_I}	$gN.gCOD_{X_I}^{-1}$	0.02
N content of X_S	i_{N,X_S}	$gN.gCOD_{X_S}^{-1}$	0.04
N content of biomass, X_H, X_A	$i_{N,X_{BM}}$	$gN.gCOD_{X_{BM}}^{-1}$	0.07
S_S to COD ratio for X_I	i_{SS,X_I}	$gSS.gCOD_{X_I}^{-1}$	0.75
S_S to COD ratio for X_S	i_{SS,X_S}	$gSS.gCOD_{X_S}^{-1}$	0.75
S_S to COD ratio for biomass, X_H, X_A	$i_{SS,BM}$	$gSS.gCOD_{X_{BM}}^{-1}$	0.09
S_S to COD ratio for X_{STO} base on PHB	$i_{SS,STO}$	$gSS.gCOD_{X_{STO}}^{-1}$	0.6

2.3.6. Components in mathematical models

Components in mathematical models are summarized in Table 2.19 and Table 2.20 [71].

Table 2.19. The AMS1 matrix

Component → i		1	2	3	4	5	6	7	8	9	10	11	12	13	Process rate
j	Process ↓	S_I	S_S	X_I	X_S	X_{BH}	X_{BA}	X_P	S_O	S_{NO}	S_{NH}	S_{ND}	X_{ND}	S_{ALK}	
1	Aerobic growth of heterotrophs		$-\frac{1}{Y_H}$			1			$-\frac{1-Y_H}{Y_H}$		$-i_{XB}$			$-\frac{i_{XB}}{14}$	$\hat{\mu}_H\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{S_O}{K_{OH}+S_O}\right)X_{BH}$
2	Anoxic growth of heterotrophs		$-\frac{1}{Y_H}$			1				$-\frac{1-Y_H}{2.86Y_H}$	$-i_{XB}$			$\frac{1-Y_H}{14\cdot 2.86Y_H}-\frac{i_{XB}}{14}$	$\hat{\mu}_H\left(\frac{S_S}{K_S+S_S}\right)\left(\frac{K_{OH}}{K_{OH}+S_O}\right)\times\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\eta_g X_{BH}$
3	Aerobic growth of autotrophs						1		$-\frac{4.57}{Y}+1$	$-\frac{1}{Y_A}$	$-i_{XB}-\frac{1}{Y_A}$			$-\frac{i_{XB}}{14}-\frac{1}{7Y_A}$	$\hat{\mu}_A\left(\frac{S_{NH}}{K_{NH}+S_{NH}}\right)\left(\frac{S_O}{K_{OH}+S_O}\right)X_{BA}$
4	Decay of heterotrophs				$1-f_P$	-1		f_P					$-i_{XB}-f_P i_{XP}$		$b_H\cdot X_{BH}$
5	Decay of autotrophs				$1-f_P$		-1	f_P					$-i_{XB}-f_P i_{XP}$		$b_A\cdot X_{BA}$
6	Ammonification of soluble organic nitrogen										1	-1		$\frac{1}{14}$	$k_a\cdot S_{DN}\cdot X_{BH}$
7	Hydrolysis of entrapped organics		1		-1										$k_h\frac{\frac{X_S}{X_{BH}}}{K_X+\left(\frac{X_S}{X_{BH}}\right)}\left[\left(\frac{S_O}{K_{OH}+S_O}\right)+\eta_h\left(\frac{K_{OH}}{K_{OH}+S_O}\right)\left(\frac{S_{NO}}{K_{NO}+S_{NO}}\right)\right]\cdot X_{BH}$
8	Hydrolysis of entrapped organic nitrogen											1	-1		$\rho_7\cdot\left(\frac{X_{ND}}{X_S}\right)$
Observed conversion rates ($ML^{-3}T^{-1}$)		$r_i=\sum_j v_{ij}\rho_j$													

Table 2.20. The AMS3 matrix

Compound i →		1	2	3	4	5	6	7	8	9	10	11	12	13
j Process		S_{O_2}	S_I	S_S	S_{NH_4}	S_{N_2}	S_{NOX}	S_{ALK}	X_I	X_S	X_H	X_{STO}	X_A	X_{SS}
↓ Expressed as →		O_2	COD	COD	N	N	N	Mole	COD	COD	COD	COD	COD	SS
1	Hydrolysis		f_{S_I}	x_1	y_1			z_1		-1				$-i_{X_S}$
Heterotrophic organisms, aerobic and denitrifying activity														
2	Aerobic storage of S_S	x_2		-1	y_2			z_2				Y_{STO,O_2}		t_2
3	Anoxic storage of S_S			-1	y_3	$-x_3$	x_3	z_3				$Y_{STO,NOX}$		t_3
4	Aerobic growth of X_H	x_4			y_4			z_4			1	$\frac{-1}{Y_{H,O_2}}$		t_4
5	Anoxic growth (denitrific.)				y_5	$-x_5$	x_5	z_5			1	$\frac{-1}{Y_{H,NOX}}$		t_5
6	Aerobic endog. respiration	x_6			y_6			z_6	f_I		-1			t_6
7	Anoxic endog. respiration				y_7	$-x_7$	x_7	z_7	f_I		-1			t_7
8	Aerobic respiration of X_{STO}	x_8										-1		t_8
9	Anoxic respiration of X_{STO}					$-x_9$	x_9	z_9				-1		t_9
Autotrophic organisms, nitrifying activity														
10	Aerobic growth of X_A	x_{10}			y_{10}		$\frac{1}{Y_A}$	z_{10}						t_{10}
11	Aerobic endog. respiration	x_{11}			y_{11}			z_{11}	f_I					t_{11}
12	Anoxic endog. respiration				y_{12}	$-x_{12}$	x_{12}	z_{12}	f_I					t_{12}
Composition matrix $i_{k,I}$														
k	Conservatives													
1	ThOD; gThOD	-1	1	1		-1.71	-4.57		1	1	1	1	1	
2	Nitrogen; gN		i_{N,S_I}	i_{N,S_S}	1	1	1		i_{N,X_I}	i_{N,X_S}	$i_{N,BN}$		$i_{N,BN}$	
3	Ionic charge; Mole +				-1/14		-1/14	-1						
Observables									i_{SS,X_I}	i_{SS,X_S}	$i_{SS,BN}$	0.60	$i_{SS,BN}$	
4	SS; gSS													

2.3.7. Comparison between ASM1 and ASM3

The main difference between ASM1 and ASM3 is the recognition of the importance of storage polymers in the heterotrophic conversions in the activated sludge processes in ASM3. The aerobic storage process in ASM3 describes the storage of the readily biodegradable substrate (S_S) into a cell internal component (X_{STO}). Substrate flows in ASM1 and ASM3 are modified from [71, 72] and illustrated in Figure 2.06.

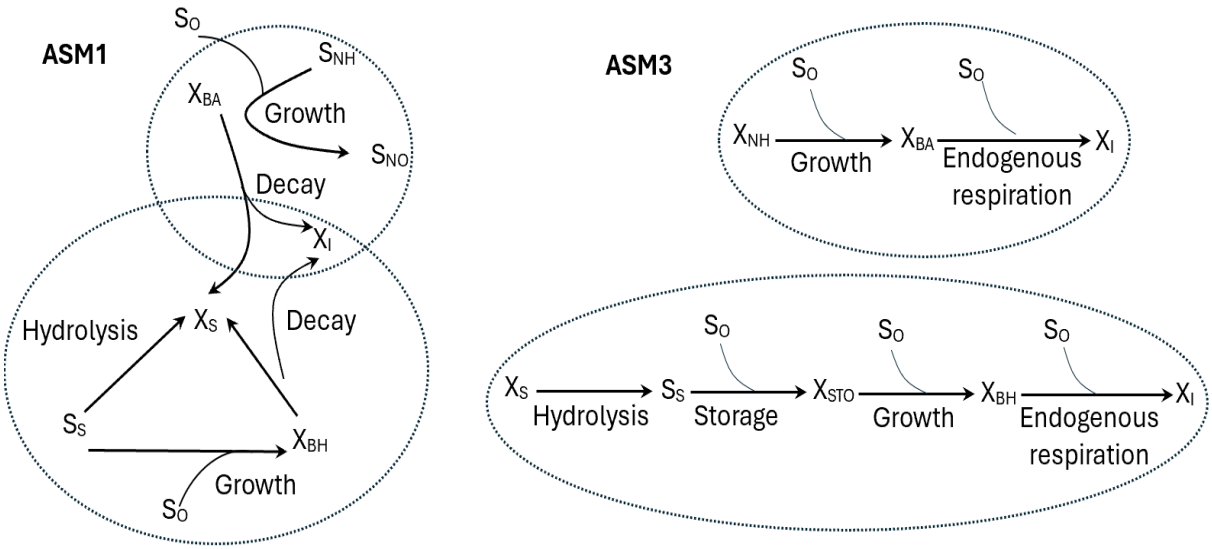


Figure 2.06. Substrate flows in ASM1 and ASM3

As compared to ASM1 this fraction has a different origin. In ASM3 all X_S is contained in the influent and none is generated in decay processes. In ASM1 a large fraction of X_S is assumed to originate from decay processes. Which also means that the X_S coming from the hydrolysis of substrate has the same biodegradability than the initial substrate in wastewater.

In the literature review we provide an overview of previous studies on landfill leachate characteristics, treatment technologies, and modelling approaches.

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CHAPTER 3: LANDFILL LEACHATE CHARACTERIZATION FOR SIMULATION OF BIOLOGICAL TREATMENT WITH ACTIVATED SLUDGE MODEL No.1 (ASM1) AND No.3 (ASM3)

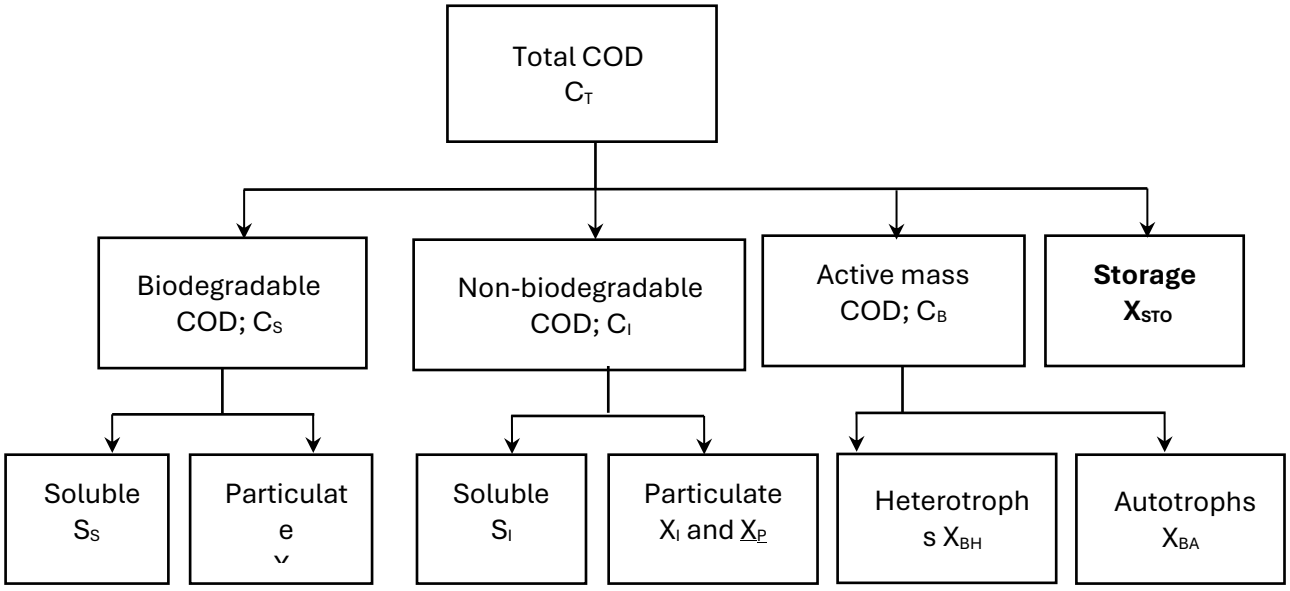
3.1. INTRODUCTION

Both biodegradable material, refractory organics and inorganics as well as a number of pathogenic bacteria are contained in leachate [1, 2]. The main pollutants in leachate include COD, BOD, ammonia, hydrocarbons, suspended solids, heavy metals, and inorganic salts [3-6]. As it is well known, leachate characteristics reflect biodegradation properties, and the age of landfill site. To treat leachate, several methods such as physicochemical and biological methods are often combined. Biological treatment methods are more common for leachate treatment [2, 7-9]. The efficiency of biological leachate treatment depends both on pollutant concentration level and on their susceptibility to decay in the biological treatment processes. Biological treatments with activated sludge can lead to high performances, due to bacterial colonies, particularly autotrophic bacteria for the ammonia consumption and heterotrophic ones for the carbon fraction [10-12]. The behavior of these micro-organisms can be mathematically described and models like the Activated Sludge Model's family have proven to simulate it correctly, at least for municipal wastewater treatment conditions [13, 14]. Nowadays, a more detailed characterization of influent wastewater is needed because they are the relevant factors to the efficient operation of wastewater treatment plants. Moreover, the increasing utilization of simulation tools such as ASMs developed by IWA requires accurate and precise description of raw wastewater, especially organic and nitrogen matter present in the influent as those values are state variables of those models. Therefore, COD and nitrogen fractions play an important role in the design of wastewater treatment plants, especially in the case of leachate.

ASM models focus on the compounds of wastewater as state variables of the models. These variables represent the organic matter present, measured as chemical oxygen demand, nitrogen forms, biomass, alkalinity and dissolved oxygen. Good model calibration requires knowledge of model parameters and influent wastewater characteristics which can significantly influence plant performance, especially for biological nutrient removal systems [15, 16].

COD is selected as the most suitable parameter for defining the carbon substrates because COD is relevant to quantify the electron equivalents in the organic substrate, the biomass and oxygen utilized. There are seven COD factions in the ASM1 based on solubility, biodegradability, biodegradation rate and viability (biomass). Moreover, COD is a conservative value, which is not the case of BOD.

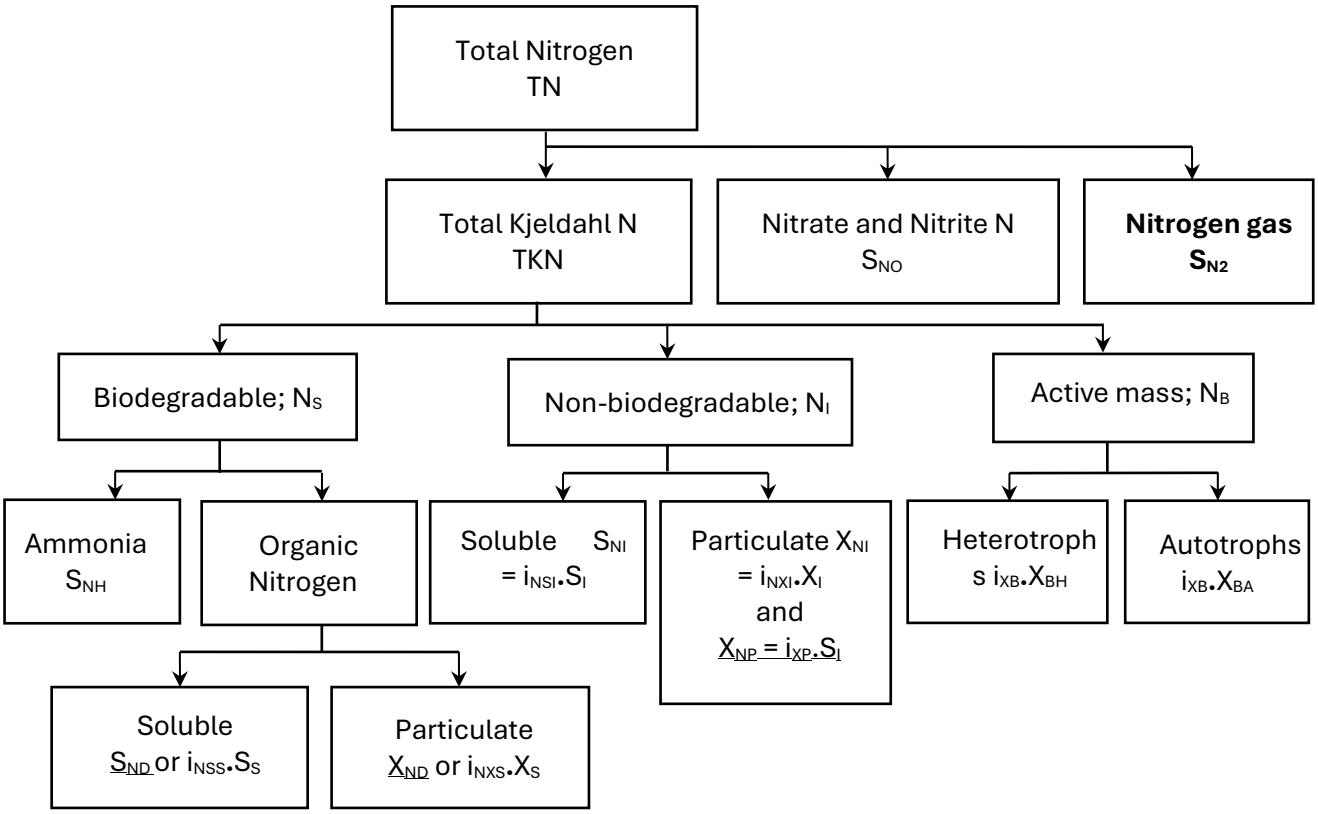
One of the most difficult parts to calibrate the ASM1 is due to the characterization of the wastewater fractions. Wastewater can be characterized either with physical-chemical methods or with biological methods. To be able to characterize all the fractions, the two methods must be combined [17]. The biological methods are based on the rates of degradation. COD fractions in ASM1and ASM3are modified from [18, 19] and presented in Figure 3.01.



Components specifically related to ASM1 are given in underline and the ones only related to ASM3 are given in bold.

Figure 3.01. COD fraction in ASM1 and ASM3

In ASM, similarly to organic matter, total nitrogen can be subdivided based on solubility, biodegradability and biodegradation rate. Summarizing, the total nitrogen balance for the components in ASM is modified from [17, 18, 20] and illustrated in Figure 3.02.



Components specifically related to ASM1 are given in underline and the ones only related to ASM3 are given in bold

Figure 3.02. Nitrogen components in ASM1 and ASM3

3.1.1. Determination COD fractions in leachate

Several methods have been applied to determine the composition of COD for simulation of biological treatment in wastewater and they can be described as follows:

- The physicochemical method is determined based on the assumption that the COD fractions can be divided by physical processes such as settling, filtration and flocculation. Then, COD fractions are measured by using standard analytical methods. This method is fast but a disadvantage of the filtration process is the difficulty to effectively separate between rapidly and slowly biodegradating fractions. The main reason is that colloidal matters may contribute to both fractions.
- The combined physical chemical and biological method of the Dutch Foundation of Applied Water Research (STOWA) includes both filtration and flocculation steps with COD and BOD measurements [21]. It is rapid and easy to use, but it completely neglects biomass fractions.

- The COD is divided into two main fractions that are biodegradable and non-biodegradable which are subdivided into particulate and soluble fractions [22, 23]. Various methods have been proposed for characterizing the readily and slowly biodegradable organic fractions [23, 24]. Biodegradable COD fractions can be determined by combining respirometric experiments in various So/Xo ratios [23, 25, 26]. A rapid physical–chemical method for determining the soluble readily biodegradable COD and the soluble non-biodegradable COD was also developed by Mamais *et al* [22, 27]. Soluble COD was determined experimentally by means of the chemical analysis of COD after pretreatment of wastewater with coagulation, flocculation with $Zn(OH)_2$ and 0.45 µm filters. According to Bilgili *et al*. (2008), the selection of the filter size is controversial for many authors and depends mainly on the quality of wastewater. After the determination of soluble COD fractions, particulate COD fractions can be calculated easily [22].
- Total biodegradable COD may be determined by using the total biochemical oxygen demand method which assumes that particulate organic materials are hydrolyzed when the biological oxidation process is completed. Thus, the Total BOD is conceptually equal to the biodegradable COD including soluble and particulate degradable COD [22]. Thus, in our case total biodegradable COD was performed by BOD test and calculated according to Thomas' method and the constant k_{BOD} can be determined by fitting the BOD curve through the measured data with the BOD_{tot} equation [28, 29]. Thomas' Equation is expressed below:

From equations:

$$\frac{dL_t}{dt} = -k' \times L_t \quad \Rightarrow \quad \frac{L_t}{L_0} = e^{-k't} = 10^{-k_1 t} \tag{3.1}$$

$$k_1 = \frac{k'}{2.303} = 0.4343k' \tag{3.2}$$

$$BOD_t = L_0 - L_t = L_0 - L_0 \times 10^{-kt} = L_0\left(1-10^{-k_1 t}\right) \tag{3.3}$$

Where

L_t amount of first stage BOD remaining in the sample at t; $mg.l^{-1}$

L_0 initial remaining BOD, total/ultimate first stage BOD present at t_0; $mg.l^{-1}$

k' rate constant (to base e); d^{-1}

k_1 rate constant (to base 10); d^{-1}

t time; d

Thomas method

$$\sqrt[3]{\frac{t}{BOD}} = \sqrt[-3]{k'L_0} + \frac{t \times \sqrt[3]{(k')^2}}{6 \times \sqrt[3]{L_0}} \quad (3.4)$$

Equation above can be put in the form of a straight line: x = a + b.t

Where

$$x = \left(\frac{t}{BOD}\right)^{1/3} \quad (3.4.1)$$

a: intercept of the line: $a = (k'L_0)^{-1/3}$ (3.4.2)

Slope b of the straight line is given by:

$$b = \frac{\sqrt[3]{k'^2}}{6 \times \sqrt[3]{L_0}} \quad (3.4.3)$$

The reaction rate constant and ultimate BOD may be determined in equations:

$$k' = \frac{6 \times b}{a} \quad (3.4.4)$$

$$L_0 = \frac{1}{k' \times a^3} \quad (3.4.5)$$

Or

$$\sqrt[3]{\frac{t}{BOD}} = \sqrt[-3]{2.3k_1L_0} + \frac{t \times \sqrt[3]{(k_1)^2}}{3.43 \times \sqrt[3]{L_0}} \quad (3.4.6)$$

$$k_1 = \frac{2.61 \times b}{a} \quad (3.4.7)$$

$$L_0 = \frac{1}{2.3k_1 \times a^3} \quad (3.4.8)$$

The calculation method to provide L_O (BOD_{tot}) and k' (k_1) have been developed in an excel sheet to treat the data collected during the respirometric BOD test.

Soluble inert COD (S_I): Soluble inert COD is due to dissolved non-biodegradable molecules. It can be determined as the soluble COD remaining after a long – term BOD test [11] where the wastewater is aerated in a batch reactor around 10 days [30]. The inert fraction was also computed as the soluble COD in the treated effluent [22]. However, according to [31], the solution COD in the effluent from a biological process includes not only biodegradable and inert compounds from the influent, but also biodegradable and inert compound produced by the microbial activities in the treatment system [32]. Thus, the effluent soluble COD of a biological reactor includes the remaining readily biodegradable in influent soluble COD, initial soluble inert COD influent, and soluble inert COD from microbial activities. In this study, S_I was determined

from difference between total soluble COD (C_S) and soluble biodegradable COD (S_S).

Soluble biodegradable COD (S_S): The concentration of the biodegradable soluble COD of leachate was estimated by soluble BOD test. Therefore, S_S was calculated from the results of soluble BOD analysis where BOD was measured as a function in the time and calculating the soluble BOD_{tot} (Lo) of wastewater via Thomas' equation. Besides, S_S can be determined through total soluble COD and S_I [11, 32].

Particulate inert COD (X_I): Particulate inert COD is created from non-biodegradable compounds, both in suspended and in colloidal forms [21]. It is calculated as the difference between total non-biodegradable COD (C_I) and soluble non-biodegradable COD (S_I). The value of X_p was neglected.

Particulate biodegradable COD (X_S): Particulate biodegradable COD is created from suspended and colloidal solids and compounds with high molecular weight that require enzymatic hydrolysis before being metabolized. It is also called "slowly biodegradable COD" and is the difference between total biodegradable COD (C_S) and soluble biological COD (S_S) [32, 33]. Besides, the particulate biodegradable COD (X_S) was measured by calculation from total COD (C_T) and the other components [11].

Heterotrophic and autotrophic active biomass (X_{BH} and X_{BA}): There is no procedure recognized to determine autotrophic biomass concentration in wastewater [11], so X_{BA} is often neglected in the COD fractionation. According to Foladori *et al*. (2010), the terms X_{BH} and X_{BA} can be considered as negligible in influent wastewater and however, their quantification is not easy [34].

X_{STO}: In ASM3 X_{STO} is storage process of S_S before being used for growth [11]. X_{STO} value is too low in wastewater and almost impossible to measure [15].

3.1.2. Determination of Nitrogen fractions in leachate

Nitrogen can be evaluated in two major groups: unoxidized nitrogen and oxidized nitrogen [35]. Besides, nitrogen can be divided including ammoniacal nitrogen, soluble inert organic, particulate inert organic nitrogen, readily biodegradable organic nitrogen, and slowly biodegradable organic nitrogen. The total unoxidized nitrogen content in wastewater sample was estimated by the total Kjeldahl test. Similarly, a specific Kjeldahl test for free and saline ammonia was used to determine the ammonia nitrogen fractions, so that the difference between the total Kjeldahl nitrogen (including N-org and ammonia) and ammonia nitrogen yields the total organic content of the sample comprising biodegradable and inert components.

There are some nitrogen fractions that can be determined by physical-chemical or biological methods. According to [11], S_{NH}, S_{ND}, S_{NO}, X_{ND} can be determined by physical – chemical methods through a combination of standard analyses of ammonium, nitrite, nitrate and Kjeldahl nitrogen on filtered and non-filtered samples.

Ammonia nitrogen (S_{NH}): S_{NH} component is obtained based on standard analyses of solution ammonium nitrogen. The samples were coagulated and filtrated with 0.45μm membrane filters before NH_3-N concentration was measured following standard methods and considered to be equal to S_{NH}.

Nitrite and Nitrate S_{NO}: S_{NO} is assumed to include nitrate as well as nitrite nitrogen, since nitrite is not as a separate model component [36].

Soluble non-biodegradable nitrogen (S_{NI}): S_{NI} is assumed to be negligible and therefore not incorporated into the model.

Particulate non-biodegradable nitrogen (X_{NI}): Organic nitrogen associated with inert organic particulate matter. X_{NI} is calculated as difference between non-biodegradable (N_I) organic nitrogen and soluble biodegradable organic nitrogen (S_{NI}) (see Table 3.01).

Soluble biodegradable organic nitrogen (S_{ND}): It is converted to ammonia by ammonification and is the result of hydrolysis of particulate organic nitrogen. Hydrogen ions consumed in this conversation process result in an alkalinity change. S_{ND} is calculated from filtered TNK of raw wastewater, filtered TNK of treated wastewater TNK filtered treated and ammonia (S_{NH}).

Particulate biodegradable organic nitrogen (X_{ND}): The nitrogen in the biomass is divided between biomass debris and particulate substrate, with the latter being called particulate biodegradable organic nitrogen.

3.1.3. Determination of other fractions in leachate

Alkalinity (S_{Alk}): Alkalinity is used to approximate the conservation of ionic charge in biological reactions.

Alkalinity of water is due primarily to the presence of bicarbonate, carbonate, and hydroxide ions. Salts of weak acids, such as borates, silicates and phosphates, may also contribute [37]. The alkalinity will affect ammonium concentration in leachate and nitrification process during removal of nitrogen by biological methods.

Total suspended solids (X_{SS}): Suspended solids are introduced into the biokinetic models. In the influent, X_{SS} include an inorganic fraction of SS and the soluble fraction, which passed membrane filters.

Dissolved oxygen (S_O): Dissolved oxygen can be directly measured and is subject to gas exchange.

The objective of this chapter is to characterize leachate from a landfill of HCMC with respect to its COD and nitrogen fractions in accordance the fractions included in ASM1 and ASM3. The study aims to apply combined physico-chemical and biological methods to determine both soluble and particulate COD fractions, and to identify and quantify the main nitrogen forms present in the leachate. This characterization provides the necessary basis for accurate simulation, design, and optimization of biological treatment processes for landfill leachate.

3.2. MATERIALS AND METHODS

3.2.1. Materials

Leachate was collected at Phuoc Hiep landfill site, located in the North East Solid Waste Treatment Complex, Phuoc Hiep Commune, Cu Chi District, about 37 km distance from the center of HCMC. Phuoc Hiep landfill covers 50 ha and has been operated since 2003. The samples were collected at two or three days intervals, in the period 2013 - 2015. The samples were collected and transported immediately to the laboratory. The samples were stored ate room temperature at about 26 - 33°C to use for experimental purposes.

3.2.2. Methods

The leachate characterization employed in this study is based on a physico-chemical method combined with a BOD analysis.

Table 3.01. Analytical methods

Parameter	Methods
Total COD (C_T)	Chemical oxygen demand (COD) was determined following Method 5220.C of Standard Methods [38].
Soluble COD ($C_{Sol.}$)	Using 2 - 5 mL zinc sulfate solution with 100 $g.l^{-1}$ concentration was added 100 ml leachate sample and mixed with magnetic stirrer for 1 – 2 min. Then the pH was adjusted to 10.5 with 6 M sodium hydroxide solution and the mixture is precipitated. Samples from clear supernatant was taken, after that passed through Whatman GF/C (0.45µm) glass fiber filters, and the COD of the filtrate was measured by Method 5220.C of Standard Methods [38].
Particulate COD ($C_{Part.}$)	After the determination of soluble COD fractions, the COD of the wastewater suspended solids was obtained by subtracting soluble COD from total COD [22].

Parameter	Methods
Total BOD (C_S)	BOD was performed by The Hach BOD Trak II Apparatus during 10 days and was calculated by Thomas method. Thus, a certain amount of ammonia was present and this has to be considered during the BOD test where an appropriate amount of ATU (Allylthiorea) has to be added to inhibit nitrifying bacteria that would otherwise affect the oxygen consumption in the BOD bottle.
Inert COD (C_I)	Non-biodegradable COD was obtained by subtracting biodegradable COD from total COD.
S_S	Soluble BOD was performed by The Hach BOD Trak II Apparatus after wastewater was pretreated with coagulation, flocculation with $Zn(OH)_2$ and 0.45 μm filters (the sample pretreatment procedure was performed the same to determination of soluble COD). Then Soluble biodegradable COD (S_S) is calculated by using Thomas method.
X_S	X_S was calculated via C_S and S_S in accordance with balance COD.
S_I	Soluble inert COD (S_I) was obtained by subtracting soluble biodegradable COD (S_S) from total soluble COD [25, 32, 33].
X_I	Particulate inert COD was calculated from difference between total non-biodegradable COD (C_I) and soluble inert COD (S_I) [32, 33].
NH_3 + NH_4^+-N (TKN)	Sample is adjusted pH to 9.5 with 6N NaOH then It is boiled by Kjeldahl test system to use mixture to steam out. Distillate is collected in Erlenmeyer flask with boric acid. The colorimetric method is used to determine ammonium. The Nessler method in which potassium, mercury, and iodine react with ammonium to react a yellow brownish colored compound is called the Nesslerization. The wavelength for the light used in the measurement was set at 425nm [39, 40].
NO_2^--N + NO_3^--N	Nitrite was measured by colorimetric method, in accordance to 4500.B of Standard Methods. Nitrate was determined by cadmium reduction method in 4500.E of Standard Methods [38].
S_{NI}	S_{NI} was estimated on the basis of filtered Kjeldahl nitrogen (TKN_F) in effluent of pilot to determine soluble residual organic nitrogen.
X_{NI}	X_{NI} was calculated as difference between non-biodegradable nitrogen (N_I) and soluble biodegradable nitrogen (S_{NI}).

Parameter	Methods
S_{ND}	S_{ND} = TNK filtered influent – TNK filtered effluent – S_{NH}.
X_{ND}	X_{ND} was calculated as difference between biodegradable organic nitrogen total (C_{ND}) and soluble biodegradable organic nitrogen (S_{ND}).
X_{SS}	The TSS was determined after filtration of sample of mixed liquor on a Whatman glass micro filter. Dry weight was determined after the filter was dried at 105°C and weighted on a microbalance in accordance 2540.D of Standard Methods [38].

3.3. RESULTS AND DISCUSSION

3.3.1. COD fractions

3.3.1.1. Result of BOD

The Thomas method was used to determine BOD. An example of BOD determination in the leachate is calculated based on Equation (3.4.1) and results are given in Table 3.02, Fig.3.03 and Fig.3.04.

Table 3.02. Result of BOD

Time; d	Mean	t/BOD	$(t/BOD)^{1/3}$	Time; d	Mean	t/BOD	$(t/BOD)^{1/3}$
1.0	188	0.0053191	0.1745611	5.0	404	0.0103762	0.2181122
1.5	235	0.0051830	0.1730586	6.0	425	0.0121176	0.2296886
2.0	275	0.0060727	0.1824433	7.0	458	0.0132838	0.2368325
2.5	303	0.0062508	0.1842097	8.0	464	0.0152414	0.2479370
3.0	331	0.0070634	0.1918693	9.0	471	0.0171083	0.2576729
3.5	365	0.0075890	0.1965150	10.0	479	0.0188768	0.2662623
4.0	376	0.0086383	0.2051836				

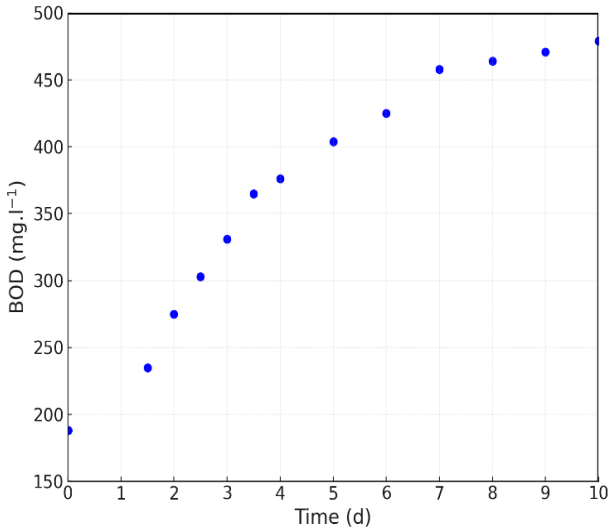


Figure 3.03. BOD vs. time

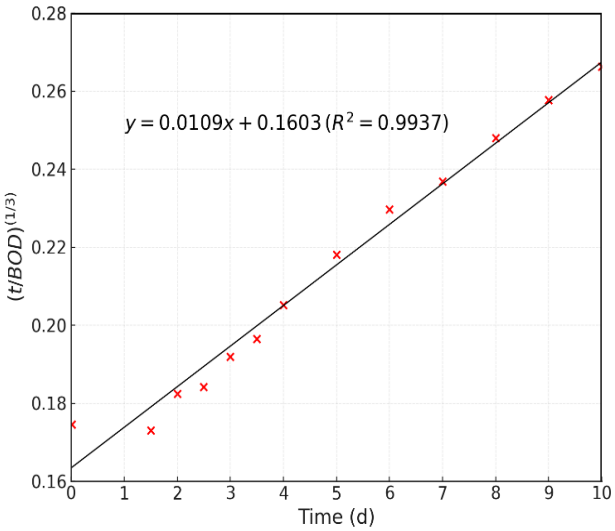


Figure 3.04. Plot a graph of $(t/BOD)^{1/3}$ vs. time

From the constructed straight line estimate, the slope and intercept.

intercept	0.1603
slope	0.0109

Using Eq. (3.4.4), (3.4.7) and (3.4.8), results are calculated below:

k'	0.41	d^{-1}
k_1	0.18	d^{-1}base 10
Lo	595	$mg.l^{-1}$

3.3.1.2. Result of COD fractional ratios

In this study, the concentrations of the biodegradable and non-biodegradable COD of leachate samples were determined. The mean concentrations of the COD fraction in landfill leachate during studying are presented in Table 3.03. It can be seen from the Table 3.03 that the concentrations of soluble biodegradable COD were the highest with various range from 1174 to 2387 $mgO_2.l^{-1}$ and from 1002 to 1877 $mgO_2.l^{-1}$ for dry and rainy seasons, respectively. On the contrary, the lowest of S_I concentrations were determined about from 44 to 502 $mgO_2.l^{-1}$ in rainy season and from 94 to 539 $mgO_2.l^{-1}$ in dry season.

Table 3.03. The COD fractional concentration in leachate

COD Fractions; $mgO_2.l^{-1}$	**Dry seasons**			**Rainy seasons**		
	min	max	average ± SD	min	max	average ± SD
S_S	1174	2387	1963 ± 356	1002	1877	1426 ± 283
S_I	44	502	293 ± 159	94	539	301 ± 150
X_I	385	1870	1089 ± 456	279	1244	630 ± 390
X_I	173	2518	1253 ± 719	45	1792	829 ± 477

The statistical analysis showed that the p-values for all individual COD fractions in the leachate were less than 0.05 ($p < 0.001$), indicating statistically significant differences among the fractions. In addition, the t-test results comparing COD fractions between dry and rainy seasons revealed significant differences in the mean values of X_I ($p = 0.036$) and S_S ($p = 0.003$), while no significant differences were observed for X_S ($p = 0.162$) and S_I ($p = 0.908$). In summary, as presented in Table 3.03, the concentrations of X_I and S_S varied significantly between the dry and rainy seasons ($p < 0.05$). These findings suggest that seasonal variation plays an important role in the distribution of COD fractions, which should be considered in wastewater treatment design and operation.

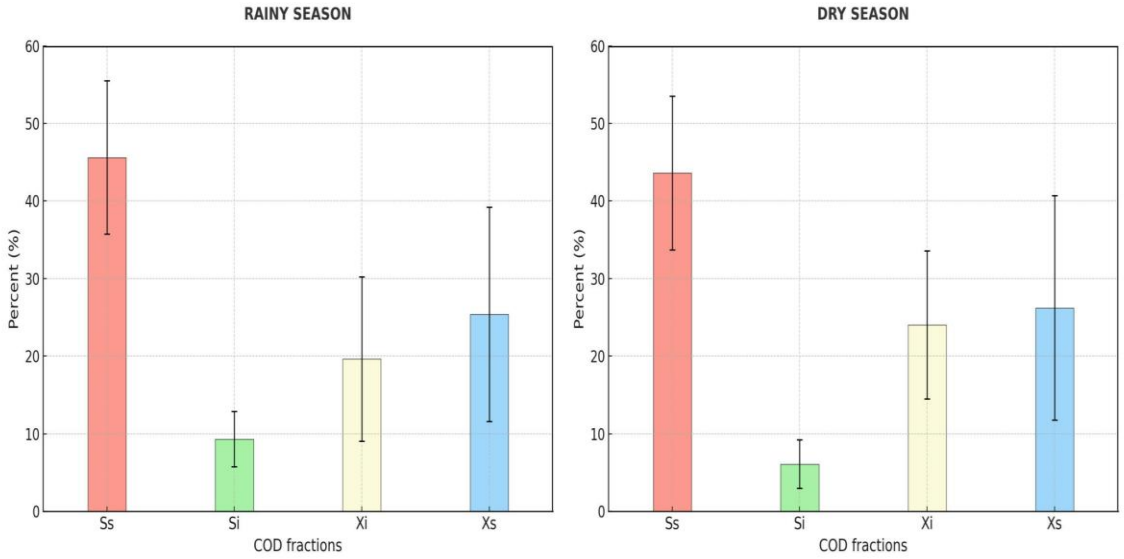
After the determination of the concentration of COD fractions, the percentage ratios of the individual fractions of total COD are calculated and presented in Table 3.04 and Figure. 3.05.

Table 3.04. Results of COD fractional ratios in leachate

COD Fractions	**Dry season** (% of total COD)	**Rainy season** (% of total COD)
S_S	43.61 ± 9.90	45.63 ± 9.87
S_I	6.11 ± 3.14	9.32 ± 3.55
X_I	24.04 ± 9.53	19.65 ± 10.62
X_S	26.24 ± 14.48	25.4 ± 13.81

In this study, the results shown that the higher concentration is soluble biodegradable fraction (S_S) in leachate. The S_S value in dry season and rainy season accounted 43.61 ± 9.90 % and for 45.63 ± 9.87 % of total COD, respectively.

The results are given as the ratio of total particulate COD fraction ($X_S + X_I$) to total COD in Figure 3.05. Evaluation of experimental study indicated that X_S value recorded 26.24 ± 14.48 % and 25.4 ± 13.81 % of total COD in dry and rainy seasons, respectively. Likewise, the results of X_I were determined 24.04 ± 11.07 % and 19.65 ± 10.62 % in dry and rainy season, respectively. The most likely reason for the relatively small? particulate fractions is the landfill leachate samples were taken from collecting pond, so part of particulate matter may have settled. Furthermore, the leachate from well operating sanitary landfill have passed the entire depth of deposits and a filtration has been done by the solid wastes.



(a) (b)

Figure 3.05. COD fractions in rainy season (a) and dry season (b)

On the other hand, the lowest were the concentrations of soluble inert COD fraction (S_I). The fraction of inert soluble (S_I) compounds in this study in dry and rainy season accounted for 6.11 ± 5.95 % and 9.32 ± 3.55 total COD, respectively.

On the whole, the change of weather affects COD fractions. Especially, it is significant difference to X_I and S_S. The seasonal changes in leachate quality have also other authors investigated on COD fractions in various wastewater. Values for the major COD fractions are listed in Table 3.05.

Table 3.05. COD fractions from other studies

Country	COD Fractions (% of total COD)				Ref.
	S_S	S_I	X_S	X_I	
Belgium[a]	11.59	60.74	-	18.84	[41]
Malaysia[a]	22.42	36.58	41 (X_S + X_I)		[42]
South Africa[b]	20	7	60	13	[43]
Hungary[c]	40.7	10.8	65.5	32.5	[21]
Poland[c]	14.0 – 22	5.5 – 8.0	26.6 – 56	16.5 – 44.3	[33]
Turkey[c]	27.9	3.2	15.1	53.8	[44]
Pakistan[c]	32.02	3.9	15.3	52.9	[45]
China – Shanghai[c]	8.1 ± 1.6	6.3 ± 2.2	45.5 ± 3.5	31.1 ± 2.1	[46]
Bailonggang[c]	11.1 ± 2.2	9.9 ± 2.0	38.9 ± 10.7	23.3 ± 9.8	

[a]leachate; [b]industrial wastewater; [c]domestic wastewater

An analysis of the results given by Galleguillos *et al.* (2011), showed that the ratio of non-biodegradable fraction in leachate was 60.74 % from old leachate in Belgium [41], in Malaysia this parameter was found to be about 36.58 % from stabilized landfill site [42]. The collected data in Table 3.05 also indicate that the highest percentage ratios in the examined domestic wastewater are those of fractions X_S or X_I, and the lowest - fraction S_I. For example, the domestic wastewater in the Hungarian study contains the highest of X_S. Otherwise, in Turkey sewage has the highest of S_I and the lowest S_I ratio.

3.3.2. Nitrogen fractions

In this study, the measured nitrogen concentrations for nitrogen components is given in Table 3.06. As can be seen from the data, the highest concentration of S_{NH} in dry and rainy season were measured from 954 mg.l^{-1} to 1621 mg.l^{-1} and from 412 mg.l^{-1} to 1334 with average value 1268 ± 198 mg.l^{-1} and 945 ± 305 mg.l^{-1}, respectively. Alternatively, the lowest ratio of nitrogen of the tested leachate was of X_{ND} fraction with concentrations from 3 to 69 mg.l^{-1} and 3 – 91 mg.l^{-1} in dry and rainy season, respectively.

Table 3.06. Results of nitrogen fractions

litrogen fractions	**Dry season**			**Rainy season**		
(mg.l^{-1})	min	max	average ± SD	min	max	average ± SD
S_{NH}	954	1621	1268 ±198	412	1334	945 ± 305
S_{NO}	1.9	4.6	2.99 ± 0.96	1.3	3.3	1.98 ± 0.69
S_{NI}	55	203	115 ± 53	35	142	83 ± 38
X_{NI}	31	394	152 ± 117	85	284	187 ± 66
S_{ND}	196	591	307 ± 123	9	74	32 ± 19
X_{ND}	3	69	30 ± 25	3	91	34 ± 33

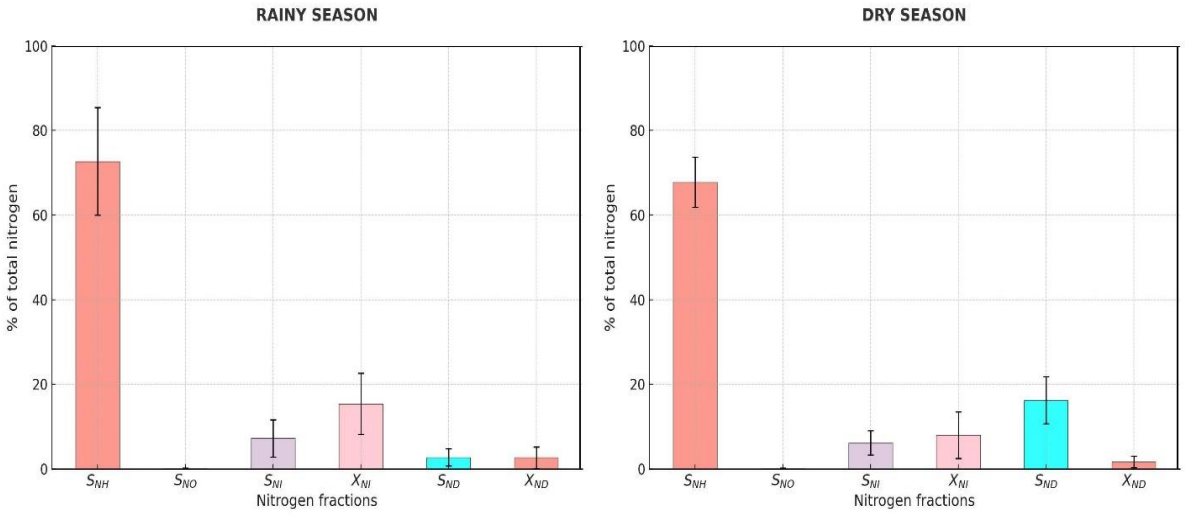
Ammoniacal nitrogen is a common constituent of landfill leachate as a result of the biological degradation of amino acids and other nitrogenous organic matter in solid waste. As can be seen from the Table 3.06, S_{NH} concentration has a significant difference between dry and rainy season. Results of t-test show that there is a statistically significant difference of S_{NH} value change due to seasons ($p = 0.019$). Mean concentrations of ammonia in the dry season are higher than during rainy season. Indeed ammonia evaporates at high pH value, and pH decreases in the rainy season. Besides, in this period, leachate is diluted with higher rainfall water volume.

The determined values of the percentage ratios of the individual nitrogen fractions differ from the values presented in Table 3.07, Figure 3.07 and Figure. 3.08.

Table 3.07. Results of nitrogen fractions

Nitrogen fractions; (% of total nitrogen)	**In this study**		**Ref.** [43]
	Dry season	**Rainy season**	
S_{NH}	67.77 ± 5.95	72.71 ±12.67	5 – 68
S_{NO}	0.16 ± 0.05	0.16 ± 0.05	6 – 9
S_{NI}	6.18 ± 2.84	7.25 ± 4.41	0.4 – 3
X_{NI}	8.01 ± 5.46	15.41 ± 7.21	0.4 – 1
S_{ND}	16.25 ± 5.52	2.74 ± 2.03	11 – 85
XND	1.63 ± 1.35	2.69 ± 2.52	5 – 20

Ammonium represents the major part of total nitrogen. Table 3.07 shows TKN composition obtained in wet weather conditions in comparison to dry weather results. As shown in this table, the ammonia nitrogen (S_{NH}) prevails in total nitrogen and amounts up to 67.77 ± 5.95 % for dry season and from 72.71 ±12.67 % in dry and wet weather, respectively.



(a) (b)

Figure 3.06. Nitrogen fractions in rainy season (a) and dry season (b)

On the contrary, the fractions of the total nitrogen showed that S_{NO} fraction accounted for a very small proportion in leachate and was only 0.16 ± 0.05 % in rainy and dry weather. However, S_{NO} fraction in domestic wastewater is higher than that in this study (between 6 and 9 % of TKN) demonstrated by Boubaker (2015) . This result can be explained because the nitrification process in domestic wastewater is easier than in landfill leachate. However, according to

Zawilski*et al*. (2009), nitrate and nitrite nitrogen as a pollutant of raw wastewater have been ignored as their total content usually does not exceed 1 % [33].

The fractions of the total nitrogen showed that landfill leachate had values of soluble organic nitrogen biologically undegradable (S_{NI}) ranging from 6.18 ± 2.84 % to 7.25 ± 4.41%. Meanwhile, Boubaker (2015), found that in domestic sewage soluble organic nitrogen biologically undegradable (S_{NI} ranges from 0.4 % to 3 % of TKN [43].

As can be seen from the Table 3.07, the particulate non-biodegradable nitrogen (X_{NI}) values are different between wet and dry seasons. Estimations obtained X_{NI} varied from 8.01 ± 5.46 % to 15.41 ± 7.21 % of the total nitrogen in dry and rainy season, respectively. In the rainy season, the non-biodegradable particular nitrogen value is twice the one in dry season because the rainwater swept particles from the landfill site On the other hand, this value is found in domestic wastewater at low ratio, ranging from 0.4 – 1% [43].

The organic fraction was divided into two compartments as S_{ND} and X_{NI}. The fraction of soluble organic nitrogen biologically degradable, S_{ND} was determined to be of 16.25 ± 5.52% and 2.74 ± 2.03 % in dry and rainy season, respectively. Meanwhile, this value was determined 11 – 85% in domestic wastewater in Table 3.07.

X_{ND} values were 1.63 ± 1.35 % (dry season) and 2.69 ± 2.52 % (rainy season). However, the value wastewater particulate organic nitrogen biologically non-degradable (X_{NI}) percent from TKN range from 5 to 20 % [43].

3.3.3. Other components

Alkalinity (S_{Alk}): In this investigation, the Phuoc Hiep landfill was found to have significantly high alkalinity values. Alkalinity during the study period in the dry and rainy season varied from 8403 ± 1327 $mgCaCO_3.l^{-1}$ and 2202 ± 744 $mgCaCO_3.l^{-1}$, respectively.

Total suspended solids (X_{SS}): X_{SS} value was determined 1300 ± 318 $mg.l^{-1}$ and 1802 ± 633 $mg.l^{-1}$ in the dry and rainy season, respectively.

Dissolved oxygen (S_O): Dissolved oxygen concentration in landfill leachate is very low. The value of measured DO is close to zero because the leachate is flowing out of an anaerobic environment.

3.4. CONCLUSIONS

The analysis of fractional composition of COD and Nitrogen for landfill leachate in dry and rainy season presented has proved that characteristics of landfill leachate are different from two seasons (dry and rainy).

Ammonia nitrogen and biodegradable, soluble nitrogen have the largest share in total nitrogen. A significant decrease of the soluble organic biological degradable fraction of nitrogen in rainy conditions was recorded. Contrary, there is a significant increase in inert organic particulate nitrogen that was also determined in this study. A slight change of the ratios of COD fractions were found between dry and rainy season.

The statistical analysis showed that the weather has an effect on the ratios of COD and nitrogen fractions include X_I, S_S and S_{NH}, S_{ND}, S_{NO}, respectively.

The percentage ratios for nitrogen and COD are quite different from the ratios observed for domestic wastewater, confirming that it was necessary to proceed at those characterizations in order to have correct values to use mathematical models.

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Chapter 4: CHARACTERIZATION OF SLUDGE FOR STOICHIOMETRIC AND KINETIC PARAMETERS FOR MODEL No.1 AND No.3 FOR LEACHATE TREATMENT

4.1. INTRODUCTION

Modelling of activated sludge processes has become a common part of the design and operation of wastewater treatment plants [1]. Activated Sludge Model No.1 has been used extensively for the design and simulation of biological treatment systems. In this model, biomass was considered to grow solely on the external substrate present and the oxygen consumption after the external substrate depletion was explained with the decay of biomass.

Activated Sludge Model No.3 (ASM3) has also introduced a number of kinetic and stoichiometric coefficients with the new processes defined in the model, also suggesting some default values for domestic wastewater. In ASM3, the decay processes are replaced by endogenous processes. The conceptual basis of ASM3 has been largely criticized and alternative models taking into account simultaneous storage and growth processes were also proposed. The main innovation of this model (model 3) is the assumption that all the readily biodegradable organic substrates taken up under feast conditions are directly converted into stored material. These stored compounds become the carbon and energy source for growth purposes.

For the development and optimization of bioprocesses by modelling, information about the kinetic and stoichiometric parameters of the microorganisms are needed [2]. There are some methods to determine these parameters including:

- *Method of direct measurement:* the conventional engineering approach for biomass quantification is to measure particulate COD or VSS [3].
- *Respirometric method:* the techniques based on oxygen uptake measurements. The principles of respirometry are thoroughly illustrated in *Spanjers et al.,* [4]. Respirometry can be used for the calibration of the activated sludge kinetic models of the IAWQ suite [5]. The batch tests were conveniently used in order to estimate ASM3 parameters and the main kinetic and stoichiometric model coefficients were successfully and uniquely determined for aerobic and anoxic conditions. Several authors have used respirometric techniques for the determination of microbial kinetic parameters [6-8].

Overview of the methods of determination of kinetic and stoichiometric parameters of the activated sludge models is presented in Table 4.01.

Table 4.01. Stoichiometric and kinetic coefficients

Group of parameters	Parameters	Unit	Ref.
Stoichiometry	Y_{HO}	$mgCOD.mgCOD^{-1}$	[9-11]
	Y_{HA}	$mgCOD.mgCOD^{-1}$	[12, 13]
	Y_A	$mgCOD.mgCOD^{-1}$	[9, 14, 15]
Kinetic growth rate constants	μ_{HO}	d^{-1}	[3, 9, 16]
	μ_A	d^{-1}	[3, 15]
Kinetic saturation constants	K_S	$mgCOD.l^{-1}$	[6, 9]
	K_A	$mgNH_4^+\text{-}N.l^{-1}$	[9, 14]
Kinetic decay rate	b_{HO}	d^{-1}	[9, 11, 17]
	b_{HA}	d^{-1}	[18, 19]
	b_A	d^{-1}	[11, 15]
Maximum rate substrate utilization	k_{HO}	d^{-1}	[9]
Decay coefficient	k_d	d^{-1}	[20]

Advantages and disadvantages of direct measurement method are discussed by former authors [21]. Limitations of traditional methods may be overcome by respirometry, which allows the indirect measurement of substrate consumption rates by monitoring the biological oxygen consumption rate, under well-defined conditions [21]. The OUR measurement and respirometry have demonstrated to be useful tools. Compared to many other methods it is relatively easy to apply and the other data could be used for simpler characterization and process control as well as for more complex task like simulation and plant design [22].

Based on the identified research gaps and the need for accurate modeling of leachate treatment, the objectives of this study are defined as follows. The study seeks to determine and calibrate the stoichiometric and kinetic parameters of ASM1 and ASM3 using respirometric experiments, to ensure reliable simulation of biological treatment processes and to compare the predictive performance of both models under leachate conditions.

4.2. MATERIALS

4.2.1. Samples of wastewater and activated sludge

The landfill leachate was collected from the landfill site in Phuoc Hiep, Ho Chi Minh City for operating the pilot. The effluent wastewater of pilot was used as the environment for the experiment during determination of kinetic and stoichiometry.

Activated sludge was collected from the landfill leachate treatment plant in Binh Duong to for operating of pilot. The value of MLSS and MLVSS concentration were 5.213 and 4.015 $g.l^{-1}$, respectively.

4.2.2. Pilot

The experimental study was conducted on a pilot shown schematically in Figure 4.01.

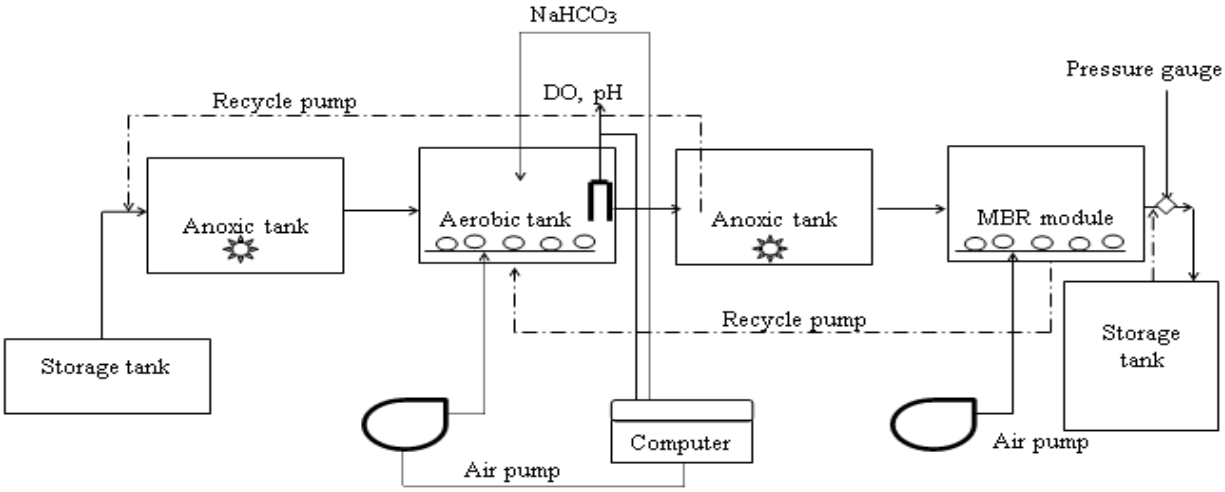


Figure 4.01. The pilot for experiment

The operating parameters of pilot are given in Table 4.02.

Table 4.02. Parameters of system operation

Parameters	Unit	AN1	AE	AN2	MBR
Volume	l	40	40	28	55
Influent	$l.h^{-1}$		2.5 ± 0.2.6		
HRT	h	5.33	1.78	1.24	2.4
Return flow	$l.h^{-1}$	5 ± 0.32	15 ± 1.3	-	-
Average flow total	l	7.5	22.5	22.5	22.5

After the pilot was operated until stable condition. The efficiency of COD and nitrogen removal achieved around 85 % and 92 %, respectively. Activated

sludge from the various tanks were used for determination of kinetic and stoichiometry.

4.2.3. Bioreactor

4.2.3.1. Respirometer

The respirometric tests were performed in 1.5 liters and 3.0 liters. The reactor with a SE 715 Model dissolved oxygen probe (Knick) was inserted into respirometer at the top and a fine bubble air diffuser was placed at the bottom. A laboratory stir plate and magnetic stir bars were used to maintain the respirometer content in suspension. To prevent air getting into the reactor, the electrode has a rubber o-ring fitted and the reactor was completely filled with no air bubbles inside. A SE 555 Model pH sensor (Knick) was installed to record the change of pH. The specific nitrate utilization rate for denitrification can be assessed by using completely mixed, closed atmosphere.

The respirometer used for our study is presented in Figure 4.02.

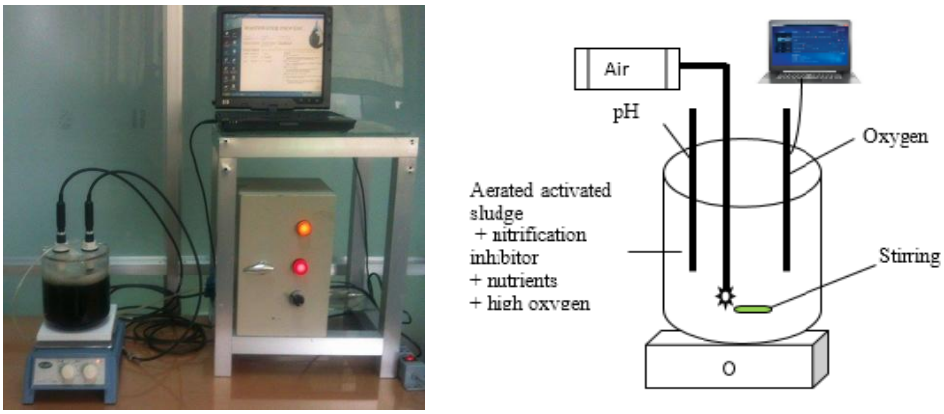


Figure 4.02. Illustration of the principle of OUR measurement

4.2.3.2. Batch reactors

Four acrylic batch reactors were constructed with a volume of 7.84 liters (for one unit) (L x W x H = 20 x 14 x 28 cm), whereas the working volume was changed depending on the purpose in experiments.

Two reactors were connected with the air diffusers placed at the bottom of each reactor. DO concentration was adjusted manually to maintain the dissolved oxygen during experiments using a SE 715 Model dissolved oxygen probe (Knick). A SE 555 Model pH sensor (Knick) was installed to record the change of pH for some experiments.

There were two reactors with caps having small holes to release the air and prevent dissolved oxygen from the air and another small hole to supply nitrogen gas. They are equipped with mixers to maintain the suspension even in anoxic

conditions. The batch reactors were used to estimate the stoichiometric and kinetic are illustrated in Figure 4.03.

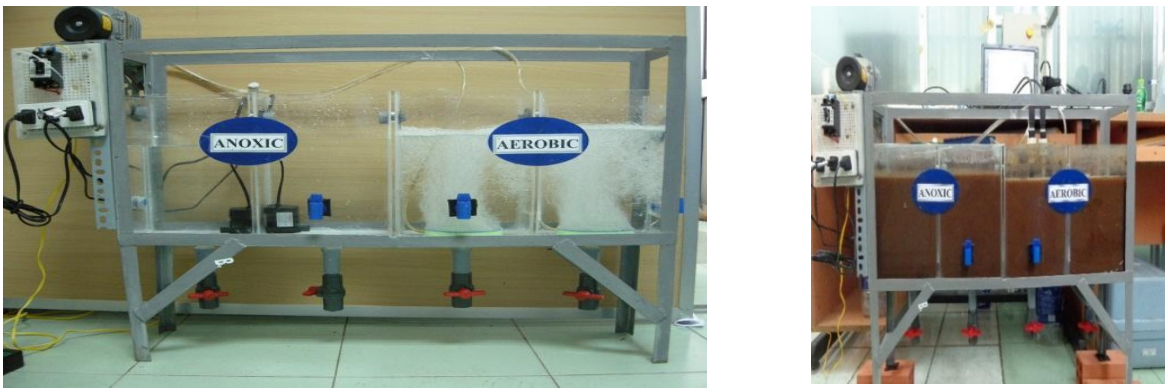


Figure 4.03. Bioreactors used in the experiments

4.2.4. Chemicals

- Allylthiourea (ATU): the inhibitor of nitrifying activity.
- $KClO_3$: to inhibit the process converting nitrite into nitrate.
- KNO_3: prevent anaerobic conditions.
- $(NH_4)_2CO_3$, $NaNO_3$, NH_4Cl, $NaHCO_3$. NaOH, HCl, $Al(OH)_3$.
- Glucose.

The nutrient solution was prepared with the following chemicals mixed in 1 liter of de-mineralized water [23, 24], is presented in Table 4.03.

Table 4.03. Nutrient solution

Nutrient solution A			**Nutrient solution B**		
Chemical	Unit	Weight	Chemical	Unit	Weight
K_2HPO_4	g	320	$MgSO_4.7H_2O$	g	15
KH_2PO_4	g	160	$FeSO_4.7H_2O$	g	0.5
NH_4Cl	g	120	$ZnSO_4.7H_2O$	g	0.5
			$MnSO_4.3H_2O$	g	0.5
			$CaCl_2$	g	2

4.3. PROCEDURE OF EXPERIMENT

4.3.1. Determination of heterotrophic stoichiometric and kinetic coefficients

4.3.1.1. Aerobic condition

a. Heterotrophic yield coefficient under aerobic condition, Y_{HO}

a.1). Preparation for the leachate and organic carbon

- The leachate was collected from the effluent of the pilot in order to experiment. The sludge and nutrients were added to the leachate and aerated for one week to remove completely the biodegradable organics in leachate. The remaining organic components were inert organic. The landfill leachate was pre-filtered through a G6 GF/C membrane filter (through a 0.45-µm) in order to remove suspended solids and a part of colloidal matter.
- The source of biodegradable organic carbon for this experiment was glucose 0.001M – 0.002M (COD approximately 192 $mgO_2.l^{-1}$ – 384 $mgO_2.l^{-1}$).

a.2). Preparation of the biomass

The activated sludge used for experiment was taken in the MBR and AE reactors from the pilot. Activated sludge was aerated for one day to remove all biodegradable COD and ammonia nitrogen contained in the sample before starting experiment. This operation allowed reaching the requested endogenous condition for biomass.

a.3). Operation

- Addition of the filtered leachate and biomass into the 1.5 L respirometer (the whole volume of the respirometer was filled by mixed sludge in order to have a low gas/liquid volume ratio, and so the oxygen transfer from the small volume of gas phase (headspace) of the reactor was considered negligible. The initial VSS was around 50 $mg.l^{-1}$ and mean temperature was maintained at 22 °C during each test.
- At the beginning of the experiment, 10 mg ATU per liter of activated sludge were added as an inhibitor of nitrification process [15].
- Continuous stirring of the reactor contents by using mixer and aeration of the content by using a stone diffuser.
- Add glucose solution according to the initial soluble COD ranging from 100 to 200 $mgO_2.l^{-1}$. After adding glucose solution, samples were aerated and collected quickly to determine exactly the initial soluble COD.

- The sludge contained in the reactors were aerated until reaching the maximum dissolved oxygen concentration. Then, the aeration was interrupted and DO concentration started to drop. Change of DO versus time was recorded.
- Soluble COD concentration was determined immediately after finishing the test.
- The experiments were implemented 3 times to calculate mean value and standard deviation.

a.4). Calculation of Y_{HO} value

- The respiration rate was estimated according to Eq. (4.1) as the slope of the diagram of the dissolved oxygen concentration versus time [23].

$$\frac{dS_{O_2}}{dt} = -r_{O_2}(t) \quad (4.1)$$

- Calculation of value of OUR from slope achieved and the COD consumed (initial sCOD minus remaining sCOD).
- Plotting the OUR versus COD consumed at different COD:VSS ratio. From slope of the graph, the value of Y_{HO} was calculated by Eq. (4.2) [16, 25].

$$Y_{HO} = 1 - \text{slope} \quad (4.2)$$

b. Endogenous decay rate for heterotrophic biomass under aerobic condition, b_{HO}

Heterotrophic decay coefficient (b_{HO}) in aerobic conditions should be estimated by respirometric test using activated sludge only. It is a standard, simple and widespread used method [3, 14, 15, 26]. The value of b_{HO} was determined by measuring the respiration rate of endogenous sludge, in the presence of nitrification inhibitor (ATU) over a period of several days until a constant endogenous respiration [3, 15] rate is reached.

b.1). Preparation of sludge

The activated sludge used was taken from the MBR and AE reactors of the pilot. The activated sludge was aerated for 1 day to starve out before starting the experiment.

b.2). Operation

- The leachate and activated sludge were mixed in vessel of 1.5 liters. The concentration of activated sludge in MBR and AE were 2.6 $g.l^{-1}$ and 2.23 $g.l^{-1,}$ respectively.
- At the beginning of the experiment, 15 mg of ATU were added to the 1.5 liters of sample to each respirometer to prevent any possible interference

induced by nitrification process [15]. No substrate was added to the bioreactors.

- The respirometer pH was maintained at 7.5 by adding sodium bicarbonate with a mean temperature of 22 ± 1.06 °C (21.2 – 23.5 °C).
- The respirometer was aerated by using a stone diffuser connected to aquarium pumps placed at the bottom of bioreactors in order to maintained DO between 2 to 4 $mgO_2.l^{-1}$. Besides, a magnetic stir bar was used to mix continuously the bioreactor.
- Measurement the oxygen uptake rate periodically for 15 days (respiration more than 60 minutes for every measuring time).
- Distilled water was used to compensate evaporative losses.

b.3). Calculate of b_{HO}

Presenting the changes of endogenous respiration rate of sludge versus time in a semi-logarithmic coordinates system, b'_{HO} can be estimated as the slope of the decreasing line. Determine the model decay coefficient by using the following Eq. (4.3) [5, 11, 27]:

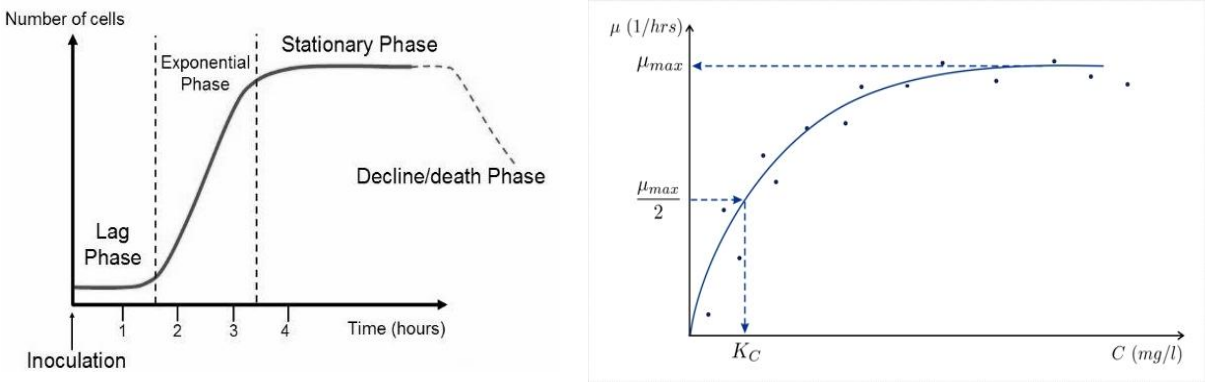
$$b_{HO} = \frac{b'_{HO}}{1-Y_{HO}(1-f_p)} \quad (4.3)$$

Where f_p fraction of biomass that forms particulate decay products. An f_p value of 0.08 was applied in the calculations IAWPRC [28]. Convert to the temperature of 20 °C dependency factor of θ of 1.029 [3, 29]. The Y_{HO} in the formula is the value calculated from Eq. (4.2).

$$b'_{HO,T} = b'_{HO,20} \times (1.029)^{(T-20)} \quad (4.4)$$

c. *Maximum specific growth rate of the heterotrophs under aerobic condition, $\mu_{max,HO}$*

The maximum specific growth rate of heterotrophs was determined by the batch respirometric method of *Ekama et al* [3] and *Kappeler et al* [9]. The oxygen respiration should increase due to unlimited heterotrophic growth until an abrupt decrease in the OUR is detected, while concentration of readily biodegradable substrate becomes limiting. Microbial growth curve and Monod kinetics graph are shown in Figure 4.04 [30].



(a) (b)

Figure 4.04. Microbial growth curve (a) and Monod kinetics graph (b)

c.1). Preparation of leachate

The effluent leachate was coagulated by $Al(OH)_3$ and centrifuged at 1560 $rpm.min^{-1}$ in order to decrease COD and suspended solids in leachate. This sample is used with soluble inert COD in wastewater.

c.2). Preparation of sludge

Withdraw a predetermined amount of mixed liquor from MBR and AE reactors of pilot. Sludge samples were aerated for 3 – 3.5 hours to achieve stable steady state respiration condition before adding glucose.

c.3). Operation

- Mix the centrifuged effluent wastewater and the mixed liquor in the respirometer having working volume of 1 liter. Initial VSS concentration were 54.5 and 52.3 $mg.l^{-1}$ for MBR and AE sludge , respectively.
- Add nitrification inhibitor according to 10 g per 1 liter of sample to each respirometer to prevent nitrification. Then glucose 0.002M was added according to a sbCOD:VSS ratio (COD from glucose) around 4:1 as discussed by *Ekama et al* [3]. Initial soluble biodegradable COD concentration was around 200 $mg.l^{-1}$.
- Place reactors into a laboratory temperature 22 ± 1.06 ^oC.
- Stir the respirometer contents by using a stir plate and a magnetic stir bar. Aeration of the respirometer contents by using a stone diffuser. Insert a calibrated dissolved oxygen probe linked to a computer. Stop aeration when the dissolved oxygen concentration of wastewater reaches around 6.7 – 7.4 $mg.l^{-1}$.
- Allow the DO probe to equilibrate for 5 minutes and then record the change in dissolved oxygen concentrations versus time.

- Turn on the air supply to re-aerate the respirometer contents to maintain a dissolved oxygen concentration above 6 $mgO_2.l^{-1}$. After 30 minutes, the aeration was stopped again to measure DO change versus time. The total measured time recorded about 8 hours.

c.4). Calculate

- OUR is presented as the negative slope of the DO concentration profile during the non-aerated period. The OUR is measured until a sharp decrease in rate is observed.
- The maximum heterotrophic growth rate $\mu_{max,HO}$ can be determined from Eq. (4.5) [17].

$$\ln\left(\frac{OUR_t}{OUR_0}\right) = \mu_{max,HO} - b_{HO} \quad (4.5)$$

Where

OUR_0 oxygen uptake rate at t_0 (first 5 minutes)

OUR_t oxygen uptake rate at t.

b_{HO} endogenous decay rate (calculated from Eq. 4.3)

The maximum specific growth rate was calculated from the slope ($\mu_{max,HO} - b_{HO}$) of the relative OUR versus time plot according to equation outline by *Kappeler and Gujer* [31]. The addition of decay coefficient and the slope of the straight line determine the maximum specific growth rate. The measured rates were corrected to a reference temperature of 20 ºC by Eq. (4.6).

$$\mu_{max,HO(T)} = \mu_{max,HO(20)} \times 1.072^{(T-20)} \quad (4.6)$$

d. Maximum rate of substrate utilization under aerobic condition, k_{HO}

The maximum rate of substrate utilization per unit mass of microorganisms is calculated from Eq. (4.7).

$$k_{HO} = \frac{\mu_{max,HO}}{Y_{HO}} \quad (4.7)$$

e. Half saturation coefficients, $K_{S,HO}$

e.1). Prepare of leachate and activated sludge

The leachate and activated sludge were prepared as for determination of $\mu_{max,HO}$

e.2). Operation

- Pour leachate and activated sludge into respirometer with 1.5 L. Initial activated sludge concentration for MBR and AE were 1.127 and 1.165 $g.l^{-1}$, respectively.
- The mixed liquid was aerated to get the highest value of DO concentration.

- Substrate solution (glucose) was added and samples were taken after 30 seconds. Initial COD concentrations were 539 $mgO_2.l^{-1}$ and 603 $mgO_2.l^{-1}$ for MBR and AE, respectively. The change of DO concentration was measured during 30 minutes or until DO concentration decreased to 3 $mg.l^{-1}$.
- Aeration was restarted and maintained DO around 4 – 5 $mgO_2.l^{-1}$. After one hour, the mixed liquid was aerated until the highest value of DO concentration. Then the aeration was stopped and DO concentration is measured and sample was taken to analyze COD concentration. The test is done several times until a total test of 5 hours.

e.3). Calculate $K_{S,HO}$

- Calculate OUR (v) from value of DO recorded and $COD_{consumed}$ versus time.
- Calculate the gradient at the reaction start as the velocity [V]. Then plot the substrate concentration [S] versus the observed velocity [V]. Lineweaver-Burk Plot: 1/[S] versus 1/v, taking the reciprocal of the Michaelis-Menten Eq. (4.8).

$$\frac{1}{v} = \frac{K_s}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \quad (4.8)$$

The slope $a = \frac{K_s}{V_{max}}$ and intercept $b = \frac{1}{V_{max}}$. From intercept and slope of the plot, the value of V_{max} and K_S were calculated by Eq. (4.9).

$$V_{max} = \frac{1}{b} \quad \text{and} \quad K_s = V_{max} \quad (4.9)$$

4.3.1.2. Anoxic condition

a. The heterotrophic yield under anoxic condition, Y_{HA}

The maximum denitrification rate and the anoxic growth yield were implemented according to Mark C. M. van Loosdrecht *et al,* [23].

a.1). Preparation of stock solution

- Nitrate solution was prepared with concentration of 10 $gNO_3^-\text{-}N.l^{-1}$ with $NaNO_3$ and glucose stock solution of 10 $gCOD.l^{-1}$ (approximately 9.372 g glucose per liter – 1g glucose represents 1.067 g thCOD).
- 1M HCl and 1M NaOH solutions were prepared for pH correction.

a.2). Preparation of sludge

- Activated sludge samples from AN1, AN2 and MBR in pilot.
- Three liters of activated sludge were transferred to the reaction test tanks and mixed some hours to ensure a deoxygenated environment and a gas outlet to limit overpressure.

- Continue to wait for approximately 120 minutes to ensure stable initial conditions. This pre-incubation phase will usually allow the removal of any residual nitrate in samples.

a.3). Operation

- Initial activated sludge were set from 2.5 – 3.0 $g.l^{-1}$.
- The mean temperature set point was set at 22 °C, the initial pH was 7.8.
- Removed any residual DO (< 0.1 $mgO_2.l^{-1}$) with nitrogen gas and ensure anoxic conditions before the nitrate addition.
- The initial nitrate concentration in activated sludge of AN1, AN2 and MBR were set 19.23, 23.01 and 24.4 mg NO_3^--$N.l^{-1}$, respectively. Nitrate stock solution was spiked through the sampling port. Then the liquid was mixed about 1 min and the first sample was taken (minute zero). Six samples were taken at 60 min intervals to assess endogenous denitrification rate.
- After reaching a stable condition (endogenous respiration), a glucose solution was added to determine the exogenous denitrification rate. The initial soluble COD concentrations in AN1, AN2, and MBR were adjusted to 236, 250, and 210 $mg{\cdot}l^{-1}$, respectively.
- COD stock solution was added and 30 seconds afterwards, a sample was taken. Then, samples must be collected every 10 minutes until the end of the test.
- These samples were used to assess the nitrate, nitrite and soluble COD concentration. The test finished after 140 minutes and final samples were taken to assess the MLVSS concentration. The activated sludge concentrations were measured to be 2.363, 2.535 and 2.867 $g.l^{-1}$ in AN1, AN2 and MBR, respectively.

a.4). *Calculate Y_{HA}*

The heterotrophic yield under anoxic condition was determined by van Loosdrecht *et al.,* [23].

Combining denitrification rates and the COD consumption rate, the biomass growth yield (Y_{HA}) can also be assessed according to the following formula:

$$Y_{HA} = 1 - 2.86 \frac{(r_{NO_X,exo} - r_{NO_X,endo})}{r_{sCOD}} \quad (4.10)$$

The maximum specific denitrification rate on readily biodegradable carbon source (rbCOD) on the tested carbon source can be computed as follows:

$$q_{NO_x,rbCOD} = 60 \frac{\left(r_{NO_x\text{-}N_2,exo} - r_{NO_x\text{-}N_2,endo}\right)}{MLVSS} \quad (4.11)$$

b. Heterotrophic decay coefficient under anoxic condition, k_{HA} ; Endogenous decay rate under anoxic condition, b_{HA}

b.1). Preparation of sludge

The activated sludge in AN1, AN2 and MBR reactors in pilot were used for this experiment. DO concentration was kept lower than 0.5 $mg.l^{-1}$.

b.2). Operation

- In AN1, AN2 and MBR from the pilot, three liters of samples were taken and poured into each reactor. The concentration of initial activated sludge (VSS) were 2.413, 2.499 and 3.726 $g.l^{-1}$, respectively.
- The biomass was stirred continuously more than 25 days (until VSS concentration changed insignificantly).
- Potassium nitrate was added into the reactor in order to prevent anaerobic conditions [32]. The pH of sample was maintained at 7.4 ± 0.6 by addition of phosphate buffer.
- The bioreactor was aerated daily for 5 min in order to prevent any shortage of energy in the form of ATP [33]. Because under anoxic conditions, autotrophic bacteria cannot gain ATP because they are obligate aerobes [18]. No substrate was added to the reactor. Samples were collected for every two days analysis.

b.3). Calculate $k_{d,HA}$

Endogenous decay coefficient under anoxic condition was determined by VSS method. The slope of plot of MLVSS versus time is the rate of endogenous decay of biomass, $R_{d,HA}$.

$$R_{d,HA} = - k_{d,HA} \times X \quad (4.12)$$

Where

$R_{d,HA}$ rate of endogenous decay of biomass; $mg.l^{-1}.d^{-1}$

$k_{d,HA}$ endogenous decay coefficient; d^{-1}

X concentration of biomass in the reactor; $mg.l^{-1}$

b.4). Calculate b_{HA}

Determination of heterotrophic decay coefficient under anoxic condition is done from the plots of $ln(MLVSS_t - MLVSS_u)$ versus time, the endogenous decay rate b_{HA} was determined from the slope of $ln(MLVSS_t - MLVSS_u)$.

Where

$MLVSS_t$ VSS concentration at time t, $mgVSS.l^{-1}$.

$MLVSS_u$ VSS concentration at the end of the batch test, $mgVSS.l^{-1}$.

4.3.2. Determination of autotrophic stoichiometric and kinetic coefficients (nitrification processes)

a. Determinate the value of ammonia half saturation coefficient, K_A

The procedure for the experiment was done according to [34, 35].

a.1). Preparation for leachate

The effluent wastewater was coagulated by $Al(OH)_3$ and centrifuged at 1560 $rpm.min^{-1}$ in order to decrease COD and suspended solids in leachate. This sample is used as inert COD in wastewater.

a.2). Prepare for activated sludge

- The activated sludge was collected at AE and MBR in pilot of landfill leachate treatment in this study.
- The value of the initial MLSS and MLVSS were analyzed at AE and MBR reactors were 3.375 and 4.217 $g.l^{-1}$, respectively.

a.3). Operation

- There are 7 bioreactors with working volume 1 liter for every bioreactor. The experiment was separated into 7 different hydraulic retention time (θ) and the activated sludge was not washout.
- The air was continuously supplied and maintained at a stable concentration between 3.0 – 4.0 $mg.l^{-1}$ during experiment.
- The mean temperature was 22 °C.
- The experiments were conducted mainly in a pH range of 7.5 to 8.5 and without alkalinity limitations by adding $NaHCO_3$ to adjust and maintain the value of pH.
- The leachate, NH_4Cl and $NaHCO_3$ were added at the end of each phase.
- The parameters including NH_4^+-N and MLVSS were analyzed at the beginning and the end of each phase.

a.4). Calculating of data from the experiment

$S = S_0 - S_t$,	$mgNH_4^+\text{-}N.l^{-1}$
$X = X_t - X_0$,	$mgVSS.l^{-1}$
$X \times \theta$;	$mg\ VSS.l^{-1}.d^{-1}$
$X \times \theta / S_0\text{-}S$	d
$1/S$	$(mg.l^{-1})^{-1}$

$1/\theta$, d^{-1}

S_0-S/ X×θ; d^{-1}

θ d

By plotting Eq. (4.13) (Plot of X×θ/S_0-S versus 1/S)

$$\frac{X\times\theta}{S_0-S} = \frac{K_A}{k} \times \frac{1}{S} + \frac{1}{k} \quad (4.13)$$

From Eq. (4.13) the slope of this plot represents K_A/k and the ordinate intercept equals 1/k.

b. Determine the value of the autotrophic yield coefficient, Y_A

b.1). Preparation for leachate

Activated sludge was collected from AE and MBR reactors and aerated to remove remaining biodegradable substrate in sludge.

b.2). Prepare for activated sludge

Leachate from effluent of pilot was added to activated sludge and nutrient. Then mixed and aerated during 20 days to remove total of remaining organic in leachate. Remaining COD is mainly inert.

b.3). Operation

- Add a known volume of leachate poured activated sludge with initial VSS concentration set around 2.2 – 2.5 $g.l^{-1}$ and record respiration rate until constant endogenous rate is reached.
- Then aerate until DO saturation.
- Add a known amount of ammonium to activated sludge and record the respiration rate until the endogenous respiration rate is reached again. Initial NH_4^+-N concentration were 210 ± 5 $mg.l^{-1}$ and 223 ± 15 $mg.l^{-1}$ for AE and MBR, respectively.
- Aeration was stopped and record the change DO until around 2 $mg.l^{-1}$.

b.4). Calculation

After calculation of OUR, the coefficient Y_A was determined Eq. (4.14).

$$Y_A = \frac{4.57 \times S_{NH} - \int OUR_{ex}(t)dt}{S_{NH}} \quad (4.14)$$

c. Determinate the value of $\mu_{max,A}$ maximum specific growth rate of active autotrophic biomass

Determine the value of the coefficient $\mu_{max,A}$ using Eq. (4.15).

$$\mu_{max,A} = k \times Y_A \quad (4.15)$$

d. Autotrophic decay rate, b_A

d.1). Preparation of sludge

The activated sludge from pilot AE and MBR reactors were added to nutrients, ammonia and aerated for one month to remove COD in activated sludge.

d.2). Operation

- From AE and MBR reactors, three liters of samples were taken and poured into every reactor. The concentration of initial activated sludge (VSS) were 2.164 and 3.217 $g.l^{-1}$, respectively.
- The biomass was aerated and mixed continuously with DO concentration around 3 – 4 $mgO_2.l^{-1}$ more than 28 days (until VSS concentration changed insignificantly)
- The pH of sample was maintained at 8.2 ± 0.31 by $NaHCO_3$.
- No substrate was added to the reactors. Samples were collected every two days for analysis.

d.3). Calculate b_A

From the results of experiment determination of autotrophic decay rate, the plots of $\ln(MLVSS_t - MLVSS_u)$ versus time, the endogenous decay rate b_A was determined from the slope of $\ln(MLVSS_t - MLVSS_u)$.

Where

$MLVSS_t$ VSS concentration at time t; $mgVSS.l^{-1}$.

$MLVSS_u$ VSS concentration at the end of the bath test; $mgVSS.l^{-1}$.

4.4. METHODS OF ANALYSIS

The MLSS and MLVSS were determined after filtration of sample of mixed liquor on a GC/F filter (Whatman glass micro fibre filter). The sample was weighed after drying at 105 °C on microbalance. The ash content was calculated after incinerating the dried filter in an oven for 1 hour at 550 °C. NH_4^+-N, NO_2^--N, NO_3^--N and COD were measured in accordance Standard Methods [36].

4.5. RESULTS AND DISCUSSION

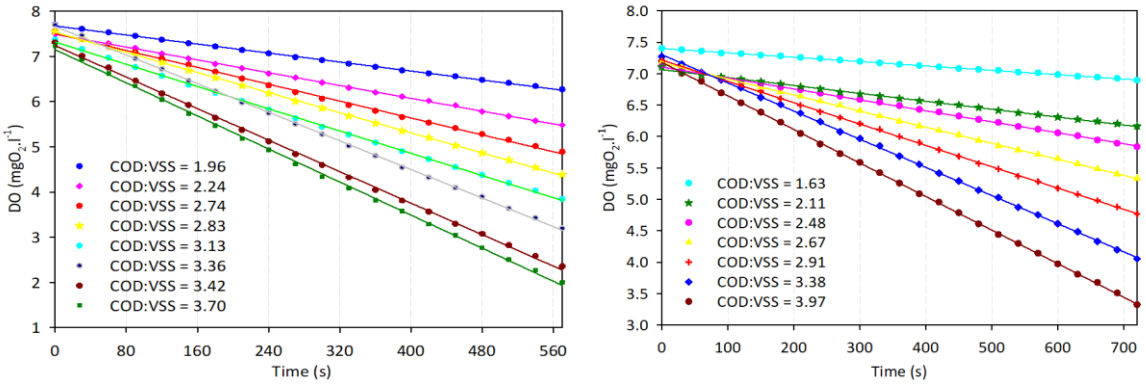
4.5.1. Determination of heterotrophic stoichiometric and kinetic coefficients

4.5.1.1. Aerobic condition

a. Heterotroph yield coefficient under aerobic condition, Y_{HO}

The heterotrophic yield coefficient is an important parameter for modelling of activated sludge [37, 38]. The coefficient of heterotrophic biomass requires an

accurate estimate of the biomass yield because it provides the stoichiometric link between biomass synthesis, substrate consumption and oxygen uptake. In this study, the value of Y_{HO} was determined by using respirometric test. The example of changing DO concentrations versus time were plotted and shown in Figure 4.05.



(a) (b)

Figure 4.05. An example of change DO at various initial COD in MBR (a) and AE (b)

The equations obtained from Figure 4.05, data were collected as Table 4.04.

Table 4.04. Example of data collected from equations of change DO versus time

Reactor	**No. test**	**a** (Slope)	**b** (Intercept)	**R^2**	**SE of slope**
MBR	1	-0.0025	7.6718	0.9993	0.00005
	2	-0.0035	7.4893	0.9996	0.00013
	3	-0.0047	7.5045	0.9989	0.00013
	4	-0.0056	7.5335	0.9998	0.00016
	5	-0.0061	7.3171	0.9995	0.00018
	6	-0.0079	7.6619	0.9997	0.00008
	7	-0.0087	7.2379	0.9993	0.00011
	8	-0.0091	7.1472	0.9993	0.00022
AE	1	-0.0007	7.4008	0.9991	0.00002
	2	-0.0013	7.0648	0.9984	0.00007
	3	-0.0017	7.1057	0.9994	0.00008
	4	-0.0026	7.1720	0.9997	0.00010
	5	-0.0034	7.2182	0.9999	0.00002
	6	-0.0045	7.3018	0.9998	0.00008
	7	-0.0053	7.1810	0.9999	0.00014

From Table 4.04, the value of OUR calculated, the data OUR obtained from experiments at different COD:VSS ratios in MBR and AE reactor are summarized in Table 4.05 and Table 4.06, respectively.

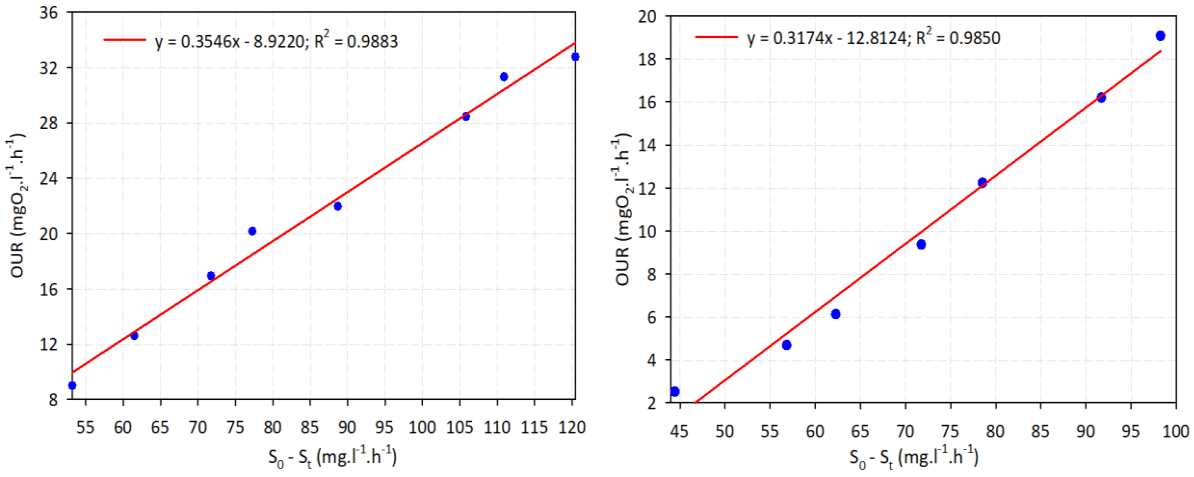
Table 4.05. Example of data of OUR obtained during determining $Y_{HO,MBR}$

COD:VSS ratio	OUR $mgO_2.l^{-1}.h^{-1}$	S_0 $mgO_2.l^{-1}$	$S_{t=570s}$ $mgO_2.l^{-1}$	$(S_0 - S_{t=570s})$ $mgO_2.l^{-1}$	$(S_0 - S_{t=1h})$ $mg\ O_2.l^{-1}.h^{-1}$
1.96	9.00	97.76	89.34	8.42	53.197
2.24	12.60	112.10	102.36	9.74	61.487
2.74	16.92	136.81	125.45	11.36	71.727
2.83	20.16	141.67	129.44	12.23	77.257
3.13	21.96	156.34	142.30	14.04	88.687
3.36	28.44	168.21	151.46	16.75	105.817
3.42	31.32	171.10	153.54	17.56	110.897
3.70	32.76	184.90	165.83	19.07	120.427

Table 4.06. Example of data OUR obtained during $Y_{HO,AE}$ quantification

COD:VSS ratio	OUR $mgO_2.l^{-1}.h^{-1}$	S_0 $mgO_2.l^{-1}$	$S_{t=720s}$ $mgO_2.l^{-1}$	$(S_0 - S_{t=720s})$ $mgO_2.l^{-1}$	$(S_0 - S_{t=1h})$ $mgO_2.l^{-1}.h^{-1}$
1.63	2.52	81.34	72.45	8.89	44.43
2.11	4.68	105.32	93.96	11.36	56.82
2.48	6.12	124.12	111.67	12.45	62.26
2.67	9.36	133.56	119.21	14.35	71.75
2.91	12.24	145.31	129.60	15.71	78.53
3.38	16.20	168.97	150.63	18.34	91.71
3.97	19.08	198.31	178.66	19.65	98.26

From data in Table 4.06, the graph $(S_0 - S_t)$ versus OUR was made and shown in Figure 4.06.



(a) (b)

Figure 4.06. Example of determination of $Y_{HO,MBR}$ (a) and $Y_{HO,AE}$ (b)

From the graphs achieved, the equations, correlation coefficient and accuracy of the slope were calculated and shown in Table 4.07.

Table 4.07. Relationship between OUR and sCOD used during determine Y_{HO}

Reactor	Test	**a** (Slope)	**b** (Intercept)	R^2	SE of slope
MBR	Test 1	0.3546	-8.9220	0.9883	0.01594
	Test 2	0.3287	-7.4215	0.9930	0.01246
	Test 3	0.3174	-27.0730	0.9889	0.01748
AE	Test 1	0.3174	-12.812	0.9850	0.01745
	Test 2	0.3017	-13.242	0.9804	0.01898
	Test 3	0.3393	-18.019	0.9655	0.02859

From equations achieved, the values of slope were found as in Table 4.07. The experiments were done three times for every reactor and achieved results with $R^2 > 0.96$ (the confidence level is 95 %) and $P < 0.0001$. By using Eq. (4.02), the values of Y_{HO} in MBR and AE were calculated and presented in Table 4.08

Table 4.08. The values of Y_{HO} (mgCOD.mgCOD^{-1})

Reactor	Test 1	Test 2	Test 3	Mean ± SD
MBR reactor	0.6454	0.6713	0.6287	0.6485 ± 0.0215
AE reactor	0.6826	0.6983	0.6607	0.6805 ± 0.0189

The values of heterotrophic yield coefficient obtained in this study and from different literature sources are presented in Table 4.09.

Table 4.09. Results of Y_{HO} achieved in this study and literature

Remark	**Y_{HO}, gCOD.gCOD^{-1}**	**Ref.**
MBR - leachate - glucose	0.6485 ± 0.0215	In this study
AE - leachate - glucose	0.6805 ± 0.0189	In this study
SBR - leachate	0.6833	[39]
Sewage wastewater	0.67	[40]
Domestic - acetate	0.61 – 0.67	[38]
Domestic - wastewater	0.70 – 0.87	[38]
Domestic - glucose	0.79 – 0.85	[38]
Dairy wastewater	0.933	[41]
Municipal wastewater	0.3 – 0.8	[20]
Pharmaceutical wastewater	0.3 – 0.72	[42]
Batch test - domestic wastewater	0.31 – 0.35	[43]
Jet loop – leachate wastewater	0.28	[44]

As shown in Table 4.09, a slightly smaller growth yield was observed for the MBR compared to the AE. From test t-test of achieved results shown that there is no statistically significant difference between values Y_{HO} achieved in MBR and AE ($P = 0.125$).

However, the slightly lower growth yield in the MBR might be attributable to differences in the microbial species present in the two processes, which can affect the growth yield. The value of obtained Y_{HO} in AE is closed to the one of Yen H. V [39] also measured on Vietnamese leachates.

Comparison of the average value of Y_{HO} obtained for leachate and various wastewaters, shows that there are significant differences between wastewaters and methods determination. According to [38], the values of heterotrophic yield coefficient varies with substrates. Some values seem unrealistic: even for easy biodegradable substrates, it is difficult to reach values > 0.7 as part of the substrate has to be converted into energy for biosynthesis.

In conclusion, the value of Y_{HO} is an important parameter for modelling degradation process with activated sludge. It is significantly affected by the type of substrate, and process during determination of heterotrophic yield coefficients. Therefore, to avoid false calculations these observations imply the

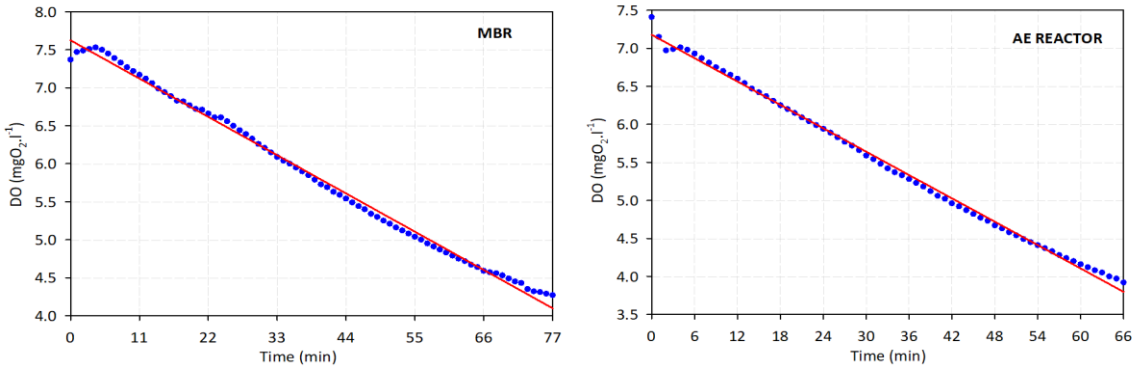
necessity for determining the Y_{HO} for each wastewater and treatment technology studied separately.

b. Endogenous decay rate for heterotrophic biomass under aerobic conditions, b_{HO}

The heterotrophic decay coefficient is one of the most important kinetic coefficient in ASM. It is used to predict the sludge production and oxygen demand of an activated sludge process. A change in b_{HO} has a large effect on the predicted biomass concentration. In this study, the decay coefficient for heterotrophic biomass was determined by measuring the respiration rate of endogenous sludge. The value of OUR was estimated as the slope of a regression line fitted to a series of DO data. The Example of the change DO versus time is demonstrated in Table 4.10 in MBR and Figure 4.07 during determination of OUR in MBR and AE.

Table 4.10. Example of change DO data versus time in MBR

Time min	**DO** $mgO_2.l^{-1}$	**Time** min	**DO** $mgO_2.l^{-1}$	**Time** min	**DO** $mgO_2.l^{-1}$	**Time** min	**DO** $mgO_2.l^{-1}$
0	7.37	*20*	6.72	*40*	5.73	*60*	4.83
1	7.47	*21*	6.71	*41*	5.69	*61*	4.79
2	7.49	*22*	6.66	*42*	5.63	*62*	4.75
3	7.51	*23*	6.61	*43*	5.59	*63*	4.72
4	7.53	*24*	6.61	*44*	5.54	*64*	4.67
5	7.50	*25*	6.56	*45*	5.49	*65*	4.64
6	7.45	*26*	6.50	*46*	5.44	*66*	4.59
7	7.39	*27*	6.44	*47*	5.40	*67*	4.57
8	7.33	*28*	6.39	*48*	5.34	*68*	4.56
9	7.27	*29*	6.33	*49*	5.30	*69*	4.53
10	7.22	*30*	6.26	*50*	5.25	*70*	4.49
11	7.17	*31*	6.21	*51*	5.21	*71*	4.45
12	7.12	*32*	6.15	*52*	5.16	*72*	4.43
13	7.06	*33*	6.09	*53*	5.12	*73*	4.35
14	6.99	*34*	6.04	*54*	5.08	*74*	4.32
15	6.94	*35*	6.00	*55*	5.04	*75*	4.31
16	6.89	*36*	5.95	*56*	5.00	*76*	4.29
17	6.83	*37*	5.90	*57*	4.95	*77*	4.27
18	6.82	*38*	5.85	*58*	4.91	-	-
19	6.77	*39*	5.79	*59*	4.87	-	-



(a) (b)

Figure 4.07. Example of changes DO versus time in MBR (a) and AE (b)

In MBR (Figure 4.07a)

$y = -0.0458x + 7.6244$; $R^2 = 0.9954$; slope = 0.0458 ± 0.00035

In AE (Figure 4.07b)

$y = -0.051x + 7.16$; $R^2 = 0.9999$; slope = 0.051 ± 0.00014

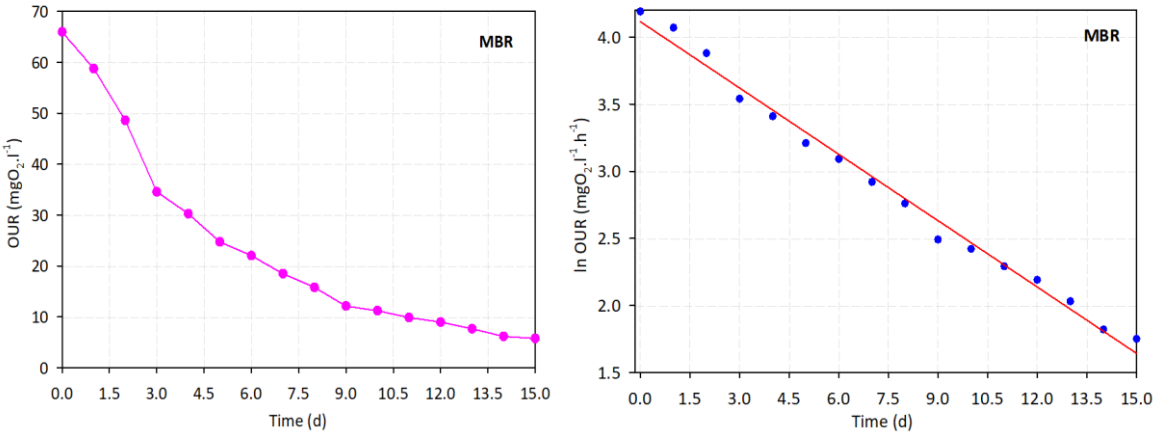
The observation was continued for several days. The loss of biomass due to endogenous decay is commonly considered as an oxidation process. Depending on the change of DO concentration versus time, the OUR curve was obtained from the recorded DO profile in Figure 4.07. The decay coefficient was calculated from the slope of a plot of the natural logarithm of respiration rate versus time [15, 45].

The Example of OUR, lnOUR versus time (t) determination for $b_{HO,MBR}$ is demonstrated in Table 4.11. The profile OUR and plot of lnOUR versus time for the determination $b_{HO,MBR}$ and $b_{HO,AE}$ are presented in Figure 4.08 and Figure 4.09.

Table 4.11. Calculate OUR and lnOUR versus time in MBR

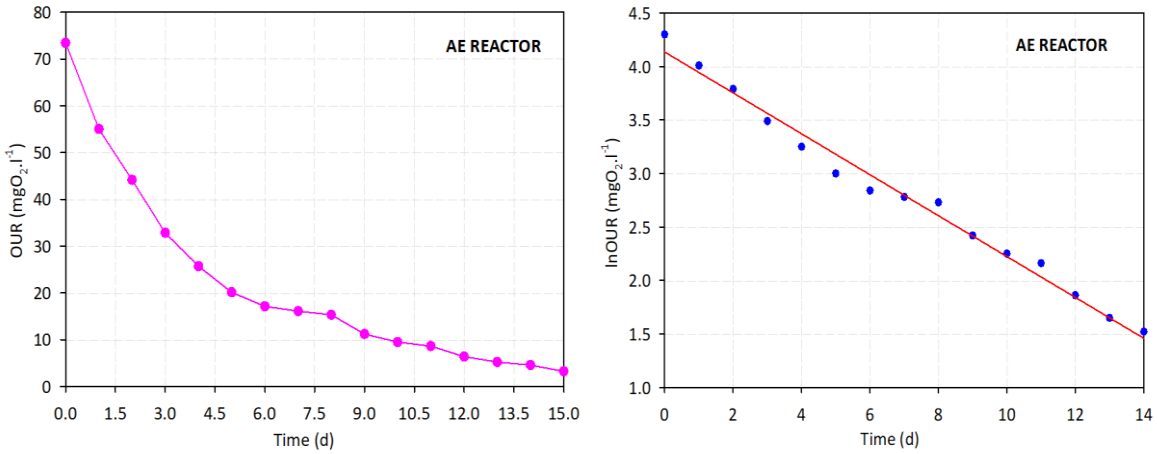
Time d	**OUR** $mgO_2.l^{-1}.d^{-1}$	**lnOUR**	**Time** d	**OUR** $mgO_2.l^{-1}.d^{-1}$	**lnOUR**
0	65.95	4.19	*8*	13.94	2.63
1	58.75	4.07	*9*	11.27	2.42
2	48.62	3.88	*10*	9.35	2.24
3	32.56	3.48	*11*	8.41	2.13
4	30.24	3.41	*12*	9.13	2.21
5	24.86	3.21	*13*	7.80	2.05
6	22.16	3.10	*14*	6.30	1.84
7	18.63	2.92	*15*	5.90	1.77

From Figure 4.08 shown that the decrease of OUR up to reach an endogenous respiration level around 15 days.



(a) (b)

Figure 4.08. Example of OUR (a) and $lnOUR_{HO,MBR}$ (b) during determine $b_{HO,MBR}$ in MBR



(a) (b)

Figure 4.09. Example of OUR (a) and $lnOUR_{HO,AE}$ (b) during determine $b_{HO,AE}$ in AE

The collected results from graphs Figure 4.08b and Figure 4.09b during determine of b_{HO} was shown in Table 4.12.

Table 4.12. Data collected from lnOUR versus time during determine b_{HO}

Reactor	**Test**	**a** (slope)	**b** (intercept)	**r^2**	**SE of slope**
MBR	Test 1	-0.1666	4.1309	0.9934	0.00372
	Test 2	-0.1565	4.117	0.9958	0.00277
	Test 3	-0.1643	4.1841	0.9915	0.00404
AE	Test 1	-0.1884	4.1207	0.9883	0.00542
	Test 2	-0.1906	4.1413	0.9947	0.00385
	Test 3	-0.1972	4.1479	0.9966	0.00309

From equations of Figure 4.08 (b) and 4.09 (b), the values of $b_{HO,T}$ were found as Table 4.12 ($R^2 > 0.98$). Using Eq. (4.03) to calculate the values of endogenous decay rate, $b'_{HO,T}$ for the MBR, AE were presented in Table 4.13. Because the decay coefficient was estimated at a various temperatures. The test results were adjusted by the Arrhenius equation. A small value of θ has been used for the effect of temperature on decay with a value of 1.029 (convert to the temperature of 20 °C dependency factor) [3]. This value was used to correct the model decay coefficient $b'_{HO,T}$ to a temperature of 20 °C. The average temperature correction of the parameters determined from the lab-scale experiments (22°C) was carried out according to standard procedures [37]. Using Eq. (4.04) to convert to the temperature of 20° C, the results were shown in Table 4.13.

Table 4.13. The values of b_{HO}

Test	MBR		AE	
	$b_{HO,T}{}^{o}C$	$b_{HO,20}{}^{o}C$	$b_{HO,T}{}^{o}C$	$b_{HO,20}{}^{o}C$
1	0.0989	0.0934	0.1183	0.1117
2	0.0967	0.0913	0.1224	0.1156
3	0.0950	0.0898	0.1199	0.1132
Mean	**0.0969**	**0.0915**	**0.1202**	**0.1135**
SD	**0.0020**	**0.0018**	**0.0021**	**0.0020**

The values of b_{HO} at 20°C achieved in MBR and AE and literature are presented in Table 4.14.

Table 4.14. The value of $b_{HO,20}{}^{o}C$ in this study and former authors

Remark	$b'_{HO;}\ d^{-1}$	Ref.
MBR - leachate	0.0915 ± 0.0018	In this study
AE - leachate	0.1135 ± 0.0020	In this study
AE – sewage wastewater	0.054 – 0.057	[18]
Biofilm - petrochemical wastewater	0.180	[46]
AE – industrial wastewater	0.08 – 0.36	[47]
AE – municipal wastewater	0.04	[15]

As shown in Table 4.14 shown results from batch decay tests using biomass from the MBR and AE reactors are in the range found in literature. During the endogenous phase testing, there was no external input of substrate and therefore all substrate was initially generated through the processes of decay

and hydrolysis. The biomass concentration and respiration rate decreased slowly and continuously, while inert particulate matter and biomass debris accumulated [10]. There is a slight difference in values of b_{HO} between MBR and AE. It can be suggested that the main reason is microbial community composition in MBR and AE. The b_{HO} values achieved in this study are higher than the values obtained on activated sludge from sewage and municipal wastewater plants. Besides, values achieved and biofilm sludge from petrochemical wastewater plant have no significant difference.

According to [48], from the result of Ramdani *et al.* showed that the endogenous decay rate obtained is 0.24 d^{-1} under aerobic condition. According to [47], Martinage and Paul, the $b_{HO,AE}$ value can be effected by environmental parameters including load, temperature, hydraulic. They observed some variation in decay rate under aerobic conditions from 0.08 – 0.36 d^{-1} due to the changes in influent quality from the same source for wastewater at 30 °C. However, this value achieved 0.36 d^{-1} when 30 % of the influent load consists of urban wastewater.

In conclusion, the variations of the aerobic endogenous decay rate found in the literature could be attributed to various factors such as characteristics of wastewater, temperature, microbial community composition, etc. Also a rather unnatural matrix such as leachates could generate a stress for bacteria and increase the decay rate.

c. *Maximum specific growth rate of the heterotrophs under aerobic condition, $\mu_{max,HO}$*

The maximum specific growth rate was evaluated using the respirometric technique of Kapperler and Gujer [9]. The experiment measurements for the assessment of the maximum specific growth rate for heterotrophs, $\mu_{max,HO}$ was conducted on activated sludge from MBR and AE. OUR is calculated from the decrease DO versus time measured during 5 minutes. The graph of $\mu_{max,HO,MBR}$ determination is presented in Table 4.15 and Figure 4.10.

Table 4.15. Example of a change DO and calculate OUR during determine $\mu_{max,HO,MBR}$

Time h	**DO_0** $mgO_2.l^{-1}$	**DO_t** $mgO_2.l^{-1}$	**OUR** $mgO_2.l^{-1}.h^{-1}$	**Time** h	**DO_0** $mgO_2.l^{-1}$	**DO_t** $mgO_2.l^{-1}$	**OUR** $mgO_2.l^{-1}.h^{-1}$
0	7.27	6.93	7.65	-	-	-	-
0.5	7.15	6.41	8.87	*4.5*	6.72	5.34	16.61
1.0	6.90	6.079	9.86	*5.0*	7.18	5.85	15.92
1.5	7.20	6.35	10.20	*5.5*	7.00	5.71	15.40

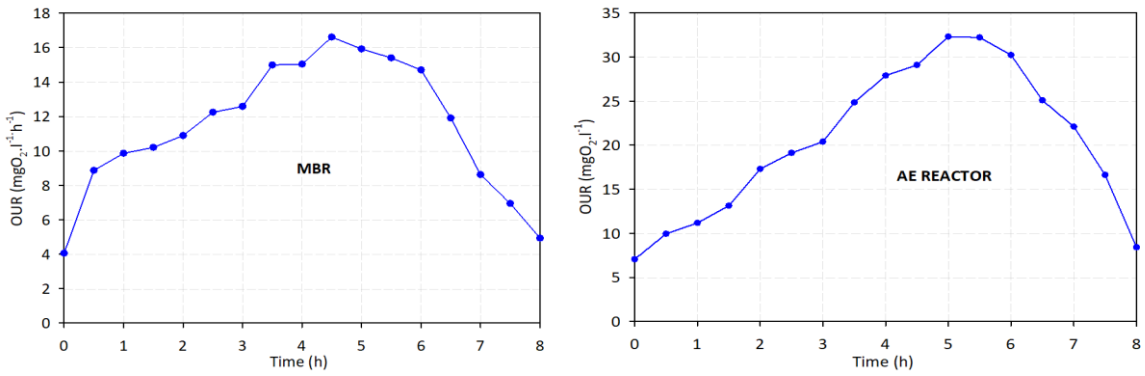
Time h	DO_0 $mgO_2.l^{-1}$	DO_t $mgO_2.l^{-1}$	OUR $mgO_2.l^{-1}.h^{-1}$	Time h	DO_0 $mgO_2.l^{-1}$	DO_t $mgO_2.l^{-1}$	OUR $mgO_2.l^{-1}.h^{-1}$
2.0	6.78	5.87	10.89	*6.0*	6.71	5.49	14.70
2.5	6.88	5.86	12.24	*6.5*	7.36	6.37	11.91
3.0	7.01	5.96	12.58	*7.0*	7.00	6.28	8.62
3.5	7.37	6.12	14.99	*7.5*	6.93	6.35	6.94
4.0	6.84	5.58	15.04	*8.0*	7.33	6.92	4.93

The values of $\ln(OURt/OUR_0)$ were calculated as Table 4.16

Table 4.16. Example of calculated $\ln(OURt/OUR_0)$ values during determine of $\mu_{max,HO,MBR}$

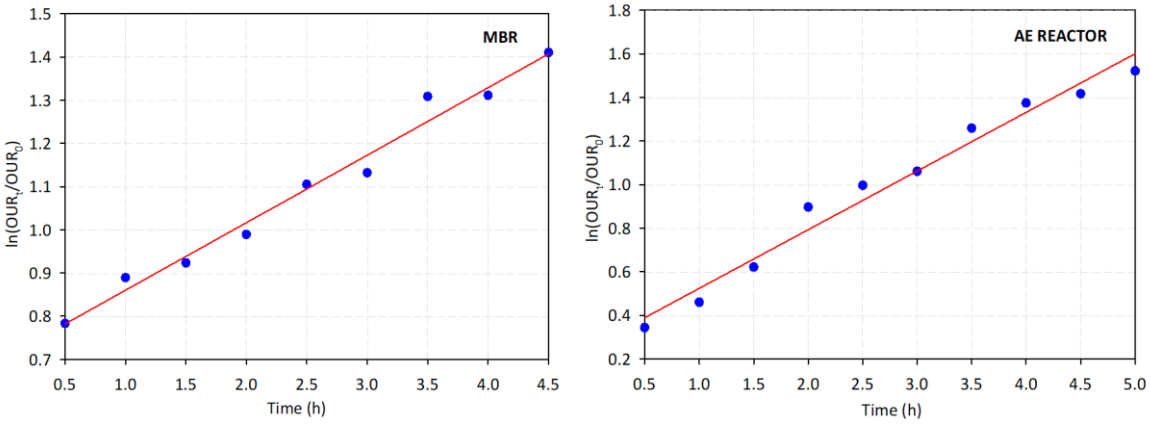
Time; h	OUR_t/OUR_0	$\ln(OUR_t/OUR_0)$	Time; h	OUR_t/OUR_0	$\ln(OUR_t/OUR_0)$
0.5	2.19	0.78	*3.0*	3.10	1.13
1.0	2.43	0.89	*3.5*	3.70	1.31
1.5	2.52	0.92	*4.0*	3.71	1.31
2.0	2.69	0.99	*4.5*	4.10	1.41
2.5	3.02	1.11	-	-	-

The respiration rate trend was as expected, the OUR increased and reached a maximum at 4.5 hours. As shown in Figure 4.10, after the readily biodegradable substrate addition, the OUR immediately increased. Besides, oxygen respiration increased due to the unlimited growth of heterotrophic biomass to a maximum during the first 4 to 5 hours of the test, followed by a sharp decrease to a low level because of limiting concentrations of readily biodegradable substrate. The oxygen respiration rate at this level was dominated by growth on substrate released by hydrolysis.



(a) (b)

Figure 4.10. Graph of determine OUR versus time in MBR (a) and AE (b)



(a) (b)

Figure 4.11. Example of determine of μ_{HO} in MBR (a) and AE (b)

From graphs in Figure 4.11, collected data are presented as Table 4.17.

Table 4.17. Data during determine μ_{HO}

Reactor	Test	**a** (slope)	**b** (intercept)	R^2	SE of slope
MBR	Test 1	0.1563	0.7038	0.9809	0.00811
	Test 2	0.1560	0.8313	0.9839	0.00763
	Test 3	0.1658	0.4416	0.9846	0.00794
AE	Test 1	0.2692	0.2545	0.9756	0.01505
	Test 2	0.2649	0.05117	0.9722	0.01584
	Test 3	0.2844	0.2735	0.9874	0.01139

The kinetic growth rate constants, $\mu_{max,HO,T}$ and maximum specific growth rate at 20 °C were calculated by using Eq. (4.5), Eq. (4.6) and previously obtained value of b_{HO} from this study. The results are shown in Table 4.18.

Table 4.18. Results of $\mu_{max,HO}$

Reactor	b_{HO,T^oC}	$\mu_{max,HO,T^oC} - b_{HO,T^oC}$	μ_{max,HO,T^oC}	$\mu_{max,HO,20^oC}$
MBR	0.0989	3.751	3.85	3.350
	0.0967	3.744	3.84	3.342
	0.0950	3.979	4.07	3.545
AE	0.1183	6.461	6.58	5.725
	0.1224	6.358	6.48	5.639
	0.1199	6.826	6.95	6.044

The values of maximum specific growth rate of heterotrophic in this study and some results from literature are presented in Table 4.19.

Table 4.19. Results for determine of maximum specific growth rate, $\mu_{max,HO}$

Remark	$\mu_{max,HO}$; d^{-1}	Ref.
MBR - glucose	3.41 ± 0.12	In this study
AE - glucose	5.80 ± 0.21	In this study
MBR - synthetic	1.28 - 6.46	[49]
Extended AE - municipal wastewater	1.96 - 3.17	[50]
AE - municipal wastewater	0.95 - 0.98	[50]
Biofilm - petrochemical wastewater	6.1	[46]
AE - acetate	3.53	[51]
AE - domestic sewage wastewater	3.4 - 6.5	[31]

As shown in Table 4.19, comparison of the average values of maximum specific growth rate obtained for activated sludge from MBR and AE in this study are in the same range than other authors for various wastewater. The mean maximum heterotrophic growth rate for the MBR and AE were 3.41 d^{-1} and 5.80 d^{-1} respectively. However, significant differences of value of maximum specific growth rate can be obtained from authors on municipal wastewater at different air supplied conditions. These values were within the range 1.28 – 6.46 d^{-1} reported in literature for synthetic wastewater [50]. According to Mardani S. *et al.*, the maximum specific growth rate of heterotrophs for municipal wastewater may vary in the range of 0.95 - 0.98 d^{-1}, with traditional aerobic but in the range of 1.96 – 3.17 for extended aeration.

d. Maximum rate of substrate utilization under aerobic condition, k_{HO}

From results achieved of Y_{HO} in Table 4.08 and $\mu_{max,HO}$ in Table 4.18, the values of maximum rate of substrate utilization are calculated by using Eq. (4.7) and shown in Table 4.20.

Table 4.20. Calculate of $k_{HO,T}$ values

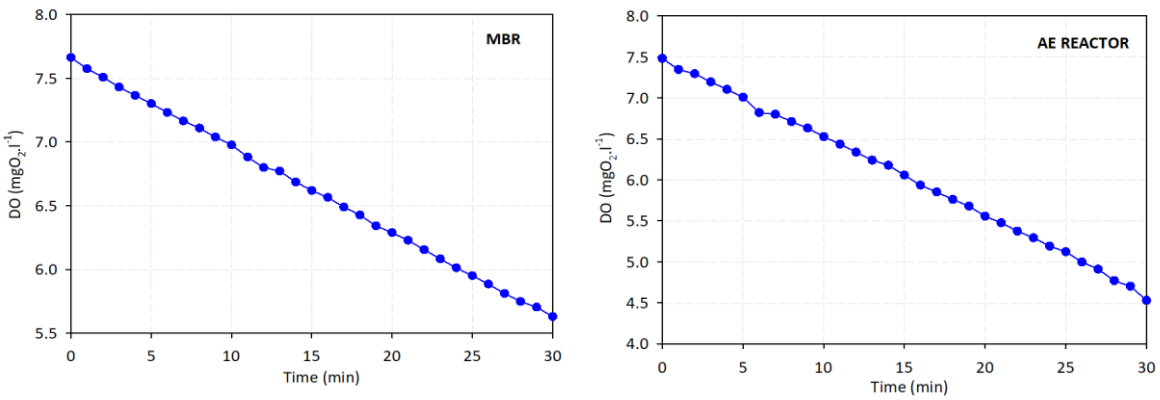
Reactor	$Y_{HO,T}$	$\mu_{max,HO,T}$	$k_{HO,T}$
MBR	0.6454	3.85	5.97
	0.6713	3.84	5.72
	0.6287	4.07	6.47
AE	0.6826	6.58	9.64
	0.6983	6.48	9.28
	0.6607	6.94	10.50

Table 4.21. Mean results of $k_{OH,T}$ in this study and former authors

Remark	**$k_{HO,T}$ gCOD.gVSS^{-1}.d^{-1}**	**Ref.**
MBR	6.05 ± 0.38	In this study
AE	9.81 ± 0.63	In this study
AE (2/3gSS.l^{-1}) – industrial wastewater	2.6/10.3	[52]
AE - municipal wastewater	2 - 10	[20]
Conventional (2/3gSS.l^{-1}) - municipal wastewater	1.95/1.22	[50]
Extended aeration (4/5 gSS.l^{-1}) – municipal wastewater	3.17/2.53	[50]
Contact stabilization (2/3 gSS.l^{-1}) municipal wastewater	0.586/0.366	[50]
Domestic wastewater	5	[53]
Cheese processing wastewater	9.3	[53]

As shown in Table 4.21, the mean value of k_{HO} in this study were 6.05 and 9.81 gCOD.gVSS^{-1}.d^{-1} for MBR and AE reactors, respectively. The value of k_{HO} for municipal wastewater found in former studies varied over a wide range and were affected by activated sludge concentration. According to Mardani S. *et al.*, [50] the value of k_{OH} decreased if activated sludge concentration increased, although this difference was not significant for municipal wastewater. However, Azimi N. *et al.*, [52] investigated that the activated sludge concentration affects significantly k_{OH} from industrial wastewaters. Besides, Ambreen L. *et al*, also found that the maximum rate of substrate utilization in domestic wastewater was lower than for cheese processing wastewater [53].

e. *Half saturation coefficients, $K_{S,HO}$*



(a) (b)

Figure 4.12. Example of changing DO during determining of $Ks_{,HO}$ in MBR (a) and AE (b)

The half saturation coefficient or the substrate concentration at which the specific growth rate is ½ the maximum specific growth rate can be obtained from the experimental oxygen uptake curve in the following manner. The results of OUR in MBR and AE are calculated and presented in Table 4.22 and Table 4.23, respectively.

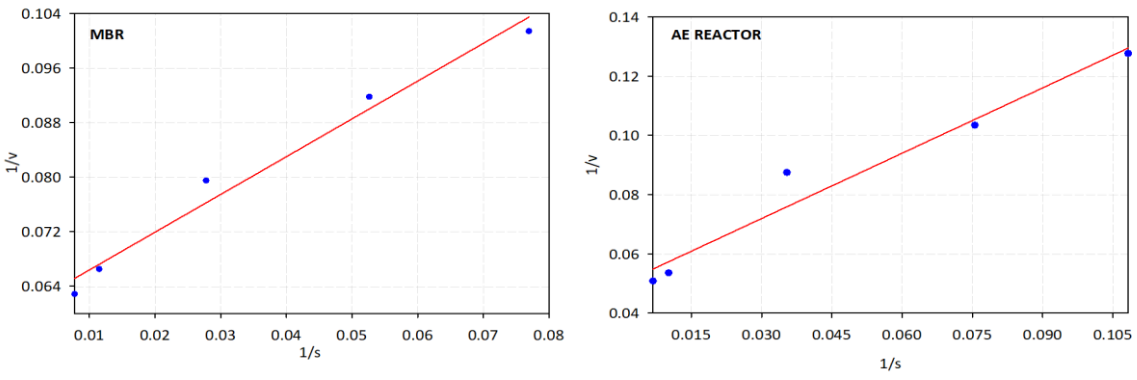
Table 4.22. Example of OUR during determination of $K_{S,HO,MBR}$

Time, h	**OUR (v),** $mgO_2.l^{-1}$	**$sCOD_t$,** $mgO_2.l^{-1}$	**$s=sCOD_{t0}-sCOD_t$** $mgO_2.l^{-1}$	**1/s**	**1/v**
0	4.05	539	0	-	0.247
1.0	9.86	526	13	0.077	0.101
2.0	10.89	520	19	0.053	0.092
3.0	12.58	503	36	0.028	0.079
4.0	15.04	452	87	0.011	0.066
5.0	15.92	410	129	0.008	0.063

Table 4.23. Example of OUR during determination of $K_{S,HO,AE}$

Time h	**OUR (v)** $mgO_2.l^{-1}$	**$sCOD_t$** $mgO_2.l^{-1}$	**$s=sCOD_{t0}-sCOD_t$** $mgO_2.l^{-1}$	**1/s**	**1/v**
0	5.74	603	0	-	0.174
1.0	7.83	594	7	0.108	0.128
2.0	9.67	590	14	0.076	0.103
3.0	11.43	575	22	0.035	0.087
4.0	18.66	505	79	0.010	0.054
5.0	19.68	457	116	0.007	0.051

Based on Table 4.22 and 4.23, a plot of the experimental data illustrates the relationship between 1/s and 1/v were done and shown in Figure 4.13.



(a) (b)

Figure 4.13. Example of $Ks,_{HO}$ determination in MBR (a) and AE reactor (b)

Table 4.24. Data during determine $K_{S,HO}$

Reactor	Test	**a** (Slope)	**b** (Intercept)	R^2	SE of slope
MBR	Test 1	0.555	0.061	0.9779	0.04821
	Test 2	0.511	0.051	0.9945	0.02187
	Test 3	0.529	0.051	0.9834	0.03960
AE	Test 1	0.736	0.050	0.9603	0.08633
	Test 2	0.755	0.050	0.9873	0.04951
	Test 3	0.734	0.052	0.9898	0.04291

The slope (a) and intercept (b) of linear curve is calculated by the least-squares method from Eq. (4.09). From equations fitted the values of V_{max} in MBR and AE were determined. The half saturation coefficients in MBR and AE were calculated in this study and authors and presented in Table 4.25.

Table 4.25. Data for calculating during determine $K_{S,HO}$

Reactor	Test	$1/V_{max}$	$K_{S,HO}/V_{max}$	V_{max}	$Ks_{,HO}$
MBR	Test 1	0.061	0.555	16.447	9.133
	Test 2	0.051	0.511	19.646	10.033
	Test 3	0.051	0.529	19.763	10.451
AE reactor	Test 1	0.050	0.736	20.080	14.783
	Test 2	0.050	0.755	20.161	15.220
	Test 3	0.052	0.734	19.305	14.160

Mean values of $K_{S,HO}$ in this study and former researchers are presented in Table 4.26.

Table 4.26. Results for half saturation coefficients, $K_{S,HO}$

Remark	$K_{S,HO}$; mgCOD.l^{-1}	Ref.
MBR - leachate	9.872 ± 0.673	In this study
AE - leachate	14.721 ± 0.532	In this study
SBR - soybean curd wastewater	9.98	[54]
MBR - municipal wastewater	6.65	[55]
AE - domestic w wastewater	85.5	[56]
Biofilm – petrochemical wastewater	9.4	[46]
IFAS[a] – industrial wastewater	54.7	[52]

[a] Integrated fixed-film activated sludge

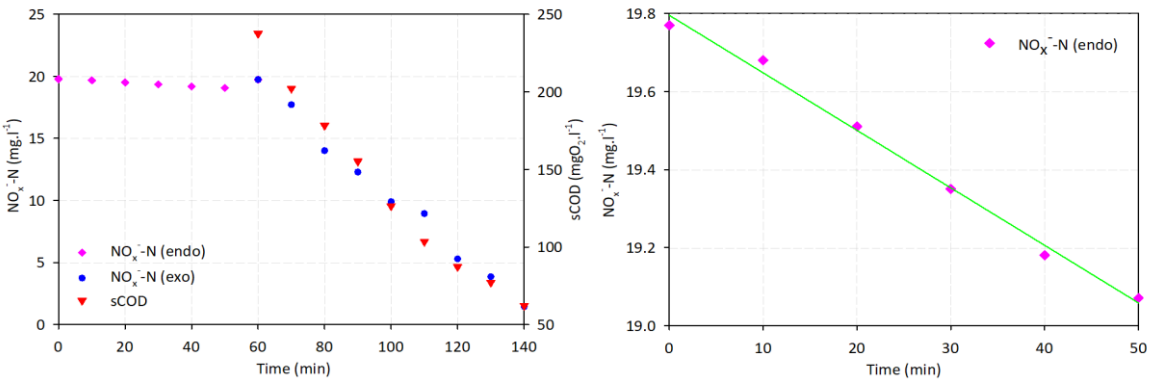
From Table 4.26, the half saturation coefficients in MBR and AE were 9.872 and 14.721 mgCOD.l^{-1}, respectively. The values reported in this study agree with the literature reported values by Gao D. *et al.*, [54] and Trojanowicz K. *et al.*, [46] from soybean curd and petrochemical wastewater, respectively. However, these values in this study are slightly higher than that reported by Naghizadeh A. *et al.*, [55] for municipal wastewater treated by MBR and the very small ones investigated by Azimi N. *et al.*, [52] for industrial wastewater. Especially, the value of $K_{S,OH}$ for domestic wastewater was found to be 85.5 mgCOD.l^{-1} by Najafpour G. [56] . Hence, the difference of values measured from different authors are probably due to the organic used, especially other components of wastewater affected the research results. This also means that those half saturation coefficients are rather low and most of the time the Monod Equation of growth for the heterotroph will be in close to a zero order kinetic, not far from the maximum growth rate.

4.5.1.2. Anoxic condition

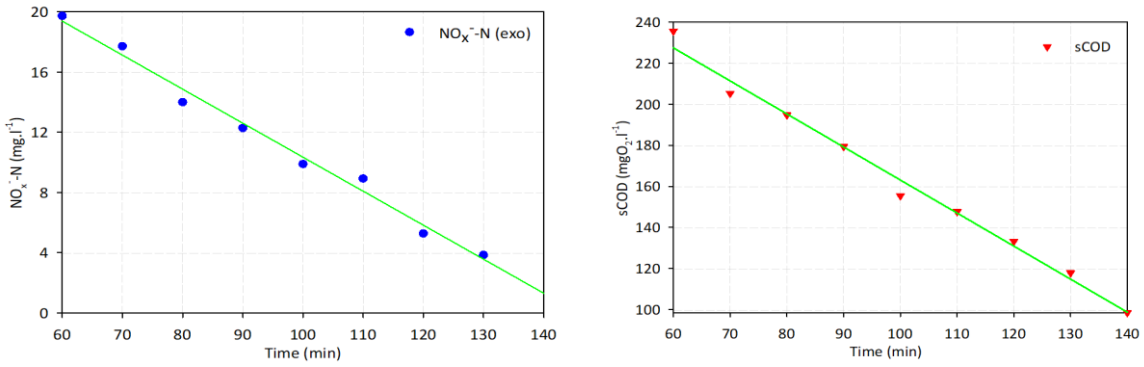
a. Heterotroph yield coefficient under anoxic condition, Y_{HA}

With denitrifying heterotrophic microorganism, the true anoxic yield Y_{HA} is important in order to determine the mass of organic carbon required to remove nitrogen and the corresponding sludge production. In anoxic condition, many heterotrophs have the capability of switching the mode of their metabolic activity, readily adapting themselves to consume nitrate as the terminal electron acceptor instead of dissolved oxygen. Experimental results of a typical denitrification batch test for AN1, AN2 and MBR reactors of the pilot are shown in Figure 4.14, 4.15 and 4.16, respectively.

In the denitrification batch tests, COD consumption rate and the nitrate utilization rate were determined from the COD and nitrate concentration versus time. Typical examples of OUR and NUR profiles obtained in anoxic are shown in Figure 4.14a, 4.15a, 4.16a.



(a) (b)



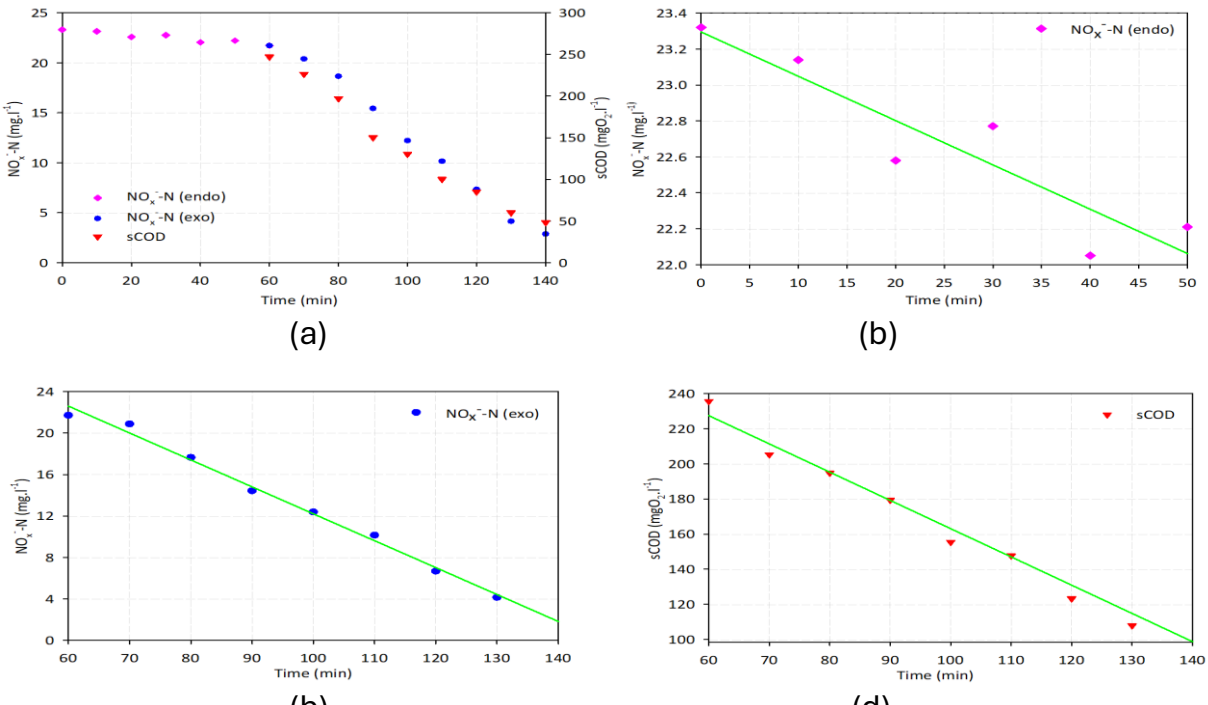
(c) (d)

Figure 4.14. Profiles obtained from example during determination of $Y_{HA,AN1}$

As shown in Figure 4.14a, it explained that after feeding period of glucose, NO_X^--N was consumed rapidly (due to acclimated biomass and exogenous substrate) denitrification started and was depleted completely after 80 minutes.

Endogenous and exogenous denitrification rates were estimated by linear regression $r_{NOX-N2,\ endo}$ and $r_{NOX-N2,exo}$ as shown in Figure 4.14b and 4.14c, respectively. Besides, the exogenous COD consumption rate was also estimated by linear regression rate of sCOD, in $mgCOD.l^{-1}.min^{-1}$. The only data obtained in the non-limiting denitrification phase were used for kinetic rates quantification of denitrification.

From data obtained, profiles for AN2 and MBR were plotted and shown in Figure 4.15 and Figure 4.16.



(a) (b)

(b) (d)

Figure 4.15. Profiles obtained from example during determination of $Y_{HA,AN2}$

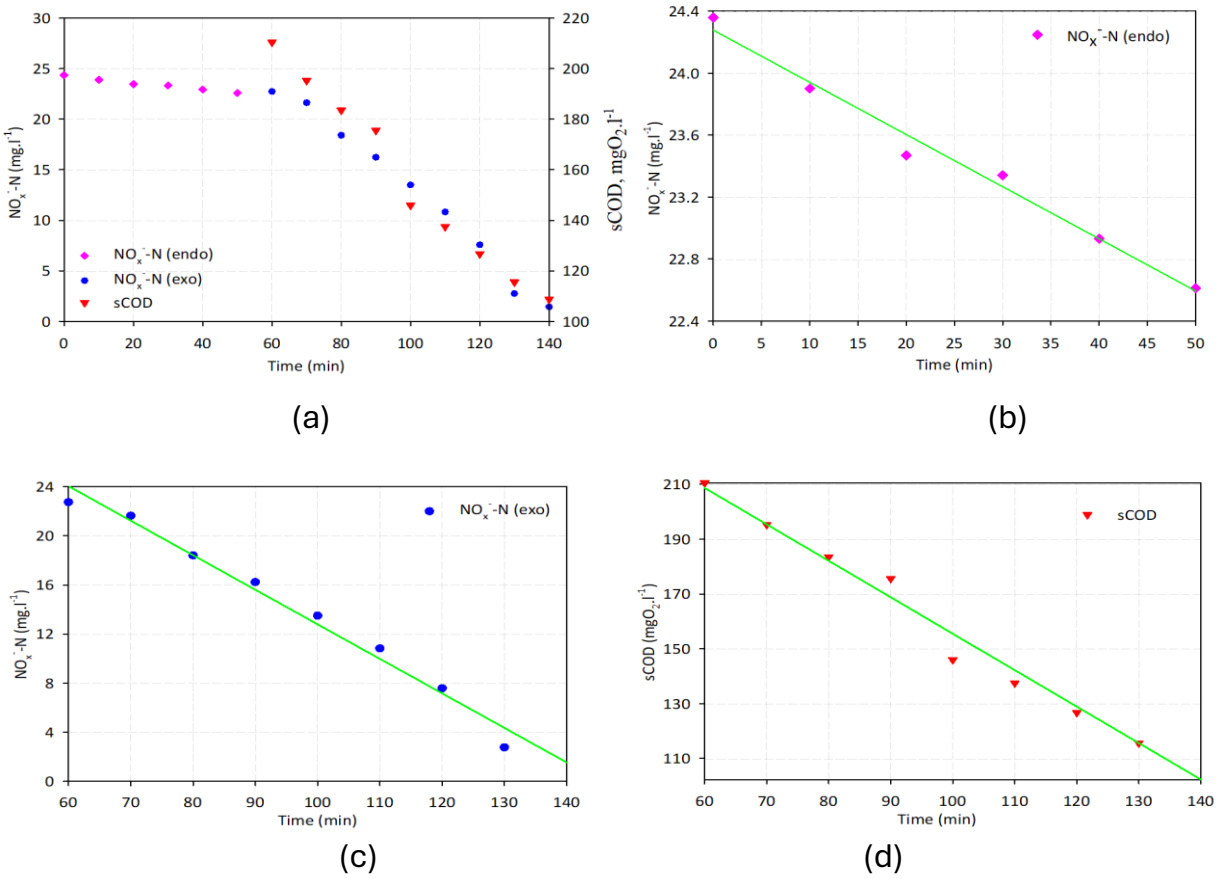


Figure 4.16. Profiles obtained from example during determination of $Y_{HA,MBR}$

Table 4.27. Data collected from examples during determine of Y_{HA}

Reactor	Phase	**A** (slope)	**B** (intercept)	R^2	SE of slope
AN1 Figure 4.14	Endo.	-0.0147	19.7952	0.9933	0.00083
	Exo.	-0.2258	32.9283	0.9915	0.00799
	sCOD	-1.7129	324.0222	0.9887	0.06518
AN2 Figure 4.15	Endo.	-0.0247	23.2948	0.8464	0.00520
	Exo.	-0.2598	38.1922	0.9984	0.00751
	sCOD	-1.4000	294.4444	0.9936	0.04260
MBR Figure 4.16	Endo.	-0.0337	24.2771	0.9842	0.00208
	Exo.	-0.2816x	40.9417	0.9872	0.00208
	sCOD	-1.3295x	288.4289	0.9804	0.07092

All of values found with $R^2 > 0.96$ (reliability of 95 %), but the endogenous stage in AN2 reactor had only a R^2 of 0.8464. However there was a very small change in total of nitrite and nitrate (ranging from 22.05 to 23.32 mg.l^{-1}) in AN2 reactor in this stage. The nitrogen utilization rate in the endogenous, exogenous and sCOD consumption rates in three tests are presented in Table 4.28.

Table 4.28. Data collected during determine of Y_{HA}

Reactor	Test	Exogenous	Endogenous	sCOD consumption
AN1	1	-0.2258	-0.0147	-1.7129
	2	-0.2533	-0.0305	-1.7782
	3	-0.2627	-0.0254	-1.6385
AN2	1	-0.2848	-0.0247	-1.6155
	2	-0.2686	-0.0333	-1.4463
	3	-0.3065	-0.0196	-1.5931
MBR	1	-0.2806	-0.0337	-1.3986
	2	-0.2719	-0.0624	-1.1579
	3	-0.2884	-0.0407	-1.2316

The linear regression can be used to assess the endogenous, exogenous denitrification rate and maximum sCOD consumption rate. The biomass growth yield on glucose under anoxic condition is assessed according to the following formula Eq. (4.10). The results of Y_{HA} calculated in this study are presented in Table 4.29.

Table 4.29. The values of Y_{HA} (mgCOD.mgCOD^{-1})

Reactor	Test 1	Test 2	Test 3	Mean ± SD
AN1	0.648	0.642	0.586	0.625 ± 0.034
AN2	0.540	0.535	0.485	0.520 ± 0.030
MBR	0.495	0.483	0.425	0.467 ± 0.037

Mean values of Y_{HA} in AN1, AN2, MBR and literature are shown in Table 4.30.

Table 4.30. Results for growth yield under anoxic conditions, Y_{HA}

Wastewater	Y_{HA}, (gCOD.g COD^{-1})	Ref.
AN1 - glucose	0.625 ± 0.034	In this study
AN2 - glucose	0.520 ± 0.030	In this study
MBR - glucose	0.467 ± 0.037	In this study
AE – sewage wastewater	0.54	[57]
Acetate	0.557 – 0.639	[58]
Municipal wastewater	0.506 – 0.584	[58]

The results of t-test showed that there is a statistically significant difference between result in AN2 and MBR (P = 0.034), but there is not a significant difference between AN1 and AN2 (P = 0.058), as well as between AN1 and MBR (P = 0.091). This indicates that the organic in leachate and DO concentration can affect the presence of microorganism population. Besides, there is a difference of denitrification microbial density in reactors, especially in MBR having the highest of activated sludge concentration but the value of Y_{HA} is the lowest due to the presence of aerobic microorganisms.

The results of growth yield under anoxic conditions in this study agree well with reported values in the literature. Compare to the default in ASM3 [57], the result achieved from AN2 is not significantly different, but the higher value measured in AN1 and lower in MBR. According to Rahman, A. *et al* [13], the value of Y_{HA} can vary based on relative acclimation to a substrate used for experiment and the authors found that the yield of organisms in sources with many biodegrade substrates (in wastewater) will have value lower than in fewer biodegradable substrates or in a single biodegradable substrate. This may explain the different anoxic yield observations in literature. Muller A. W. *et al.,* [58] investigated that the amount of COD for biomass synthesis will depend on the chemical structure of the COD compounds. Christensson M. *et al.,* [59] found that ethanol was considerably more readily available as a carbon source for denitrification than was methanol and the growth rate of denitrifiers with ethanol as carbon source was 2–3 times higher than with methanol.

In nitrogen removal systems, under anoxic conditions, nitrate is used as the final electron acceptor instead of dissolved oxygen and is reduced to dinitrogen. Theoretically, 10 mole NAD (energy transport molecule) are required to synthesize 1 mole $C_5H_7NO_2$ and energy required for anabolism is 201 kcal/mole. Each ATP molecule has an available free energy of approximately 10 kcal. So 20.1 moles of ATP need to be generated to synthesize 1 mole of $C_5H_7NO_2$ [60]. With oxygen as terminal electron acceptor, 3 ATP are formed per pair of electrons transferred. If nitrate is used instead of oxygen, only 2 ATP are formed per pair of electrons transferred to the nitrate. In the calculations, the total energy required to synthesize 1 mole $C_5H_7NO_2$ in aerobic conditions is lower than in anoxic conditions, corresponding to 33.4 e^- eq. (e^- equivalent) and 40.l e^- eq, respectively. Therefore, the yield with nitrate as terminal electron acceptor (0.35 gVSS/gTh.COD) is lower than that with oxygen (0.42 gVSS/gTh.COD). Besides, the Y in anoxic conditions is a little bit smaller than in aerobic conditions as the final part of the electron transfer chain is not used.

b. Maximum specific denitrification rate, q_{HA}

The experiments implemented for AN1, AN2 and MBR with VSS concentration were 2.363 $g.l^{-1}$, 2.535 $g.l^{-1}$ and 2.867 $g.l^{-1}$, respectively. Using the data of VSS concentration obtained and $r_{NOx,exo}$, $r_{NOx,endo}$, the maximum specific denitrification rate on tested carbon source can be computed from Eq. (4.11) and shown in Table 4.31.

Table 4.31. The values of q_{HA}

Reactor	Test 1	Test 2	Test 3	Mean ± SD
AN1	5.360	5.657	6.025	5.681 ± 0.333
AN2	6.156	5.569	6.791	6.172 ± 0.611
MBR	5.167	4.384	5.184	4.912 ± 0.457

Mean values of q_{HA} in this study and former authors are presented in Table 4.32.

Table 4.32. Results for maximum specific denitrification rate, q_{HA}

Substrate	$q_{HA,NO,rbCOD}$ $mgNO_x^-$-N.$gVSS^{-1}.h^{-1}$	Ref.
AN1 - glucose	5.681 ± 0.333	In this study
AN2 - glucose	6.172 ± 0.611	In this study
MBR - glucose	4.912 ± 0.457	In this study
Sugar/acetate/methanol/ethanol (11-16 °C)	1/2/5/6	[61]
Acetate	6.51	[62]
Ethanol/methanol/acetate	30.4/9.2/31.7	[63]
Ethanol/methanol	10/3	[64]
Ethanol/methanol/acetate/starch wastewater	9.6/3.2/12/0.74	[65]

Experimental results from this study showed similar maximum specific denitrification rate values found by Yin F.F *et al.*, who observed to be 6.51 $mgNO_3^-$-N.$gVSS^{-1}.h^{-1}$ at mean temperature 15.4 ± 0.8 °C [62].

A wide range of values have been reported in literature for observed maximum specific denitrification rate using different external substrates found by Mokhayeri Y. *et al*, [63]. Their experiments were implemented at 13 °C and Sequencing batch reactors were set up for experiment. Besides, these authors suggested that acetate and ethanol were equally effective external carbon sources with 30.4 $mgNO_x^-$-N.$gVSS^{-1}.h^{-1}$ for ethanol and 31.7 $mgNO_x^-$-N.$gVSS^{-1}.h^{-1}$ for acetate. The maximum specific denitrification rate estimated for ethanol

substrate by Nyberg *et al.*, [64] and Peng *et al.*, [65] has no significant difference. Similarly, these authors found that there was light difference from results for methanol substrate. Otherwise Mokhayeri *et al*, [63]. Obtained q_{HA} values for ethanol 3.3 times higher than for methanol. The lowest rates of around 0.74 $mgNO_x^-$-N.$gVSS^{-1}.h^{-1}$ was measured with starch wastewater by Peng *et al.*, [65].

c. Heterotrophic decay coefficient under anoxic condition, $k_{d,HA}$

The VSS concentration in AN1, AN2 and MBR were 2.413, 2.499 and 3.726 $g.l^{-1}$, respectively. The graphs of change VSS concentration versus time were measured as Figure 4.17.

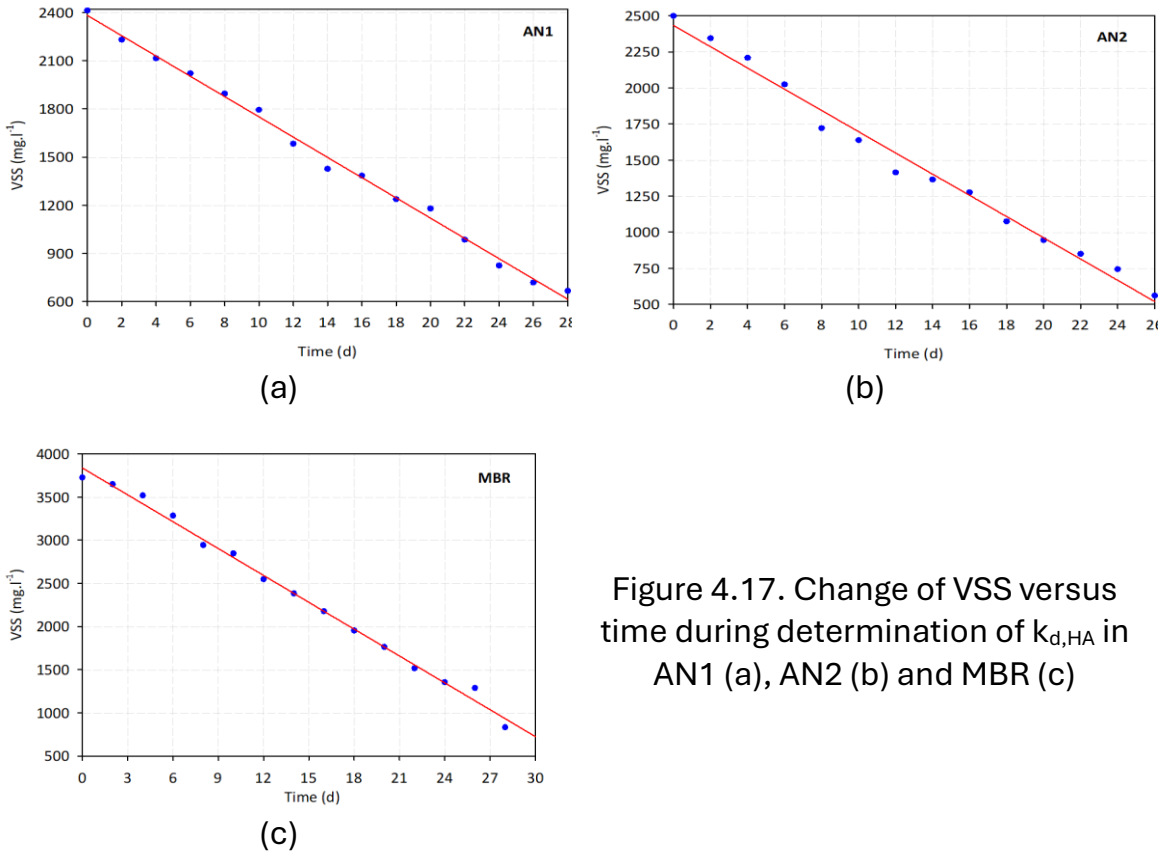


Figure 4.17. Change of VSS versus time during determination of $k_{d,HA}$ in AN1 (a), AN2 (b) and MBR (c)

From the slopes of plots achieved, data are shown in Table 4.33.

Table 4.33. Data collected during determine of $k_{d,HA}$

Reactor	Test	a (Slope)	b (Intercept)	R^2	SE of slope
AN1	1	-64.01	2390	0.9956	2.64957
	2	-64.40	2483	0.9931	5.62781
	3	-62.59	2428	0.9831	3.01410
AN2	1	-73.61	2433	0.9874	2.40030
	2	-80.48	2411	0.9938	1.83849
	3	-82.38	2496	0.9955	1.60792

Reactor	Test	a (Slope)	b (Intercept)	R^2	SE of slope
MBR	1	-103.74	3837	0.9953	1.90607
	2	-106.57	3681	0.9935	2.31829
	3	-97.62	3889	0.9945	1.93751

The rate of endogenous decay of biomass was calculated by using Eq. (4.12) and shown in Table 4.34.

Table 4.34. The values of $k_{d,HA}$

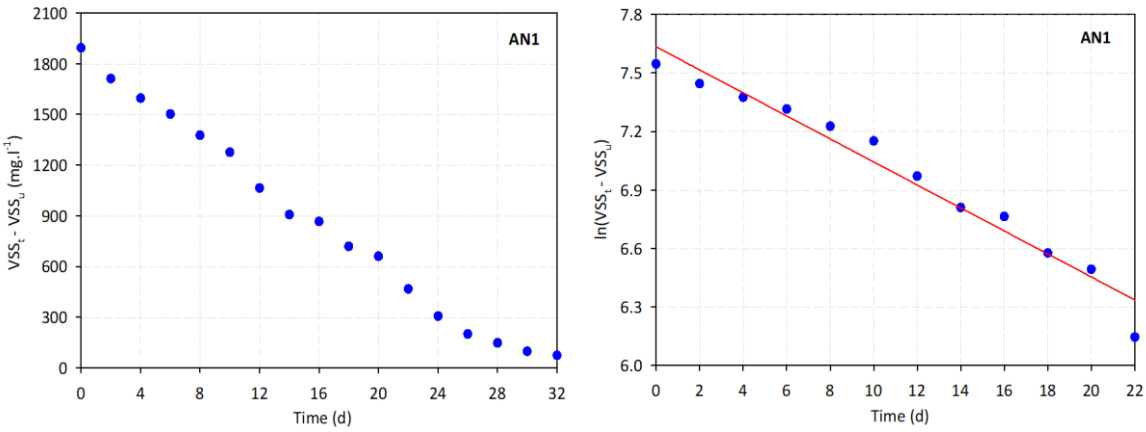
Reactor	Test 1	Test 2	Test 3	Mean ± SD
AN1	0.026	0.028	0.026	0.027 ± 0.001
AN2	0.029	0.032	0.033	0.032 ± 0.002
MBR	0.028	0.029	0.026	0.028 ± 0.001

Heterotrophic decay coefficient $k_{d,HA}$ represents the proportion of the total mass of microorganisms that self-degrades (endogenous respiration) per unit time under anoxic conditions. By using Eq. (4.12) for calculation, the obtained values of $k_{d,HA}$ were 0.027, 0.032 and 0.028 d^{-1} for AN1, AN2 and MBR, respectively. The obtained decay coefficients under anoxic conditions were compared with reported value in literature to be 0.05 with methanol substrate at 20 °C [20]. Therefore, these values are lower than literature value because of substrate used for heterotrophic decay coefficient under anoxic conditions.

d. Heterotrophic decay rate under anoxic conditions, b_{HA}

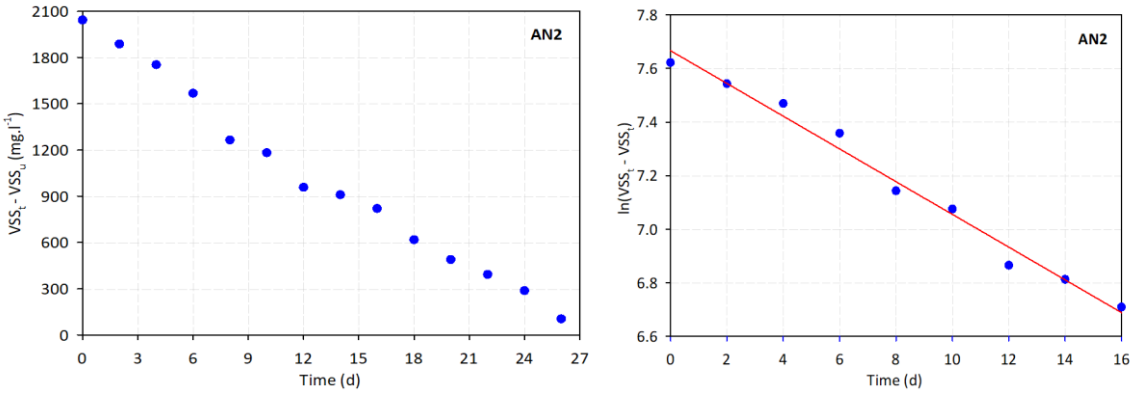
The decrease in total inorganic nitrogen in the anoxic batch assays could have been due to heterotrophic decay under anoxic conditions. This is essential digestion of the biomass using nitrite or nitrate as electron acceptor. The endogenous decay under anoxic condition was determined by VSS method. [18]. The rate of digestion of microbial protoplasm was determined through the oxidation of cell tissue and the formation of new cellar materials using the volatile suspended solids method. Endogenous respiration process of denitrifying bacteria under anoxic conditions was investigated in a batch study using 3 liters bioreactor. It was observed that from time 0 – t, a significant amount of active biomass disappeared during the anoxic digestion. Under anoxic sludge digestion, the anoxic cell decay rate followed a first – order decay with respect to VSS, but with a decreasing rate. In the endogenous nitrate respiration system, nitrate instead of oxygen is utilized as the terminal electron acceptor for the microorganisms involved in biomass destruction under anoxic conditions.

The biomass concentration in all reactors of the activated sludge was observed and measured during the experiment (up to 28 days). The decrease of the volatile suspended solids concentration is illustrated in Figure 4.18, 4.19, 4.20.



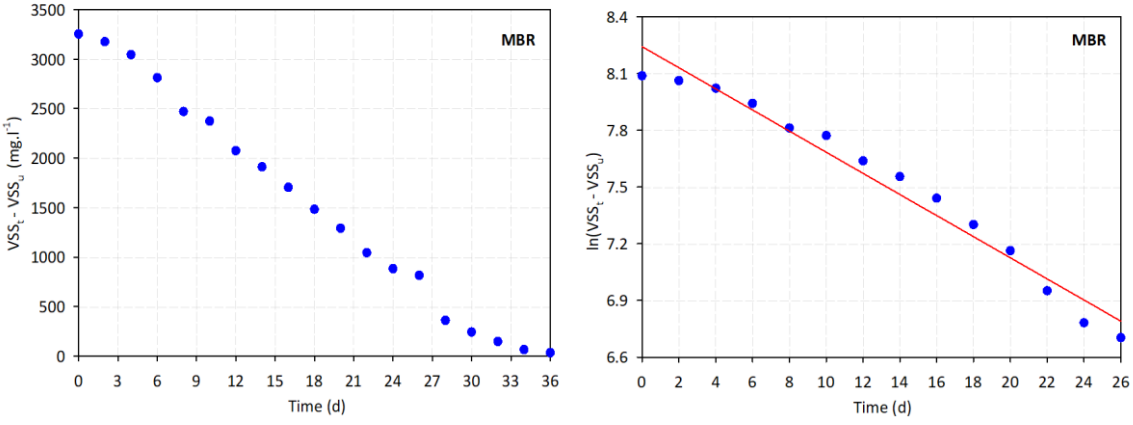
(a) (b)

Figure 4.18. Change of VSS concentration (a) and VSS digestion plot (b) for determination of the value of $b_{HA,AN1}$



(a) (b)

Figure 4.19. Change of VSS concentration (a) and VSS digestion plot (b) for determination of the value of $b_{HA,\ AN2}$



(a) (b)

Figure 4.20. Change VSS concentration (a) and VSS digestion plot (b) during determination of the value of $b_{HA,MBR}$

Table 4.35. Data collected during determine of $k_{d,HA}$

Reactor	Test	**a** (Slope)	**b** (Intercept)	R^2	SE of slope
AN1	1	-0.0591	7.6343	0.9636	0.00357
	2	-0.0579	7.7099	0.9654	0.00350
	3	-0.0573	7.6680	0.9788	0.00257
AN2	1	-0.0611	7.6640	0.9844	0.00299
	2	-0.0559	7.7545	0.9872	0.00236
	3	-0.0552	7.7322	0.9918	0.00172
MBR	1	-0.0558	8.2426	0.9689	0.00294
	2	-0.0553	8.3227	0.9715	0.00266
	3	-0.0540	8.2307	0.9548	0.00341

The decay rate was estimated from the slope of a plot the natural logarithm of decrease rate of VSS versus time. The overall results from batch tests for the assessment of endogenous decay rate with biomass in AN1, AN2 and MBR are summarized in Table 4.36.

Table 4.36. The values of b_{HA}

Reactor	Test 1	Test 2	Test 3	Mean ± SD
AN1	0.0591	0.0579	0.0573	0.0581 ± 0.0009
AN2	0.0571	0.0559	0.0552	0.0561 ± 0.0010
MBR	0.0580	0.0553	0.0540	0.0558 ± 0.0020

Results of b_{HA} in this study and former authors are presented in Table 4.37.

Table 4.37. Results of b_{HA} in this study and former authors

Wastewater	b_{HA}, d^{-1}	Ref.
AN1 - leachate	0.0581 ± 0.0009	In this study
AN2 - leachate	0.0561 ± 0.0010	In this study
MBR - leachate	0.0558 ± 0.0020	In this study
SBR – synthetic ww containing beef	0.097	[19]
Sewage/digestion method	0.053	[18]

ww: wastewater

It was observed that a significant amount of active biomass disappeared during experiments. After an extended digestion time, the changing of concentration of the biomass is not significant. Thus, the VSS concentration of digested sludge

in AN1, AN2 and MBR reached an ultimate value. As listed in Table 4.37, the values of b_{HA} found in AN1, AN2 and MBR were 0.0581, 0.0561 and 0.0558 d^{-1}, respectively.

According to statistical results with P of 0.250, there is no significant difference between results found for the various reactors in this study. These results in this study are in good agreement with the literature value [18]. However, a higher value was found by Lee Y., *et al*. to be 0.097 d^{-1}.

4.5.2. Determination of autotrophic stoichiometric and kinetic coefficients

a. Saturation coefficient for nitrification, K_A

The example for determination of autotrophic parameters in an aerobic reactor.

- *Setup data*

Experimental data obtained at different hydraulic retention time. A data set collected from the experiment, calculated and presented in Table 4.38 and Table 4.39.

Table 4.38. Example of the set data collected from the experiment

Test No	θ, d	S_0	S_t	$S = S_0 - S_t$	X	X
			$mgNH_4^+-N.l^{-1}$		$mgVSS.l^{-1}$	$mgCOD.l^{-1}$
1	2.42	331.5	1.1	330.4	134.8	191.5
2	2.21	343.5	5.3	338.2	118.2	167.8
3	1.92	323.1	11.2	311.9	120.4	171.0
4	1.71	335.6	18.9	316.7	132.4	188.1
5	1.50	326.4	25.7	300.7	142.7	202.7
6	1.33	307.5	34.3	273.2	146.9	208.6
7	1.17	301.3	41.8	259.5	152.2	216.1

The formation of sludge during the experiment is shown in Table 4.38. A decreasing trend of ammonium concentration with time resulted mainly from the prolongation of hydraulic retention time from experiments 1.2 to 2.5 days.

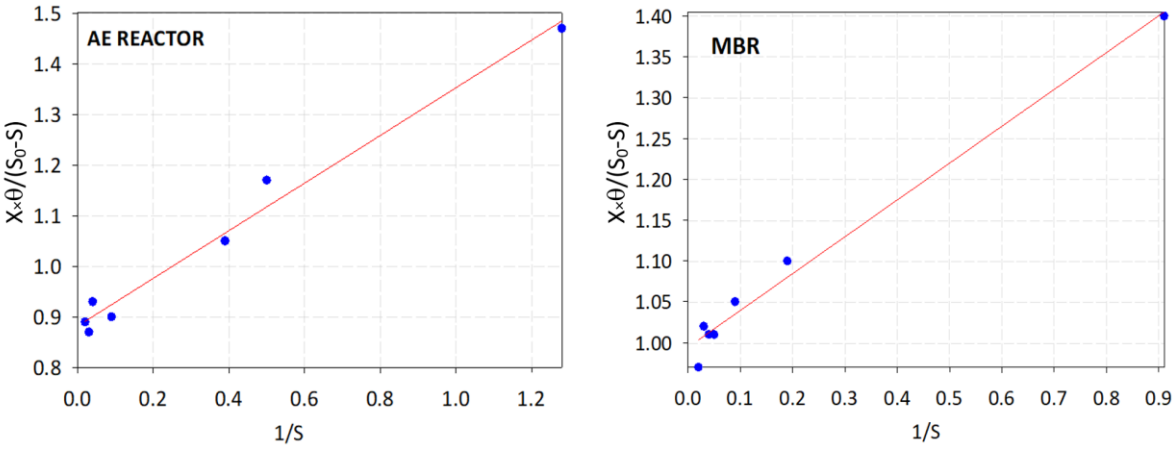
Table 4.39. Data calculated to determine kinetic coefficients for nitrification process

Test No	**X×θ;** $mg\ COD.l^{-1}.d^{-1}$	**X×θ/S_0-S;** d	**1/S;** $(mg.l^{-1})^{-1}$
1	463	1.40	0.71
2	371	1.10	0.91
3	328	1.05	0.95

Test No	**X×θ;** mg COD.l^{-1}.d^{-1}	**X×θ/S_0-S;** d	**1/S;** (mg.l^{-1})$^{-1}$
4	321	1.01	0.99
5	304	1.01	0.99
6	278	1.02	0.98
7	252	0.97	1.03

Determination the constant of saturating substrate (K_A) and maximum rate of use of the substrate with the data of the initial subtract of influent (S_0), final substrate (S), concentration of biomass (X) and time of hydraulic retention (θ), made a table to calculate based on Eq. (4.13).

Graphically the relation between θ/S_0-S with respect to 1/S has an intersection point with the ordinate axis that corresponds to the value of k and the graphic corresponds to the value of K_A/k yielding the value of coefficient K_A.



(a) (b)

Figure 4.21. Plot of X×θ/S_0-S versus 1/S in AE reactor (a) and MBR (b)

Table 4.40. Data collected during determination of K_A

Reactor	Test	**a** (Slope)	**b** (Intercept)	R^2	SE of slope
AE	1	0.4684	0.8827	0.9820	0.02828
	2	0.4288	0.9240	0.9640	0.03712
	3	0.4595	0.9284	0.9596	0.04206
MBR	1	0.4512	0.9944	0.9861	0.02387
	2	0.3931	0.8257	0.9833	0.02290
	3	0.4102	0.8666	0.9727	0.03065

From the plots shown that the results obtained have high reliability (> 95 %) due to small change of microorganism concentration during the experiment while analytical methods may have errors. However, the results obtaines show no significant difference compared to previous studies.

From Eq. (4.13), the term $X \times \theta/(S_0-S)$ was plotted versus 1/S as shown in the Figure 4.20 to determine k and K_A. The slope of this plot represents K_A/k and the ordinate intercept equals 1/k [43]. Results of parameters for nitrification process are shown in Table 4.41.

Table 4.41. Calculation of parameters for the nitrification process

Reactor	**Test**	1/k	k	K_A/k	K_A
AE	1	0.468	2.135	0.883	1.885
	2	0.429	2.332	0.924	2.155
	3	0.460	2.176	0.928	2.020
	Mean	**0.452**	**2.214**	**0.912**	**2.020**
	SD	0.021	0.104	0.025	0.095
MBR	1	0.451	2.216	0.994	2.204
	2	0.393	2.544	0.826	2.100
	3	0.410	2.438	0.867	2.113
	Mean	**0.418**	**2.399**	**0.896**	**2.139**
	SD	0.030	0.167	0.088	0.057

Mean values of K_A in this study and former authors are shown in Table 4.42.

Table 4.42. Results of parameters for nitrification process

Wastewater	**K_A, NH_4^+-N.l^{-1}**	**Ref.**
AE - leachate	2.020 ± 0.095	In this study
MBR - leachate	2.139 ± 0.057	In this study
SBR - leachate	1.016	[66]
Biofilter	5.14	[67]
AE - domestic wastewater	0.912	[68]
AE - domestic wastewater (default values)	1	[5]

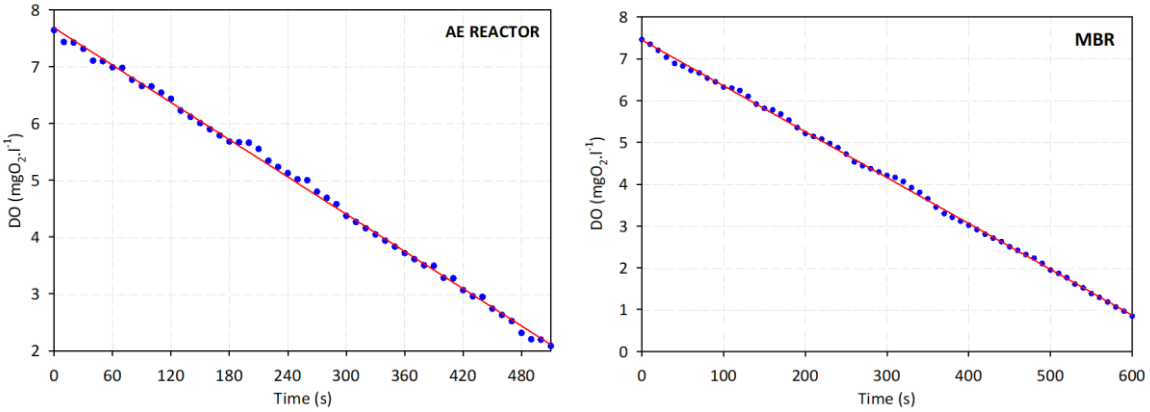
As shown in Table 4.42, K_A value obtained at AE reactor and MBR have no significant difference. This can be explained because the autotrophic microorganism populations are similar in the MBR and AE reactors due to the

sludge circulation from MBR to AE in the pilot. Compared to results measured by Vivekanandan *et al.* [66] and Liwarska-Bizukojc *et al.*, [68], the values obtained in this study are higher. Besides, these values are also higher than the default value indicated by Henze *et al.* for domestic wastewater in ASM. On contrary, the value of K_A in biofilter found by Dinçer *et al.*, [67] is higher than in this study.

Again this value indicates that those K_A values are rather small. The consequence is that Monod equation for autotroph will be close to a zero order kinetic for NH_4^+-N and thus close to the maximum growth rate.

b. Coefficient of autotrophic yield, Y_A

Sludge activity in AE and MBR for nitrification are quantified by measuring oxygen uptake rate of activated sludge sample in close batch respirometer. Collected data, are shown in Figure 4.22. The slope of the curve presented the DO decrease versus time allows the calculation of autotrophic yield coefficient of biomass. The values of autotrophic yield coefficient can be determined by Eq. (4.14).



(a) (b)

Figure 4.22. The change DO versus time in AE (a) and MBR (b)

From Eq. (4.14) the results of Y_A are calculated and presented in Table 4.43.

Table 4.43. Data collected during determination of Y_A

Reactor	Test	**a** (slope)	**b** (intercept)	R^2	SE of slope
AE	1	-0.0109	7.6840	0.9982	0.00014
	2	-0.0103	7.7468	0.9968	0.00008
	3	-0.0106	7.5302	0.9971	0.00010
MBR	1	-0.0110	7.4503	0.9992	0.00009
	2	-0.0107	7.3700	0.9972	0.00007
	3	-0.0120	7.8019	0.9969	0.00008

The mean values of Y_A in the AE reactor, MBR and former researchers are presented in Table 4.44.

Table 4.44. Results of Y_A

Wastewater	**Y_A,** mgCOD.mgN^{-1}	**Ref.**
AE - leachate	0.22 ± 0.02	In this study
MBR - leachate	0.21 ± 0.02	In this study
AE - municipal wastewater	0.23 - 0.27	[16]
AE - sewage wastewater	0.22	[66]
AE - sewage wastewater	0.24	[57]
AE - sewage wastewater	1	[45, 69]
AE *- sewage wastewater	0.0072 ± 0.01	[70]

* Carbon Dioxide Uptake

The default values used in the ASMs were identical and equal to 0.24 mgN.l^{-1} [57]. There is no difference between obtained value of Y_A in AE and MBR. This can be explained due to the return of wastewater led to mix activated sludge from MBR to AE reactor. The lowest mean value of Y_A of 0.072 by using carbon dioxide uptake rate methods at temperature of 21 ± 2 °C was found by Blackburne *et al.* [70] . The difference of Y_A value depends on test method, condition operation and the source of activated sludge. Vivekanandan *et al.* measured a Y_A of 0.22 by using respiration rates in a respirometer operated at a temperature of 23 °C [66].

c. Maximum specific growth rate of the autotrophs, $\mu_{max,A}$

As most biological reactions nitrification kinetics are generally influenced by temperature. Using Eq. (4.15), the $\mu_{max,A}$ value was calculated and presented in Table 4.45.

Table 4.45. Results of $\mu_{max,A}$

Wastewater	**$\mu_{max,A}$,** d^{-1}	**Ref.**
AE - leachate	0.50 ± 0.05	In this study
MBR - leachate	0.48 ± 0.07	In this study
SBR - leachate	1.25	[39]
AE - municipal wastewater	0.45	[71]
AE - sewage wastewater	0.47	[66]
AE - sewage wastewater	1	[57]
AE - domestic wastewater	0.675	[68]

As shown in Table 4.45, most of the previous researchers provided maximum specific growth rates close to those found in this study. However, these values are lower than values found by Yen H.V. The maximum specific growth rate for sewage wastewater was 1 d^{-1} and for leachate with SBR technology was 1.25 [39]. The effect of temperature on the maximum specific growth rate, $\mu_{max,N}$ and the endogenous decay coefficient, b_A was observed by Orhon *et al.,* [24].

It is reported that environmental parameters such as COD/TNK ratio of the influent can affect the value of maximal nitrification rate. Choubert *et al*. [71] obtained a value of 0.45 d^{-1}.

d. Autotrophic decay coefficient, b_A

The value of b_A is important in assessing nitrification behavior for purpose of plant design. Decay rate of ammonium oxidizing bacteria, b_A was obtained by plotting ln(VSSt – VSSu) versus time.

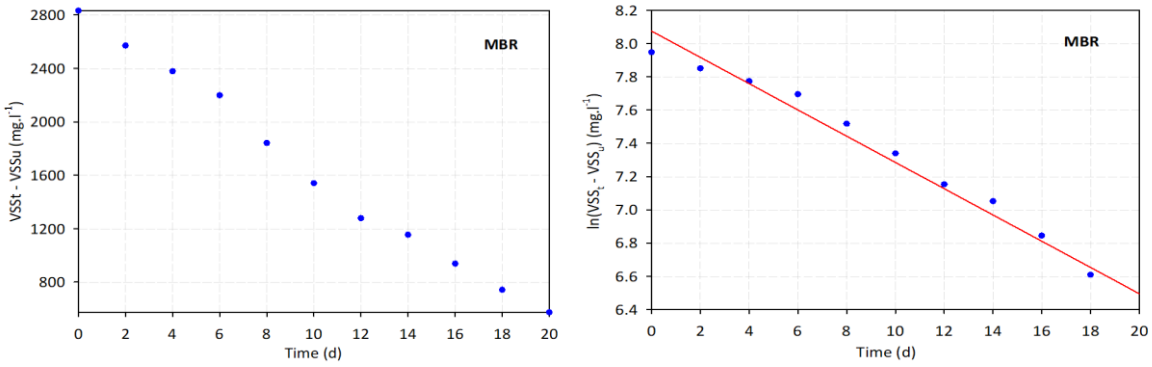


Figure 4.23. Example of changing ln(VSS_t-VSS_u) versus time in MBR

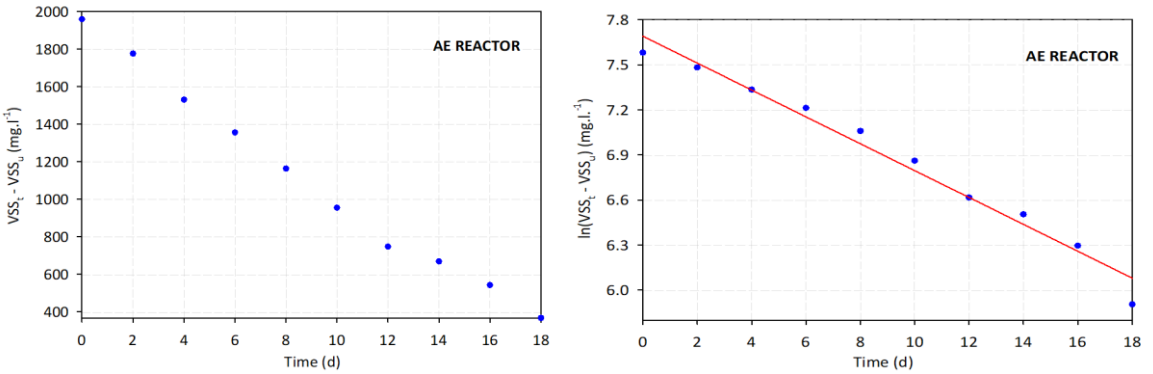


Figure 4.24. Example of changing ln(VSS_t-VSS_u) versus time in AE

Table 4.46. Data collected during determination of b_A

Reactor	Test	**a** (slope)	**b** (intercept)	R^2	SE of slope
AE	1	-0.0894	7.6898	0.9763	0.00501
	2	-0.0951	8.1631	0.9615	0.00641
	3	-0.0867	7.7207	0.9554	0.00658

Reactor	Test	**a** (slope)	**b** (intercept)	R^2	SE of slope
MBR	1	-0.0791	8.0755	0.9756	0.00412
	2	-0.0870	8.1234	0.9562	0.00632
	3	-0.0845	7.7748	0.9696	0.00520

The plot shows that the results have high reliability (R^2 > 95 %). In this study, the mean b_A value obtained in AE and MBR reactors is presented in Table 4.47.

Table 4.47. The values of b_A

Reactor	Test 1	Test 2	Test 3	Mean ± SD
AE reactor	0.0894	0.0951	0.0867	0.0904 ± 0.0043
MBR	0.0791	0.0870	0.0845	0.0835 ± 0.0040

The results of b_A in this study compared to the literature given in Table 4.48.

Table 4.48. Results of b_A

Wastewater	b_A, d^{-1}	Ref.
AE - leachate	0.0904 ± 0.0037	In this study
MBR - leachate	0.0835 ± 0.0040	In this study
SBR - leachate	0.0628	[39]
AE - sewage wastewater	0.08 – 0.16	[66]
AE - municipal wastewater	0.13	[71]
AE - municipal wastewater	0.0361– 0.0561	[16]
Biofilter	0.021	[67]

The value of b_A is important in assessing the nitrification behavior for the purpose of plant design. In this study, the b_A values obtained were 0.0904 and 0.0835 d^{-1} in AE and MBR, respectively. The results obtained were slightly different from the results of Yen. with a value of 0.0628 d^{-1} [39] on SBR treating leachates. The literature of autotrophic cellular decay rate was reported of 0.0361 and 0.0561 d^{-1} for municipal wastewater at 20 °C and 26.5 °C, respectively [16]. Meanwhile, a value of 0.13 d^{-1} was found by Choubert *et al.,* for municipal activated sludge from the continuous flow reactor and using batch test reactor at 10 °C [71]. Meanwhile, *Martinage and Paul* investigated that changes in the quality of an industrial effluent from the same source induced b_A variations from 0.08 to 0.16 d^{-1} [47]. The b_A value doubled when small amounts of urban wastewater were added to the pilot plant influent. When 30 % of the load was composed of urban wastewater, b_A reached a value of 0.36 d^{-1} [47].

4.6. CONCLUSION

The experimental procedures presented in this study for determination of heterotrophic and autotrophic yielded, decay coefficient, maximum growth rate, half saturation coefficient, etc. The results found are summarized in Table 4.49.

Table 4.49. Summary of results in this study

Parameters	Symbol	Value
1. Stoichiometric and kinetics in ASM1		
a. Stoichiometric parameters		
Heterotrophic yield coefficient under aerobic conditions; $gCOD.gCOD^{-1}$	$Y_{HO,MBR}$	0.649
	$Y_{HO,AE}$	0.681
Heterotrophic yield coefficient under anoxic condition; $gCOD.g\ COD^{-1}$	$Y_{HA,AN1}$	0.652
	$Y_{HA,AN2}$	0.520
	$Y_{HA,MBR}$	0.467
b. Kinetic parameters		
Heterotrophic decay rate under aerobic condition; d^{-1}	$b'_{HO,MBR}$	0.0915
	$b'_{HO,AE}$	0.1135
Heterotrophic decay rate under anoxic condition; d^{-1}	$b_{HA,AN1}$	0.0581
	$b_{HA,AN2}$	0.0561
	$b_{HA,MBR}$	0.0558
Autotrophic decay rate; d^{-1}	$b_{A,MBR}$	0.08
	$b_{A,AE}$	0.09
Maximum specific growth rate of the heterotrophs under aerobic condition; d^{-1}	$\mu_{max,HO-MBR}$	3.14
	$\mu_{max,HO-AE}$	5.80
Maximum specific growth rate of the autotrophs; d^{-1}	$\mu_{maxA,MBR}$	0.48
	$\mu_{maxA,AE}$	0.53
Maximum rate of substrate utilization under aerobic condition; $gCOD.gVSS^{-1}.d^{-1}$	$k_{HO,MBR}$	6.05
	$k_{HO,MBR}$	9.81
Half saturation coefficients; $mgCOD.l^{-1}$	$K_{S,HO-MBR}$	9.12
	$K_{S,HO-AE}$	14.78
Half saturation coefficient for nitrification; $mgNH_4^+-N.l^{-1}$	$K_{A,MBR}$	2.02
	$K_{A,AE}$	2.14
Heterotrophic decay coefficient under anoxic condition; d^{-1}	$k_{d,HA-AN1}$	0.027
	$k_{d,HA-AN2}$	0.032
	$k_{d,HA-MBR}$	0.028

Parameters	Symbol	Value
2. Stoichiometric and kinetics in ASM3		
Coefficient of autotrophic yield; gCOD/gN[1]	$Y_{A,MBR}$	0.21
	$Y_{A,AE}$	0.22
Maximum specific denitrification rate; $mgNO_x^-$-N.$gVSS^{-1}.h^{-1}$	$q_{HA,NO,rbCOD,AN1}$	5.68
	$q_{HA,NO,rbCOD,AN2}$	6.17
	$q_{HA,NO,rbCOD,MBR}$	4.91

The determination of these coefficients will be helpful in understanding the kinetics of substrate utilization and design of biological treatment plants for leachate treatment.

Some results achieved are different from other studies, mainly due to the method of implementation, research conditions, substrate properties, treatment technology, studying scale. The research results can be applied to design treatment system for leachate in Vietnam.

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CHAPTER 5: MODELLING OF PARTIAL NITRIFICATION AND DENITRIFICATION

5.1. INTRODUCTION

Several treatment methods were applied and developed to remove wastewater with high nitrogen concentration in recent years, including biological and physico-chemical. Among these methods, biological treatment of wastewater can be a cost-effective and environmental-friendly method. Biological removal of nitrogenous compounds from wastewater involves combination two processes of nitrification and denitrification [1-3]

In activated sludge, nitrifying bacteria convert ammonium to nitrate under aerobic conditions as shown in Eq. (5.1) [4, 5].

$$\begin{aligned} NH_4^+ &\xrightarrow{\text{ammonia monooxygenase}} NH_2OH \\ NH_2OH &\xrightarrow{\text{hydroxylamine oxidoreductase}} NO_2^- \\ NO_2^- &\xrightarrow{\text{Nitric oxide reductase}} NO_3^- \end{aligned} \quad (5.1)$$

Then, denitrifying bacteria continue to convert nitrate to nitrogen gas under anoxic conditions as shown in Eq. (5.2) [6, 7]

$$\begin{aligned} NO_3^- &\xrightarrow{\text{Nitrate reductase}} NO_2^- \xrightarrow{\text{Nitric oxide reductase}} NO \\ NO &\xrightarrow{\text{Nitrite reductase}} N_2O \xrightarrow{\text{Nitrous oxide reductase}} N_2 \end{aligned} \quad (5.2)$$

In nitrification, ammonia is oxidized to nitrate by two different groups of bacteria. The first group of bacteria, AOB, converts ammonia to nitrite; then, the second group, NOB, further oxidizes the intermediate product to nitrate [1, 8-10].

Nitrosomonas type : $2NH_4^+ + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 4H^+$ (5.3)

Nitrobacter type: $2NO_2^- + O_2 \longrightarrow 2NO_3^-$ (5.4)

Total Reaction $NH_4^+ + 2O_2 \longrightarrow NO_3^- + H_2O + 2H^+$ (5.5)

Theoretically, 4.57g of oxygen are required to completely oxidize 1g ammonia into nitrate, with $3.43gO_2.gN^{-1}$ for the first step nitrification (ammonia oxidation) and $1.14g\ O_2.gN^{-1}$ for second step nitrification [10-12].

During the nitrification process, these species of bacteria are most active in the pH range of 7 to 8 and hydrogen ions are released. According to Halling-Sarensen *et al.* [7] and Henze *et al.* [5] the optimum pH value for the nitrification process varies between 8 and 9.

Alkalinity is consumed to neutralize the produced acid from nitrification process (from Eq. (5.3)). Therefore, the alkalinity consumption by nitrification may result in a significant reduction of pH. Theoretically, approximately 7.14 mg of alkalinity as $CaCO_3$ is destroyed for every milligram of ammonium ions oxidized [13-16].

$$H^+ + HCO_3^- \longrightarrow H_2O + CO_2 \quad (5.6)$$

Assimilative reactions are also occurring during nitrification according to: [7, 10, 15].

$$NH_4^+ + 4CO_2 + HCO_3^- + H_2O \longrightarrow C_5H_7NO_2 + 5O_2 \quad (5.7)$$

Nitrate removal process:

$$6NO_3^- + 5CH_3OH \longrightarrow 3N_2 + 5CO_2 + 7H_2O + 60H^- \quad (5.8)$$

$$8NO_3^- + 5CH_3COOH \longrightarrow 4N_2 + 10CO_2 + 6H_2O + 8OH^- \quad (5.9)$$

Nitrite removal process:

$$2NO_2^- + CH_3OH \longrightarrow N_2 + CO_2 + H_2O + 20H^- \quad (5.10)$$

However, the occurrence of nitrification in the reactor of a treatment system will not remove nitrogen in wastewater if it is not followed by denitrification to remove nitrogen.

Denitrification is a process in which heterotrophic microorganisms use organic carbon as electron donor to reduce nitrate under the condition of low dissolved oxygen. In the denitrification process nitrate is converted to nitrite and then to nitrous oxide and nitric oxide and finally to nitrogen gas [15-17] . In addition, nitrous oxide is one of the obligatory intermediates in denitrification [16]. Complete denitrification consists of four steps transforming nitrate to nitrogen gas, in which nitric oxide and nitrous oxide are an intermediate as described in Eq. (5.11), (5.12), (5.13) and (5.14).

$$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O \quad (5.11)$$

$$NO_2^- + 2H^+ + e^- \longrightarrow NO + H_2O \quad (5.12)$$

$$2NO + 2H^+ + 2e^- \longrightarrow N_2O + H_2O \quad (5.13)$$

$$N_2O + 2H^+ + 2e^- \longrightarrow N_2 + H_2O \quad (5.14)$$

Usually, it is modeled as one or two-step model ($NO_3^- \longrightarrow NO_2^- \longrightarrow N_2$) as the production of nitric oxide and nitrous oxide does not contribute significantly to the total mass –flow of nitrogen in the biological system [18]. The nitrous oxide modeling has recently received more attention as nitrous oxide is a powerful greenhouse gas [19-21]. The strong greenhouse gas (nitrous oxide) can be emitted from wastewater treatment systems as a byproduct of ammonium

oxidation and as the last intermediate in the stepwise reduction of nitrate to nitrogen gas by denitrifying organisms [19, 22].

Therefore, denitrification will be considered as a two-step process in which the first step is a conversion of nitrate into nitrite. The second step carries nitrite through two intermediates to nitrogen gas. This two-step process is normally termed "dissimilation" presented in Eq. (5.15) and (5.16).

$$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O \quad (5.15)$$

$$2NO_2^- + 8H^+ + 2e^- \longrightarrow N_2 + 4H_2O \quad (5.16)$$

From Eq. (5.15) and (5.16) denitrification process increases the alkalinity of the wastewater. Hence, alkalinity affects considerably to nitrification process and alkalinity produced by denitrification also partially compensates its consumption by nitrification.

To overcome these constraints, some alternatives to the conventional BNR have been developed which resulted in the development of partial nitrification [9, 23]. The term “partial nitritation” refers to the partial oxidation of ammonium into nitrite, with a fraction of ammonium remaining non converted [24].

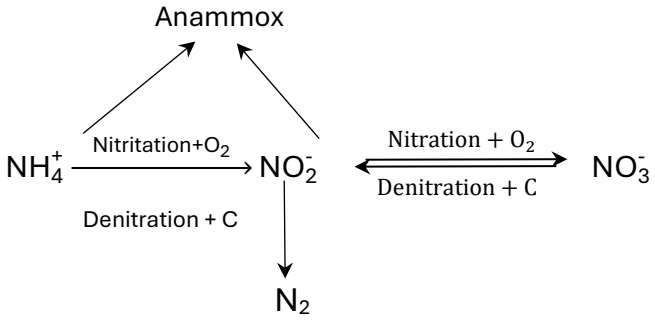


Figure 5.01. Biological nitrification – denitrification via nitrite pathway

Partial nitrification to nitrite is the primary step to achieve nitrogen removal via nitrite. Partial nitrification has gained a lot of interest among researchers in the last years especially in the field of high-strength ammonium wastewater treatment. Partial nitrification to nitrite and nitrite denitrification was reported to be technically feasible and economically favorable, especially when wastewater with high ammonium concentrations or low C/N ratios is treated [24]. Successful partial nitrification processes recorded are obtained in sequencing operation process, and few are achieved in a continuous-flow process [25]. This partial nitrification – denitrification of single stage bio-denitrification has many advantages such as decreasing the energy for aeration and saving carbon source.

Sustained nitrite accumulation via the nitrite pathway ($NH_4^+ \longrightarrow NO_2^- \longrightarrow N_2$) offers several benefits for nitrogen removal of wastewater, compared to the nitrate pathway ($NH_4^+ \longrightarrow NO_2^- \longrightarrow NO_3^- \longrightarrow NO_2^- \longrightarrow N_2$) [26-31].

The optimum temperature for achieving partial nitrification i.e. for the growth of aerobic AOB is also believed to be in between 30 and 35°C [32]. However, AOB can also grow properly at low temperature (11-16 °C) and mean temperature range from 20 to 25 °C [32, 33]. Besides, to achieve partial nitritation it is necessary to reduce the activity of NOB but not the activity of AOB [34].

According to the basic research work by Anthonisen [29], the non-ionized forms of the ammonium and of the nitrite have - as ammonia and as nitrous acid - an inhibition effect on *Nitrosomonas* and on *Nitrobacter*.

The value of pH is one of the most important parameters to affect partial nitrification. The effect of pH on undissociated ammonia was investigated by Jianlong *et al.*[1], the pH can affect nitrification in two ways:

- directly by changing the enzyme's reaction mechanism [35].
- indirectly by changing the speciation of total ammonium and total nitrite to the inhibiting forms as FA and FNA [36-39].

The FA concentration increases in a basic condition, but the FNA concentration increases in acidic condition [40].

Alkalinity plays an important role in terms of both growth substrate (inorganic C) and environment for microorganisms, especially in establishing a balanced system for the growth of AOB and NOB to control nitritation [41]. The alkalinity produced by denitrification also partially compensates its consumption by nitrification [15]. Thus, the alkalinity is one of the most important parameters to affect partial nitrification.

Dissolved oxygen is known to have an important effect on the activity and settling ability of the sludge [42]. Generally, in overall nitrification process it is recommended to keep DO levels higher than 2 – 3 $mgO_2.l^{-1}$ to obtain nitrate as final product. Several authors observed that working with lower DO they were able to stop the process at the intermediate nitrite [43].

The application of the ANAMMOX process in the removal of ammonium in wastewater treatment and consists of two separate processes.

The first step is partial nitrification (nitritation) of half of the ammonium to nitrite by ammonia oxidizing bacteria. Then ammonium and nitrite are converted in the ANAMMOX process to dinitrogen gas by ANAMMOX bacteria.

$$NH_4^+ + NO_2^- \longrightarrow N_2 + 2H_2O \qquad (5.17)$$

The application of the ANAMMOX process in wastewater systems has resulted in higher rates of nitrogen removal and lower energy requirements than those for conventional nitrogen removal. However, the ANAMMOX bacteria grow too slow, thus this makes it difficult to grow enough sludge for a wastewater treatment reactor. Besides, ANAMMOX and some denitrifying bacteria are coupled under harsh living conditions, certain operating conditions and mechanisms of the coupling process are not clear; thus, it is more difficult to control the process, which is why the process has not been widely applied [44].

The objective of this study is to investigate the effects of key operational parameters on partial nitrification and to evaluate the influence of different carbon sources and sCOD/N ratios on denitrification, in order to optimize biological nitrogen removal for high-strength ammonium wastewater.

5.2. MATERIALS AND METHODS

5.2.1. Batch reactors

The batch reactors used in this study are described in Chapter 4.

5.2.2. Activated sludge

The activated sludge with initial MLVSS of 1.87 $g.l^{-1}$ was collected from the plant of leachate treatment in Hiep Phuoc, Ho Chi Minh City. The activated sludges were operated in aerobic and anoxic reactor before using as seed sludge.

- For nitrification process, the activated sludge was aerated continuity with old leachate with adding NH_4Cl for 3 weeks to get the best nitrification conditions. The alkalinity, pH and DO were ranging from (1832 – 2276 $mgCaCO_3.l^{-1}$), (7.42 – 8.31) nd (3.02 – 4.17 $mgO_2.l^{-1}$), respectively.
- For denitrification process, the activated sludge was first aerated to convert ammonia into nitrite or nitrate for 3 days, then activated sludge was mixed and stirred (without aeration) and glucose solution, nitrates were added for 3 weeks to obtain the best denitrification process.

5.2.3. Leachate

The old leachate for the experimental investigation was collected at Go Cat landfill site (closed landfill site) in Southern Vietnam for a background environment. The characteristics of leachate for this study are presented in Table 5.01.

Table 5.01. Parameters of leachate for study

Parameters	Unit	Range
pH	-	8.53 – 8.73
COD	$mgO_2.l^{-1}$	307 - 1916
Alkalinity	$mgCaCO_3.l^{-1}$	1349 - 1934
NH_4^+- N	$mg.l^{-1}$	254 - 717
NO_2^-- N	$mg.l^{-1}$	0.9 – 6.2
NO_3^-- N	$mg.l^{-1}$	6.3 – 18.3

The specific composition of the synthetic wastewater for partial nitrification process used in this experiment.

5.2.4. Chemicals

- For nitrification: NH_4Cl, glucose
- For denitrification: glucose; $NaNO_2$ and $NaNO_3$
- $NaHCO_3$, NaOH, H_2SO_4.
- Nutrient solution (Table 4.03, Chapter 4)

5.2.5. Operation of the reactor

5.2.5.1. Effects on partial nitrification

a. Effect of DO concentration on partial nitrification

- *Operation*

Ammonium in samples was measured before adding ammonium solution. The experiment was investigated at the same conditions with initial VSS and ammonium concentration of 0.843 – 0.859 $g.l^{-1}$ and 99.8 – 103.5 $mg.l^{-1}$, respectively. The experiments were operated at pH of 7.5 – 8.0, but mean dissolved oxygen was changed from 0.5 ± 0.1 to 3.5 ± 0.3 $mgO_2.l^{-1}$. DO concentrations were measured using specific electrodes (DO sensor SE 7115 Model, Knick), connected with computer to record and adjust airflow. The concentrations of NH_4^+- N, NO_2^-- N and NO_3^-- N were collected and analyzed for 5 hours. The lost water due to evaporation and drawn samples were added by tap water to maintain constant volume during experimental tests.

- *Calculation*

Calculations are based on slopes of graphs of changes of ammonium, nitrite and nitrate versus time to determine AUR, NPR1 and NPR2. The value of specific ammonia uptake rate, specific nitrite production rate and specific nitrate production rate are calculated as Eq. (5.18).

$$qAUR = \frac{AUR}{MLVSS};\ qNPR1 = \frac{NPR1}{MLVSS} \text{ and } qNPR2 = \frac{NPR2}{MLVSS} \quad (5.18)$$

Where

qAUR	specific ammonia uptake rate; $mgNH_4^+$-N.$gVSS^{-1}$.h^{-1}
qNPR1	specific nitrite production rate; $mgNO_2^-$-N.$gVSS^{-1}$.h^{-1}
qNPR2	specific nitrate production rate; $mgNO_3^-$-N.$gVSS^{-1}$.h^{-1}
AUR	ammonia uptake rate; $mgNH_4^+$-N.l^{-1}.h^{-1}
NPR1	nitrite production rate; $mgNO_2^-$-N.l^{-1}.h^{-1}
NPR2	nitrate production rate; $mgNO_3^-$-N.l^{-1}.h^{-1}
MLVSS	mixed liquor volatile suspended solids in reactor; $g.l^{-1}$

b. Effect of Alkalinity/NH_4^+-N on partial nitrification

- *Operation*

The influent average NH_4^+-N was around 101 - 104 $mg.l^{-1}$. The *Alkalinity/NH_4^+-N* ratio was gradually increased from 5.08 ± 0.11 to 10.46 ± 0.25 by addition of $NaHCO_3$. mean MLVSS and DO concentration were maintained at 1.205 $g.l^{-1}$ and (2.56 ± 0.22 $mgO_2.l^{-1}$), respectively. The test was implemented at temperature ranging from 26.32 ± 0.49 °C. pH fluctuated in the range 7.54 ± 0.08 and the duration of the experiment was fixed at 7 hours. The initial concentrations of ammonia, nitrite, nitrate and alkalinity in both initial and after finishing of experiment were measured.

- *Calculation*

The nitrification and nitrite accumulation ratio percentages were calculated according to the following Eq. (5.19) and Eq. (5.20) [45].

$$NIT = \frac{NH_4^+\text{-}N_i - NH_4^+\text{-}N_e}{NH_4^+\text{-}N_i} \times 100 \quad (5.19)$$

Where

NIT	nitrification ratio; %
NH_4^+- N_i	concentrations of ammonia in the influent; $mgNH_4^+$- $N.l^{-1}$
NH_4^+- N_e	concentrations of ammonia in the effluent; $mgNH_4^+$- $N.l^{-1}$

$$NAR = \frac{NO_2^-\text{-}N_e}{NO_3^-\text{-}N_e + NO_2^-\text{-}N_e} \times 100 \quad (5.20)$$

Where

NAR	nitrite accumulation ratio, %
NO_2^--N_e	concentrations of nitrite and nitrate in the effluent; $mgNO_2^-$- $N.l^{-1}$
NO_3^--N_e	concentrations of nitrite and nitrate in the effluent; $mgNO_3^-$- $N.l^{-1}$

c. The effect of pH on partial nitrification

- *Operation*

The test was operated at initial NH_4^+- N of 100 – 106 $mg.l^{-1}$. DO and alkalinity were controlled ranging of 1.8 to 2.02 $mgO_2.l^{-1}$ and 850 ± 34 $mgCaCO_3.l^{-1}$. In the reactors, the average MLVSS concentrations ranged from 1.171 to 1.195 $g{\cdot}L^{-1}$, with the operational temperature controlled at 24.13 ± 0.4 °C. pH increased from 6.12 ± 0.08 to 10.01 ± 0.04 by using sulfuric acid or sodium hydroxide. The samples were collected once thirty minutes within 3.5 hours to measure NH_4^+-N, NO_2^--N and NO_3^--N concentrations.

- *Calculation*

The dissociation balance for free ammonia (FA) is dependent on the temperature and on the pH value. It can be calculated by using the equation given below [28, 46, 47].

$$FA\left(mg.l^{-1}\right)=\frac{17}{14}\times\frac{TAN\times10^{pH}}{\frac{K_b}{K_w}+10^{pH}} \tag{5.21}$$

Where

FA Free ammonia, $mg.l^{-1}$

$\frac{K_b}{K_w} = e^{6344/(273+T(℃))}$

K_b ionization constant of the ammonia equilibrium equation

K_w ionization constant of water

TAN total ammonia nitrogen concentration in the reactor; $mg.l^{-1}$

pH is the pH value in the reactor

t celsius degrees is the temperature in the reactor

Concentration of free nitrous acid as Eq. (5.22)

$$FNA\ as\ HNO_2\left(mg.l^{-1}\right)=\frac{47}{14}\times\frac{NO_2^-\text{-}N\left(mg.l^{-1}\right)}{K_a\times10^{pH}} \tag{5.22}$$

Where

FNA free nitrous acid, $mg.l^{-1}$

$K_a = e^{\frac{-2300}{273+t}}$ ionization constant of nitrous acid

NO_2^--N is the nitrite nitrogen concentration in the reactor; $mg.l^{-1}$

5.2.5.2. Effects of COD on denitrification

To test for denitrification, various carbon sources were used by several investigators [48, 49]. In this research, carbon sources from leachate and

glucose were used to investigate the effect of sCOD on nitrite and nitrate denitrification.

- *Operation*
  - + Measured nitrite, nitrate, MLVSS in reactors.
  - + Leachate was precipitated by $Al(OH)_3$ and filtrated through Whatman GF/C (0.45µm) glass fibre filters to analyze sCOD concentration.
  - + Calculated volume of leachate to add reactors.
  - + Activated sludge was settled and withdrawn settled wastewater in reactor with volume equal volume of leachate added.
  - + Leachate, nitrite and nitrate were fed in the first AN1 and nitrate into second AN2. Samples were mixed quickly, then collected and analyzed sCOD, nitrite and nitrate.
  - + Initial nitrite and nitrate concentration in reactors were 50.77 ± 0.41 $mg.l^{-1}$ and 50.68 ± 0.69 $mg.l^{-1}$, respectively. Leachate was added and increased gradually sCOD/NO_2^--N ratio, corresponding to sCOD/NO_2^--N and to sCOD/NO_3^--N from 2.89 to 9.11 and from 6.23 to 14.65, respectively.
  - + Like leachate, glucose source was implemented at sCOD/NO_2^--N ratio from 4.32 to 11.5 and sCOD/NO_3^--N ratio from 3.02 to 15.05 with initial nitrite and nitrate to be 100.54 ± 0.19 $mg.l^{-1}$.
  - + The pH and temperature were maintained ranging from 7.0 to 7.3 and from 24.6 to 26.32, respectively.
- *Calculation*

Nitrite and nitrate uptake rate were determined from slopes of graph of changes of nitrite and nitrate versus time. Specific denitrification is calculated by dividing NUR to MLVSS concentration.

$$qNUR1 = \frac{NUR1}{MLVSS} \text{ and } qNUR2 = \frac{NUR2}{MLVSS} \quad (5.23)$$

Where

qNUR1 specific nitrite uptake rate; $mgNO_2^-\text{-}N.gVSS^{-1}.h^{-1}$
qNUR2 specific nitrate production rate; $mgNO_3^-\text{-}N.gVSS^{-1}.h^{-1}$
NUR1 nitrite uptake rate; $mgNO_2^-\text{-}N.l^{-1}.h^{-1}$
NUR2 nitrate uptake rate; $mgNO_3^-\text{-}N.l^{-1}.h^{-1}$
MLVSS mixed liquor volatile suspended solids in reactor; $g.l^{-1}$

5.2.6. Analytical methods

All chemical analyses were performed in accordance with standard method [49].

5.3. RESULTS AND DISCUSSION

5.3.1. Effects on partial nitrification

5.3.1.1. Effect of DO on partial nitrification

Dissolved oxygen is a substrate (electron acceptor) in the nitrification reaction in activated sludge. Therefore, the oxygen concentration is also mentioned as a very important limiting factor and could be used as a tool to control partial nitrification. Theoretically, the oxygen requirements are 3.43 $mgO_2.l^{-1}$ for oxidation of 1mg ammonia to nitrite and 1.14 mg for oxidation of 1 mg nitrite to nitrate and DO is also used in the equations used to describe the kinetic of autotrophic nitrifiers. The experiment to determine the effect of DO concentration on partial nitrification was implemented in this study. Example of changes of ammonium concentration and nitrite and nitrate production versus time at various DO concentration are presented on Figure 5.02.

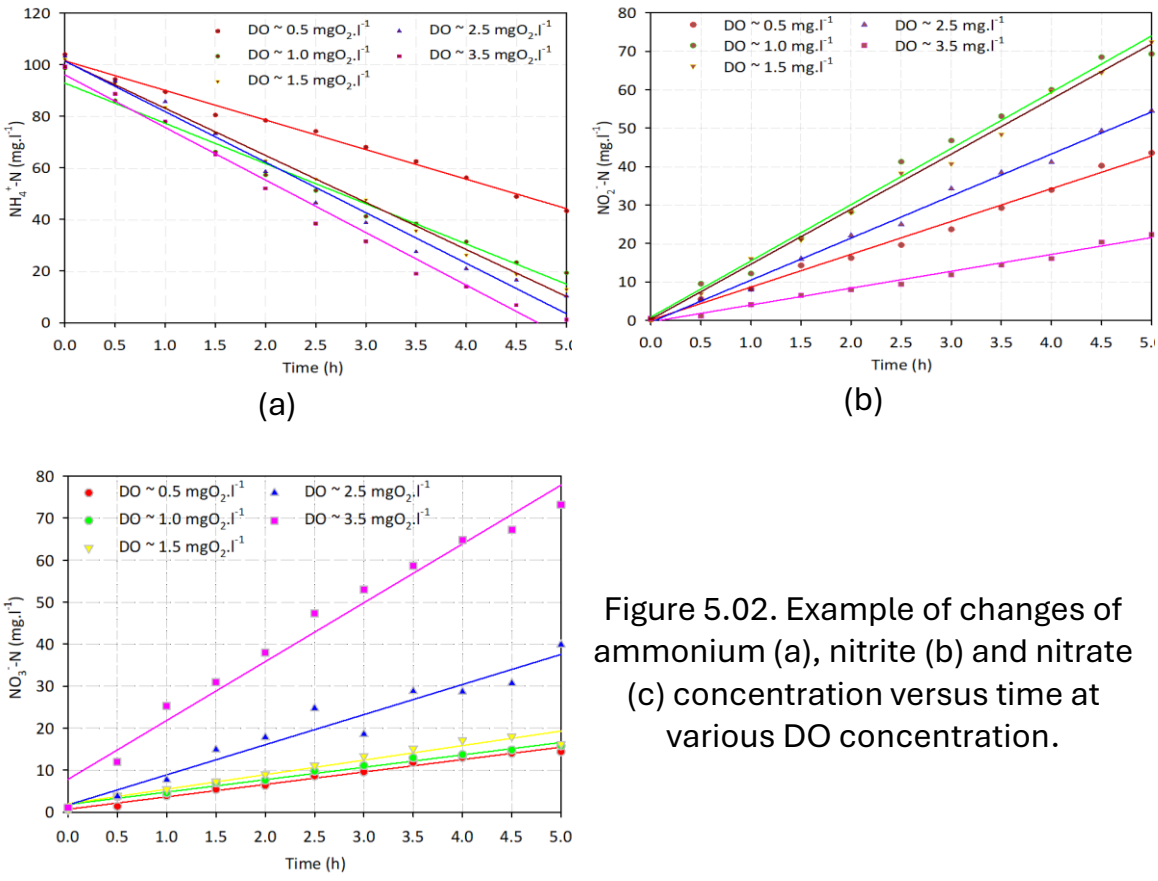


Figure 5.02. Example of changes of ammonium (a), nitrite (b) and nitrate (c) concentration versus time at various DO concentration.

From Figure 5.02, the data from simple linear regression equations found in Table 5.02.

Table 5.02. Collected data from example determining effect of DO on AUR, NPR1 and NPR2

Nitrogen		DO concentration, $mgO_2.l^{-1}$				
		0.6	1.1	1.6	2.4	3.7
Ammonium	**a** (slope) - **AUR**	-11.466	-15.588	-18.245	-19.592	-20.376
	b (intercept)	101.510	92.836	101.328	101.398	96.029
	R^2	0.9921	0.983	0.9975	0.9834	0.9847
Nitrite	**a** (slope) - **NPR1**	8.537	14.626	14.321	10.938	4.380
	b (intercept)	0.1100	0.8535	0.2859	-0.4755	-0.3840
	R^2	0.9921	0.9892	0.9959	0.9940	0.9880
Nitrate	**a** (slope) – **NPR2**	2.9547	2.9516	3.4583	7.210	14.017
	b (intercept)	0.6794	1.8267	2.0042	1.7904	7.8083
	R^2	0.9874	0.9852	0.9544	0.9538	0.9740

As shown in Table 5.02, the relationship between ammonium consumption and nitrite and nitrate production versus time were linearly fitted with $R^2 > 0.97$ (accepted $R^2 > 95\%$). The AUR, NPR1 and NPR2 could be obtained from the corresponding slope of the linear regressions. MLVSS concentration at DO of 0.6, 1.1, 1.6, 2.4 and 3.7 $mgO_2.l^{-1}$ were 0.846, 0.856, 0.834, 0.857 and 0.859 $g.l^{-1}$, respectively. The value of the specific ammonium uptake rate, specific nitrite production and specific nitrate production rate are calculated by using Eq. (5.18) and shown in Table 5.03.

Table 5.03. Example for the calculation of the effect of DO concentration on qAUR, qNPR1, and qNPR2

DO $mgO_2.l^{-1}$	**qAUR** $mgNH_4^+\text{-}N.gVSS^{-1}.h^{-1}$	**qNPR1** $mgNO_2^-\text{-}N.gVSS^{-1}.h^{-1}$	**qNPR2** $mgNO_2^-\text{-}N.gVSS^{-1}.h^{-1}$
0.6	13.553	11.028	2.955
1.1	18.210	15.243	3.448
1.6	21.876	17.214	3.768
2.4	22.861	13.845	9.167
3.7	23.721	4.717	16.318

The experiment on influence of DO concentration on AUR, NPR1 and NPR2 was implemented 3 times and the mean values are presented in Table 5.04.

Table 5.04. Effect of DO concentration on qAUR, NPR1 and qNPR2

	DO concentration, $mgO_2.l^{-1}$				
	0.5 ± 0.1	1.0 ± 0.2	1.5 ± 0.1	2.5 ± 0.1	3.5 ± 0.3
AUR $mgNH_4^+$-N.l^{-1}.h^{-1}	-10.98 ± 0.50	15.03 ± 0.49	18.05 ± 0.63	19.70 ± 0.15	20.77 ± 0.38
qAUR $mgNH_4^+$-N.$gVSS^{-1}$.h^{-1}	-13.04 ± 0.60	17.68 ± 0.46	21.38 ± 0.77	22.93 ± 0.15	24.07 ± 0.32
NPR1 $mgNO_2^-$-N.l^{-1}.h^{-1}	8.74 ± 0.52	13.73 ± 0.83	14.36 ± 0.29	11.06 ± 0.79	4.24 ± 0.17
qNPR1 $mgNO_2^-$-N.$gVSS^{-1}$.h^{-1}	10.38 ± 0.56	16.15 ± 0.86	17.01 ± 0.31	12.87 ± 0.92	4.92 ± 0.20
NPR2 $mgNO_3^-$-N.l^{-1}.h^{-1}	2.77 ± 0.16	3.11 ± 0.18	3.36 ± 0.13	7.70 ± 0.43	15.04 ± 0.96
qNPR2 $mgNO_3^-$-N.$gVSS^{-1}$.h^{-1}	3.28 ± 0.18	3.65 ± 0.23	3.99 ± 0.20	8.97 ± 0.48	17.43 ± 1.09

The results are given in Table 5.04, demonstrated that the ammonia oxidation rate increased if DO concentration increased from 0.5 to 3.5 $mgO_2.l^{-1}$. The results indicate that AUR and NPR1 and NPR2 are influenced by DO concentration. With the increase of DO concentration, a more significant increase in NH_4^+- N removal was observed. At DO concentration of 0.5 $mgO_2.l^{-1}$, AUR and NPR1 were 10.98 $mgNH_4^+$-N.l^{-1}.h^{-1} and 8.74 $mgNO_2^-$-N.l^{-1}.$h^{-1}$and 2.77 $mgNO_3^-$-N.l^{-1}.h^{-1}, respectively. At low DO concentrations can affect the specific growth rate of both AOB and NOB. However, at DO concentration of 1.0 $mgO_2.l^{-1}$, the AUR and NPR1 increased considerably, corresponding 15.03 $mgNH_4^+$-N.l^{-1}.$h^{-1}$and 13.73 $mgNO_2^-$-N.l^{-1}.h^{-1}, respectively. Meanwhile, the NPR2 value rose slightly to around 0.4 $mgNO_3^-$-N.l^{-1}.h^{-1}.

Although there was a significant increase of DO concentration, the AUR grew slightly from 18.05 to 20.77 $mgNH_4^+$-N.l^{-1}.h^{-1} at DO concentration of 1.5 and 3.5 $mgO_2.l^{-1}$, respectively. In contrast, the results for NPR1 dropped dramatically from 14.36 to 4.24 $mgNO_2^-$-N.l^{-1}.h^{-1} at DO concentration of 1.5 and 3.5 $mgO_2.l^{-1}$, respectively, due to the convert nitrite to nitrate in activated sludge at high DO concentration. A significant increase in NPR2 values was observed, from 3.36 to 15.04 $mgNO_3^-$-N.l^{-1}.h^{-1}, when the DO concentration was raised from 1.5 mg $O_2.l^{-1}$ to 3.5 mg $O_2.l^{-1}$. Therefore, a sharp rise in dissolved oxygen concentration will not increase ammonium removal efficiency but remaining dissolved oxygen will delay the denitrification process in the next stage.

To estimate ammonium removal efficiency and nitrite accumulation ratio due to DO concentration, the Eq. (5.19) and (5.20) is used to calculate as in Table 5.05 and Figure 5.03.

Table 5.05. Effect DO concentration on NIT and NAR

DO	NH_4^+-N_i	NH_4^+-N_e	NO_2^--N_e	NO_3^--N_e	NIT	NAR
$mgO_2.l^{-1}$	$mgN.l^{-1}$				%	%
0.5 ± 0.1	99 ± 4.2	43.2 ± 1.4	45.3 ± 2.1	13.1 ± 1.2	56.6 ± 1.7	77.5 ± 2.4
1.0 ± 0.2	102 ± 3.2	24.1 ± 4.3	66.3 ± 2.7	16.4 ± 1.2	76.5 ± 3.5	80.1 ± 1.6
1.5 ± 0.1	101.0 ± 3.1	14.0 ± 1.2	71.7 ± 1.3	16.8 ± 0.8	86.3 ± 0.9	81.0 ± 0.7
2.5 ± 0.1	103 ± 1.5	7.7 ± 2.6	53.4 ± 1.1	40.1 ± 0.5	92.6 ± 2.5	57.1 ± 0.7
3.5 ± 0.3	103 ± 3.00	2.5 ± 1.1	22.2 ± 1.0	74.3 ± 1.2	97.6 ± 1.0	23.0 ± 1.0

As can be seen in Figure 5.03, the DO concentration increased ranging from 0.5 to 3.5 $mgO_2.l^{-1}$. A nitritation percentage increase was noticed with a DO concentration growth at value of 1.0 $mgO_2.l^{-1}$, at which a nitritation percentage of 76.5 % was attained. The nitratation percentage increased gradually from 76.5 % to 97.6 % with the increase of DO concentration from 1.0 to 3.5 $mgO_2.l^{-1}$. The metabolic activity of AOB will enhance high DO concentration, thus NIT is also increased.

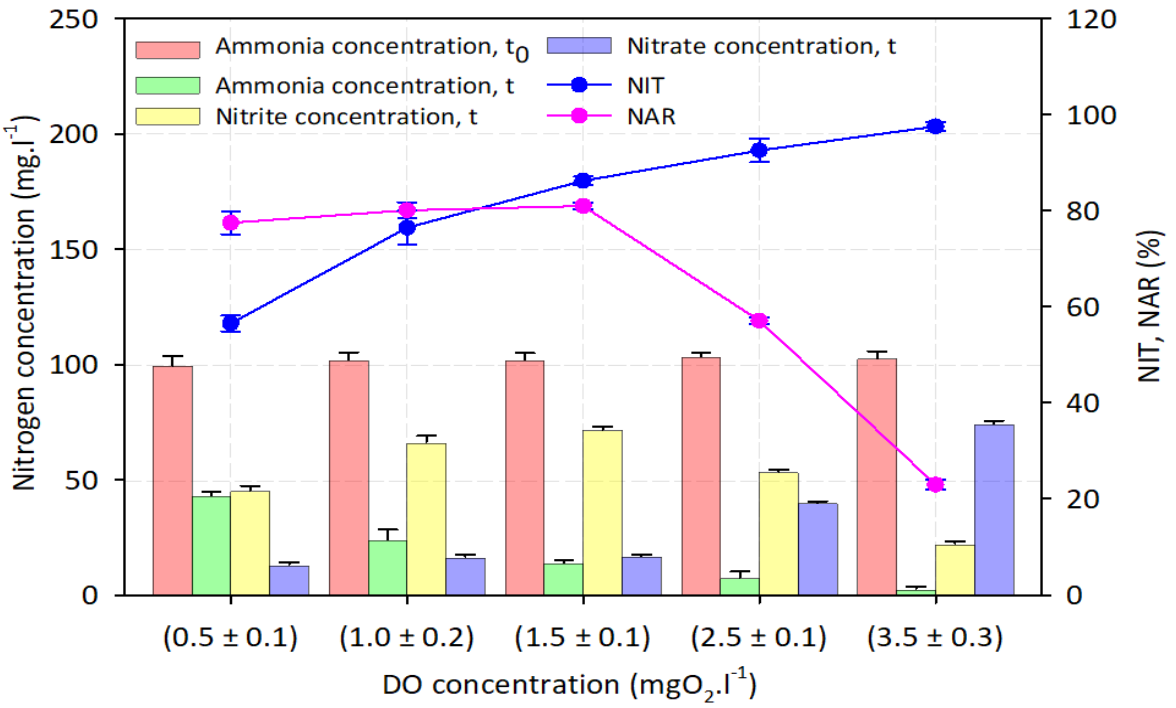


Figure 5.03. Effect of DO concentration on NIT and NAR

In contrast with AUR, when the dissolved oxygen concentration increased from 0.5 to 1.5 $mgO_2.l^{-1}$, the value of NPR1 went up slowly from 77.5 % to 80.1 %, respectively. When DO concentrations in the range of 2.5 to 3.5 $mgO_2.l^{-1}$, nitrite accumulation rate reduced remarkably. The results indicated that in the range, from DO of 0. 5 to 1.5 there was no significant affection into nitrite accumulation rate, but at DO concentration higher than 1.5 $mgO_2.l^{-1}$ the simulation of *Nitrobacter* activity took place. The NAR decreased and got the lowest at DO concentration of 3.5 $mgO_2.l^{-1}$ to be 23 % due to nitrite was converted to nitrate at high DO concentration.

Therefore, controlling the dissolved oxygen concentration in the reactor is a key parameter for enhancing the nitrite accumulation and inhibiting its further oxidation to nitrate. Thus, low DO concentration might be more restrictive for the growth of NOB than AOB due to the higher affinity for oxygen of AOB.

The results obtained in this research are in agreement with the results obtained Park *et al.*[50] who observed changes in the bacterial population structure at low oxygen concentrations, which could affect nitrite accumulation rates. Ruiz G., *et al.*, [43] also indicated that ammonia was accumulated at DO concentration below 0.5 $mgO_2.l^{-1}$ and complete nitrification to nitrate was achieved at DO over 1.7 $mgO_2.l^{-1}$. Guo X., *et al.* also demonstrated that dissolved oxygen concentration of 1.0 mg $O_2.l^{-1}$ was needed for satisfactory partial nitrification [51]. Meanwhile, the DO concentration from 0.8 to 2.3 $mgO_2.l^{-1}$ was achieved steady partial nitritation found by Liang Zhu *et al.* [3].

5.3.1.2. Effect of alkalinity on partial nitrification

Alkalinity is one of influencing factors for conversion of ammonia to nitrite and nitrate. The alkalinity form of carbonate and bicarbonate is not only a nutrient (inorganic carbon) element for nitrifying bacteria, but has also a buffering capacity for preventing change of pH during the nitrification process. So, the impact of alkalinity on the nitrification rate is related to the pH. Gujer *et al.*, indicated that 8.64 $mg.l^{-1}$ of bicarbonate will be utilized for each $mg.l^{-1}$ of ammonia - nitrogen oxidized [52]. The experiment of effect of alkalinity on partial nitrification was also implemented in this study. From initial and finish concentration of ammonia, nitrite and nitrate were measured, then using Eq. (5.19) and Eq. (5.20), the nitrification (NIT) and nitrite accumulation ratio (NAR) percentages are calculated as in Table 5.06. Table 5.06 indicates the effect of the ratio of Alkalinity/NH_4^+-N on removal efficiencies of NH_4^+-N and NO_x^--N.

Table 5.06. Effect alkalinity/ammonium on NIT and NAR

Alkalinity / NH_4^+-N	NH_4^+-N_i	NH_4^+-N_e	NO_2^--N_e	NO_3^--N_e	NIT	NAR
	mg.l^{-1}				%	%
5.08 ± 0.11	101.3 ± 1.5	49.7 ± 4.1	40.2 ± 4.0	11.1 ± 1.6	50.6 ± 3.8	78.3 ± 3.5
6.32 ± 0.08	102.9 ± 2.8	22.3 ± 2.9	67.1 ± 4.7	17.2 ± 2.5	82.3 ± 2.7	79.6 ± 3.4
7.36 ± 0.14	101.3 ± 0.6	9.3 ± 1.9	44.2 ± 1.5	47.5 ± 3.5	90.9 ± 2.0	48.2 ± 2.5
9.03 ± 0.23	101.7 ± 3.5	9.0 ± 1.1	19.7 ± 2.4	75.9 ± 5.5	95.1 ± 2.6	20.7 ± 3.1
10.46 ± 0.25	103.7 ± 7.6	5.2 ± 2.4	6.3 ± 1.8	93.3 ± 4.4	97.9 ± 0.5	6.3 ± 1.7

As shown in Table 5.06, the efficiency of ammonium conversion was low if the alkalinity addition was insufficient. When we increased the ratio of Alkalinity/NH_4^+-N, the ammonium removal, production rates of nitrite and nitrate raised. Alkalinity/NH_4^+-N ratio increased gradually from 5.08 to 7.36, the efficiency of ammonium removal rate ascended rapidly from 50.6 to 90.9 % respectively and reached a peak 97.9 % at Alkalinity/NH_4^+-N ratio of 10.46. According to Xiaojing *et al*. [53], when the alkalinity/ammonia ratio was 10,7, ammonia was almost completely converted with an ammonia removal efficiency of nearly 100 %.

The increase in the ammonium removal rate is explained by the activity of *Nitrosomonas* which was stimulated with the increase in the Alkalinity/NH_4^+-N ratio during the nitrification. When Alkalinity/NH_4^+-N ratio was lower than 7.36, there was a significant growth in the ammonia efficiency removal because the alkalinity was enough for consumption of *Nitrosomonas* to convert ammonia to nitrite. However, when Alkalinity/NH_4^+-N was higher the conversion of nitrite to nitrate by *Nitrobacter* leading to NAR percent, declined remarkably. From Table 5.06, the graph of the relationship between NIT (%) and NAR (%) versus Alkalinity/NH_4^+-N ratio is shown in Figure 5.04.

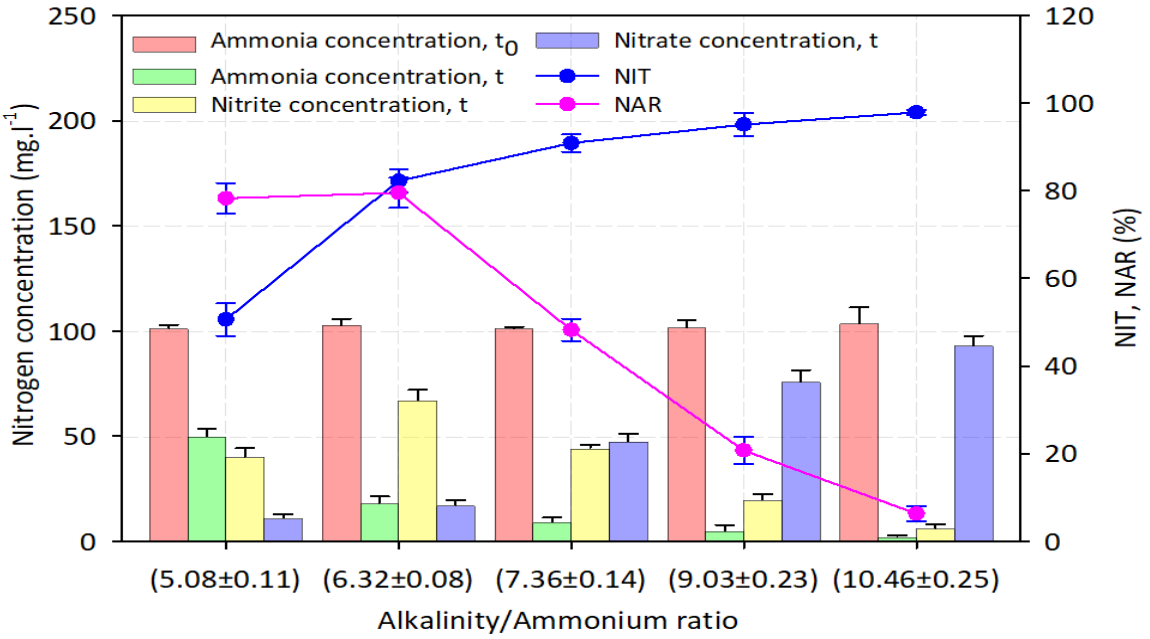


Figure 5.04. Effect of Alkalinity/NH_4^+-N ratio on NIT and NAR

Figure 5.04. presents the relationship between NIT and NAR versus mean Alkalinity/NH_4^+-N ratio. The ammonium removal rate and nitrate production rate represent the activities of *Nitrosomonas* and *Nitrobacter*, respectively. As the Alkalinity/NH_4^+-N ratio increased from 5.08 to 6.32, the nitrite accumulation gradually increased. In this study, the nitrite accumulation rate was highest at ratio of Alkalinity/NH_4^+-N of 6.32, i.e. 79.6 %. As the ratio of Alkalinity/NH_4^+-N increased from 6.32 to 7.36, the nitrite build-up dropped quickly, the mean percent of nitrite compare with total NO_x^--N decreased from 79.6 % to 48.2 %, respectively. With Alkalinity/NH_4^+-N ratio over 7.36, the percent of nitrite concentration went down significantly and hit the lowest point 6.3 % of NO_x^--N at Alkalinity/NH_4^+-N ratio of 10.46. These nitrite accumulations are mainly due to a difference in the reaction rates of ammonium and nitrite oxidizers. An increase ratio of Alkalinity/NH_4^+-N led to increases of pH and FA concentration which caused enhanced nitrite build-up or inhibitor for *Nitrobacter*.

The results obtained in this research are in agreement with the results obtained by Hwang *et al*, who observed an increase of nitrite build-up in biofilm reactors. The authors found that effluent nitrite concentration reached up to 70% of NO_x^--N at Alkalinity/NH_4^+-N ratio of 7.1.

Besides, FA is an important parameter to control partial nitrification. The high concentration of FA caused enhanced nitrite accumulation because FA is an inhibiting factor for *Nitrobacter* found by Hwang *et al*. [54] and former authors.

The increase in the ammonium removal rate with the increase in the Alkalinity/NH_4^+-N ratio is explained as *Nitrosomonas* consumes most of alkalinity during the nitrification. The investigation also demonstrated that nitrite accumulation increased with sufficient alkalinity addition which suggested that alkalinity was not only essential for ammonia removal but also played a vital role in nitrite accumulation.

In conclusion, alkalinity plays an important role in terms of both growth substrate and environment for microorganisms, especially in establishing a balanced system for the growth of AOB and NOB in the control of nitritation.

5.3.1.3. Effect of pH on partial nitrification

The experiments were done to investigate the effect of pH on nitrite accumulation during nitrification. With an initial NH_4^+-N concentration around 100 - 106 mg.l^{-1} and initial MLVSS concentration ranging from 1.171 g.l^{-1} to 1.195 g.l^{-1} were tested. The pH was adjusted from 6.12 to 10.01 by using acid sulfuric or sodium hydroxide solution. The DO, temperature and alkalinity were 2.8 – 3.02 mgO_2.l^{-1}, 24 – 26 °C and 750 - 792 mg$CaCO_3$.l^{-1}, respectively. Example the effect of pH on ammonia oxidation rate and nitrite production are shown in Figure 5.05.

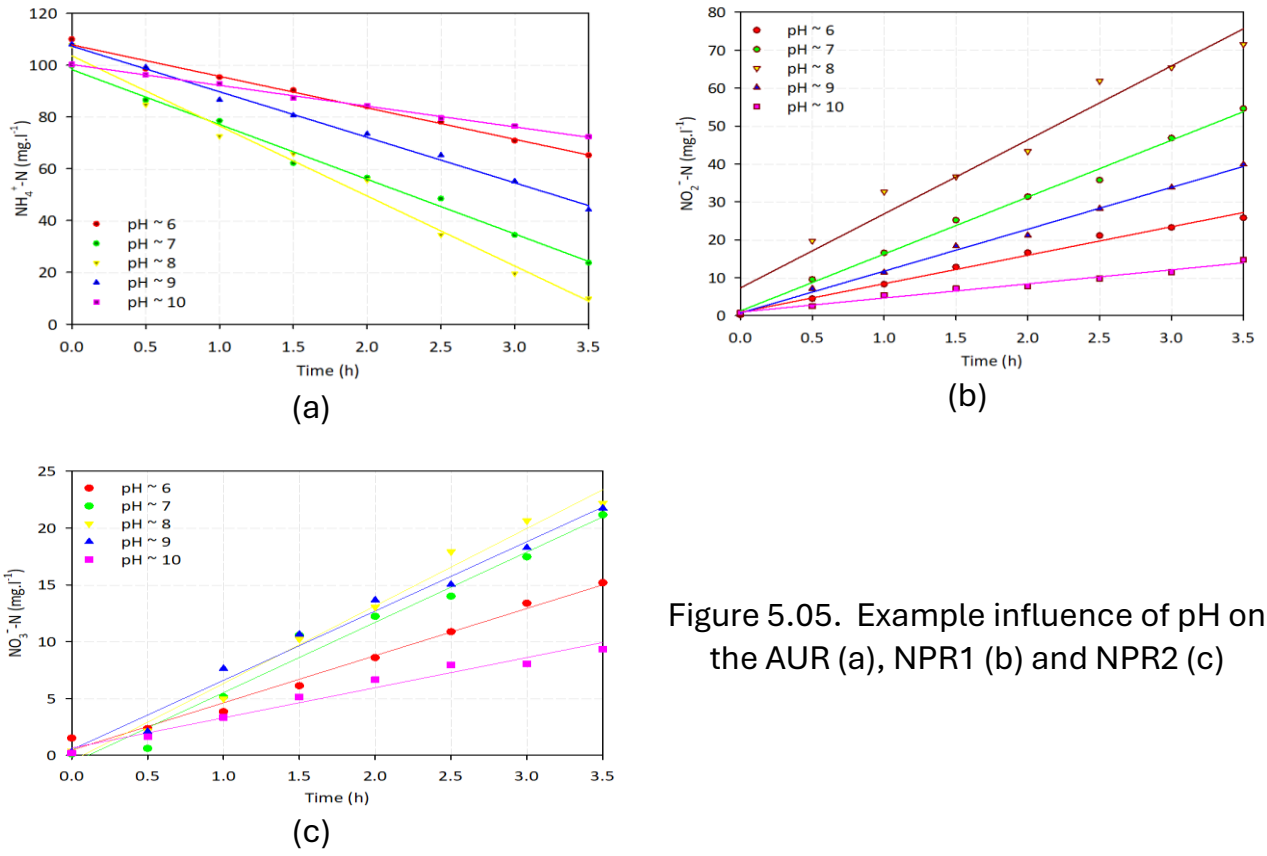


Figure 5.05. Example influence of pH on the AUR (a), NPR1 (b) and NPR2 (c)

The ammonia utilization rate, nitrite and nitrate production rate were determined from the slope of the equations of simple linear regressions as

illustrated in Figure 5.05. The achieved values from equations are shown in Table 5.07.

Table 5.07. Collected data from example determining effect of pH on AUR, NPR1 and NPR2

Nitrogen		pH				
		6.21	**7.16**	**8.17**	**9.10**	**9.98**
Ammonium	**a** (slope) - **AUR**	-12.202	-21.856	-27.215	-17.536	-8.0195
	b (intercept)	107.97	100.32	95.984	107.22	100.23
	R^2	0.9887	0.9851	0.9888	0.9944	0.9979
Nitrite	**a** (slope) - **NPR1**	7.5014	15.5650	19.452	11.044	3.7150
	b (intercept)	0.9150	3.3829	3.5442	0.6807	0.9317
	R^2	0.9910	0.9891	0.9927	0.9960	0.9811
Nitrate	**a** (slope) – **NPR2**	4.1555	6.1864	6.8157	6.0996	2.6526
	b (intercept)	0.4595	-0.6690	-0.4878	0.4979	0.6442
	R^2	0.9872	0.9783	0.9854	0.9849	0.9712

Linear regression was applied to quantify the slopes. The ammonia oxidation rate, the nitrite and nitrate production rate were determined from the slopes. The values of the coefficient of determination (R^2) in the regression were above 0.97, the reaction was virtually zero-order. MLVSS concentration in reactor were 1.171, 1.182, 1.192, 1.188 and 1.184 $g.l^{-1}$ corespongding to mean pH 6.12, 7.16, 8.17, 9.10 and 9.98, respectively. From slopes and MLVSS concentration, the specific ammonia oxidation and nitrite production rates at various pH are calculated by Eq. (5.18) and presented in Table 5.08.

Table 5.08. Example for the calculation of the effect of pH on qAUR, qNPR1, and qNPR2

pH	**VSS** $g.l^{-1}$	**qAUR** $mgNH_4^+\text{-}N.gVSS^{-1}.h^{-1}$	**qNPR1** $mgNO_2^-\text{-}N.gVSS^{-1}.h^{-1}$	**qNPR2** $mgNO_3^-\text{-}N.gVSS^{-1}.h^{-1}$
6.21	1.171	-10.420	6.406	3.549
7.16	1.182	-18.491	13.168	5.234
8.17	1.192	-22.831	16.319	5.718
9.10	1.188	-14.761	9.296	5.132
9.98	1.184	-6.773	3.138	2.240

The value of the specific ammonium uptake rate, specific nitrite production and nitrate production are calculated by using Eq. (5.18) and shown in Table 5.09.

Table 5.09. Effect of pH on qAUR, qNPR1 and qNPR2

Kinetic parameters	pH				
	6.12 ± 0.08	7.15 ± 0.05	8.22 ± 0.07	9.11 ± 0.09	10.01 ± 0.04
AUR $mgNH_4^+$-N.l^{-1}.h^{-1}	-12.54 ± 0.30	-21.51 ± 0.36	-27.27 ± 0.26	-18.39 ± 0.84	-8.26 ± 0.29
qAUR $mgNH_4^+$-N.$gVSS^{-1}$.l	-10.71 ± 0.25	-18.25 ± 0.25	-22.90 ± 0.12	-15.50 ± 0.66	-6.91 ± 0.18
NPR1 $mgNO_2^-$-N.l^{-1}.h^{-1}	7.73 ± 0.21	15.15 ± 0.37	19.86 ± 0.65	11.52 ± 0.43	3.63 ± 0.18
qNPR1 $mgNO_2^-$-N.$gVSS^{-1}$.l	6.60 ± 0.21	12.85 ± 0.28	16.68 ± 0.48	9.72 ± 0.42	3.04 ± 0.17
NPR2 $mgNO_3^-$-N.l^{-1}.h^{-1}	4.06 ± 0.10	6.31 ± 0.10	6.80 ± 0.29	5.80 ± 0.28	2.69 ± 0.14
qNPR2 $mgNO_3^-$-N.$gVSS^{-1}$.l	3.47 ± 0.08	5.35 ± 0.08	5.71 ± 0.25	4.89 ± 0.25	2.25 ± 0.10

As shown in Table 5.09, when the value of initial mean pH increased from 6.12 to 7.15, AUR and qAUR increased more than two times, from 12.54 to 21.51 $mgNH_4^+$-N.l^{-1}.h^{-1} and 10.71 to 18.25 $mgNH_4^+$-N.$gVSS^{-1}$.h^{-1}, respectively. The AUR and qAUR increased and reached maximum value as pH increased to 8.22, yielding 27.27 $mgNH_4^+$-N.l^{-1}.h^{-1} and 22.90 $mgNH_4^+$-N.$gVSS^{-1}$.h^{-1} , respectively. However, when the pH is further increased from 8.22 to 9.11, AUR and qAUR decreased slightly and pH went up to 10.01, the AUR and qAUR dropped significantly. The lowest value of AUR and qAUR was found at pH of 10.01 to be 8.26 $mgNH_4^+$-N.l^{-1}.h^{-1} and 6.91 $mgNH_4^+$-N.$gVSS^{-1}$.h^{-1}, respectively.

Likely AUR, NPR1 and q_{NPR1} also climbed drastically from 7.73 to 15.15 $mgNO_2^-$-N.l^{-1}.h^{-1} and from 6.60 to 12.85 $mgNO_2^-$-N.$gVSS^{-1}$.h^{-1}, respectively, corresponding pH of 6.12 and 7.15. The highest value of AUR and qNPR1 achieved at pH 8.22 to be 19.86 $mgNO_2^-$-N.l^{-1}.$h^{-1}$and 16.68 $mgNO_2^-$-N.$gVSS^{-1}$.h^{-1}, then there was a tremendous drop with pH increasing and reached the lowest value of 3.63 $mgNO_2^-$-N.l^{-1}.h^{-1} for NPR1 and 3.04 $mgNO_2^-$-N.$gVSS^{-1}$.h^{-1} for q_{NPR1} at pH of 10.

Burton *et al.* [55] found that pure cultures of autotrophic ammonia-oxidizing bacteria in liquid culture was optimal within the pH range 7.0 to 8.5 and typically does not occur below a pH of 6.5. According to Allison *et al.* [56] ammonia

oxidation occurred at a pH value of 6, even though the pH minimum for growth of the organism in liquid batch culture was 7.

In the literature, the optimum pH value for the nitrification process varies between

8 and 9. Claros J- *et al.,* [57] indicated that the optimal pH for AOB was found to be in the range from 7.4 to 7.8. In addition to, Chen *et al*. [58] also found the optimum pH for *Nitrosomonas sp*. ranging from 7.2 to 8.8 and for *Nitrobacter sp*. from 7.2 to 9.0. Burton *et al*. [55] suggested that the growth and nitrite production from ammonium did not occur at pH values below 7. Ammonium oxidation, on the other hand, was completely inhibited at pH of 5 while the nitrite oxidation was strongly inhibited at pH of 8.5 in tropical rivers investigated by Le *et al*.[59].

The ammonia oxidation rate was considered as the production rate of nitrite and nitrate. Nitrites accumulate when the ammonia oxidation rate is higher than the nitrite oxidation rate. Using Eq. (5.19) and Eq. (5.20) for calculating NIT and NAR percentages is shown in Table 5.10.

Table 5.10. Effect of pH on NIT and NAR

pH	NH_4^+-N_i	NH_4^+-N_e	NO_2^--N_e	NO_3^--N_e	NIT	NAR
-	mgN.l^{-1}				%	%
6.12 ± 0.08	106.6 ± 4.2	61.0 ± 5.0	27.5 ± 1.7	14.5 ± 0.9	42.9 ± 2.5	65.5 ± 2.8
7.15 ± 0.05	100.0 ± 1.4	24.9 ± 2.1	54.6 ± 1.5	22.5 ± 1.3	75.1 ± 1.8	70.8 ± 1.7
8.22 ± 0.07	102.9 ± 4.6	8.5 ± 1.3	70.1 ± 1.4	23.3 ± 1.0	91.7 ± 0.9	75.1 ± 0.5
9.11 ± 0.09	103.8 ± 3.4	37.8 ± 5.7	41.3 ± 1.9	20.6 ± 1.1	63.7 ± 4.2	66.7 ± 2.2
10.01 ± 0.04	101.6 ± 2.7	72.5 ± 3.2	13.8 ± 0.8	9.8 ± 0.4	28.6 ± 1.5	59.8 ± 2.5

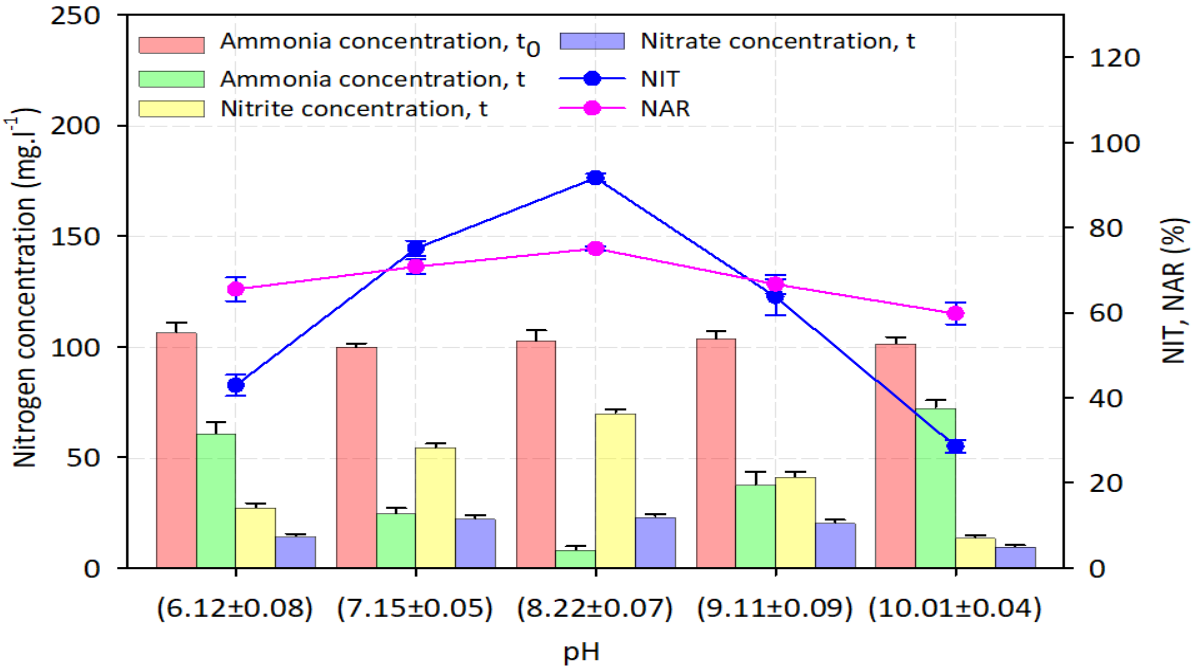


Figure 5.06. Influence of pH on the NIT and NAR

The concentrations of both nitrate and nitrite increased in the reactor when pH went up from 6.12 to 8.22. As shown in Table 5.09, ammonia was oxidized to nitrite and only part of the nitrite was further oxidized into nitrate. The ratio of oxidized ammonia increased with increasing of pH from 6.12 to 8.22 and got a maximum value at pH of 8.22 to be 91.7 %. However, NIT reduced considerably and hit a low 28.6 % when pH increased until 10.01. Nitrite accumulation could easily reach 65.5 % in the system when the pH was around 6.12. There was a gradual growth of NAR with climbing pH from 7.15 to 8.22, the nitrite concentration was observed from 70.8 % to 75.1 %, respectively. At higher pH, the nitrite accumulation declined markedly and achieved the lowest value at pH of 10.01 to be 59.8 %.

In general, nitrite can accumulate relatively due to the rate differences between nitritation and nitratation. The effects of pH on rate of converting nitrite to nitrate is lower rate of oxidized ammonia during nitrification process. The effects of pH on nitrification rate have been observed by several authors. Most of the literature suggests that pH in the range of 7.5 - 8.5 is most suited to inhibit NOB. Besides, pH affects also indirectly nitrification process through chemical equilibriums, the most important being the ammonium/ammonia and nitrite/nitrous acid equilibriums. Therefore, the pH affects nitrification through FA and FNA, which are known to inhibit nitrification.

The FA and FNA concentrations were also considered to estimate their effects on NIT and NAR. The initial FA and FNA at different pH levels were calculated by using Eq. (5.21) and Eq. (5.22), respectively. However, initial nitrite concentration was too low, all values of FNA found were < 0.003 $mg.l^{-1}$, therefore Table 5.11 only shows the value of FA.

Table 5.11. Effect of pH on FA

pH	6.12 ± 0.08	7.15 ± 0.05	8.22 ± 0.07	9.11 ± 0.09	10.01 ± 0.04
FA, $mg.l^{-1}$	0.09 ± 0.02	0.88 ± 0.11	9.92 ± 1.19	49.93 ± 4.86	103.60 ± 1.87

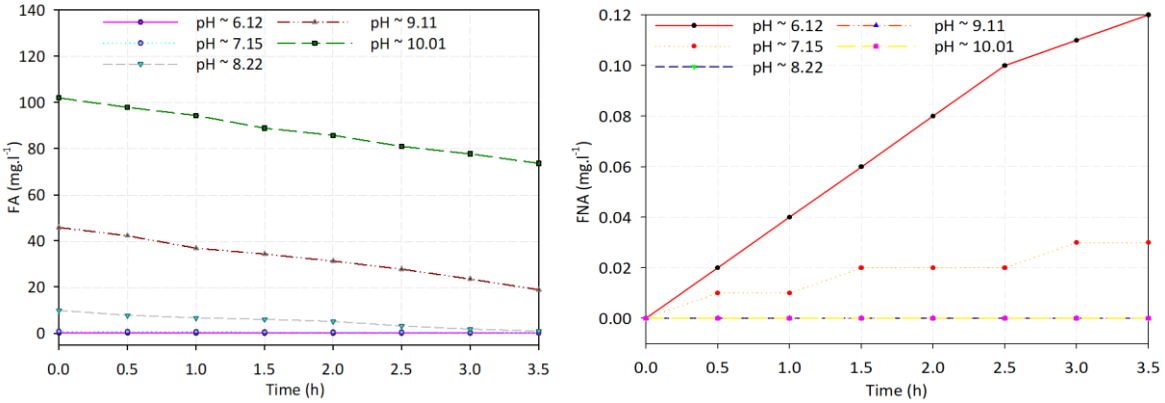
Table 5.10 shows that the FA concentration is strongly dependent on pH. The ammonia in solution includes ammonium ion NH_4^+ and un-ionized ammonia (NH_3) [50]. The equilibrium ammonium and ammonia depends on pH of solution. Therefore, the value of FA increased considerably with increasing pH in solution. The trends indicated that the ammonia oxidation decreased with the increase of free ammonia concentration. Figure 5.06 shows that ammonia removal efficiency increased with taking off pH from 6.12 to 8.22, then reduced with increasing pH until 10.01.

From this study, results indicated that FA concentration went up from 0.09 to 9.92, there was an increase of nitrification rate, but at FA higher than 9.92 there was inhibition of nitritation. Therefore, when pH in the influent rose and FA reached the concentration range that inhibited AOB, inducing the decrease of ammonia oxidation rate. The ammonia oxidation rate dropped dramatically within the free ammonia concentration range of 49.93 $mg.l^{-1}$, corresponding to NIT of 63.7 %. The lowest value of NIT of 28.6 % found at FA mean concentration of 103.6 $mg.l^{-1}$.

The rate of nitrite and nitrate production were quite low at FA of 0,09 due to inhibiting of pH to AOB and NOB. When the FA increased from 0.08 to 9.92, corresponding pH from 7.15 to 8.22, the rate of nitrite production went up rapidly. Although there was an increase of nitrite concentration, the nitrate concentration rose insignificantly. Thus, FA concentration inhibited on NOB, reducing nitratation process. However, when FA continued to increase to 49.93 $mg.l^{-1}$, nitrite concentration decreased rapidly but nitrate declined very slowly. But when FA climbed up over 49.93, both nitrite and nitrate went down remarkably. This is explained that FA not only inhibited nitritation but also nitratation.

In general, the highest value of nitrite accumulation ratio was achieved in this study at FA concentration of 9.92 $mg.l^{-1}$. Nitrite accumulation could be achieved by inhibiting NOB by FA.

The value of initial FNA is low, and it can be neglected. Producing nitrite in the nitrification process will increase FNA. On the contrary, FA concentration will decrease due to ammonia removal. Examples of the variation of FA and FNA concentrations with time in the reactor at various pH, corresponding to temperature, alkalinity and initial total ammonia nitrogen were 23.6 °C, 1050 $mgCaCO_3.l^{-1}$ and 103 ± 4.3 $mg.l^{-1}$, respectively as in Figure 5.07.



(a) (b)

Figure 5.07. Examples of variation of FA (a) and FNA (b) at various pH during nitrification

As mentioned, FA and FNA concentrations depend on pH, so that at high pH a large

part of the ammonium will be present as ammonia and at low pH most of the nitrite will be present in the form of nitrous acid.

Although there was a decrease in FA concentration versus time, the FA concentration at pH of 10 was very high. As seen in Table 5.10, the mean FA concentration declined from 103.6 to 73.96 (occupied 28.6 %) in 3.5 hours at pH of 10.01. At this pH, the nitritation was very low as seen in Table 5.09. At pH of 9.11, the FA concentration decreased from 49.93 to 18.04 (occupied 63.9 %), the ammonium oxidation rate was improved. The FA inhibition was estimated by many authors. According to Anthonisen A. C., *et al.*, [46] FA concentration will inhibit of *Nitrosomonas* and *Nitrobacter* in the ranges in 10 – 150 $mg.l^{-1}$ and 0.1 – 1.0 $mg.l^{-1}$, respectively.

In conclusion, FA and FNA concentrations depend on pH, so that at high pH a large

part of the ammonium will be present as ammonia and at low pH most of the nitrite will be present in the form nitrous acid.

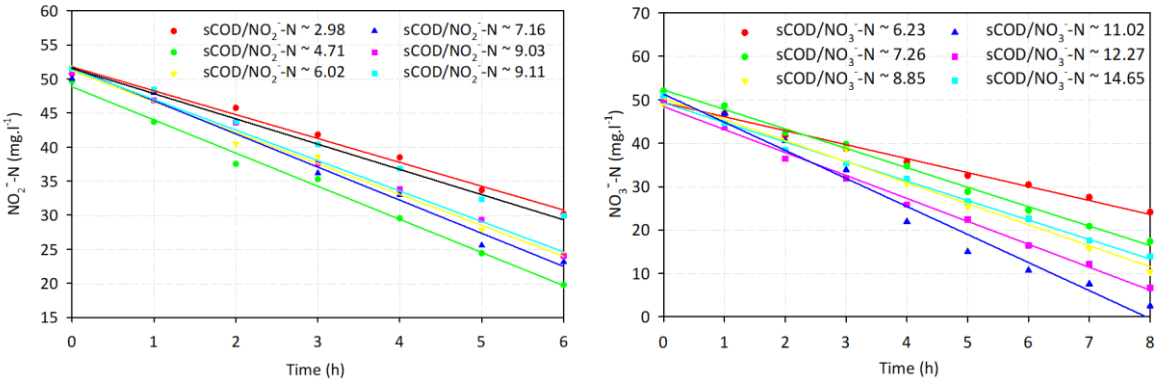
5.3.2. Effects on sCOD/N ratio on denitrification

During the denitrification process nitrate and nitrite in wastewater are reduced by many organisms that use nitrate, nitrite as electron acceptors for energy metabolism. Besides nitrate or nitrite, a source of organic carbon is required for the reduction of these oxidized inorganic compounds into nitrogen gas. The sCOD/N ratio is one of the most critical parameters for wastewater nitrogen removal process, because it directly affects functional microorganism populations, especially heterotrophic denitrifiers. Denitrifying bacteria can reduce nitrate through nitrite, nitric oxide, and nitrous oxide to nitrogen gas. In this study various soluble COD to nitrogen ratios in anoxic conditions were tested for AN1 with nitrate and AN2 with nitrite.

5.3.2.1. Effect of sCOD from leachate on denitrification

- *Effect of sCOD/nitrite ratio on denitrification (sCOD from leachate)*

Nitrate uptake rate was measured in the anoxic reactor experiments to verify the denitrification potential. The carbon source was used from filtered leachate to estimate for the experiment. The average initial sCOD/nitrite ratios were changed between 2.89 and 9.11 in AN1. The average initial sCOD/nitrate varied between 6.23 and 14.65 in AN2. The average initial nitrite and nitrate concentrations were around 50.77 ± 0.41 $mg.l^{-1}$ and 50.68 ± 0.69 $mg.l^{-1}$, respectively. MLVSS concentration was 1780 $mg.l^{-1}$ for every anoxic reactor. Examples of the changes of nitrite and nitrate concentration versus time at various sCOD/N ratios were presented in Figure 5.08.



(a) (b)

Figure 5.08. Example of changes nitrite (a) and (b) nitrate versus time at various sCOD/N ratios from leachate

The denitrification rate was determined from the slope of nitrite removal and is illustrated at Figure 5.08.

Table 5.12. Collected data from example of NUR_L at various sCOD/N from leachate

sCOD/N	**a** (Slope)	**b** (Intercept)	**R^2**	**SE of slope**
sCOD/nitrite				
4.02	-5.003	49.410	0.9853	0.27342
7.25	-7.044	49.669	0.9822	0.42351
8.91	-7.224	53.534	0.9920	0.28929
10.56	-8.330	52.339	0.9910	0.35456
11.92	-8.602	50.610	0.9904	0.37879
13.54	-8.743	52.947	0.9871	0.44742
sCOD/nitrate				
6.23	-5.534	50.751	0.9944	0.18546
7.23	-6.298	50.074	0.9873	0.31966
9.08	-6.678	50.336	0.9958	0.19321
10.96	-8.079	51.933	0.9847	0.45016
11.90	-8.651	51.688	0.9951	0.27211
14.50	-8.988	54.270	0.9845	0.50445

All R^2 values were above 0.98 in all sets of experiment (accepted > 0.95). Specific denitrification rates for nitrite ($qNUR1_L$) was determined based on nitrite response curve (nitrite reduction with time) and MLVSS concentration [60]. By using Eq. (5.23), the results were calculated as in Table 5.13 and shown in Figure 5.09.

Table 5.13. Effect of sCOD on denitrification for nitrite (leachate)

sCOD/nitrite	**MLVSS** $g.l^{-1}$	**$NUR1_L$** $mgNO_2^-\text{-}N.l^{-1}.h^{-1}$	**$qNUR1_L$** $mgNO_2^-\text{-}N.gVSS^{-1}.h^{-1}$
4.25 ± 0.28	1.575 ± 0.02	5.28 ± 0.29	3.35 ± 0.17
7.03 ± 0.20	1.448 ± 0.04	6.91 ± 0.18	4.77 ± 0.18
9.02 ± 0.09	1.316 ± 0.03	7.44 ± 0.19	5.65 ± 0.14
10.76 ± 0.19	1.351 ± 0.01	8.37 ± 0.09	6.19 ± 0.02
12.02 ± 0.11	1.325 ± 0.01	8.57 ± 0.05	6.47 ± 0.07
13.49 ± 0.24	1.311 ± 0.02	8.75 ± 0.10	6.68 ± 0.06

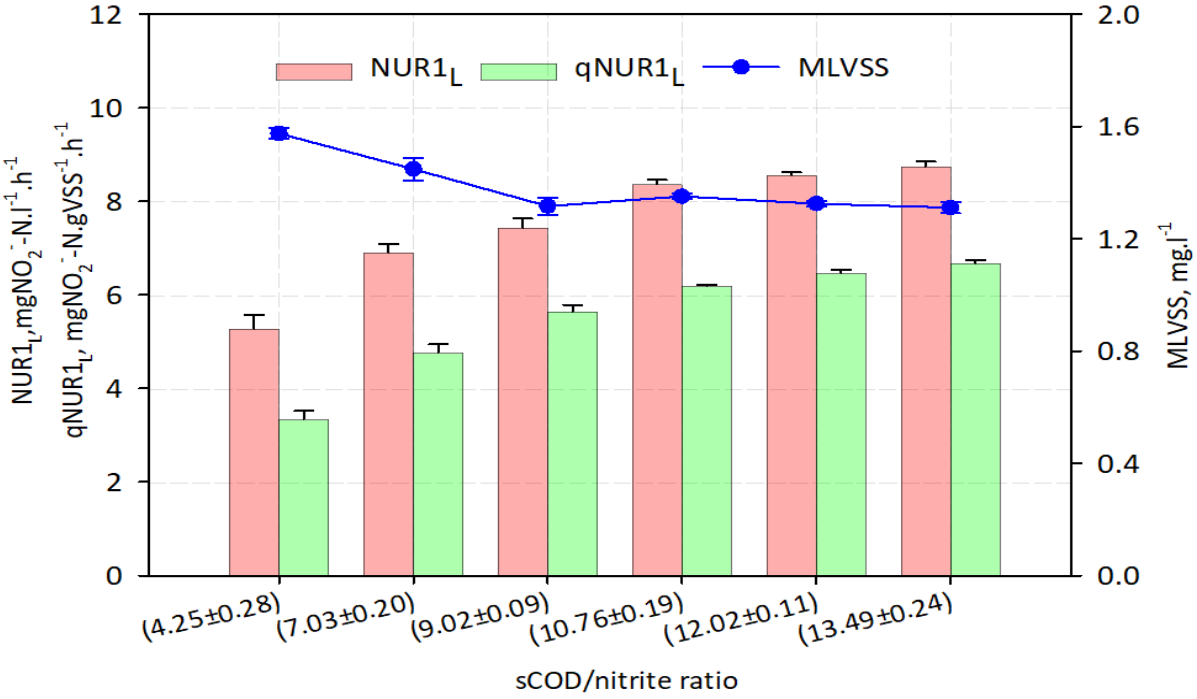


Figure 5.09. Effect of sCOD/nitrite ratio on denitrification (leachate)

Table 5.13 shows that it is important to point out that the effect of carbon source from leachate on denitrification process was similar at different sCOD/nitrite ratio. The $qNUR1_L$ with leachate increased slightly when the sCOD/nitrite ratio increased from 2.25 to 9.02. However, $qNUR1_L$ increased marginally when COD/nitrite ratio increased from 10.76 to 13.49.

In general, there were small changes of qNUR1 with changing sCOD/nitrite ratio. This can be explained by the composition of soluble organic in leachate. From results in Chapter 3 shown that mean ratio sbCOD in leachate accounted for 44.62 % of total COD, corresponding 82 % of sCOD. Thus in those conditions sbCOD is not the limiting factor of denitrification.

- *Effect of sCOD/nitrate ratio on denitrification (sCOD from leachate)*

In this study, under the condition of maintaining the nitrate nitrogen concentration invariable, by changing the concentration of organic matter in leachate to investigate the effect of different sCOD/nitrate ratio on nitrate uptake rate ($NUR2_L$) and specific denitrification rates for nitrate ($qNUR2_L$) was implemented. Using Eq. (5.23), the results were calculated as in Table 5.14 and Figure 5.10.

Table 5.14. Effect of sCOD on denitrification for nitrate (leachate)

sCOD/nitrate	MLVSS $g.l^{-1}$	$NUR2_L$ $mgNO_3^-\text{-}N.l^{-1}.h^{-1}$	$qNUR2_L$ $mgNO_3^-\text{-}N.gVSS^{-1}.h^{-1}$
6.23 ± 0.06	1.280 ± 0.01	5.47 ± 0.17	4.27 ± 0.17
7.26 ± 0.08	1.301 ± 0.02	5.98 ± 0.30	4.55 ± 0.20
8.85 ± 0.21	1.284 ± 0.03	6.74 ± 0.11	5.44 ± 0.28
11.02 ± 0.19	1.307 ± 0.04	7.98 ± 0.08	5.97 ± 0.24
12.20 ± 0.36	1.384 ± 0.04	8.57 ± 0.08	6.22 ± 0.13
14.65 ± 0.13	1.364 ± 0.01	8.92 ± 0.09	6.53 ± 0.07

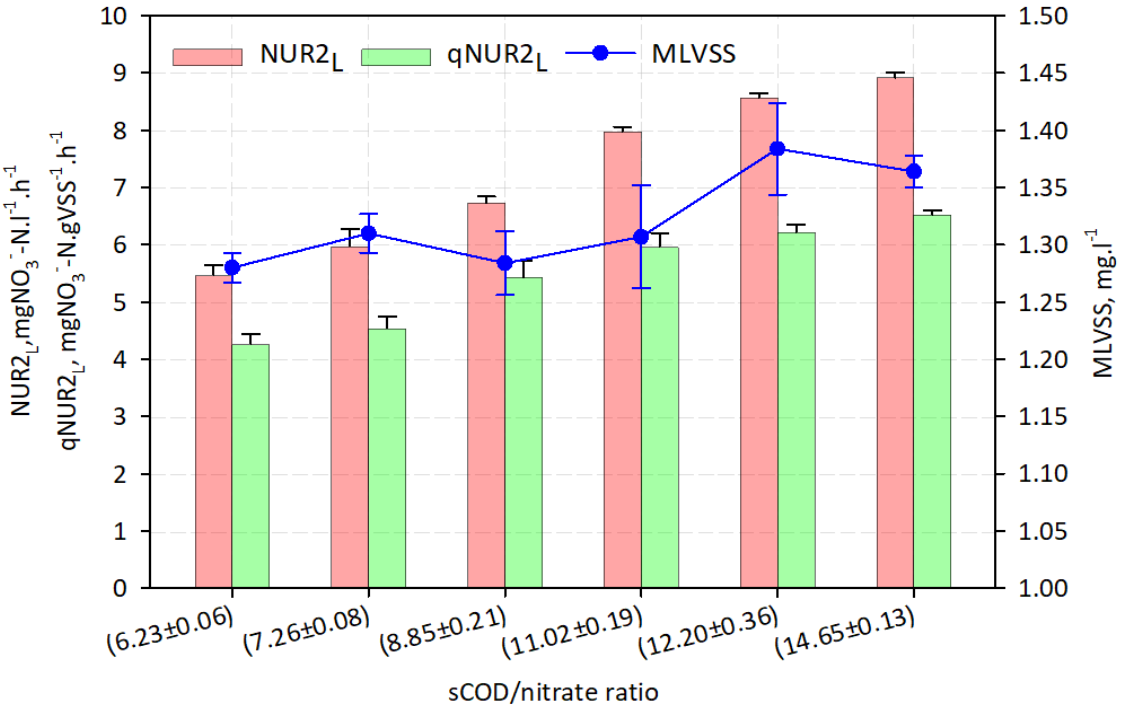


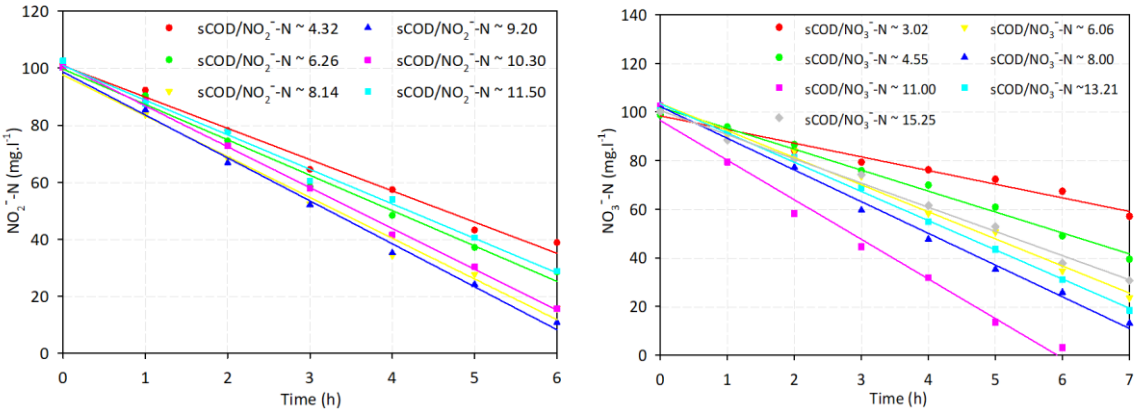
Figure 5.10. Effect of sCOD/nitrate ratio on denitrification (leachate)

From Table 5.14 the test results shown that the sCOD/nitrite ratio has important influence on NUR2 also qNUR2.

5.3.2.2. Effect of external sCOD (glucose) on denitrification

- *Effect of sCOD/nitrite ratio on denitrification (sCOD from glucose)*

In the denitrification tank test, nitrite and nitrate were added under anoxic conditions at the beginning of the experiment in order to determine the denitrification capacity of the sludge with an external carbon source. Different sCOD/nitrite and sCOD/nitrate ratios ranged from 4.32 to 11.15 and from 3.02 to 15.25, respectively. Examples of the experiment are shown in Figure 5.11 (a) and Figure 5.11 (b).



(a) (b)

Figure 5.11. Example of change of nitrite (a) and nitrate concentration (b) versus time at ratios of various sCOD/N from glucose

Table 5.15. Collected data of determine NUR with carbon source from glucose

sCOD/N	**a** (Slope)	**b** (Intercept)	**R^2**	**SE of slope**
sCOD/nitrite				
4.33	10.962	100.83	0.988	0.53868
6.39	12.414	99.7	0.9907	0.53806
8.40	14.301	97.575	0.9878	0.71018
9.30	15.064	98.654	0.9958	0.43945
10.45	14.333	101.13	0.9984	0.25108
11.57	12.118	100.89	0.9940	0.41894
sCOD/nitrate				
3.15	5.624	98.44	0.9752	0.36621
4.62	8.622	101.96	0.9905	0.34472
5.93	11.156	1103.55	0.9912	0.42787
7.8	13.050	102.25	0.9948	0.38386
11.13	16.303	96.49	0.9867	0.84448
13.39	12.022	103.38	0.9975	0.24500
15.30	9.956	100.63	0.9923	0.35786

In order to achieve a total denitrification very often other external carbon sources than glucose are used. In this study, mean nitrite concentration of 101 $mg{\cdot}L^{-1}$ was applied by adding glucose, sCOD/nitrite was adjusted from 4.32 to 11.50. Under different sCOD/nitrate conditions, the effect on denitrification

were studied. The experiment was also implemented with nitrite. Results are calculated and presented in Table 5.16 and Figure 5.12.

Table 5.16. Effect of sCOD on denitrification for nitrite (glucose)

sCOD/nitrite	**MLVSS** $g.l^{-1}$	**$NUR1_G$** $mgNO_2^-\text{-}N.l^{-1}.h^{-1}$	**$qNUR1_G$** $mgNO_2^-\text{-}N.gVSS^{-1}.h^{-1}$
4.32 ± 0.12	1.664 ± 0.03	10.35 ± 0.75	6.21 ± 0.36
6.26 ± 0.15	1.694 ± 0.01	12.37 ± 0.47	7.30 ± 0.26
8.18 ± 0.19	1.690 ± 0.02	14.22 ± 0.35	8.41 ± 0.13
9.20 ± 0.14	1.682 ± 0.04	15.64 ± 0.86	9.33 ± 0.31
10.30 ± 0.14	1.697 ± 0.02	14.63 ± 0.43	8.62 ± 0.15
11.50 ± 0.16	1.683 ± 0.03	12.08 ± 0.04	7.18 ± 0.10

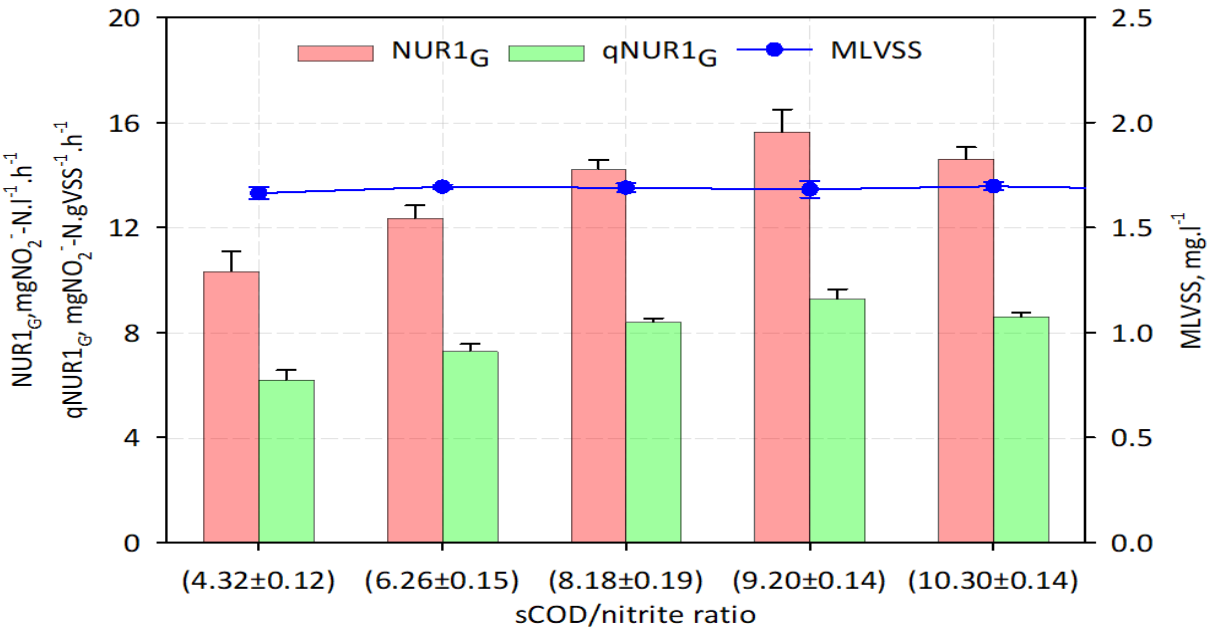


Figure 5.12. Effect of sCOD/nitrite ratio on denitrification (glucose)

As shown in Table 5.16 and Figure 5.12, $qNUR1_G$ achieved at high value at low ratio of sCOD/nitrite (10.35 $mgNO_2^-\text{-}N.l^{-1}.h^{-1}$). There was a small increase of $NUR1_G$ with the increase sCOD/nitrite. The $NUR1_G$ value was highest at a 9.20 ratio of sCOD/nitrite yielding 15.64$mgNO_2^-\text{-}N.l^{-1}.h^{-1}$. However, when sCOD/nitrite increased, $NUR1_G$ and $qNUR_G$ declined due to effecting of activity of heterotrophic bacteria.

From Table 5.16 indicated that when the sCOD/nitrate ratio increased from 4.32 to 9.20, the rate of denitrification increased and the rate of specific "N" consumption also increased. This may be explained due to carbon source was

supplied enough for heterotrophic denitrifying bacteria. However, if sCOD/nitrite ratio continued to rise, the rate of denitrification as well as specific denitrification rate decreased. This can explain that when sCOD concentration is too high, it stimulates heterotrophic microorganisms to decompose carbon. There are a change predominant bacteria population or environmental condition. For example, organic matter is decomposed under anaerobic conditions which leads to reducing the carbon source for denitrifying bacteria.

- *Effect of sCOD/nitrate ratio on denitrification (sCOD from glucose)*

The initial nitrate concentration was adjusted to be 100.54 ± 0.19 $mg.l^{-1}$ by adding nitrate solution. The MLVSS was maintained at 1.796 ± 0.03 $g.l^{-1}$. The nitrate concentration was monitored during the batch reaction process. The effect of sCOD/nitrate ratio on denitrification for nitrate by using glucose as carbon source were calculated as in Table 5.17 and Figure 5.13.

Table 5.17. Effect COD/Nitrate ratio on denitrification for nitrate (glucose)

sCOD/nitrate	**MLVSS** $g.l^{-1}$	**$NUR2_G$** $mgNO_3^-\text{-}N.l^{-1}.h^{-1}$	**$qNUR2_G$** $mgNO_3^-\text{-}N.gVSS^{-1}.h^{-1}$
3.02 ± 0.12	1.775 ± 0.01	4.54 ± 0.94	2.56 ± 0.54
4.55 ± 0.19	1.764 ± 0.05	8.15 ± 0.55	4.62 ± 0.18
6.06 ± 0.13	1.785 ± 0.04	12.12 ± 0.89	6.78 ± 0.36
8.00 ± 0.20	1.793 ± 0.02	13.53 ± 0.42	7.55 ± 0.16
11.00 ± 0.18	1.824 ± 0.01	16.40 ± 0.17	8.99 ± 0.04
13.21 ± 0.34	1.850 ± 0.03	11.53 ± 0.43	6.29 ± 0.23
15.25 ± 0.24	1.783 ± 0.04	9.77 ± 0.28	5.48 ± 0.16

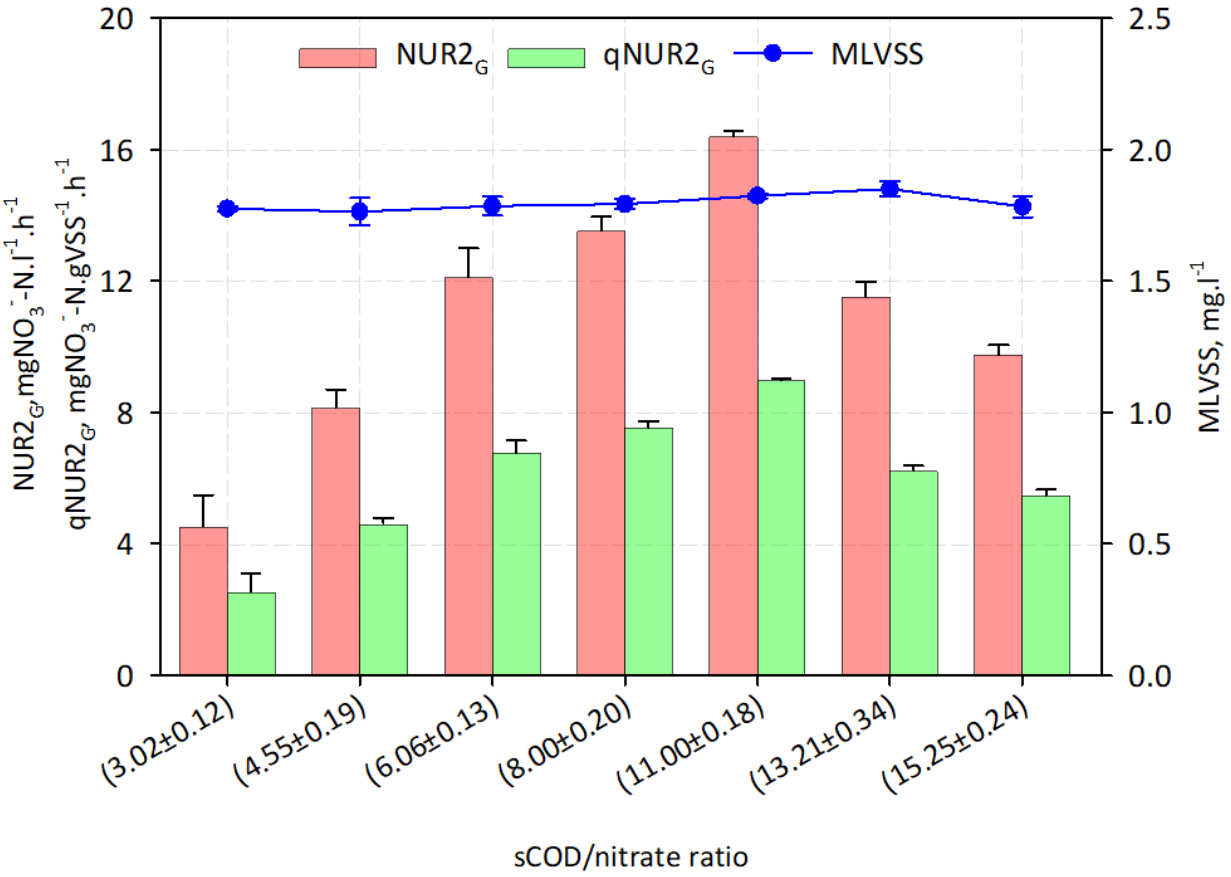


Figure 5.13. Effect of sCOD/nitrate ratio on denitrification (glucose)

With the increase of COD/nitrate, the removal rate of nitrate gradually increased and shown in Table 5.17. When sCOD/nitrate was adjusted to 3.02, the specific denitrification rates of nitrate was the lowest because the carbon source required by microorganisms was insufficient to convert nitrate to nitrite. Specific denitrification rate at this ratio was only 2.56 mg NO_3^--N.$gVSS^{-1}$.h^{-1}. When sCOD/nitrate was 4.55 the specific denitrification rate of nitrate was obviously improved and achieved 4.62 mg NO_3^--N.$gVSS^{-1}$.h^{-1}. Because the COD concentration in the reactor was higher, the conversion rate of nitrate to nitrite increased. When sCOD/nitrate increased, the carbon source in the system was sufficient, so the specific denitrification rates increased and was further increased to sCOD/nitrate of 11.00, the specific denitrification rates climbed to 8.99 mg NO_3^--N.$gVSS^{-1}$.h^{-1}

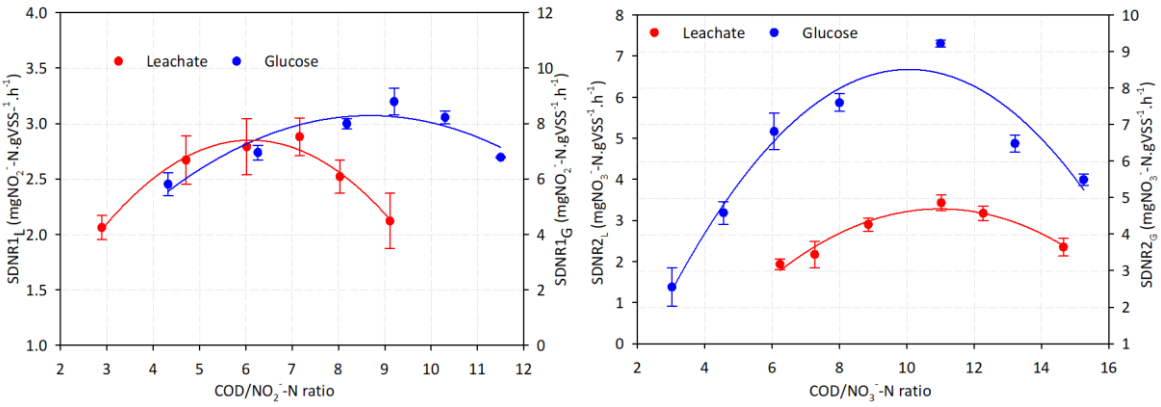
However, if sCOD/nitrate continued to rise over 11.00, specific denitrification rates reduced markedly until 6.29 mg NO_3^--N.$gVSS^{-1}$.h^{-1} at 13.21 ratio and 5.48 mg NO_3^--N.$gVSS^{-1}$.h^{-1} at 15.25 ratio.

A higher organic carbon concentration can accelerate the activity of heterotrophic bacteria that utilize oxygen for COD oxidation. For this reason,

high initial COD concentration may result in oxygen depletion in activated sludge that supports nitrogen removal. However, when this ratio is increased higher, the rate of denitrification decreased significantly. The negative effect of high sCOD/nitrate ratio on qNUR2 was reported in very few studies. Ge *et al*. [61] conducted the tests at high substrate concentrations, the decrease in qNUR2 at high sCOD/N ratios could be attributed to organic substrate inhibition. Meanwhile, Badia *et al*., [62] reported a decrease in qNUR2 with glucose from 0.64 mg NO_3^--N.gVSS^{-1}.d^{-1} at COD/N of 15 to 0.04 mg NO_3^--N.gVSS^{-1}.d^{-1} at COD/N of 25. Kuba *et al*. [63] indicated that, denitrification process needs external sCOD to remove nitrate with an ratio of influent sCOD/N lower 3.4 gCOD.g N^{-1}.

Theoretically, it has been shown that, under anoxic conditions and with a biodegradable organic substrate present in the wastewater, COD needs are 2.86 mg to reduce 1 mg of N-nitrate but the value measured in this study is higher. Katarzyna *et al*. [64] also indicated that for the reduction of 1 g of NO_x^--N an average amount of 3.73 g COD. Besides, denitrification rates depend on carbon sources indicated by Lee *et al*. [65]. Cherchi *et al*. [66] reported that the maximum specific denitrification rates obtained with methanol was higher denitrification rate with acetate.

As shown in Figure 5.14, the qNURs values obtained with glucose as carbon source were higher than those with landfill leachate. The results indicated that support of carbon from glucose leads to higher carbon in leachate in denitrification processbecause soluble carbon from leachate contains an inert or slowly biodegradable carbon part.



(a) (b)

Figure 5.14. Effect of initial sCOD to qNURs for nitrite (a) and nitrate (b)

Besides, denitrification of nitrites requires a lower amount of organic compounds for the reduction of each gram of nitrites in comparison with

denitrification. It is considered that specific consumption rate of denitrification process is not only associated to the concentration of substrate, but is also associated to the type of substrate. With similar trend as found by Badia *et al*., [62] who investigated effect of sCOD/N ratio from municipal wastewater and acetate on nitrite denitrification. According to these authors, there was a difference between two kinds of substrates due to higher and faster biodegradability of acetate, meanwhile, organics for municipal wastewater are typically a complex mix of carbohydrates, proteins, lipids, and VFA. Swinarski *et al*. [66] found that there were an different enhance on denitrification process between external carbon sources. The characteristics of the carbon source had a significant influence on the denitrification process as also been demonstrated by Lee *et al*. [64].

5.4. CONCLUSIONS

Partial nitrification is sensitive to operational conditions, such as temperature, pH, alkanility and DO levels, ect. Results in this study show that the partial nitrification process is influenced by the following factors:

- High DO concentrations could enhance the ammonia oxidation rate significantly but ammonia is converted to nitrate completely, leading to a decrease in nitrite built up. In this study, DO concentration yielded high NPR1 from 1.0 to 1.5. $mgO_2.l^{-1}$, coresponding to 16.16 and 17.01 mg NO_2^--N.$gVSS^{-1}.h^{-1}$, respectively.
- Another important parameter for partial nitrification is the alkalinity/ammonia ratio. Alkalinity is effective for controlling partial nitrification by adjusting alkalinity/ammonia ratio around 6.32 (insufficient alkalinity to implement completely the nitrification process).
- The optimal pH value was found in the range from 7.15 to 8.22 with NAR of 75.1%.
- Using carbon source from leachate for denitrification showed that ratio of sCOD/nitrite and sCOD/nitrate of 13.49 and 14.65, respectively achieved the highest value of qNUR. With glucose as carbon source $qNUR1_G$ and $qNUR2_G$ reached the highest at sCOD/nitrite and sCOD/nitrate ratio, corresponding to 9.2 and 11, respectively, coresponding to 9.33 mg NO_2^--N.$gVSS^{-1}.h^{-1}$ and 8.99 mg NO_3^--N.$gVSS^{-1}.h^{-1}$.

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CHAPTER 6: NITROGEN REMOVAL IN LANDFILL LEACHATE TREATMENT WITH MEMBRANE BIOREACTOR IN VIETNAM

6.1. INTRODUCTION

Biological methods to remove nitrogen in wastewater is now common practice [1]. The nitrogen compounds are removed by combining two processes: nitrification and denitrification [2]. Actually, nitrogen removal from wastewater by combining two steps of nitrification and denitrification is necessary [3]. Nitrification and denitrification processes are very contrasting ones as the type of bacteria and required conditions are very different.

Nitrification transforms ammonia into an oxidized nitrogen compound such as nitrite or nitrate by two different groups of microorganisms that are AOB and NOB. The denitrification process in which organic compounds are used as electron donors, is the most common form of denitrification [4].

Nitrification step

Biological nitrogen removal in wastewater treatment process is carried out by microbial nitrification. Nitrification is the biological process where ammonium (ammonia) serves as a substrate for nitrifying bacteria that oxidize ammonium to nitrite then nitrate in two steps [5-8]. Both groups of bacteria are autotrophic, utilizing carbon dioxide as the carbon source for biosynthetic processes and oxidation of reduced nitrogen compounds as energy source [8].

Potential factors affecting the nitrification are the dissolved oxygen concentration, solid retention time, pH and presence of organic matters that are degraded by heterotrophic organisms competitively consuming oxygen and toxic compounds. Conversion of ammonia to N_2O can reach as high as 8 percent of conversion when DO is lower than 1 $mgO_2.l^{-1}$ [3, 9].

$$NH_4^+ \longrightarrow NO_2^- \longrightarrow N_2O \qquad (6.1)$$

Some of the ammonium ions are used as nutrient source and are assimilated into new cellular material. The growth of new cells in the activated sludge process is referred to as an increase in the mixed liquor volatile suspended solids [7, 9, 10]:

$$4CO_2 + HCO_3^- + NH_4^+ + 4H_2O \longrightarrow C_5H_7O_2N + 5O_2 + 3H_2O \qquad (6.2)$$

Denitrification step

Low COD/NO_3^--N ratio in the wastewater, short SRT and low level of pH promoted the N_2O production by denitrifying activated sludge [11, 12]. Among these parameters, COD/NO_3^--N is a significantly important factor that governs

completeness of denitrification. Conversion from nitrate to N_2O can be higher than 10 percent at short SRT and low pH value [3, 9].

$$NO_3^- \longrightarrow NO_2^- \longrightarrow N_2O \quad (6.3)$$

The overall biochemical pathway for denitrification from nitrate to dinitrogen gas involves steps: nitrate reductase, nitrite reductase, nitric oxide reductase and nitrous oxide reductase [13-15].

$$NO_3^- \longrightarrow NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \quad (6.4)$$

Nitric oxide and nitrous oxide were identified as intermediates, so the denitrification process can be represented as a two-step (neglecting synthesis) [13]. Biological nitrogen removal has been widely adopted in preference to physicochemical processes because of its higher effectiveness and relative cheapness [16, 17]. Biological nutrient removal from wastewater may be performed by adopting various process configurations. One of them that has recently demonstrated a significant potential for biological nutrient removal is MBR. The MBR can separate sludge from effluent instead of a clarification tank, due to the combination of an activated sludge process with a membrane filtration. In MBR, the important steps can occur concurrently in the same reactor that has been termed simultaneous nitrification and denitrification. MBR process has recently been developed for wastewater treatment [18-20]. MBR can be operated at very long sludge ages and applied for wastewater with high concentrations of pollutants , such as leachate [21]. Nitrogen removal from wastewater using a membrane aeration bioreactor is a commonly adopted technology. Biofilm systems have several advantages, such as a very large surface area for biofilm attachment, the protection of microorganisms in the biofilm against unfavorable surroundings and the achievement of very high sludge age, which is important for nitrifying bacteria [22].

Until now, most of successful SND have been obtained in sequencing operation process, but a few studies were achieved in continuous-flow processes [23]. SND implies that nitrification and denitrification occur concurrently in the same reaction vessel under identical overall operating conditions. SND can ensured that a considerable amount of denitrification takes place together with nitrification in aerated tank.

The objective of this study is to evaluate the performance of a pilot-scale AN1/AE/AN2/MBR system for landfill leachate treatment, focusing on the effects of dissolved oxygen, sCOD/N ratios, and aeration regimes on nitrification and denitrification processes, in order to optimize nitrogen removal efficiency.

6.2. MATERIALS AND METHODS

6.2.1. Experimental set-up

The pilot for this part of the study consists of four units: an AN1, AE, AN2 and MBR compartments.

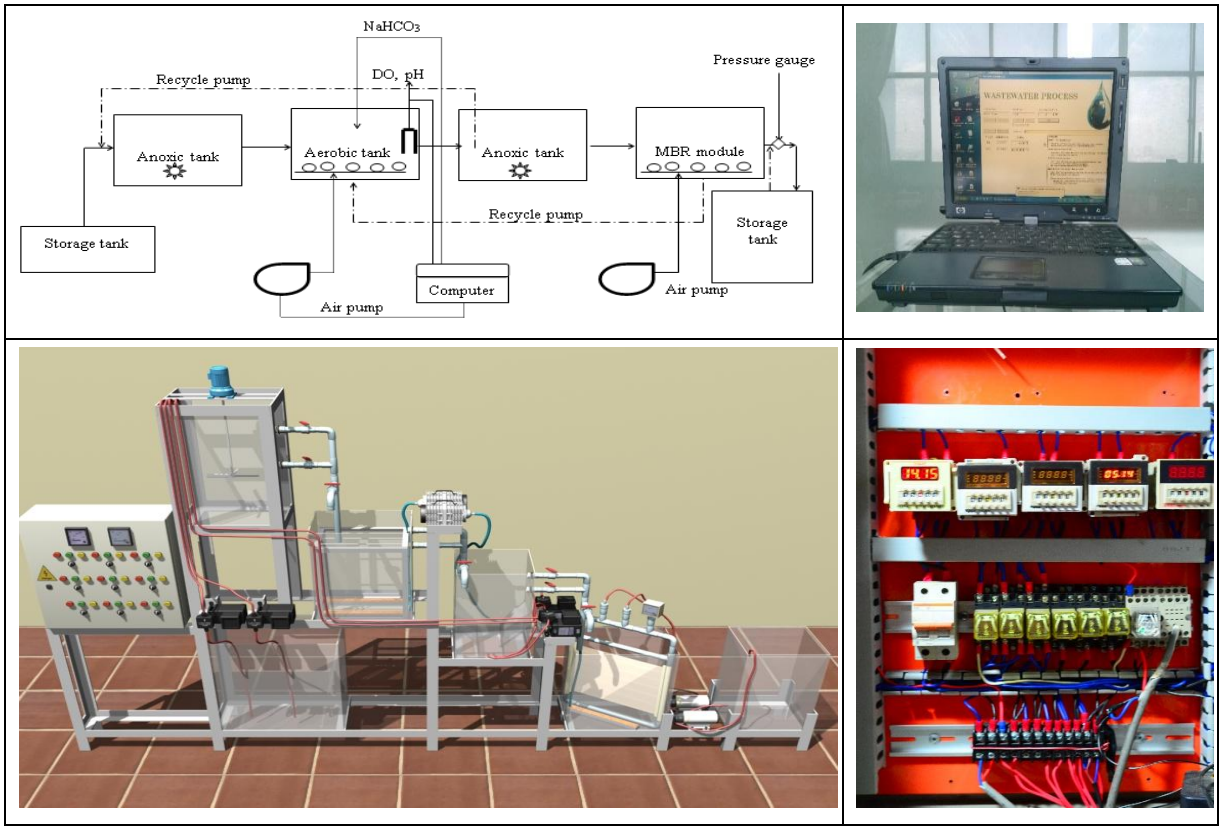

Figure 6.01. The schematic diagram of experimental set up

Fig 6.01 shows the schematic diagram of the experimental setup design consisting of a system including an AN1, AE, AN2 and a MBR. The characteristics of the MBR are shown in Table 6.01.

Table 6.01. MBR parameters

Property	Unit	Description	Property	Unit	Description
Material	-	Polyvinylidene fluoride (PVDF)	Operating temperature	°C	5 – 45
Membrane configuration	-	Hollow fiber	pH range	-	2 – 10
Pore size	µm	0.2	Designed flux	l/m^2.h	10 – 20
Membrane surface area	m^2	1	Maximum TMP[26]	KPa	50
Manufacturer	-	Motimo – China	Suggest TMP	KPa	< 40

[26] TMP: transmembrane pressure

Anoxic tank was provided only with a submerged mixer. The aeration is supplied continuously from the bottom of the AE and MBR to provide oxygen for microbial activities, including organic carbon oxidation and nitrification. Meanwhile, strong fluid turbulence brought by aeration induces membrane cleaning. Biological denitrification is achieved in AN1, which received both the raw influent and a recycled flow containing NO_x^--N (nitrite and nitrate) for denitrification from AN2. The sludge is returned to maintain the appropriate concentration of biomass in the reactors. All reactors were operated at 31 ± 3 °C. In AE or MBR DO and pH electrode were installed and these probes were connected to a computer to control dissolved oxygen and pH. The working volumes and parameters of the system operation are shown in Table 6.02.

Table 6.02. Parameters of system operation

Parameters	Units	AN1	AE	AN2	MBR
Volume	l	40	40	28	55
Influent	$l.h^{-1}$	2.5 ± 0.2.6			
Return flow	$l.h^{-1}$	5 ± 0.32	15 ± 1.3	-	-
Average flow total	$l\ l.h^{-1}$	7.5	22.5	22.5	22.5

6.2.2. Landfill leachate and inoculum

6.2.2.1. Landfill leachate

Leachate was collected at Phuoc Hiep landfill site, located in the North East Solid Waste Treatment Complex, Phuoc Hiep Commune, Cu Chi District, about 37 km distance from the center of HCM City. Phuoc Hiep landfill covers 50 ha and has been operated since 2003 [24]. It is well-known that leachate characteristics are important in reflecting biodegradation properties and can vary drastically with time. Therefore, the leachate was monitored to observe components and characteristics. The characteristics of leachate in this study are described in Table 6.03.

Table 6.03. Characteristics of the landfill leachate during this study

Parameters	Units	Mean ± SD	Parameters	Units	Mean ± SD
Temperature	°C	31.6 ± 1.3	NH_4^+-N	$mg.l^{-1}$	1435 ± 681
pH	-	7.83 ± 0.3	NO_2^--N	$mg.l^{-1}$	2.5 ± 0.7
Alkalinity	$mgCaCO_3.l^{-1}$	7639 ± 527	NO_3^--N	$mg.l^{-1}$	1.1 ± 0.6
Hardness	$mgCaCO_3.l^{-1}$	2815 ± 906	TNK	$mg.l^{-1}$	1016 ± 384
COD	$mgO_2.l^{-1}$	3518 ± 1016	TN	$mg.l^{-1}$	1962 ± 454

Parameters	Units	Mean ± SD	Parameters	Units	Mean ± SD
BOD_5	$mgO_2.l^{-1}$	2692 ± 417	Ca^{2+}	$mg.l^{-1}$	916 ± 262
SS	$mg.l^{-1}$	1414 ± 463	Cl^-	$mg.l^{-1}$	2078 ± 311
VSS	$mg.l^{-1}$	826 ± 389	TP	$mg.l^{-1}$	54.5 ± 16.4

6.2.2.2. Inoculum

The activated sludge to seed the pilot was collected at SBR tank of landfill leachate treatment plant in Binh Duong, Vietnam. The MLSS and MLVSS concentration in activated sludge were 4102 and 2932 $mg.l^{-1}$, respectively.

6.2.3. Reactor operating conditions

At the beginning of the operation, fresh activated sludge was diluted with wastewater and tap water to obtain mixed liquor. The activated sludge was settled, then diluted with leachate. The actual MLSS and MLVSS concentration were measured at the beginning of the operation approximately 3110 $mg.l^{-1}$ and 2148 $mg.l^{-1}$.

The influent was continuously fed into the AN1, AE, AN2 compartments and then flow to the MBR. The permeate was continuously extracted by a pump. Hydraulic retention time in the system was kept as 2.72 days. The influent flow rate was fixed to 2.5 liters per hour per square meter of membrane ($l.h^{-1}.m^{-2}$). In this study, the membrane suction pressure was found to increase slowly, therefore, it was not necessary to clean it chemically during operation. Biological denitrification was achieved in the AN1 compartment, which received both the raw influent, AN2 and MBR.

The active sludge and wastewater were returned from AN2 to AN1 (returned flows was twice the influent) for and from MBR to AE (returned flows was six times the influent flowrate). These recycled flows ensure the recirculation of nitrite, nitrate and the homogenization of mixed liquor micro-organism concentration in the system. A high ratio of activated sludge returned for purpose enough mass to convert ammonia to AE and remove nitrite and nitrate in AN1 and AN2. The pilot was operated with 2 periods.

In the first period, the pilot scale experimental investigation was run for approximately 290 days to remove nitrogen from leachate. The continuous operation periods were divided into seven phases with different influent NH_4^+-N concentrations. The DO in the reactor was changed and controlled with auto-DO meter. The pH was controlled by the auto-pH controller to keep values between 8.0 and 8.5 by using $NaHCO_3$. Alkalinity and ammonium in AE were collected and measured regularly to maintain ratio alkalinity/NH_4^+-N

($mgCaCO_3.l^{-1}$/$mg.l^{-1}$) from 7.1 to 8.36. The system was operated at the room's temperature (range 26 – 33°C) without wasting biomass. Yellow sugar was added to AN2 and MBR in order to stimulate the denitrification process:

- *The fist experimental phase* (1^{st} – 31^{st} day) was necessary for the start-up of the pilot. DO in AE was maintained 1.5 – 2.5 $mgO_2.l^{-1}$.
- *The second experimental phase* (32^{nd} – 70^{th} day) was divided into three runs and dissolved oxygen concentration in AE was changed as presented in Table 6.04 in order to study the effect of DO on the nitrification process in aeration tank while DO in MBR was maintained at 1.5 - 2 $mgO_2.l^{-1}$.

Table 6.04. The periods of second experimental phase

Run	R (2-1)	R (2-2)	R (2-3)
Day	32^{nd} – 45^{th}	46^{th} – 58^{th}	59^{th} – 70^{th}
DO in AE, $mgO_2.l^{-1}$	0.9 ± 0.3	1.8 ± 0.2	2.6 ± 0.1

- *The third experimental phase* (71^{st} – 113^{th} day), was divided in three runs where dissolved oxygen concentration in MBR was changed in Table 6.05 in order to study the effect of DO to nitrification process in MBR. Meanwhile, DO concentration in AE was maintained 1.5 – 2.0 $mgO_2.l^{-1}$.

Table 6.05. The periods of third experimental phase

Run	R (3-1)	R (3-2)	R (3-3)
Day	71^{st} – 87^{th}	88^{th} – 102^{nd}	103^{rd} – 113^{th}
DO in MBR, $mgO_2.l^{-1}$	1.1 ± 0.2	2.2 ± 0.1	2.9 ± 0.4

- *The fourth experimental phase* (114^{th} – 178^{th} day), was designed to compare the effect of $sCOD/NO_x^-$-N ratio on nitrogen removal. In this study, yellow sugar was added to AN2 tank to boost the denitrification starting on 114^{th} day to increase the $sCOD/NO_x^-$-N ratio. Samples were collected and analyzed sCOD, BOD_5, NO_2^--N and NO_3^--N. $sCOD/NO_x^-$-N ratio was calculated from sCOD and total nitrite and nitrate in AN2. DO concentrations in AE and MBR were from 1.5 to 2 $mgO_2.l^{-1}$ and from 2.5 to 3.0 $mgO_2.l^{-1}$, respectively. The operation condition in this phase is presented in Table 6.06.

Table 6.06. The periods of fourth experimental phase

Run	R (4-1)	R (4-2)	R (4-3)	R (4-4)
Day	114^{th} – 130^{th}	131^{st} – 142^{nd}	143 - 160	161^{st} – 178^{th}
$sCOD/NO_x^-$-N (AN2)	4.2	6.7	7.8	10.5
BOD_5/NO_x^--N (AN2)	2.5	3.8	5.5	6.4

- *The fifth experimental phase* (179^{th} – 225^{th} day), was designed to compare the effect of sCOD/NO_x^--N ratio on nutrient removal in MBR. In the fifth phase, yellow sugar was added into MBR to boost denitrification. The other parameters were operated based on the highest results from study. sCOD/NO_x^--N ratio was calculated from sCOD and total nitrite and nitrate in MBR. The sCOD/NO_x^--N ratio is presented in Table 6.07.

Table 6.07. The periods of fifth experimental phase

Run	R (5-1)	R (5-2)	R (5-3)
Day	179^{th} – 199^{th}	200^{th} – 214^{th}	215^{th} - 225^{th}
sCOD/NO_x^--N (MBR)	14.2	16.4	18.6
BOD_5/NO_x^--N (MBR)	3.4	6.2	8.5

- *The sixth experimental phase*, (226^{th} – 257^{th} day): changed the time of non-aeration time and maintained aeration time at MBR to achieve simultaneous denitrification presented in Table 6.08. In these periods, DO in AE and MBR were from 1.5 to 2 $mgO_2.l^{-1}$ and from 2.5 to 3.0 $mgO_2.l^{-1}$, respectively. Yellow sugar was used to boost denitrification in AN2 and without adding external carbon source for MBR.

Table 6.08. The periods of sixth experimental phase

Run	R (6-1)	R (6-2)	R (6-3)
Day	226^{th} – 236^{th}	237^{th} – 247^{th}	248^{th} – 257^{th}
Aeration/non-aeration (minutes) in MBR	180 – 30	180 – 60	180 – 90

- *The seventh experimental phase*, (258^{th} – 290^{th} day), changed the time of aeration time but unchanged non-aeration time in MBR as presented in Table 6.09. The other operating parameters were the same as in the sixth period.

Table 6.09. The periods of seventh experimental phase

Run	R (7-1)	R (7-2)	R (7-3)
Day	258^{th} – 268^{th}	269^{th} – 279^{th}	280^{th} – 290^{th}
Aeration/non-aeration (minutes) in MBR	60 – 60	120 – 60	150 – 60

The experiment duration was determined depending on the results achieved in previous tests.

In the second section, the pilot was operated during 160 days based on results of the first section. In this experiment there was no sludge discharged from MBR, as well as for the whole system. In this section, the DO in AE is maintained between 1.5 and 2 $mgO_2.l^{-1}$. While DO in MBR was 2.5 – 3 $mgO_2.l^{-1}$. Yellow sugar was added and maintained sCOD/NO_x^--N ratio of 11.16 ± 1.39 in AN2 to boost denitrification process. No sugar was used for denitrification during this period in MBR. The aeration and non-aeration time are 120 and 60 min, respectively. pH was the same as in the first section but temperature was done at room temperature (range from 22 to 29°C). The experiment in this section was divided four ORL of COD total, including:

- ORL_1: 0.250 ± 0.032 $g.l^{-1}.d^{-1}$ (COD of 675.9 ± 85.4 $mgO_2.l^{-1}$).
- ORL_2: 0.334 ± 0.062 $g.l^{-1}.d^{-1}$ (COD of 902.3 ± 168.1 $mgO_2.l^{-1}$).
- ORL_3: 0.470 ± 0.080 $g.l^{-1}.d^{-1}$ (COD of 1268.6 ± 216.7 $mgO_2.l^{-1}$).
- ORL_4: 0.698 ± 0.080 $g.l^{-1}.d^{-1}$ (COD of 1883.8 ± 217.3 $mgO_2.l^{-1}$).

6.2.4. Analytical methods

For leachate, analytical methods were done according to "Standard methods for examination of water and wastewater" [25]. The samples for the determination of soluble components were immediately filtered by using Whatman GF/C (0.45µm) glass fiber filters. Analytical methods are presented in Table 6.10.

Table 6.10. Analytical methods used during the study

Parameters	Analytical methods
Temperature	2550B. Laboratory and Field Method
pH	4500 - H^+ .B Electrometric Method (pH meter - Hanna 211)
EC	2510 B. Laboratory Method (EC Hanna – Hi98303)
Alkalinity	2320 B. Titration Method
TSS	2540 B. Total Solids Dried at 103–105 °C
VSS	2540 E. Fixed and Volatile Solids Ignited at 550 °C
COD	5220 C. Closed Reflux, Titrimetric Method
BOD	5210 B. 5-Day BOD test (BOD Track II)
NH_4^+-N	4500 - NH_3 D. Ammonia-Selective Electrode Method
NO_2^--N	4500 - NO_2^- B. Colorimetric Method
NO_3^--N	4500 - NO_3^- B. Ultraviolet Spectrophotometric Screening Method

Parameters	Analytical methods
Kjeldahl-N	4500 - N C. Semi-Micro-Kjeldahl
T-N	4500 - N-org B. Macro Kjeldahl Method
Hardness	2340 C. EDTA Titrimetric Method
Ca^{2+}	2340 C. EDTA Titrimetric Method
Cl^-	4500 - Cl^- B. Argentometric Method
SO_4^{2-}	4500 - SO_4^{2-} E. Turbidimetric Method
T-P	4500 - P. D Stannous Chloride Method
Fe	3500 - Fe. Phenanthroline Method

To identify microorganisms, several techniques have been applied for identification of the main bacterial population isolated from activated sludge. In this study, the test was done at industrial university of Ho Chi Minh City, combining various methods to identify microorganism, including Plate Count Agar, isolation (bacteria, yeast, fungi, actinomycetes), Gram stain [26]. Some samples with strains appearing frequently, were determined by gene sequencing at Nam Khoa Biotek Company in Ho Chi Minh City, Vietnam. Then, the results were adjusted by FINCHTV and SEEVIEW bioinformatic softwares, followed the sequences of the species specific primers were submitted to the BLAST search program of the NCBI (National Center for Biotechnology Information). Finally, CLUSTAL W software from the European Bioinformatics Institute (Welcome Trust Genome Campus, Hinxton, United Kingdom)[27] and MEGA6 were used for the analysis, comparison of sequences, and phylogenetic tree establishment. The other samples, having microorganism strain appearing rarely and were almost all positive Gram microorganism, were tested for citrate utilization, indole acetic acid, catalase activity, hydro sulfur production, fermentation sugar activity, movement of bacteria, ammonization, nitrification, and denitrification. From tested results, Bergey's manual making classification tree was used to identify microorganism [27].

SigmaPlot software 14.0 was used for drawing analyses of variance (t-test). A 95% significance level ($p < 0.05$) was selected for data analysis.

[27] http://www.genome.jp/tools/clustalw/

6.3. RESULTS AND DISCUSSION

6.3.1. The effects on nitrification and denitrification process

In landfill leachate treatment processes, nitrogen can be removed in two ways: (1) assimilation into biomass (2) biological nitrification under AE conditions and denitrification process under depleted oxygen levels or anoxic conditions [28, 29]. Besides, during metabolism under aerated conditions, some mineral nitrogen is consummed [30]. Therefore, the effects of DO, COD and aeration time on nitrogen removal efficiency were considered in this study.

6.3.1.1. Effect of dissolved oxygen on nitrification process in AE and denitrification system

The second phase was operated under various DO conditions in AE. This operation was to encourage simultaneous nitrification. As shown in Table 6.11 and Figure 6.02, by increasing average DO from 0.9 ± 0.3 $mgO_2.l^{-1}$ to 2.6 ± 0.1 $mgO_2.l^{-1}$, the response was increased in the system with the increasing trend for nitrification but decreasing trend for total nitrogen removal efficiency at R2-3 (2.6 ± 0.1 $mgO_2.l^{-1}$).

Table 6.11. Ammonium and nitrogen removal efficiencies in AE

Run	**R2-1**	**R2-2**	**R2-3**
$mgO_2.l^{-1}$	(0.9 ± 0.3)	(1.8 ± 0.2)	(2.6 ± 0.1)
Ammonium (AE)			
Influent; $mg.l^{-1}$	149.07 ± 45.65	134.45 ± 58.09	123.13 ± 18.93
Effluent; $mg.l^{-1}$	90.66 ± 21.18	61.46 ± 20.08	57.72 ± 13.05
Efficiency; %	37.02 ± 11.58	49.31 ± 19.27	52.85 ± 9.97
Nitrogen Total (AE)			
Influent; $mg.l^{-1}$	182.49 ± 52.66	190.53 ±55.92	172.83 ± 25.81
Effluent; $mg.l^{-1}$	105.58 ± 18.45	95.04 ± 19.97	125.49 ± 27.66
Efficiency; %	38.75 ± 15.73	46.41 ± 18.79	27.30 ± 11.21

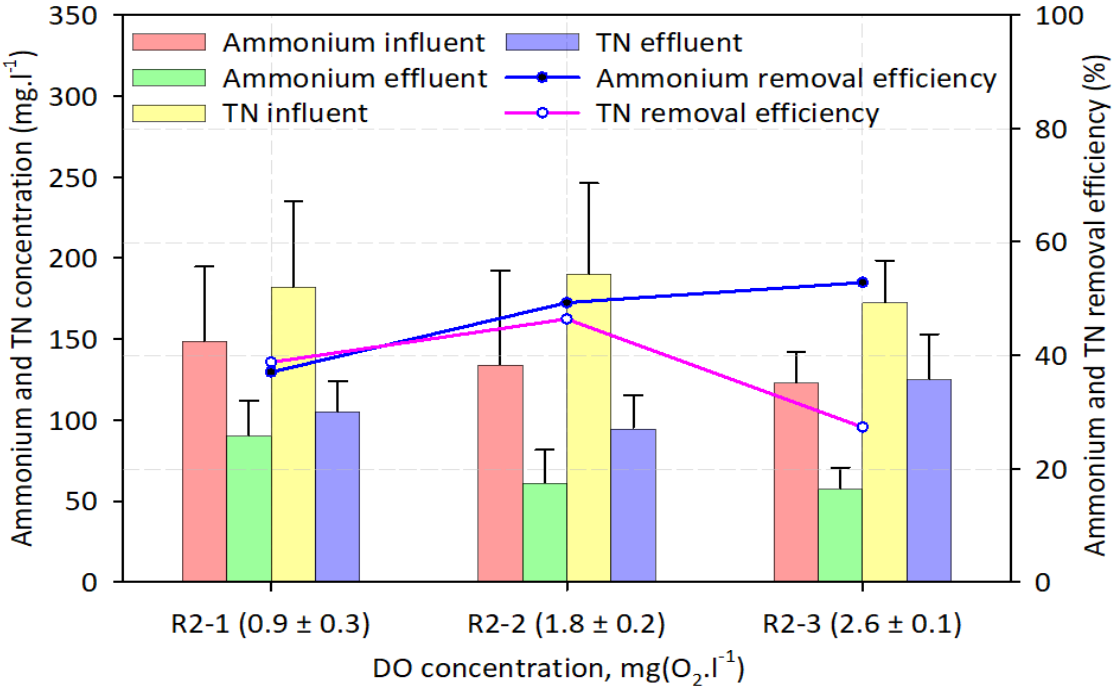

Figure 6.02. Effect of dissolved oxygen to nitrification process and nitrogen removal efficiency in AE

In this study, inlet flowrates were adjusted from 2.5 – 2.6 $l.h^{-1}$. However, ammonium loads were increased gradually from 0.073 ± 0.019, 0,095 ± 0.016 and 0.111 ± 0.015 $gNH_4^+.l^{-1}.d^{-1}$ at R2-1, R2-2 and R2-3, respectively. From results shown that there was a decrease in ammonium concentration influent AE with increasing of ammonium loads in leachate. This can be explained that activity of biomass for nitrification process increased, therefore ammonium removal in system rose up.

As shown in Table 6.11 and Figure 6.02, In run R2-1, the DO was maintained at 0.9 ± 0.3 $mgO_2.l^{-1}$. The influent and effluent NH_4^+-N concentration ranged at 149.07 ± 45.65 $mg.l^{-1}$ and 90.66 ± 21.18 $mg.l^{-1}$, respectively. There was a low NH_4^+-N removal efficiency in the initial operation. The average ammonium removal efficiency was only 37.02 ± 11.58%, corresponding to 38.75 ± 15.73% for total nitrogen removal. In these conditions, the nitrification process was hampered because of oxygen limitation and the slow growth rate of the nitrifiers.

To improve the NH_4^+-N removal, the operating DO was further increased to 1.8 ± 0.2 $mgO_2.l^{-1}$ in run R2-2. The results demonstrate that nitrification was enhanced, with NH_4^+-N removal efficiency increasing rapidly over 49.31 ± 19.27 % and the average effluent TN concentration remaining at a level of 46.41 ± 18.79 $mg.l^{-1}$.

However, in run R2-3, when DO was increased to 2.6 ± 0.1 $mgO_2.l^{-1}$. The efficiency of NH_4^+-N removal was increased to 52.85 ± 9.97 % but TN removal efficiency gradually decreased to 27.30 ± 11.24 % because high DO inhibited the denitrification process in AN1 and AN2.

From the achieved results it was demonstrated that the changes of the aeration regime in the reactor neither significantly decrease nor improve nitrification. Conversely, the TN removal efficiency was decreased. The increase of DO in AE caused an increase of oxygen flux in AN2. Besides, an increase of oxygen flux AN1 occurred due to the returned wastewater with high DO concentration. Thus, this condition was disadvantageous for denitrification in both AN1 and AN2. When the DO concentration in the AE was approximately 1.8 ± 0.2 $mgO_2.l^{-1}$, the effect of nitrogen removal yielded the highest value. The DO concentration was never greater than 1.5 $mgO_2.l^{-1}$ that is compatible with simultaneous nitrification and denitrification in the literature [31].

In this condition, although the increase of the nitrification variables is encouraged, denitrification process is limited as a result of high DO. If excessive oxygen is provided, heterotrophic bacteria will grow aerobically and the organic carbon for heterotrophic denitrification will be poorly supplied; otherwise, the heterotrophic bacteria will compete oxygen with nitrifying microorganisms. It indicates that the supply of oxygen should be controlled to optimize the operation of the pilot. In another major study, Oguz, *et al.,* demonstrated that denitrification by autotrophic ammonia oxidizers occurs when ammonia and nitrite are present and DO is absent [32].

In general, complete nitrification is the oxidation of ammonia to nitrate via nitrite, which can be limited to the nitrite step under oxygen – limited condition as affinity for oxygen of ammonium oxidizing bacteria is better than that of nitrite oxidizing bacteria. Thus, the amount of supplied oxygen is the most important control factor for operation of the pilot.

Besides, the results t-test analysis for efficiencies indicated that p value was less or equal to 0.001 for all three runs and showed that all the experimental data were statistically significant.

6.3.1.2. Effect of dissolved oxygen on nitrification and denitrification process in MBR compartment

To determine the effect of DO to nitrification and denitrification in MBR, the DO concentration changed in MBR from 1.1 ± 0.2 $mgO_2.l^{-1}$ to 2.9 ± 0.4 $mgO_2.l^{-1}$ and was divided into three runs. The concentration in AE was controlled ranging

from 1.5 to 2.0 $mgO_2.l^{-1}$. The results of the research are presented in Table 6.12 and Figure 6.03.

Table 6.12. Ammonium and nitrogen removal efficiencies in MBR

Run; $mgO_2.l^{-1}$	**R3-1** (1.1 ± 0.2)	**R3-2** (2.2 ± 0.1)	**R3-3** (2.9 ± 0.4)
Ammonium (MBR)			
Influent; $mg.l^{-1}$	67.38 ± 19.97	83.35 ± 12.84	69.51 ± 19.34
Effluent; $mg.l^{-1}$	38.53 ± 21.78	41.53 ± 18.88	25.59 ± 16.81
Efficiency; %	42.92 ± 22.24	51.01 ± 18.55	65.17 ± 17.99
Nitrogen Total (MBR)			
Influent; $mg.l^{-1}$	154.34 ± 41.84	146.67 ±27.20	164.09 ± 41.31
Effluent; $mg.l^{-1}$	100.67 ± 24.63	86.84 ± 21.58	92.50 ± 25.67
Efficiency; %	32.46 ± 16.26	39.44 ± 15.44	43.00 ± 13.75

From the data in Table 6.13 and Figure 6.03, it can be seen that ammonium and total nitrogen removal efficiencies increased with a DO concentration increase in the MBR compartment. In general, in these operation runs, nitrification improved and NH_4^+-N removal efficiency increased from 42.92 ± 22.24% to 65.17 ± 17.99%.

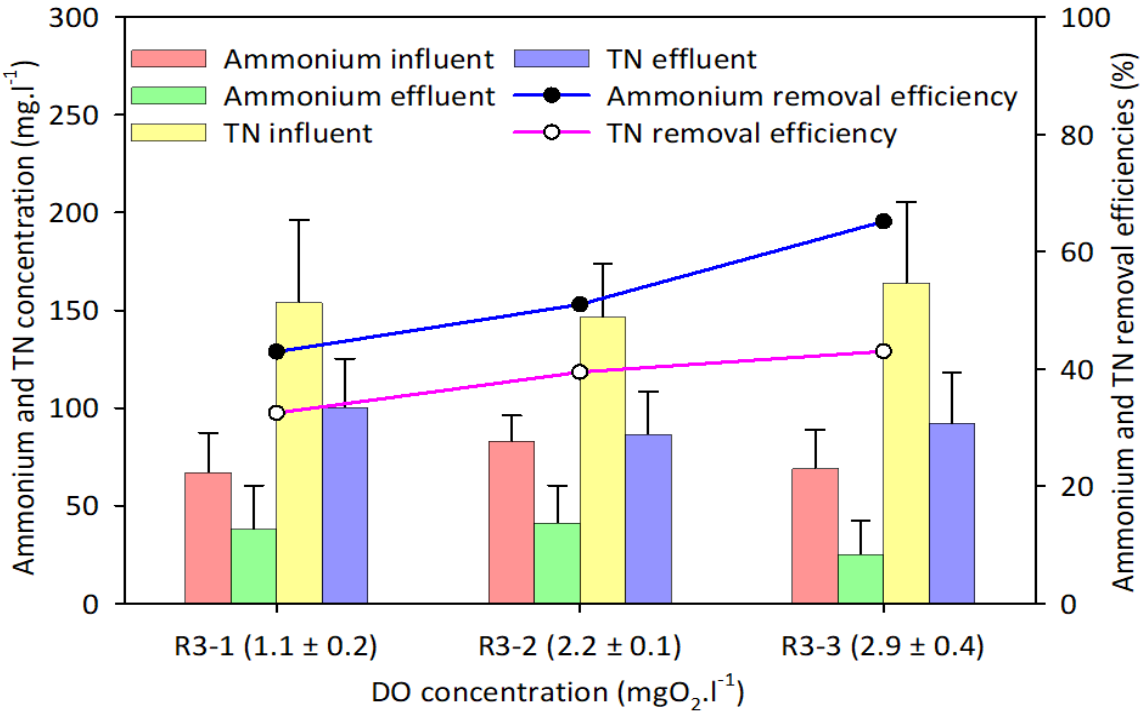

Figure 6.03. Effect of dissolved oxygen to nitrification process and nitrogen removal efficiency in MBR

The biofilm close to the membrane is aerobic and contains small amount of organic substrate. Those conditions have favored the growth of nitrifiers and the production of nitrate. In the center of the biofilm, DO is still present and the higher organic substrate concentrations encourage the growth of the faster growing aerobic heterotrophs. In the outer regions of the biofilm, high concentration of organic substrate and the absence of DO encourages denitrification and the growth of anaerobes. The fast-growing heterotrophs are located at the outer layers, where both substrate concentration and detachment rate are high while the slow-growing autotrophs (nitrifying bacteria) stay deeply inside the biofilm [33].

According to [34], the aerobic area in MBR biofilms, nitrifying bacteria are positioned near the membrane, where oxygen concentrations are the highest. For the biofilms, the competition between autotrophs and heterotrophs for substrates (oxygen and ammonia) and space in biofilm are practically important ones as previously studied by Tijhuis *et al*. [35].

6.3.1.3. Effect of sCOD/NO_x^--N to denitrification process in AN2

The denitrification ability of the AN2 compartment was also investigated. Typically, the ratio of sCOD/NO_x^--N in AN2 has to be high enough to sustain the nitrogen removal, otherwise the addition of a carbon source will become necessary. sCOD/NO_x^--N ratio was determined by collecting and analysis sCOD, nitrite and nitrate in AN2. The results obtained in this phase are summarized in Table 6.13 and Figure 6.04.

As shown in Figure 6.04, when the average sCOD/NO_x^--N ratio in AN2 went up from 4.2 to approximately 10.5 (BOD_5/NO_x^--N ratio ranged in 2.5 – 6.4), the TN removal efficiency of system started a sharp increase and the maximum TN removal efficiency reached 71 ± 12 % at run R4-4. It can be seen that there was an increase of denitrification in AN2 due to COD affection.

Table 6.13. Effects of sCOD/NO_x^--N ratio to denitrification process in AN2

Run[28]	**R4-1** (4.2/2.5)	**R4-2** (6.7/3.8)	**R4-3** (7.8/5.5)	**R4-4** (10.5/6.4)
NO_x^--N (AN2)				
Influent; mg.l^{-1}	71.35 ± 23.44	64.84 ± 15.78	57.19 ± 16.34	63.35 ± 10.54
Effluent; mg.l^{-1}	49.37 ± 17.36	31.60 ± 9.00	16.89 ± 4.81	17.72 ± 8.90
Efficiency; %	30.59 ± 9.99	50.51 ± 11.09	69.54 ± 8.13	70.98 ± 18.24

[28] Mean COD:NO_x-N/BOD:NO_x-N

Run[28]	**R4-1** (4.2/2.5)	**R4-2** (6.7/3.8)	**R4-3** (7.8/5.5)	**R4-4** (10.5/6.4)
Nitrogen Total (AN2)				
Influent; $mg.l^{-1}$	130.76 ± 24.24	128.27 ± 25.16	113.63 ± 15.37	116.35 ± 13.24
Effluent; $mg.l^{-1}$	90.12 ± 16.16	85.14 ± 19.06	55.34 ± 5.77	51.72 ± 9.87
Efficiency; %	30.56 ± 9.13	38.11 ± 9.72	50.58 ± 6.22	54.80 ± 11.98

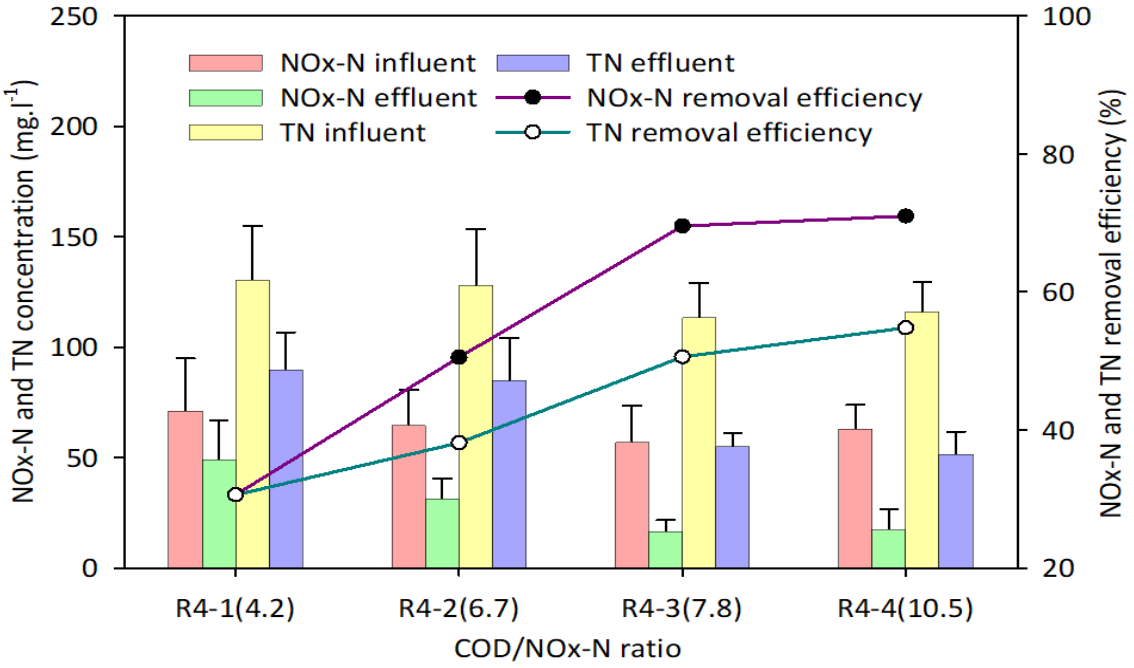

Figure 6.04. Effects of sCOD/NO_x^--N ratio to denitrification process in AN2

For run R4-1, a low denitrification efficiency was obtained at 30.59 ± 9.99 % since the organic matter requirement for denitrification was not fulfilled. At a low sCOD/NO_x^--N ratio, the transformation of nitrogen was limited, because there was no carbon source enough for the denitrification process, which is required for this reaction (based on the theoretical 1g N nitrate equivalent of 2.86g sbCOD). The denitrification efficiency increased (50.51 ± 11.09 %) as sCOD/NO_x^--N ratio increased up to 6.7 at run R4-2. However, the denitrifying activity increased further at run R4-3, NO_x^--N and TN removal efficiencies reached 69.54 ± 8.13 % and 50.58 ± 6.22 %, respectively. At 10.5 of sCOD/NO_x^--N ratio, NO_x^--N and total nitrogen removal efficiencies increased slightly, corresponding to 70.98 ± 18.24 %. 54.80 ± 11.98 %, respectively. Therefore, the NO_x^--N removal efficiency only increased approximately 1.05 % at 10.5 ratio of sCOD/NO_x^--N . These results indicated that a favorable nitrogen removal could not be reached under a low sCOD/NO_x^--N ratio. Therefore, 7.8 was considered as the optimum sCOD/NO_x^--N ratio value for SND in the AN2

reactor in this study, (equivalent of 5.5 for BOD_5/NO_x^--N). Besides, the nitrogen removal efficiency had a tendency towards a reduction because a high concentration of carbon restricts the nitrification reaction. The effects of sCOD/N for removing nitrogen were cited in previous studies [36-40].

6.3.1.4. Effect of sCOD/NO_x^--N ratio to denitrification process in MBR

In this phase, the denitrification process was performed at different sCOD/NO_x^--N (from 14.2 to 17.6) whereas the DO was operated at 1.0 ± 0.5 $mgO_2.l^{-1}$ in AE and MBR. The removal rates of nitrate, nitrite and TN by the MBR during this phase are shown in Table 6.14 and Figure 6.05.

Table 6.14. Effects of sCOD/NO_x^--N ratio to denitrification process in MBR

Run[29]	**R5-1** (14.2/3.4)	**R5-2** (16.4/6.2)	**R5-3** (18.6/8.5)
NO_x^--N (MBR)			
Influent; $mg.l^{-1}$	62.48 ± 21.47	51.05 ± 21.72	57.91 ± 19.97
Effluent; $mg.l^{-1}$	33.08 ± 11.34	22.50 ± 5.09	28.48 ± 3.76
Efficiency; %	42.66 ± 21.41	45.21 ± 35.00	45.63 ± 18.49
Nitrogen Total (MBR)			
Influent; $mg.l^{-1}$	89.09 ± 23.57	87.27 ± 21.16	87.19 ± 20.86
Effluent; $mg.l^{-1}$	43.41 ± 11.94	38.21 ± 8.21	40.59 ± 4.15
Efficiency; %	53.82 ± 10.89	53.73 ± 17.77	50.41 ± 12.11

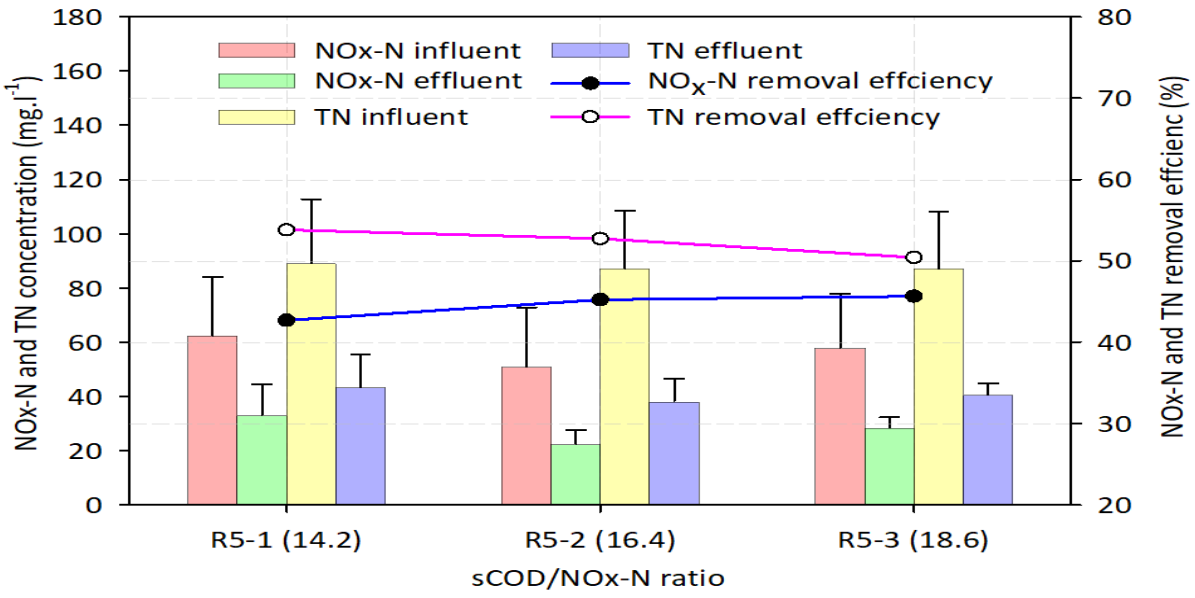

Figure 6.05. Effects of sCOD/NO_x^--N ratio to denitrification process in MBR

[29] Mean COD: NO_x^--N /BOD: NO_x^--N

The operation of the MBR system was observed during steady state and for 3 separated experimental runs where sCOD/NO_x^--N levels in the reactor was gradually increased from 14.2 in run R5-1, to 16.4 in run R5-2 and to 18.6 in run R5-3. During this period, the average level of the influent average NO_x^--N concentration fluctuated in a narrow range of 51.05 – 62.48 $mg.l^{-1}$. The NO_x^--N and TN removal efficiency was slightly changed by increasing sCOD/NO_x^--N but the difference was not significant.

The added yellow sugar to boost the denitrification could also be consumed by the aerobic heterotrophs if oxygen is available, while the denitrifying heterotrophic bacteria dominate throughout the biofilm [41]. In this phase, high biomass concentration is beneficial to nitrogen removal because longer SRT can support the increase of slow – growing autotrophic nitrifiers. Therefore, the excess sludge at high biomass concentration may create carbon for nitrification process through endogenous denitrification. The results show that the use of external carbon to boost the denitrification process did not improve nitrogen removal efficiency.

6.3.1.5. Effect of on/off aeration time on nitrification and denitrification process in MBR

In MBR, the surface of the biofilm structure offers conditions for simultaneous nitrification and denitrification. Supplying continuous aeration could be an advantage for nitrification process but disadvantage for denitrification. To facilitate the removal of nitrogen by the denitrification process, the aeration in MBR may be interrupted was modified during the experimental sixth phases as in Table 6.08. During non-aeration stage, the liquid and sludge were mixed with a mixer. Initially, the on and off time for MBR was set at 180 min and 30 min, respectively with online control system, then on/off aeration time changed according to Table 6.08 and Table 6.09. Concentration of DO was maintained ranging from 2.3 to 3.2 $mgO_2.l^{-1}$ during the aeration time in MBR. The other operational parameters were selected from research results with highest nitrogen removal efficiency of former phase.

- *Effect of non-aeration time on denitrification*

The results recorded during research show that after on average 17 minutes of non-aeration time, DO concentration dropped down to < 0.5. Table 6.15 and Figure 6.06 present the concentration of NH_4^+-N , NO_2^--N and NO_3^--N under different non-aeration conditions. Results for the removal efficiency of total nitrogen was 49.88 ± 16.20 %, 68.07 ± 6.87 % and 45.75 ± 10.70 % at on/off aeration times of 180/30 min, 180/60 min and 180/90 min, respectively.

The highest total nitrogen removal efficiency of 68.07 ± 6.87 % was achieved at an aeration on/off time of 180/60 minutes. Corresponding total nitrogen concentrations of influent and effluent in MBR were 59.66 ± 22.38 $mg.l^{-1}$ and 18.17 ± 5.03 $mg.l^{-1}$, respectively. Thus, the concentration of the dissolved oxygen in water might be low and enough time during the stop of aeration cycle to implement denitrification process.

Table 6.15. Effect of aeration-non aeration time on denitrification process in MBR

Run[30]	**R6-1** (180 – 30)	**R6-2** (180 – 60)	**R6-3** (180 – 90)
Ammonium (MBR)			
Influent; $mg.l^{-1}$	21.69 ± 6.71	19.07 ± 11.37	20.66 ± 5.12
Effluent; $mg.l^{-1}$	5.53 ± 2.90	4.80 ± 1.59	10.24 ± 4.42
Efficiency; %	73.06 ± 13.64	70.72 ± 10.30	50.73 ± 16.84
NO_x^--N (MBR)			
Influent; $mg.l^{-1}$	16.89 ± 5.55	17.48 ± 2.36	15.52 ± 3.91
Effluent; $mg.l^{-1}$	17.01 ± 5.32	10.08 ± 2.91	7.67 ± 2.64
Nitrogen Total (MBR)			
Influent; $mg.l^{-1}$	57.64 ± 6.68	59.66 ± 22.38	64.53 ± 5.82
Effluent; $mg.l^{-1}$	28.82 ± 9.55	18.17 ± 5.03	34.82 ± 6.48
Efficiency; %	49.88 ± 16.20	68.07 ± 6.87	45.75 ± 10.70

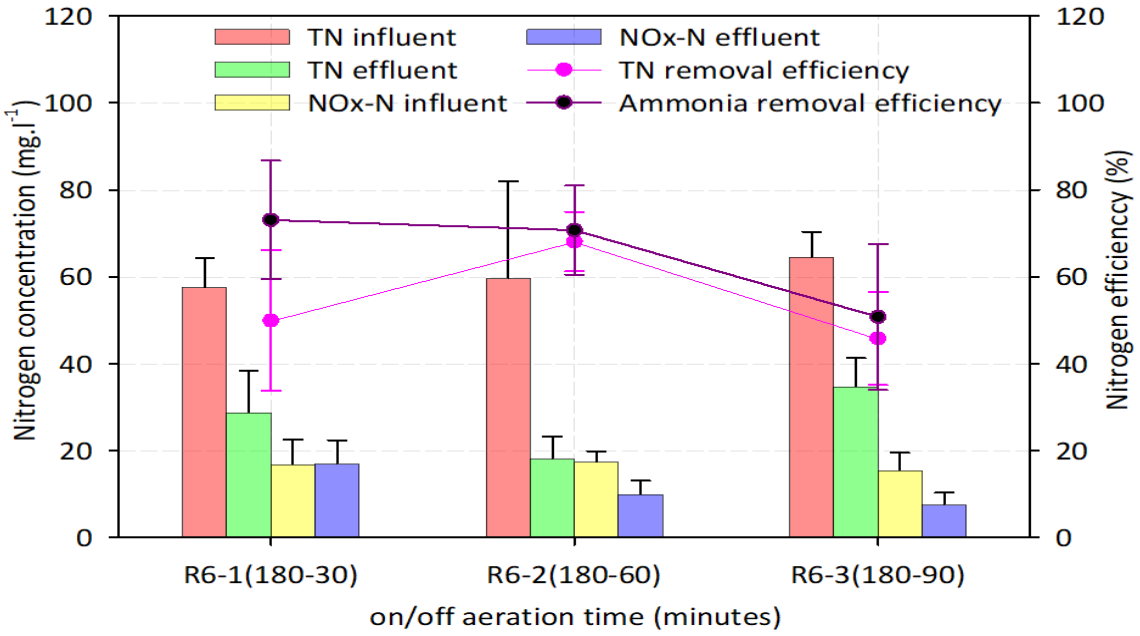

Figure 6.06. Nitrogen efficiency during on/off aeration time in MBR

[30] On/off aeration time (minute)

At lower non-aeration time (30 min), the efficiency of total nitrogen removal was only 49.88 ± 16.20 %, the denitrification efficiency was lower due to the higher remaining concentration of NO_x^--N in effluent and in mixed liquor recycled from MBR to AE. Otherwise, the experimental results also demonstrated that the increase of no-aeration time could decrease the nitrification efficiency. Besides, ammonia and total nitrogen Kjeldahl from AN2 reactor flowed into MBR without nitrification during off aeration time.

The removal efficiency of total nitrogen was 45.75 ± 10.70 % at 180/90 min aeration on/off times, corresponding influent and effluent were 64.53 ± 5.82 $mg.l^{-1}$ and 34.82 ± 6.48 $mg.l^{-1}$, respectively. Those results suggest that nitrate can be reduced to nitrogen gas under expanded anoxic conditions. The lowest value of NO_x^--Nwas recorded around 7.67 ± 2.64 % in this phase. These results were comparable with previous studies for domestic wastewater in which total nitrogen removal found higher than 82% at aeration off time to be more than 70 min in the reactor [42].

- *Effect of aeration time on nitrification process*

The effects of the aeration time on the nitrogen removal at various time ratios were also investigated. The results for effect of aeration time on nitrogen removal efficiency are presented in Table 6.16 and Figure 6.07.

Table 6.16. Effect of time on aeration on nitrification process in MBR

Run[31]	**R7-1** (60 – 60)	**R7-2** (120 – 60)	**R7-2** (150 – 60)
Ammonium (MBR)			
Influent; $mg.l^{-1}$	19.47 ± 2.83	19.80 ± 7.41	18.28 ± 3.07
Effluent; $mg.l^{-1}$	11.22 ± 3.32	5.55 ± 1.80	4.30 ± 1.62
Efficiency; %	41.95 ± 15.49	70.13 ± 10.39	76.96 ± 6.56
NO_x^--N (MBR)			
Influent; $mg.l^{-1}$	13.58 ± 6.49	14.56 ± 6.91	16.13 ± 7.36
Effluent; $mg.l^{-1}$	6.50 ± 1.68	3.06 ± 0.76	5.91 ± 1.01
Nitrogen Total (MBR)			
Influent; $mg.l^{-1}$	50.04 ± 13.92	65.07 ± 9.65	60.64 ± 11.47
Effluent; $mg.l^{-1}$	16.12 ± 7.25	11.62 ± 2.69	12.20 ± 0.82
Efficiency; %	68.09 ± 8.49	81.92 ± 4.37	79.35 ± 4.05

[31] On/off aeration (minute)

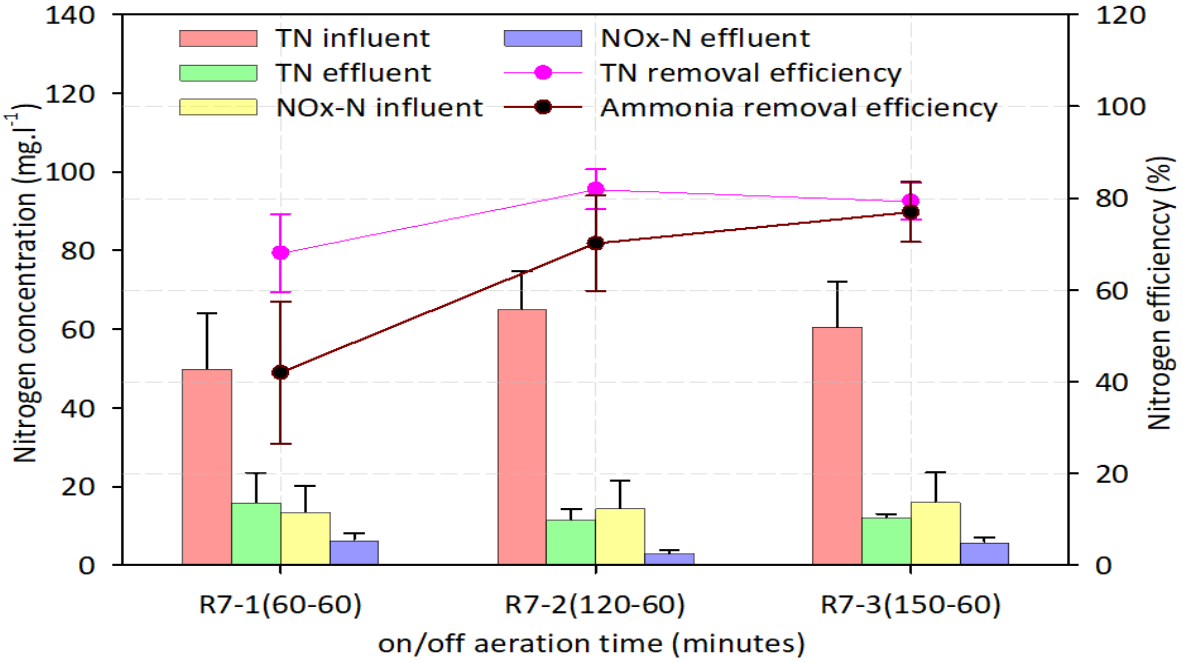

Figure 6.07. Nitrogen removal efficiency during changed DO in MBR

From research results suggest that there was a significant increase of efficiency of total nitrogen removal from 60/60 to 120/60, then slight decreasing trend at 150/60 aeration on/off time, corresponding R7-1, R7-2, and R7-3, respectively.

The unsatisfying nitrification efficiency in R7-1 was likely due to the high ammonia concentration remaining in the effluent (11.22 ± 3.32 $mg.l^{-1}$) at R7-1. The efficiency of total nitrogen removal achieved at 68.09 ± 8.49 % in this run. It was observed that as the aeration time increased, the average removal of ammonia nitrogen showed an increasing trend.

The nitrogen removal efficiency improved significantly at run R7-2 and founded 81.92 ± 4.37 % and ammonia removal efficiency achieved about 70.13 ± 10.39 %. It was also noted that the rate of the ammonia removal generally increased with the increase of the on aeration time.

The longer the aeration times, the higher ammonia removal efficiency but efficiency of total nitrogen removal slightly declined, as also founded in this study. As seen from Table 6.16 and Figure 6.07, a removal rate of 76.96 ± 6.56 % for ammonia was achieved in R7-3, and the concentration of mean ammonia nitrogen in the effluent was observed at 4.30 $mg.l^{-1}$, although the concentrations in the influent was from 18.28 ± 3.07 $mg.l^{-1}$. Compared to R7-2, the efficiency of total nitrogen removal declined slightly from 81.92 ± 4.37 % to 79.35 ± 4.05 %. Because more nitrate remained in effluent. Besides, the results in R7-3 suggest that there is an increase in DO concentration for AN1 and anoxic 2 reactors, which led to cause disadvantage for denitrification process in these reactors.

Overall, the experimental results found that both nitrification and denitrification may successfully be operated with aeration times longer than 60 minutes but less than 150 minutes in order to achieve high efficiency of nitrification and denitrification. In operational conditions of 60/75 min aeration on/off time, Chang *et al.*, reported that the efficiency of maximum total nitrogen removal was 70.7 % [43].

6.3.2. Pilot operation

6.3.2.1. Nitrogen removal efficiency

- Ammonium removal efficiency and nitrite and nitrate variations

The ammonium removal efficiency versus time was shown in Figure 6.08.

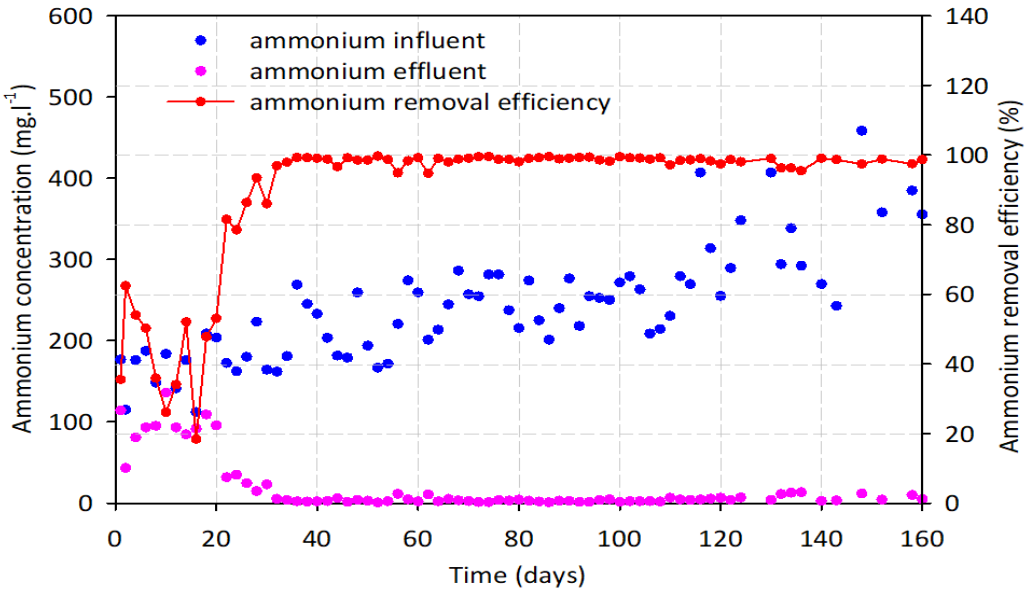

Figure 6.08. Ammonia removal efficiency

From the Figure 6.07, effluent ammonium concentration decreased from the 1st day to the 40th. After 40th day, effluent ammonium concentration was very low and stable although there was an increase influent ammonium concentration. Influent, effluent and efficiency value of ammonium were presented in Table 6.17.

Table 6.17. Efficiency of ammonium removal

ORL, g.l^{-1}.d^{-1}	**Influent**, mg.l^{-1}	**Effluent**, mg.l^{-1}	**Efficiency**, %
0.250 ± 0.032	181.86 ± 39.09	56.16 ± 44.79	66.07 ± 27.44
0.334 ± 0.062	229.02 ± 39.42	3.72 ± 2.86	98.32 ± 1.39
0.470 ± 0.080	244.81 ± 27.20	2.32 ± 0.96	99.05 ± 0.37
0.698 ± 0.080	321.78 ± 63.98	6.62 ± 3.50	97.92 ± 11.08

At ORL of 0.25 $g.l^{-1}.d^{-1}$, efficiency of ammonium removal achieved 66.07 ± 27.44 %. However, the ammonium removal efficiency climbed up significantly with increasing ORL and got the highest efficiency at ORL of 0.470 $g.l^{-1}.d^{-1}$, corresponding to 99.05 ± 0.37 %. The main reason was an MLVSS increase in the system, the existence of aerobic microorganisms and aerobic condition boost nitrification. At higher of ORL, there was a slight decline in ammonium removal efficiency.

The results of the nitrite and nitrate variations are also shown as Figure 6.09.

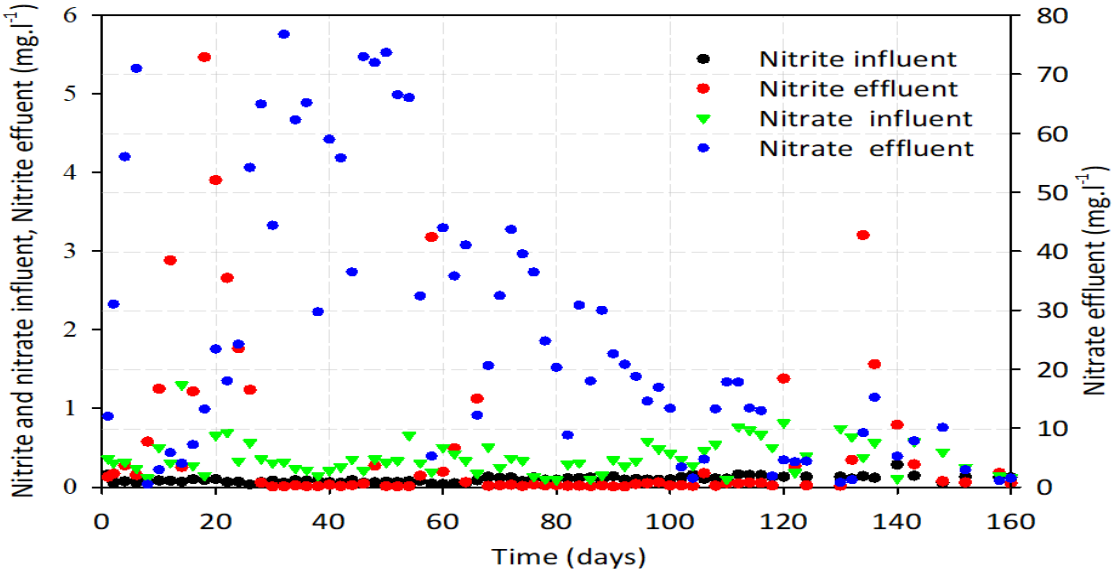

Figure 6.09. Nitrite and nitrate variations

From the results, nitrite variations were insignificant, and most of the nitrite concentrations in the influent and effluent were very low. On the contrary, influent mean nitrate concentrations were lower than 1 $mg.l^{-1}$, and effluent nitrate concentrations changed noticeably at different ORL. The results of nitrate measured at ORL_1, ORL_2, ORL_3 and ORL_4 were 34.55 ± 25.93, 41.59 ± 20.51, 15.52 ± 9.01 and 7.35 ± 5.91 $mg.l^{-1}$, respectively. Results show that effluent nitrate concentrations decreased with increasing ORL.

Total Nitrogen removal efficiency

Nitrogen removal efficiency is illustrated in Figure 6.10.

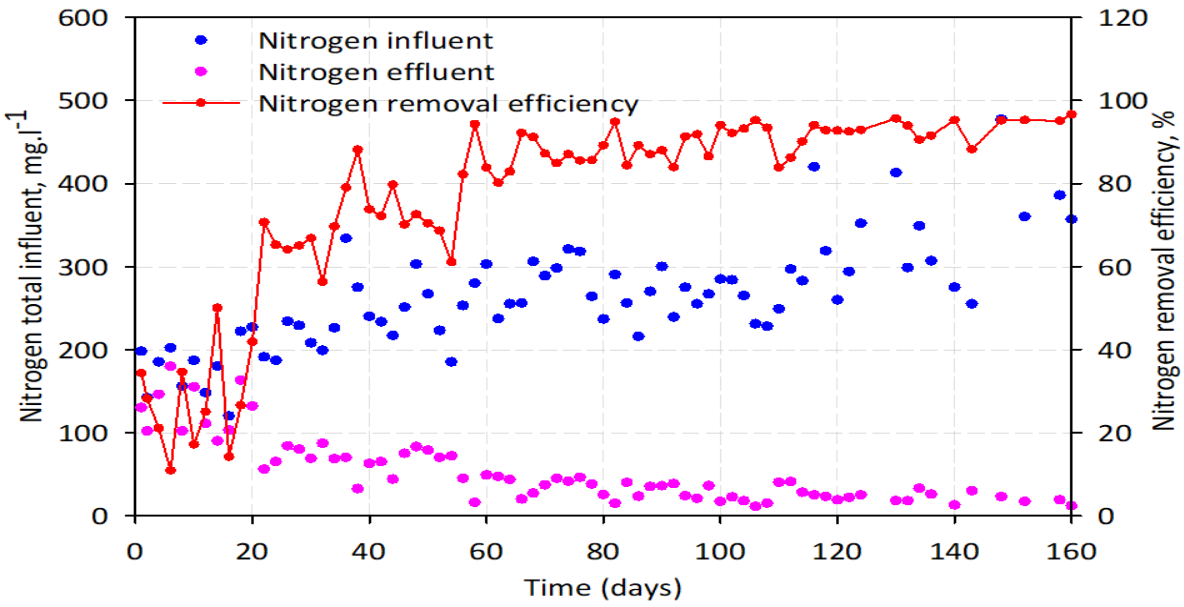

Figure 6.10. Nitrogen removal efficiency

Results of the experiment in Figure 6.10 showed that the total nitrogen removal efficiency of the system was close to 47.74 ± 23.96 % in ORL_1. The total nitrogen removal efficiency continued the remark increase and the average value reached from 81.05 ± 9.03 % to 90.35 ± 3.84 % at ORL_2 and ORL_3, respectively. There was a slight increase in nitrogen removal at ORL_4 compared to ORL3 with 92.36 ± 3.46 %. The increase of efficiency of total nitrogen removal with rising of ORL can be explained by the increase in MLVSS concentration and the formation of an anoxic biofilm layer in MBR, leading to advantage for the development of denitrification bacteria in nitrite and nitrate removal.

The nitrogen removal efficiency remained high from days 110^{th} to 160^{th}. A possible reason is that some nitrites were denitrified inside the cake layer on the membrane surface (in the biomembrane). In the deepest layers of the biomembrane, anoxic conditions were maintained, since DO concentration in the bulk of the liquid is lower than 2 $mgO_2.l^{-1}$. Nitrogen removal efficiency increased with operation time because of the increase in volatile suspended solid concentration and nitrification were achieved in the MBR. In this study, the ammonium removal was higher than 99%, indicating that nitrification was complete in the system.

6.3.2.2. COD removal efficiency

After the system was started-up, samples of leachate of influent settled and effluent were collected and analyzed routinely for COD and illustrated in Figure 6.11. As can be seen on Figure 6.11, the system showed good performances for

organic carbon removal. An average organic total removal of over 73 % was achieved through the experiment.

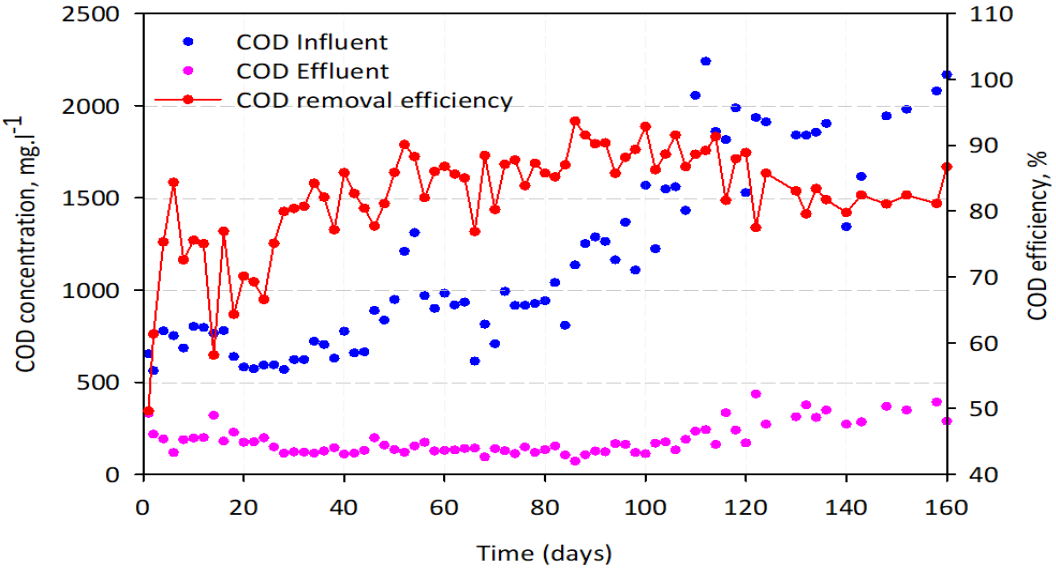

Figure 6.11. COD removal efficiency in the system

Figure 6.11 showed that COD removal efficiency of this system changed at various ORL. After day 40th of the experiment, COD concentration dropped from 675.9 ± 85.4 $mgO_2.l^{-1}$ to 176.8 ± 61.8 $mgO_2.l^{-1}$ (COD removal efficiency was about 73.5 ± 9.4 %). From the days 41th to 80th, COD concentration in the influent and the effluent was 902.3 ± 168.1 $mgO_2.l^{-1}$ and 136.3 ± 22.9 $mg.l^{-1}$ respectively (COD removal effciency reached about 84.4 ± 3.7 %). From the days 81th to 108th, COD values at the influent and the effuent were 1268.6 ± 216.7 $mgO_2.l^{-1}$ and 124.7 ± 25.9 $mgO_2.l^{-1}$ (COD removal efficiency about 89.9 ± 2.7%). There was a drop of COD removal efficiency observed from days 109th to 160th. The results showed that from the days 109th to 160th, COD values in the influent, the effluent and COD efficiency were 1883.8 ± 217.3 $mgO_2.l^{-1}$ and 299.2 ± 73,8 and 84.0 ± 3.9 %, respectively. Generally, high removal of COD was observed in the system but COD removal efficiency will also depend on the remaining refractory COD.

6.3.3. Sludge and microorganisms in the system

The changes of MLSS and MLVSS in the system were determined. The initial MLSS concentration and MLVSS/MLSS ratio were 3.11 $g.l^{-1}$ and 0.69, respectively. During the operation, the MLSS concentration and the MLVSS/MLSS ratio increased slightly, and then remained at relatively constant levels of 5.3 $g.l^{-1}$ and 0.62, respectively.

Ammonia-oxidizing bacteria and nitrobacteria of the activated sludge were characterized in the pilot. The populations of AOB and NOB were determined and shown in Figure 6.12 after finishing operating the system.

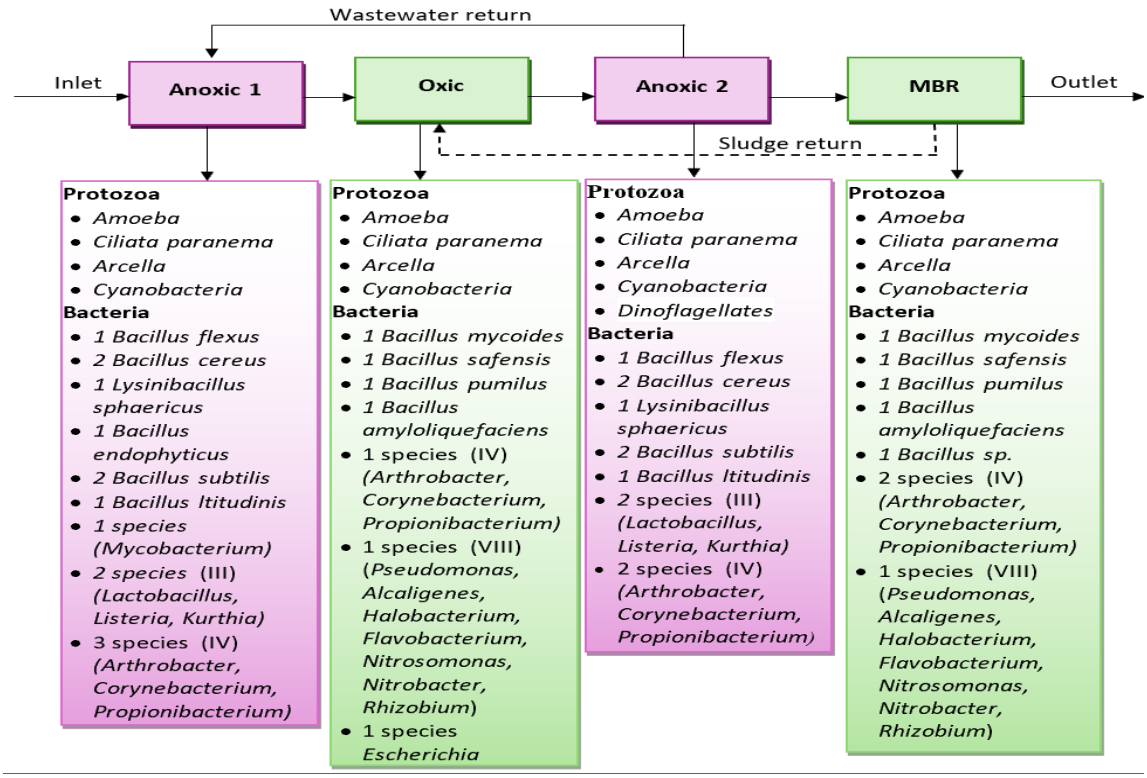

Figure 6.12. Microorganisms in the system

Biomass concentration in the system indicates treatment capacity of the system removing carbon and nitrogen. The nitrifying populations present in the pilot showed a high correlation to corresponding nitrification performance and can be a good indicator of stable nitrification.

It can be seen that the efficiency of wastewater treatment by activated sludge was related to the protozoa and bacterial population in pilot. The protozoa appeared in all tanks in and some species have been found in very large numbers (*Amoeba, Ciliata paranema, Arcella, Cyanobacter)*. According to [44, 45], protozoans play important roles in wastewater treatment processes. The positive effects of protozoa on carbon mineralization by bacteria in activated sludge are well known [46]. Conversely, *Fairbrother & Renshaw* were able to assess that protozoa were originally thought to be harmful to the activated-sludge process [47]. Protozoa, are in fact considered to be the most important bacterivorous grazers [47]. It is well known that the activated-sludge process is able to reduce the population of fecal bacteria [48].

Bacteria were found in the pilot with various species, these microorganisms are broadly distributed in the four reactors and usually use aerobic metabolism to degrade the biological matter in the liquid sludge.

Bacillus species may use inorganic and organic sources of nitrogen. Many species will utilize an ammonium salt as their sole nitrogen source, amino acids are widely utilized, and strains of some species can use urea. Nitrate respiration is a common property in the genus. Although *Bacillus subtilis* has long been regarded as a strict aerobe, it will however, like many *Bacillus* species, grow anaerobically (anoxic) using nitrate or nitrite as electron acceptor. It can grow anaerobically, not only with nitrate as electron acceptor but also by fermentation in the absence of electron acceptors. *Bacillus* reduces nitrate to nitrite and ammonium, and, unlike the denitrifying *Bacillus licheniformis*, it does not produce the gaseous products NO, N_2O and N_2 [27].

The metabolism of *Pseudomonas* is typically respiration with oxygen as the terminal electron acceptor, but some species also can use nitrate as an alternate electron acceptor and can carry out oxygen-repressible denitrification (dissimilatory reduction of nitrate to N_2O or N_2). The microorganisms should be found in four tanks because of returned sludge such as facultative group (IV) (*Arthrobacter, Corynebacterium, and Propionibacterium*).

6.4. CONCLUSIONS

This study demonstrated that successful nitrification can be achieved in AN1/AE/AN2/MBR system for treating wastewater with high nitrogen content like leachate. DO in AE and MBR were 1.8 ± 0.2 $mgO_2.l^{-1}$ and 2.9 ± 0.4 $mgO_2.l^{-1}$, respectively, corresponding to ORL of ammonium in AE and MBR to be 0.383 ± 0.066 and 0.467 ± 0.071 $gNH_4^+\text{-}N.l^{-1}.d^{-1}$, respectively.

The carbon source in AN2 needs to remain with COD/N ratio around 8 or BOD/N ratio about 5.5. For MBR, the denitrification can occur without external carbon source. The best condition for the removal of nitrogen was obtained with an aeration time of 120 minutes and non-aeration of 60 minutes.

The rate of consumption of dissolved oxygen by biological oxidation is rather low due to easy degradable organic compounds removed before flowing into MBR. Therefore, on/off aeration could be considered beneficial as it decreases the cost of aeration.

Results of the pilot run indicate that at an ORL of COD of 0.470 $g.l^{-1}.d^{-1}$ the system is appropriate to remove COD and nitrogen.

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CHAPTER 7: REFRACTORY COD REMOVAL IN LANDFILL LEACHATE TREATMENT WITH MEMBRANE BIOREACTOR IN VIETNAM

7.1. INTRODUCTION

The landfill leachate with high loads of organic and ammonia without treatment has caused serious pollution of the environment. The organic pollutant load of leachates generally reaches maximum values during the first years of operation of a landfill site and then gradually decreases over succeeding years [1]. The leachate from municipal landfill contains xenobiotic organic compounds or XOCs originating from hazardous household products such as pesticides, PPCPs, paints, detergents, etc., in addition to naturally occurring dissolved and suspended organic matter. Occurrence of XOCs such as aromatic hydrocarbons (e.g. benzene, toluene), halogenated hydrocarbons (e.g. trichloroethylene, dichloroethanes), pharmaceuticals (e.g. propyphenazone, ibuprofen), and pesticides (e.g. atrazine, simazine) has been widely reported in leachate of municipal solid waste [2]. Combining both chemical and biological treatments of landfill leachate has also been investigated. Biological processes are quite effective in removing organic matter when applied to relatively young leachates. The refractory organic contaminants (low ratios of BOD_5/COD) contained in biologically pretreated leachate and old landfill leachates are not amenable to conventional biological processes, so they must be treated by a physico-chemical process [3]. Biologically treated leachate usually contains considerable amount of refractory organics and trace concentrations of xenobiotic pollutants [4]. Some methods are used to remove refractory COD in wastewater such as Fenton [5], combined electro-Fenton [6, 7], combined conventional Fenton and photo-Fenton processes [8], using an integrated hydrogen peroxide oxidation and granular activated carbon (GAC) adsorption [9], air oxidation or biological treatment [4]. The biologically treated leachates contain significant amount of biorefractory organic matters, such as fulvic and humic-like compounds [8, 10]. Thus, the treatments of landfill leachate that has both cost – effective and high efficiency for landfill leachate are currently of great interest.

For MBR, in the past, most of the studies about MBR focused on operational stable drinking water treatment [11-13]. However, in recent years, MBR has been proposed as an alternative for conventional activated sludge process. MBR technology is very efficient and it might provide an effluent quality that is

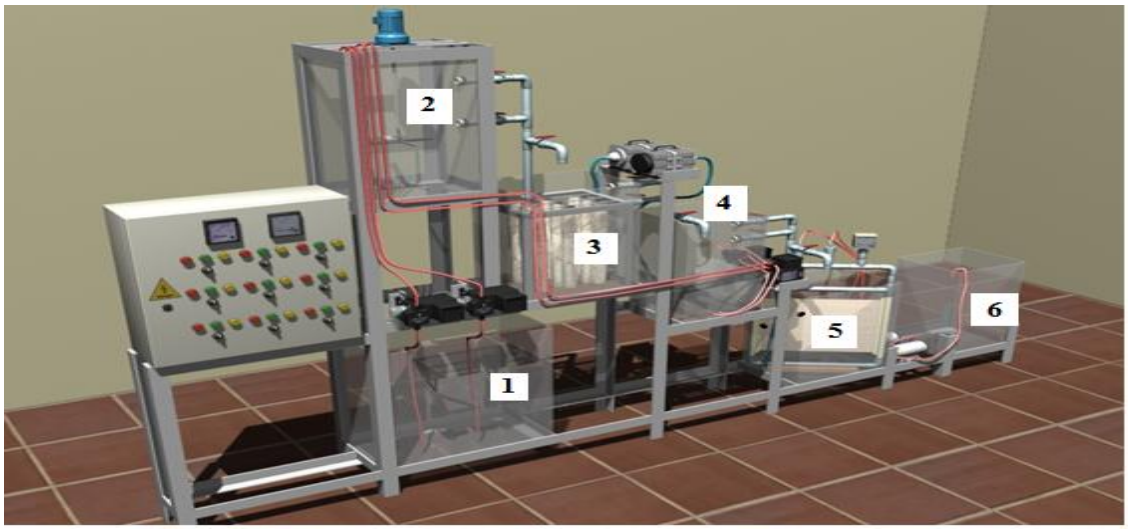
far beyond the current regulatory requirements for discharge in the environment [14-17]. MBR has attracted treatment systems in wastewater treatment works around the world due to its advantages such as compactness, high treated effluent quality and less sludge production [18]. Most of the studies about MBR have focused on the operational stability and treatment of various wastewaters. The MBR has applied successfully to treatment of various wastewaters [14], such as domestic wastewater, landfill leachate [19-22], industrial wastewater [23], dairy industrial wastewater [24, 25], pharmaceutical wastewater [26], piggery wastewater [27], and tannery wastewater [28]. The efficiency of the membrane also depends on the size of pores, types of materials, types of wastewater to be treated, solubility and retention time [29].

Given these challenges, this study aims to evaluate refractory COD removal from landfill leachate using a pilot-scale membrane bioreactor (MBR) system. Specifically, the research focuses on assessing the effects of organic loading rate (OLR), hydraulic retention time (HRT), and sludge retention time (SRT) on COD removal efficiency and COD fractions, as well as clarifying the role of MBR in improving the biodegradation and retention of refractory organic compounds.

7.2. MATERIALS AND METHODS

7.2.1. Experimental setup

The model of the laboratory scale MBR for the treatment of landfill leachate has been used to study COD removal. A pilot scale MBR was carried out in a system with four reactors including anoxic, Bio-cord (attached-growth), settling and MBR. Their working volumes are 25, 43, 15, and 35 liters, respectively. The schematic diagram is showed in Figure 7.01.



Numbers mean: (1) Feed tank, (2) Anoxic tank, (3) Bio-Cord tank, (4) Settling tank, (5) MBR, (6) Treated wastewater tank.

Figure 7.01. The schematic diagram of experimental set up

7.2.2. Landfill leachate and inoculum

7.2.2.1. Landfill leachate

The leachate used in this study was taken from a solid waste landfill site (Phuoc Hiep landfill), which has been in operation since 2003 in Vietnam. In this study, the physical, chemical and biological characteristics of the leachate were monitored throughout the study and presented in Table 7.01.

Table 7.01. Characteristics of Phuoc Hiep landfill leachate during study

Parameters	Units	Mean ± SD	Parameters	Units	Mean ± SD
Temperature	^oC	31.5 ± 1.3	NH_4^+-N	$mg.l^{-1}$	961 ± 241
pH	-	8.32 ± 0.28	NO_2^--N	$mg.l^{-1}$	1.55 ± 0.97
Alkalinity	$mgCaCO_3.l^{-1}$	9784 ± 841	NO_3^--N	$mg.l^{-1}$	3.16 ± 2.42
SS	$mg.l^{-1}$	891 ± 168	Kjeldahl-N	$mg.l^{-1}$	1028 ± 243
VSS	$mg.l^{-1}$	657 ± 143	T-N	$mg.l^{-1}$	1091 ± 246
COD	$mgO_2.l^{-1}$	4216 ± 827	Cl^-	$mg.l^{-1}$	3277 ± 227
BOD_5	$mgO_2.l^{-1}$	3482 ± 712	Fe	$mg.l^{-1}$	6.05 ± 2.18

7.2.2.2. Inoculum

The activated sludge was taken from the SBR reactor of the Binh Duong leachate treatment plant. The SS and VSS values of the sludge after settling were 7.08 $g.l^{-1}$ and 6.15 $g.l^{-1}$, respectively.

7.2.3. Reactor operating conditions

In the experiment, the influent wastewater was obtained by mixing the operating landfill leachate and tap water in various ratios to start up the system. The reactor was initially fed with landfill leachate and tap water at low COD the proportion of the leachate influent was increased by steps until 100 % raw leachate. The experimental campaign was divided into four phases with different COD concentration. The biomass was returned from settling reactors to anoxic to maintain biomass in system.

- *The fist experimental phase (1^{st} – 24^{st} day) was necessary for the start-up of the pilot.*
- *The second experimental phase (25^{th} – 109^{th} day)* was divided into four runs in Table 7.02. The aim of this study was mainly to find out the effect of OLR on organic removal efficiency.

Table 7.02. Effect of OLR on COD removal efficiency

Run	R2-1	R2-2	R2-3	R2-4
Time, d	$25^{th} – 45^{th}$	$46^{th} – 65^{th}$	$66^{th} – 87^{th}$	$88^{th} – 109^{th}$
ORL, ($kgCOD/m^3.d^{-1}$)	0.60 ± 0.06	0.82 ± 0.08	1.08 ± 0.10	1.32 ± 0.06

- *The third experimental phase ($110^{th} – 185^{th}$ day)* was operated with four different HRTs. The main purpose of this phase was to research the effect of HRT on COD removal efficiency. The HRT was changed as presented in Table 7.03.

Table 7.03. Effect of HRT on COD removal efficiency

Run	R3-1	R3-2	R3-3	R3-4
Time, d	$110^{th} – 129^{th}$	$130^{th} – 147^{th}$	$178^{th} – 17^{th}$	$172^{nd} – 185^{th}$
HRT, h	20	15	10	5

- *The fourth experimental phase ($186^{th} – 238^{th}$ day)*. The study aimed at determining the effect of SRT on COD removal efficiency in MBR. This phase was divided into four runs presented in Table 7.04.

Table 7.04. Effect of SRT (in MBR) on COD removal efficiency

Run	R4-1	R4-2	R4-3	R4-4
Time, d	$186^{th} – 199^{th}$	$200^{th} – 214^{th}$	$215^{th} – 223^{rd}$	$224^{th} – 238^{th}$
SRT, d	20	40	60	80

Usually it is recommended to study the influence of some parameters on the steady state behavior of the system, to wait around 3 times the SRT to check the effect the SRT of our system is already long (total for the 4 runs) is 200 d. Waiting would take nearly 2 years (3 times × 200 d), so we decided to wait shorter periods but we know that our results will be less reliable.

7.2.4. Analytical methods

During the whole phase of pilot operation, the characteristic of leachate, the influent leachate, the mixed liquor and the effluent permeate have been sampled and analyzed for SS, VSS, COD, BOD, NH_4^+-N, NO_2^--N, NO_3^--N, etc. according to Standard Methods [30]. The pilot monitoring scheme is described in Table 7.05.

Table 7.05. Parameters of monitoring

Parameters	Influent	Anoxic	Bio-Cord	Influent MBR (After Settling tank)	MBR Effluent	
TCOD	x	x*	x*	x	x*	x
sCOD	x	x	x	x	x	x
BOD_5	x	x	x	x	x	x

* samples filtered through filter paper (20–30 µm)

7.2.5. Calculations

- *Organic loading rate*

$$OLR = \frac{S_0 \times Q}{V} \quad (7.1)$$

Where

ORL organic loading rate; $g.l^{-1}.d^{-1}$

S_0 influent substrate concentration; $g.l^{-1}$

Q flow rate; $l.d^{-1}$

V total volume of system; l

- *Sludge retention time in MBR*

$$SRT = \frac{V \times MLSS}{Q_{WA} \times X + Q_e \times X_e} \quad (7.2)$$

Where

SRT total solids retention time; d

V volume total of the reactor; m^3

MLSS concentration of MLSS maintained in the tanks; $mg.l^{-1}$

X concentration of waste sludge suspended solids; $mg.l^{-1}$

Q_{wa} flow of waste sludge removed from the tanks; $m^3.d^{-1}$

Q_e flow of treated effluent; $m^3.d^{-1}$

X_e microorganism concentration VSS in effluent; $mg.l^{-1}$

The values of Q_e and X_e were ignored in this study.

- *Hydraulic retention time in system*

$$HRT = \frac{V}{Q} \quad (7.3)$$

Where

HRT hydraulic retention time; d^{-1}

V volume total of the reactor; m^3

Q influent flowrate; $m^3.d^{-1}$

- *COD fractions*

COD fractions of influent, after setting and effluent at the end of runs were collected, analyzed and calculated. Analytical methods and calculation have been described in more details in Chapter 3.

7.3. RESULTS AND DISCUSSION

The system started-up during 24 days to get stable (steady state) conditions before the experiments were done to study the effects on COD removal efficiency. The COD removal efficiency reached 63 % in this phase. It was observed that the nonwoven fiber fixed on the outer surface of the membrane could enhance the growth of the active biomass.

7.3.1. Effect of OLR on COD removal efficiency

After 24 days, the influence of organic load on the performance of pilot was investigated. During this phase, the mean OLR was gradually increased from run R2-1 to run R2-4, corresponding to mean OLR of 0.61 kgCOD.m^{-3}.d^{-1} and 1.32 kgCOD.m^{-3}.d^{-1}, respectively. The average influent concentration of COD increased from 881 mgO_2.l^{-1} to 1919 mgO_2.l^{-1} to check the robustness of the system until it was overloaded. The results of the amounts of COD removed from the reactor are presented in Table 7.06 and Figure 7.02.

Table 7.06. Effect of OLR on COD removal efficiency

OLR, kg,m^{-3}.d^{-1}	**COD,** mgO_2.l^{-1}			**Efficiency,** %		
	Influent	After settling	Eff. (after MBR)	Total	After settling	Eff. (after MBR)
R2-1 (0.61 ± 0.06)	881 ± 86	464 ± 70	252 ± 72	71 ± 9	46 ± 11	25 ± 12
R2-2 (0.82 ± 0.08)	1182 ± 116	576 ± 128	259 ± 36	78 ± 4	50 ± 13	27 ± 9
R2-3 (1.10 ± 0.10)	1569 ± 146	730 ± 85	286 ± 38	82 ± 3	53 ± 7	28 ± 4
R2-4 (1.32 ± 0.06)	1919 ± 91	950 ± 130	315 ± 21	84 ± 2	50 ± 7	33 ± 7

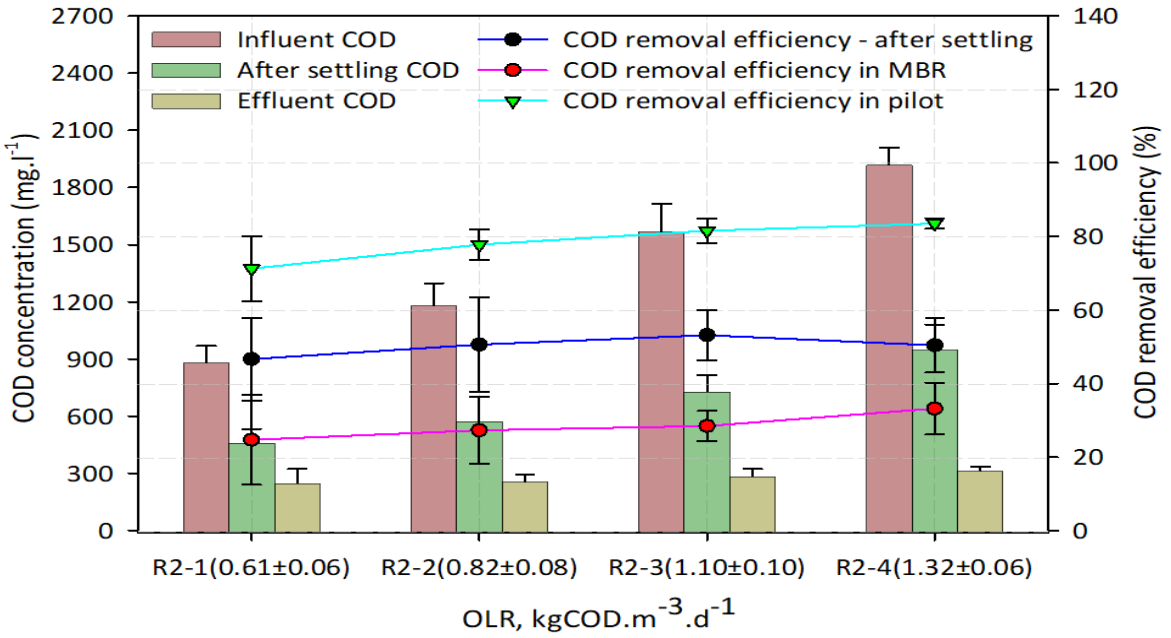


Figure 7.02. Effect of OLR on COD removal efficiency

As shown in Table 7.06 the influent average COD concentration of 881 $mgO_2.l^{-1}$ at R2-1 was reduced to an effluent concentration below 252 $mgO_2.l^{-1}$, with a COD removal percentage of 46 % after settling tank and 25 % after MBR, corresponding to 71 % for the whole pilot. In run R2-2, OLR increased to 0.82 $kgCOD.m^{-3}.d^{-1}$ and COD efficiency also increased in MBR. The results showed the mean efficiency of COD removal reached 78 % for the whole pilot. In which the mean efficiency of COD removal in MBR was 27 %. In run R2-3 (1.1 $kgCOD\ m^{-3}.d^{-1}$), COD removal efficiency achieved 82 %, but in MBR, the COD removal efficiency was 28 %. The COD removal efficiency increased slowly of around 1% compared to R2-2. In run R2-4, OLR increased to 1.32 $kgCOD\ m^{-3}.d^{-1}$. The efficiency increased to 84 % for the whole pilot. The decreasing of COD removal occurred in bio-cord tank (53 % at R2-3 and 50 % at R2-4). However, the efficiency of COD removal increased in MBR and achieved 33 % because membrane could filtrate microorganisms within the system, which were able to degrade more organic matter.

In general, the results demonstrated that the increase in organic loading rate had a positive impact on the removal efficiency of COD in MBR. Besides, as can be seen from Figure 7.02, the effluent COD remained rather stable even after a sudden increase of influent COD, suggesting that the system in this study could operate steadily under the limited shocks of organic loading. According to [31], MBR can operate at high OLR because of dissolved and colloid organic concentration accumulation on the membrane surface. The effect of initial

COD concentration on COD removal performance of biological treatment was studied by Jianlong, W *et al.,* [32] and COD removal efficiency recorded 94 % in MBR system from the former research [33]. The influence of the ORL on fouling has been studied together with the permeate flux [34]. It has been found that a minimum of 3 hours is needed to allow for the adsorption of influent colloidal matter by flocs and can depends on many parameters [35].

To estimate the role of MBR in COD removal, the major COD fractions of influent, after setting and effluent at the end of runs were analyzed and listed in Table 7.07 and Figure 7.03.

Table 7.07. Effect of OLR on COD fractions

Run	COD Fraction	COD Influent		Settled (MBR Influent)		MBR Effluent	
		Average Conc.	Percent	Average Conc.	Percent	Average Conc.	Percent
		$mgO_2.l^{-1}$	%	$mgO_2.l^{-1}$	%	$mgO_2.l^{-1}$	%
R2-1	**Total COD**	**881**	**100**	**464**	**100**	**252**	**100**
	S_S	453	51.40	98	21.14	56	22.23
	S_I	224	25.41	210	45.30	183	72.65
	X_S	108	12.25	86	18.55	9	3.57
	X_I	96	10.94	70	15.01	4	1.55
R2-2	**Total COD**	**1182**	**100**	**576**	**100**	**259**	**100**
	S_S	536	45.36	78	13.54	72	27.81
	S_I	303	25.64	272	47.22	172	66.43
	X_S	205	17.35	165	28.65	12	4.63
	X_I	138	11.65	61	10.59	3	1.12
R2-3	**Total COD**	**1569**	**100**	**730**	**100**	**286**	**100**
	S_S	769	49.01	103	14.11	122	42.68
	S_I	366	23.33	331	45.35	146	51.08
	X_S	241	15.36	211	28.91	11	3.85
	X_I	193	12.30	85	11.63	7	2.39
R2-4	**Total COD**	**1919**	**100**	**950**	**100**	**315**	**100**
	S_S	1030	53.68	345	36.31	81	25.71
	S_I	425	22.15	402	42.31	224	71.11
	X_S	236	12.30	118	12.42	7	2.22
	X_I	228	11.87	85	8.96	3	0.95

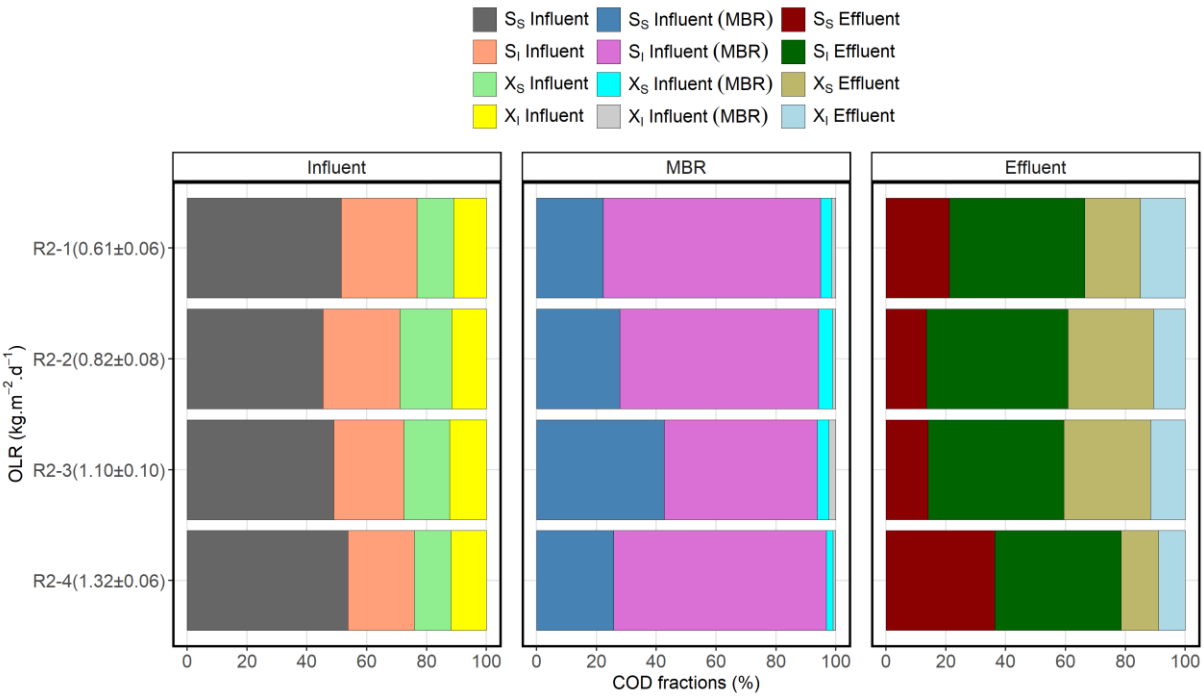


Figure 7.03. COD fractions at various OLRs

As the results, the S_S is biodegradable organic removed by the activated sludge process. The average efficiencies of S_S removal in the system ranged from 84 % to 92 % for this phase. The remaining soluble matter was mainly non-biodegradable organic. Therefore, the measured concentration of S_I in effluent is higher than the other fractions.

There was a significant increase in percentage of S_I on the treated leachate. The fraction of S_I was found ranging from 22.15 % (R2-4) to 25.64 % (R2-2) and from 51.08 % (R2-3) to 72.65 % (R2-1) in influent and effluent, respectively. Membrane separation also enable to adapt and develop the capability of microorganisms to degrade less biodegradable compounds in the leachate.

The results showed that the most concentrated are the S_S and S_I fractions in the leachate. The concentrations of X_I and S_I fraction are definitely lower. The highest mean ratio of total COD of tested inlet was fraction S_S, ranging of 45.36 % - 53.68 %. The mean value of S_I is found from 22.15 % to 25.64 %. The most important item that can be noticed from Table 7.07 is an increase of soluble inert S_I and decrease of biodegradable S_S fraction after the settling reactor. In general, a significant majority of soluble biodegradable organic is removed before flowing into MBR.

The results show that the value of X_S increased due to the biomass produced from biological reactions. This value increased according to increase of OLR and measured from 18.55 % (R2-1) to 28.91 % (R2-3). However, it decreased drastically at R2-4 (12.42 %) due to increased biomass on bio-cord surface in

this run. Alternatively, there was a slight decrease trend of ratio of X_I fraction during experimentation and recorded from 15,01 % to 8.96 %, corresponding to R2-1 to R2-4.

The research has shown that the highest concentrations into MBR are, in descending order, those of fractions S_I, then X_S, S_S and S_I. It can been written that S_I fraction is present in raw leachate and may be formed in process of hydrolysis of fraction X_S [36]. The outlet soluble inert COD concentration decreased significantly, but it was the highest ratio of total influent COD. An important role of cake layer deposited over the membrane surface in the soluble rejection was investigated by Chang *et al*. [37]. According to these authors, solute removals were attributed to the adsorption and/or sieving onto the cake layer.

Figure 7.03 shows the variation of particulate COD concentration before and after MBR in different runs. The low values found in outlet because of the removal of particulate by MBR. To determine the inert COD removal efficiency, the results were calculated and presented in Table 7.08 and Figure 7.04.

Table 7.08. Effect of OLR on removal efficiency of S_I and X_I

OLR kgCOD.m^{-3}.d^{-1}	**Total removal efficiency;** %		**Removal efficiency in MBR;** %	
	S_I	X_I	S_I	X_I
R2-1 (0.61 ± 0.06)	18.30	95.95	12.05	68.15
R2-2 (0.82 ± 0.08)	43.23	97.89	33.00	35.68
R2-3 (1.10 ± 0.10)	60.11	96.47	50.55	40.46
R2-4 (1.32 ± 0.06)	47.29	98.68	41.88	36.08

The results of the statistical analysis (t-test) showed p-values < 0.05, fluctuating in the range of 0.001 to 0.018. Therefore, the differences among the tested conditions (e.g., COD removal efficiencies at different OLRs/HRTs/SRTs) were statistically significant.

In general, the elimination of organic matter by combined bio-cord and MBR system was very efficient, ranging from 95.95 % to 98.68 % for inert particulate and from 18.30 % to 60.11 % for inert soluble, in which the MBR enabled to remove to 50.55 % for S_I at R2-3 and 68.15 % for X_I at R2-1.

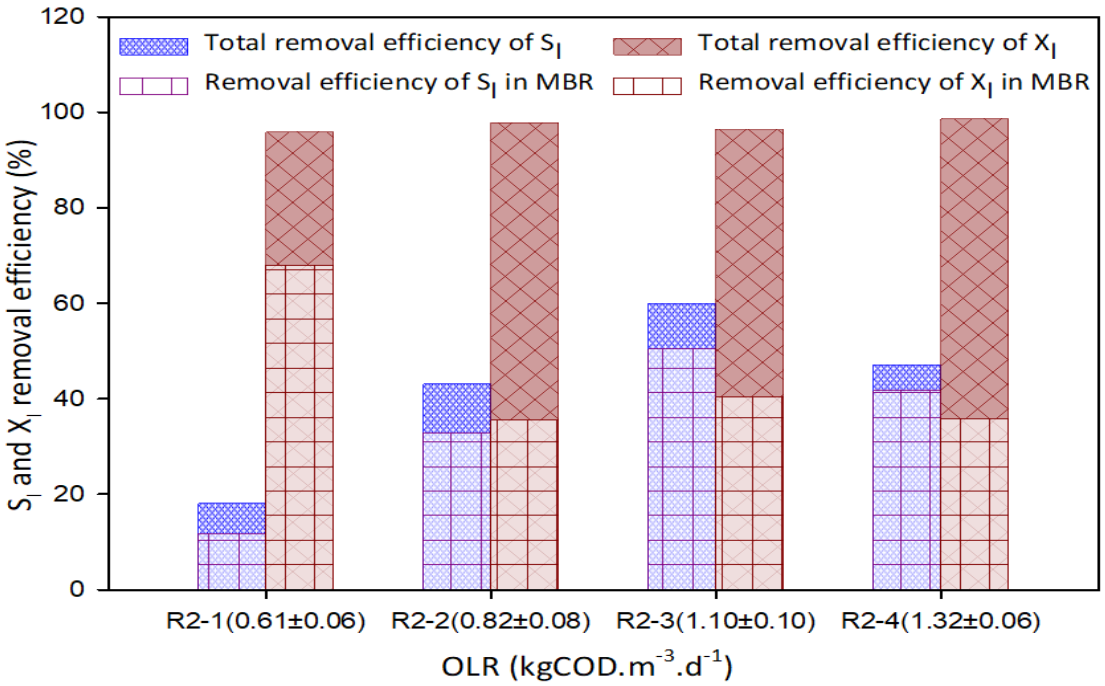


Figure 7.04. S_I and X_I removal efficiency

The results of Figure 7.04 clearly indicated that the MBR can remove inert COD in the system. In general, the removal efficiency of soluble inert organic matter increased with increasing OLR because MBR enabled to retain microorganisms able to perform the degradation of the slow biodegradable organic. Besides MBR supported the prolonged contact time between the organic matter and the biomass [38]. Particularly, the parts including slow biodegradable and particulate inert, the membrane enabled complete filtration of these parts. However, at higher OLR, the S_I removal efficiency increased because of higher adsorption of soluble matter during former runs when increasing S_I influent.

7.3.2. Effect of HRT on COD removal efficiency

The HRT is an important parameter for MBR design. In order to investigate the effect of HRT on the COD removal efficiency, various HRT (20, 15, 10 and 5 h) were selected. Table 7.09 shows that the COD removal efficiency decreased when the HRT decreased gradually from 20 to 5 h. The effect of HRT on the COD removal efficiency is described in Table 7.09 and Figure 7.05.

Table 7.09. Effect of HRT on COD removal efficiency

HRT, h	**COD,** $mgO_2.l^{-1}$			**Efficiency,** %		
	Influent	After settling	Eff. (after MBR)	Total	After settling	Eff. (after MBR)
R3-1(20±2.1)	2010 ± 247	769 ± 150	150 ± 62	93 ± 3	61 ± 11	32 ± 11
R3-2(15±1.5)	2207 ± 130	911 ± 55	264 ± 55	89 ± 2	59 ± 3	29 ± 4
R3-3(10±1.6)	2118 ± 178	876 ± 65	304 ± 37	86 ± 2	58 ± 5	27 ± 5
R3-4(5±1.3)	2254 ± 125	1046 ± 244	542 ± 107	76 ± 5	53 ± 12	23 ± 9

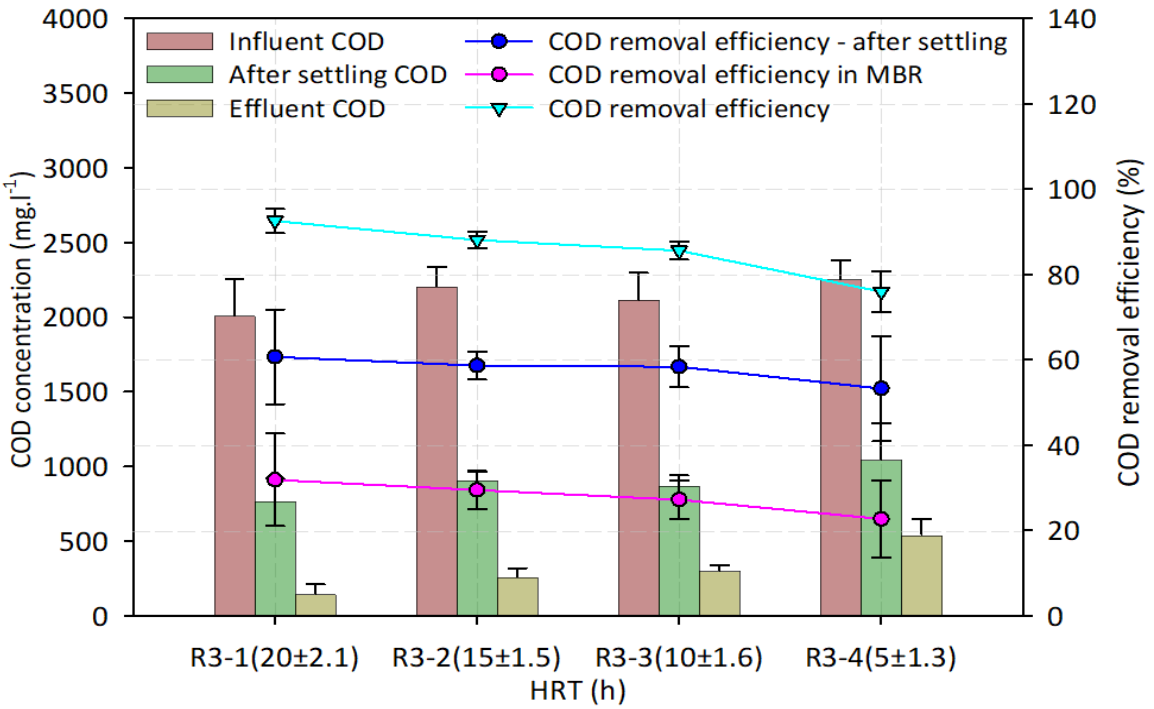


Figure 7.05. Effect of HRT on COD removal efficiency

The above results show that the contribution of HRT becomes important, a decrease of HRT yields a decrease in COD removal efficiency. When HRT declined from 20 h to 15 h, the mean efficiencies of total COD removal in MBR decreased from 32 % to 29 % corresponding to the efficiencies of COD removal on the whole system were 93 % and 89 %, respectively.

The same trends were observed at HRT of 10 h and 5 h. When the HRT at the lowest value was 5 h, the mean value of COD concentration in the effluent was 542 $mgO_2.l^{-1}$, corresponding to 76 % removal. According to Isma *et al*. [39], the lower HRT values can have the result in higher OLRs, which result in reduction of reactor volumes required to achieve a specified removal performance.

The results achieved in Figure 7.05, indicate that longer HRT leads to lower effluent COD concentration, implying a higher activity in the bioreactors and a significant contribution of MBR. However, Nagaoka *et al*. [34], demonstrated that shorter HRT results in the acceleration of membrane fouling. It should be noted that the fouling layer developed in MBR operation is composed of living and dead microbes, biopolymers and inorganic compounds [39].

The results of determining the effect of HRT on the change of COD fractions were also implemented in this phase. The COD fractions in third phase are presented in Table 7.10 and Figure 7.06.

Table 7.10. Effect of HRT on COD fractions

Run	COD Fraction	COD Influent		Settled (MBR Influent)		MBR Effluent	
		Average Conc.	Percent	Average Conc.	Percent	Average Conc.	Percent
		$mgO_2.l^{-1}$	%	$mgO_2.l^{-1}$	%	$mgO_2.l^{-1}$	%
R3-1	**Total COD**	**2010**	**100**	**769**	**100**	**149.8**	**100**
	S_S	841	41.83	152	19.77	22.0	14.69
	S_I	365	18.16	280	36.41	118.0	78.78
	X_S	412	20.49	163	21.20	7.0	4.67
	X_I	392	19.52	174	22.63	2.8	1.85
R3-2	**Total COD**	**2207**	**-**	**911**	**-**	**264.8**	**-**
	S_S	974	44.13	117	12.84	123.0	46.51
	S_I	397	17.99	332	36.45	124	56.89
	X_S	376	17.03	202	22.18	10	3.78
	X_I	460	20.85	260	28.53	7.8	2.82
R3-3	**Total COD**	**2118**	**100**	**876**	**100**	**303.9**	**100**
	S_S	987	46.60	162	18.50	105	34.55
	S_I	406	19.17	374	42.71	186	61.20
	X_S	315	14.87	123	14.05	10	3.29
	X_I	410	19.35	217	24.74	2.9	0.96
R3-4	**Total COD**	**2254**	**100**	**1046**	**100**	**541.7**	**100**
	S_S	1032	45.79	212	20.26	183	33.78
	S_I	563	24.98	482	46.07	345	63.69
	X_S	447	19.83	220	21.03	9	1.66
	X_I	212	9.41	132	12.64	4.7	0.87

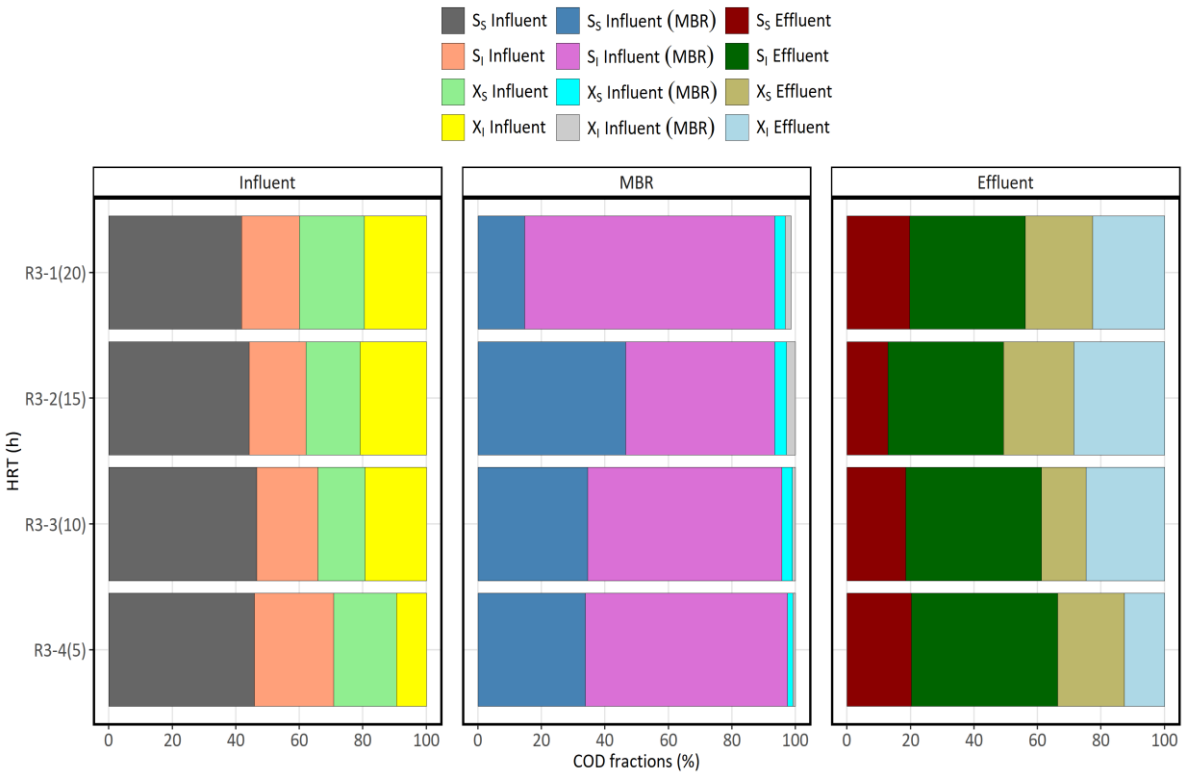


Figure 7.06. Effect of HRT on COD fractions

Figure 7.06 represents the efficiency of COD removal evaluated at four different HRTs. Results show that most of the soluble biodegradable COD was decomposed in bio-cord tank. The removal of S_S was higher than other fractions and amounted to more than 97.38 % at HRT of 20 h, corresponding to an effluent S_S concentration of 22 $mgO_2.l^{-1}$. This indicates a nearly complete removal of biodegradable organic matter in the system. The results calculated from Table 7.11 shows that the efficiencies of S_S removal decreased noticeably. The values achieved at HRT of 15h, 10 h and 5 h were 87.37 %, 89.36 % and 82.27 %, respectively.

The particulate components (X_S and X_I) removal was calculated at 98.78 % whereas 40.60 % of particulate components are removed in MBR at HRT 20 h. Therefore, the MBR gave a significant positive contribution to particulate COD removal. There were not significant changes on the removal efficiency of X_S and X_I in the whole system if HTR decreased. The results calculated from Table 7.11 were 97.91 % at HRT of 15 h, 98.22 % at HRT of 10 h and 97.92 % at HRT of 5 h. The efficiencies of inert COD in system and MBR were calculated and presented in Table 7.11.

Table 7.11. Effect of HRT on removal efficiency of S_I and X_I

HRT, h	**Total removal efficiency** %		**Removal efficiency in MBR** %	
	S_I	X_I	S_I	X_I
R3-1(20±2.1)	67.67	99.29	44.38	43.63
R3-2(15±1.5)	68.77	98.38	52.39	54.83
R3-3(10±1.6)	54.19	99.29	46.31	52.14
R3-4(5±1.3)	38.72	97.78	24.33	60.18

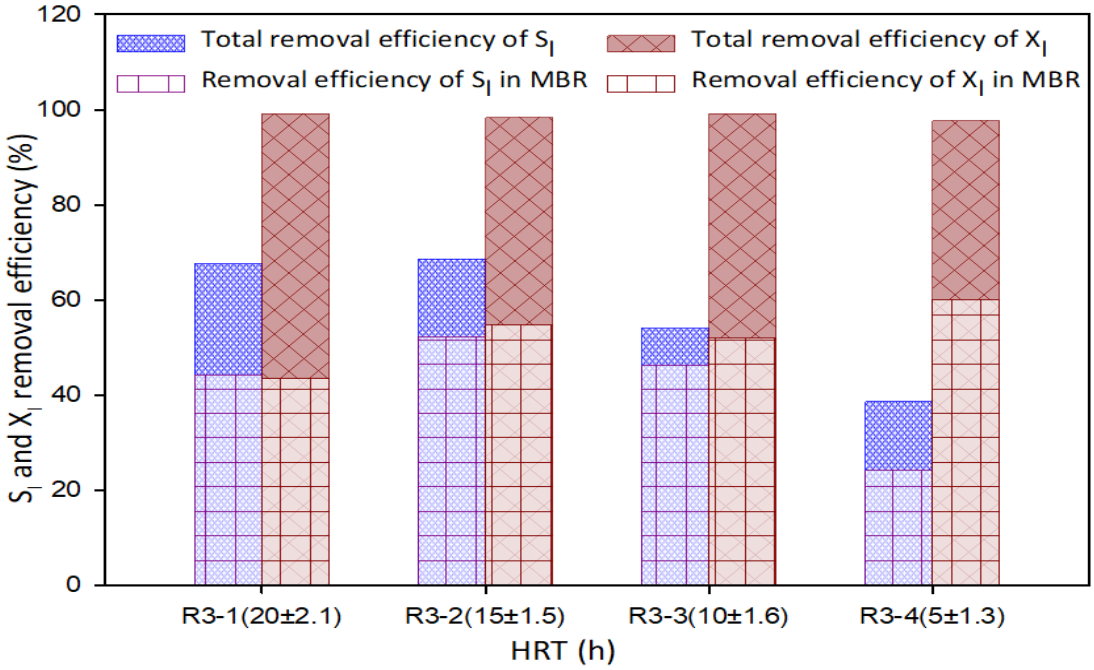


Figure 7.07. Effect of HRT on removal efficiency of S_I and X_I

The membranes performed very well throughout the experimental period, producing good quality of effluent with almost complete rejection of suspended solids. As can be seen in Figure 7.07, the non-biodegradable matter, such as parts of the cell wall remained as particulate within the bioreactor.

The COD fractions pattern of R3-1 were found to be approximately 18.16 % and 19.52 % for S_I and X_I components, respectively in influent (in Table 7.10). A satisfactory X_I removal of over 99.29 % was achieved throughout the experiment at HRT of 20 h, whereas the percentage of X_I removal efficiency was 43.63 % in MBR. There was an increase in the efficiency of particulate matter removal in MBR. As can be seen in Table 7.11, these values at HRT of 15 h and of 10 h were 54.83 % and 52.14 %, respectively. This trend increased slightly at HRT of 5 h with 60.18 % of X_I removed in MBR.

Contrary, the critical component was the inert soluble COD, which passed into the effluent. At HRT of 20h, the S_I concentration of effluent was found 118 $mgO_2.l^{-1}$ with a S_I removal efficiency lower than for X_I (67.67 % for the whole system, with 44.38 % for MBR). The results demonstrated that the role of MBR in S_I removal is very important. At HRT of 15 h, the effluent S_I concentration was 124 $mgO_2.l^{-1}$ with removal efficiency of 52.39 %. However, the efficiency of S_I decreased gradually if HRT decreased.

The t-test analysis this experiment shows that the value of p ranging of ≤ 0.001 – 0.03 (see detail in Appendix), so the results measured are statistically significant.

7.3.3. Effect of SRT on COD removal efficiency

As mentioned previously the durations of the testing periods have been shortened. Thus the reliability of our comments is lower than for the other testing periods.

SRT is one of the most important parameters in the design and operation in biological reactors. In this study, the effect of SRT on COD removal efficiencies in the system and in MBR was investigated. The SRT was changed from 20 days to 80 days and a period of approximately 8 days to 14 days was allowed for the system to achieve steady state at the new SRT. The COD concentrations were measured in the influent, after settling and in the effluent of system.

The results of total COD removal from landfill leachate in this phase are presented in Table 7.12 and Figure 7.08. It can be seen that removal efficiency for the organic matter was quite high under all of the investigated experimental conditions.

The COD removal efficiency increased from the SRT of 20 to 40 days but decreased slightly at SRT of 60 days and reached the lowest value at SRT of 80 days.

Table 7.12. Effect of SRT on COD removal efficiency

SRT, d	**COD,** $mgO_2.l^{-1}$			**Efficiency,** %		
	Influent	After settling	Eff. (after MBR)	Total	After settling	Eff. (after MBR)
R4-1(20)	2639 ± 167	862 ± 157	302 ± 85	88 ± 4	67 ± 6	21 ± 4
R4-2(40)	2465 ± 274	888 ± 229	158 ± 67	94 ± 2	64 ± 10	30 ± 11
R4-3(60)	2741 ± 312	1125 ± 152	221 ± 40	92 ± 2	58 ± 8	34 ± 7
R4-4(80)	2664 ± 215	1078 ± 62	519 ± 113	81 ± 4	59 ± 5	21 ± 8

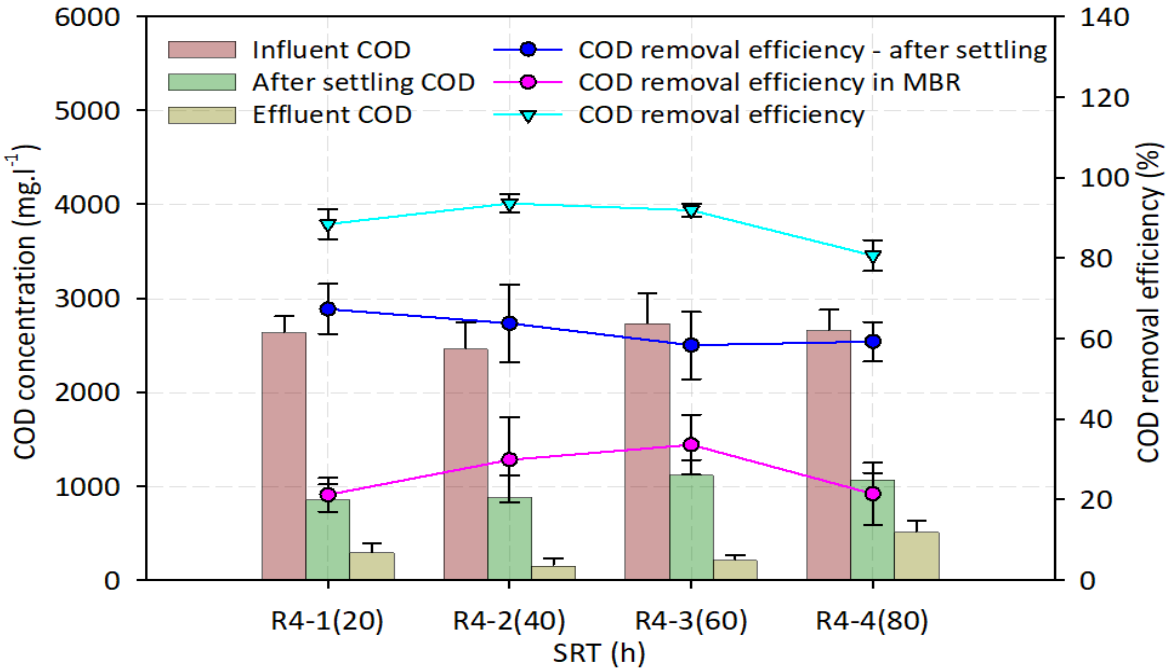


Figure 7.08. Effect of SRT on removal of COD fractions

As the Figure 7.08 indicates, the SRT varied from 20 to 80 days to optimize SRT for treatment of COD in leachate. The average concentration of influent COD ranged from 2465 ± 274 to 2741 ± 312 $mgO_2.l^{-1}$ during this phase. Considering that the best operational parameter was at SRT 40 days, experimental results showed that the average COD removal efficiencies for the whole system was 94 % with 30 % in MBR.

When SRT is higher, the biodegradation of this fraction is improved. Better removal rates can be found for soluble compounds too, since some particulate matter is adsorbed and retained in MBR. Although there was a small reduction of efficiency at SRT 60 days (92%) in the system but it increased slightly in MBR (34%). At SRT of 80 days, the lowest removal efficiency achieved only 81 % corresponding to 21 % in MBR because of self-degradation in bioreactors.

High removal efficiency is usually explained by good microbial activity and efficient removal of suspended solids by membrane filtration. How *et al*. [40], studied the performance of MBR at low SRT (0.25–5 days) and they indicated that modification of sludge morphology, i.e., proliferation of non-flocculating microorganisms, could have a positive impact on removal performance [40]. The research results were t-test analyzed and ranged of ≤ 0.001 – 0.028 ($p < 0.05$). The change of COD fraction at various SRT are presented in Table 7.13 and Figure 7.09.

Table 7.13. Effect of SRT on COD fractions

Run	COD Fractions	**COD Influent**		**Settled** (MBR Influent)		**MBR Effluent**	
		Average Conc.	Percent	Average Conc.	Percent	Average Conc.	Percent
		$mgO_2.l^{-1}$	%	$mgO_2.l^{-1}$	%	$mgO_2.l^{-1}$	%
R4-1	**Total COD**	**2639**	**100**	**862**	**100**	**302.0**	**100**
	S_S	1214	46.00	106	12.30	32.0	10.60
	S_I	675	25.58	568	65.92	175.0	57.95
	X_S	532	20.16	75	8.70	43.0	14.24
	X_I	218	8.27	113	13.07	52.0	17.22
R4-2	**Total COD**	**2465**	**100**	**888**	**100**	**158.4**	**100**
	S_S	1424	57.78	231	26.02	56	35.5
	S_I	518	21.02	425	47.87	86	54.28
	X_S	373	15.13	124	13.97	9	5.68
	X_I	150	6.07	108	12.15	7	4.69
R4-3	**Total COD**	**2741**	**100**	**1125**	**100**	**221.0**	**100**
	S_S	1564	57.07	267	23.74	32.0	14.48
	S_I	594	21.67	489	43.47	169.0	76.47
	X_S	456	16.64	263	23.38	8.0	3.62
	X_I	127	4.62	106	9.41	12.0	5.43
R4-4	**Total COD**	**2664**	**100**	**1078**	**100**	**519**	**100**
	S_S	1352	50.76	326	30.24	212	40.81
	S_I	712	26.73	485	44.99	246	47.36
	X_S	384	14.42	157	14.56	15	2.89
	X_I	216	8.09	110	10.20	46	8.94

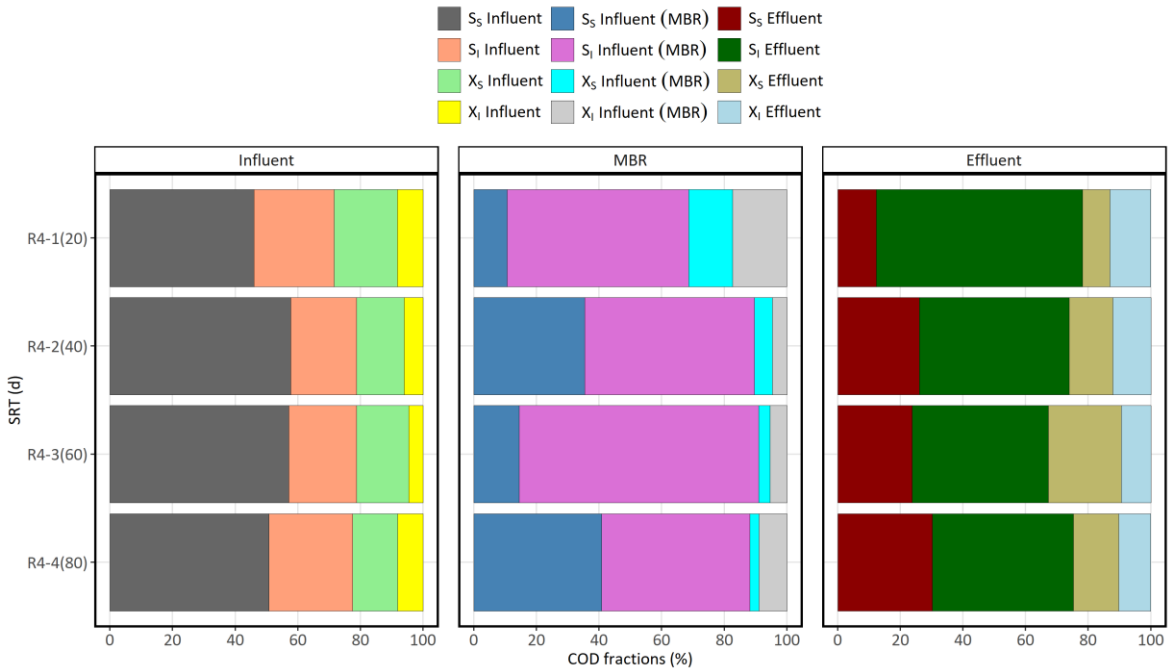


Figure 7.09. Effect of SRT on COD fractions

The results indicated the values of inert COD concentration in the influent, after settling and in the effluent. The average concentration of soluble inert COD changed in ranging of 518 – 712 $mgO_2.l^{-1}$ at R4-2 and R4-4, respectively. The anoxic/bio-cord reactors can remove soluble organic matter, so the S_S in wastewater after settling tank is reduced from 192 to 402 $mgO_2.l^{-1}$, corresponding to SRT of 20 days and 80 days. As was expected, the particulate matter decrease in the effluent for all four SRTs. Hence, the membrane can be considered as an absolute physical barrier for suspended solids. Besides, biological flocculation plays a major role in removing suspended solids and the particulate COD in the activated sludge process. The effect of SRT on the bioflocculation of particulate substrate, in the form of suspended solids and colloidal substrate could be related to the hydrolysis of these particles aggregated into the biofloc by the exocellular polymers excreted by bacteria.

Table 7.14. Effect of SRT on removal efficiency of S_I and X_I

SRT, d	**Total removal efficiency;** %		**Removal efficiency in MBR;** %	
	S_I	X_I	S_I	X_I
R4-1(20)	74.07	76.16	58.22	27.79
R4-2(40)	83.40	95.03	65.44	67.14
R4-3(60)	71.55	90.53	53.87	74.07
R4-4(80)	65.45	78.45	33.57	29.48

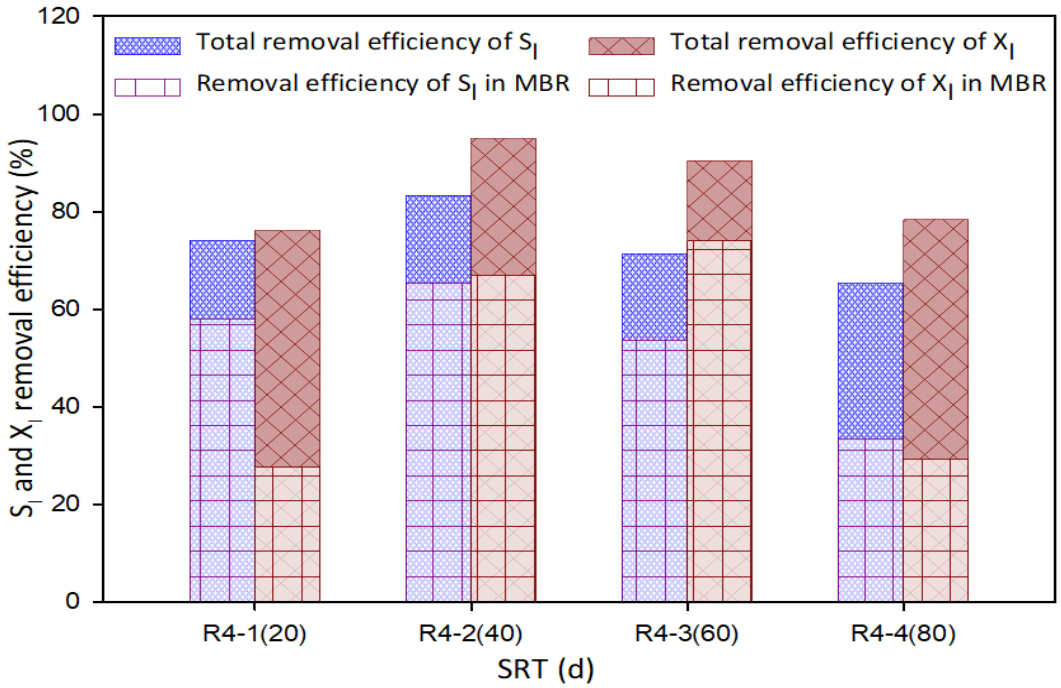


Figure 7.10. S_I and X_I removal efficiency

From the results of our study, there was an efficiency increase of inert COD removal from SRT of 20 days to 40 days. The efficiency of inert COD removal reached the highest point of 83.40 % for S_I and 95.03 % for X_I at SRT of 40 days. This value decreased slightly to 71.55 % at SRT of 60 days for S_I and 90.53 % for X_I. However, the removal efficiency of S_I with trend reduced markedly at SRT of 80 days because of the amount of non-biodegradable soluble COD produced in the reactors.

Le-Clech *et al.* [41] showed that the high sludge ages can increase of non-biodegradable compounds due to the amount of old and dead microorganisms they contain [42]. The inert COD components may be of influent origin or they may be generated as residual microbial products by means of growth or decay-associated processes [43].

The effect of SRT on COD removal was investigated in previous work by Jimenez *et al.*, [44]. Hasar *et al.* [45], indicated MBR could not retain all of non-biodegradable compounds and that most microorganisms can produce non-biodegradable soluble compounds.

7.4. CONCLUSIONS

The MBR can retain a part of the soluble refractory COD, certainly due to adsorption, precipitation, bioaccumulation, degradation, etc. Biodegradable compounds are also produced by microorganisms in the MBR. The results demonstrated also that increases in OLR had a positive impact on MBR. The

optimum OLR was found to be 1.32 kg $COD.m^{-3}.d^{-1}$ for the MBR but 1.1 kg $COD.m^{-3}.d^{-1}$ for anoxic/bio-cord used in this study. A high HRT is necessary for contacting between microorganisms and organic matter. The highest efficiency of COD removal was found at HRT of 20 h and the optimum SRT was 40 days for COD sanitary landfill leachate removal.

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GENERAL CONCLUSIONS AND PERSPECTIVES

A. GENERAL CONCLUSIONS

Here are the main steps of the thesis and the milestones of this research on leachates treatment processes, especially Membrane BioReactors (MBR) that could be useful to manage the problem of leachate from sanitary landfills which is an important issue for Vietnam.

In chapter 1 (31p) Introduction (Landfill leachate are defined and characterized). Leachate are liquids effluents flowing from “sanitary” landfills sites. Some landfill sites are built in accordance with international standards, some are just places where solid wastes were dumped. The quantity but also the quality (composition) of leachates depends on various factors such as level of life of the inhabitants, climate (temperature and pluviometry), type of waste, management of the landfill cells, and the age of the landfill. A very important consequence of those features is that the quantity but also the quality of leachates will change drastically with time. It is absolutely necessary to take this into account to avoid the many failures of leachate treatment plants in the world. The design of a leachate treatment plant cannot be done as for domestic wastewaters.

In this first chapter the properties, main components, evolution are described. The composition of leachates from various landfill sites in Vietnam are described. They have characteristics quite similar to southeast Asia similar sites: high quantity of leachate due to the climate (pluviometry), rapidly high concentration of pollutants (due to the climate). But high concentrations are associated with large flowrates, generating large fluxes requiring efficient treatment systems. One of the more challenging pollutants is nitrogen (reduced form N-NH_3), because the concentration is high, usually higher than 1 g /L and decreases slowly, much more slowly than biodegradable organic matter. Thus the research will have to focus on an efficient treatment of nitrogen. In the last part of the chapter various leachate treatment systems are presented and compared.

Chapter 2 title is called Literature review (44 p). It presents the knowledge about the main issues of leachate treatment which is COD removal and even more Nitrogen removal and the biological processes involved. The Biological Membrane Bioreactor technology which has been selected for our research is also presented. It is considered that to get an optimal treatment it will be

important to try to optimize various parts of the reactor (mainly the aerobic and anaerobic parts) and the exchange fluxes between those parts, it will be important to use tools such as mathematical models that were developed more and more in the last decades. Here the various types of models are presented to explain which type of models should be chosen to develop an appropriate model to describe the behavior of MBR treating leachates.

Chapter 3: Landfill leachate characterization for simulation of biological treatment with activated sludge model no.1 (ASM1) and no.3 (ASM3) (20p). It is a very important part of the work, even if not always well understood by non-specialists. The mathematical models of wastewater treatment plants are rather sophisticated and use many state variables most of them expressed in COD (Chemical Oxygen Demand). Thus when we take a sample of liquid in the bioreactor, its total COD is the summation of the COD of various components (state variables), biodegradable COD and non-biodegradable COD, solid or dissolved, but also the various biomasses: heterotrophs but also autotrophs... Thus in order to use the model correctly it is necessary to perform the fractionation of the "substrates" (COD, N-NH_4^+...) which means to quantify the various fractions of those substrates by appropriate methods. In this chapter various methods of fractionation were used (and compared) to characterize complex wastewater such as leachates. Probably this was the first time this procedure was made for leachates, but this is a crucial milestone in order to apply the ASM type models correctly. In addition, the effects of seasons (dry and rainy seasons) were studied on the composition of leachates and statistical differences were observed for various components including N forms, which will add to the difficulties to design and operate wastewater treatment plants focusing on nitrogen removal. For the main components average values and standard deviations were provided for both seasons and compared with values from literature, (most of those values for industrial wastewaters, not leachates as mentioned).

Chapter 4: Characterization of sludge for stoichiometric and kinetic parameters for model no.1 and no.3 for leachate treatment (54p). As mentioned previously it is necessary to avoid the many errors made in the use of mathematical models in the case of leachates treatment it is necessary to adopt a correct procedure. This procedure has mainly two important steps : to characterize (fractionation) the substrate that is not an usual domestic wastewater. This has been done in the chapter 3 where we have seen that leachates are very different from domestic wastewater, their components have usually much higher concentrations but have also seasonal variation and a tendency to decrease

with time but not at the same speed for all the components. This is a part of the difficulties in designing efficient systems that have to adapt to those changes. The other step is about the parameters (stoichiometric and kinetic parameters of the model). The equations of the ASM (Activated Sludge Models) can be considered but the values indicated in the books describing those models are for domestic wastewaters and cannot be used as such. In our case the values of the main (most sensitive) parameters have to be fitted with "activated sludge" treating leachates and on samples of Vietnamese leachates. This means that tests on pilot plants using the same technology that expected at full scale have to be designed (here MBR-Membrane Bioreactors) and operated to collect the data needed to fit the parameters of the models. This is a huge work that represents the core of the thesis. A first type of MBR bioreactor was designed and operated to get many operational data on which the fitting of the parameters was done in accordance with some of the methods described in literature. It can be noticed that for most of those parameters the quality of the fitting was good.

Chapter 5: Modelling of partial nitrification and denitrification (37p). As mentioned in the first chapter the main issue of leachate treatment is the treatment of high nitrogen fluxes. As the C/N ratio of leachate is much lower than for domestic wastewater, especially for old leachate, it is important to manage the process very carefully as there is not enough biodegradable carbon to realize the classical denitrification process. In order to save energy (for nitrification) and an external carbon source (for denitrification) the partial nitrification process is very promising. It is also a required preliminary step if the anamox process is the objective, which is not our case. As the partial nitrification process stops at the level NO_2^- instead that NO_3^- it requires less oxygen (thus energy) and carbon source to reduce the oxidized form of nitrogen into N_2. But the formation of NO_2^- is an intermediate step in the classical nitrification process, it means that stopping at this nonstable point is challenging and requires a very good choice of the needed operating conditions. The usual ASM3 mathematical model had to be modified in order to distinguish the two steps of the nitrification process. Again, this implied to make tests in a modified lab scale pilot to obtain experimental data and fit the new stoichiometric and kinetic parameters associated with the partial nitrification and denitrification metabolisms. The effects of various factors such as DO, pH,alkalinity, were studied carefully providing comprehensive data and parameters to control the partial nitrification step. The conclusions of the chapter provide the ranges of main operating factors to obtain an efficient

partial nitrification step followed by denitrification. Again, the numerous experimental data collected for this task are presented in various figures.

Chapter 6: Nitrogen Removal in landfill leachate treatment with membrane bioreactor in Vietnam (29p). In this chapter the principles and tests done in chapter 5 were applied to check the validity and performances of those concepts in real conditions. To realize this, another type of MBR had to be designed and constructed in which the volumes of aerobic, anoxic and "membrane" parts where modified, as well as the possible ranges of flowrates between the various compartments of the bioreactors. This offers many possibilities of treatment schemes which means also numerous experimental data. Taking into account the previous results some promising scenarios have been tested. Very good results could be obtained at rather high nitrogen loads, demonstrating the coherence of the research procedure.

Chapter 7: Refractory COD removal in landfill leachate treatment with membrane bioreactor in Vietnam (23p). Even if, for the reasons that have been explained in chapter 1, we focused on the efficiency of nitrogen treatment of leachate by the MBR technology, it remains that the removal of refractory COD can be an issue in some countries. The concept of refractory COD in the context of leachate treatment has to be explained. As presented in chapter 1 the organic carbon substrate is expressed as COD (particulate or dissolved) but also biodegradable or not. In this last case it can be called refractory COD (refractory to biological treatment). But we have to keep in mind the response time of the treatment system. So, if we speak of refractory compounds it is for a treatment time of some hours, maybe some days. But if we consider geological times then most of those "refractory" systems would be degraded by geobiological processes. So what can occur to refractory compounds from leachates in a MBR reactor where the residence time of the sludge (sludge age) is higher and sometimes much higher than for conventional Activated Sludge Systems? We have to consider that sanitary landfill sites are anaerobic bioreactors degrading solids and liquid organic wastes where the solids stay several years. It is not surprising that the leachates that have been in contact with those wastes represent like a picture of the decomposition state of the landfill. Thus, the leachates, especially leachates issued from old landfill sites, contain compounds generated from a long residence time in an anaerobic reactor, especially "refractory" compounds such as fulvic and humic like substances. In some countries the question arise to define if waters containing those substances have to be treated, as those compounds are present in natural waters especially flowing from wooded areas and even explain the names of

those rivers as the yellow river in China, the red river in Vietnam, and also in Malaysia,...Those molecules are natural polymers quite difficult to degrade by biological methods even if some species of mushrooms can degrade some humic acids in very specific conditions. In Vietnam the authorities decided that the leachates treatment plants have to match the same effluent standards than the effluents of industrial wastewater treatment plants, which means that some specific physical and (photo)-chemical methods could be required. Those methods are rather sophisticated and costly. Thus, it is important to check if some removal of the "refractory" COD can be obtained in the WWTP mostly focusing on nitrogen removal. Chapter 7 responds to this question; tests were made in various operating conditions on the MBR described previously on which detailed mass balances on total COD were performed. From those tests it can be concluded that part of the "refractory" COD can be removed in the MBR system treating leachates. Various mechanisms are presented that could explain this observation including the fact that the membranes keep the biomass in the reactor increasing sludge age which could help slow-growing strains and maybe humic acid degrading microorganisms to develop. The work did not try to determine what was the active mechanism in the present case, but we know that a good choice of the operating conditions can get up to 83,4% for S_I and 95,03% for X_I removal of "refractory" COD and approach the Vietnamese standards which would reduce the costs of the treatment.

B. PERSPECTIVE

This study still has some limitations and perspectives to improve to get at higher of results and practical application.

- Compare the result of COD and nitrogen fractions of non domestic wastewater by direct measurements including respirometric methods.
- Use various methods to determine stoichiometric and kinetic parameters of ASM (Activated Sludge Models) type and compare the results obtained. Even if it was not possible determine all stoichiometric and kinetic parameters. We focused on the most sensitive ones.
- Studying the effect of different external sources on denitrification.
- Trans-membrane pressure variations (linked to the energy consumption) could also be compared under different aerated volumes and various operational conditions.
- Even with rather short SRT (Sludge Retention Time), good results were obtained.

During this research various papers have been published:

- Dieu T T N., J-L. Vasel., and Canh T T., *Landfill leachate characterization for simulation of biological treatment with activated sludge model No.1 (ASM1) and No.3 (ASM3)*. APLAS Tokyo 2018 The 10th Asia-Pacific Landfill Symposium - The tenth anniversary - 24 – 26, November 2018: p. 264-273.
- Dieu T T N., J-L. Vasel., and Canh T T., *Removal of COD and nitrogen in leachate by using combined anoxic/attached-growth bioreactor - Sardinia 2017* - Sixteenth International Waste Management and Landfill Symposium. (232).
- Dieu T T N., J-L. Vasel., and Canh T T., *Removal of COD and nitrogen in leachate in a MBR by using attached growth combined with anoxic process* - Sardinia 2015 - Sixteenth International Waste Management and Landfill Symposium (537).
- Dieu T T N., J-L. Vasel., and Canh T T., *Removal of nitrogen in leachate by using a membrane bioreactor combined with anoxic process* - APLAS Ho Chi Minh 2014 - The 8th Asian-Pacific Landfill Symposium, at Ho Chi Minh City, Vietnam (29).
- Dieu T T N., J-L. Vasel., and Canh T T., *Characteristics of landfill leachate and COD fraction at landfills in Ho Chi Minh City, Vietnam* - APLAS Ho Chi Minh 2014 - The 8th Asian-Pacific Landfill Symposium, at Ho Chi Minh City, Vietnam (28).