REMOVAL OF PHENOLIC COMPOUNDS AND PHTHALIC ACID ESTERS IN TWO-STAGE MEMBRANE BIOREACTOR TREATING MUNICIPAL SOLID WASTE LANDFILL LEACHATE

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Thesis Title	REMOVAL OF PHENOLIC COMPOUNDS AND PHTHALIC ACID ESTERS IN TWO-STAGE MEMBRANE BIOREACTOR TREATING MUNICIPAL SOLID WASTE LANDFILL LEACHATE
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วรินธร บุญยะโรจน์ : การบำบัดสารฟีโนลิกและพธาลิกเอซิดเอสเตอร์ในน้ำชะมูลฝอยชุมชนด้วยระบบถังปฏิกรณ์เยื่อกรองเมมเบรนแบบสองขั้น. (REMOVAL OF PHENOLIC COMPOUNDS AND PHTHALIC ACID ESTERS IN TWO-STAGE MEMBRANE BIOREACTOR TREATING MUNICIPAL SOLID WASTE LANDFILL LEACHATE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร.ชาติ เจียมไชยศรี, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : รศ.ดร.วิไล เจียมไชยศรี, 135 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพในการบำบัดน้ำชะมูลฝอยชุมชนจากสถานที่กำจัดมูลฝอยชุมชนในประเทศไทยด้วยระบบบำบัดน้ำเสียถังปฏิกรณ์เยื่อกรองเมมเบรนแบบสองขั้น จากการศึกษาพบว่า สารกลุ่มฟีโนลิกที่พบในน้ำชะมูลฝอย ได้แก่ บีเอชที บีพีเอ โดยมีความเข้มข้นของสารมากกว่า 100 ไมโครกรัมต่อลิตร และสารกลุ่มพธาลิกเอซิดเอสเตอร์ ได้แก่ ดีเอ็มพี ดีอีพี ดีเอ็นโอพี ดีอีเอชพี เป็นต้น โดยระบบถังปฏิกรณ์เยื่อกรองเมมเบรนแบบสองขั้นสามารถบำบัดสารกลุ่มดังกล่าวได้ร้อยละ 77- 96 ซึ่งประสิทธิภาพในการบำบัดสารต่างๆ ขึ้นอยู่กับสมบัติทางเคมีของสารนั้นๆ เป็นหลัก โดยเมื่อทำการศึกษาในห้องปฏิบัติการโดยเติมสารที่ความเข้มข้นเริ่มต้น 1,000 ไมโครกรัมต่อลิตร พบว่า สาร บีพีเอ บีเอชที และ ดีอีเอชพี สามารถบำบัดได้ร้อยละ 65, 70 และ 72 ตามลำดับ โดยถังแอโรบิกมีบทบาทสำคัญในการบำบัดสารกลุ่มดังกล่าว ด้วยกลไกการบำบัดหลักสามารถแบ่งได้เป็น 3 กลไกคือ การดูดซับที่ผิวของตะกอน การย่อยสลายด้วยจุลชีพในระบบ และการกรองผ่านเมมเบรน นอกจากนี้พบว่า การเพิ่มจำนวนไนตริไฟอิงแบคทีเรียในระบบมีส่วนช่วยให้การบำบัดสารบีพีเอ และบีเอชทีสูงขึ้นเมื่อเปรียบเทียบกับตะกอนที่ไม่มีการเพิ่มจำนวนไนตริไฟอิงแบคทีเรีย แต่ในทางกลับกันสารดีอีเอชพีสามารถบำบัดได้ด้วยกลไกการดูดซับที่ผิวของตะกอน และการกรองผ่านเมมเบรน สำหรับการศึกษาการมีชีวิตรอดของสิ่งมีชีวิตในน้ำที่ผ่านการบำบัดได้ทำการทดสอบการทนต่อความเป็นพิษต่อสิ่งมีชีวิต ทั้งนี้ระบบบำบัดน้ำเสียถังปฏิกรณ์เยื่อกรองเมมเบรนแบบสองขั้น มีส่วนสำคัญอย่างมากในการช่วยลดสารอินทรีย์ที่มีความเป็นพิษต่อสิ่งมีชีวิต

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ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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VARINTHORN BOONYAROJ: REMOVAL OF PHENOLIC COMPOUNDS AND PHTHALIC ACID ESTERS IN TWO-STAGE MEMBRANE BIOREACTOR TREATING MUNICIPAL SOLID WASTE LANDFILL LEACHATE. ADVISOR: ASSOC. PROF. CHART CHIEMCHAISRI, D.Eng., CO-ADVISOR: ASSOC. PROF. WILAI CHIEMCHAISRI, D. Tech. Sc., 135 pp.

Two-stage membrane bioreactor (MBR) was applied to the treatment of leachate from a solid waste disposal site in Thailand. Priority micropollutants in landfill leachate were phenolics 4-methyl-2,6-di-tert-butylphenol (BHT), bisphenol A (BPA) at higher concentrations above 100 μgL^{-1}, PAEs i.e. dimethyl phthalate (DMP), diethyl phthalate (DEP), di-n-butyl phthalate, di-n-octyl phthalate (DnOP), and di(2-ethylhexyl) phthalate (DEHP). It was found that MBR could remove phenolic compounds and PAEs by 77–96%.The removal efficiency of micropollutants are depends on their log K_{OW} as well as their speciation behaviour. In laboratory scale experiment, the removals of BPA, BHT, and DEHP were 65%, 70%, 72%, respectively at initial concentration of 1,000 $\mu g.L^{-1}$. The removal mechanisms can be classified into adsorption, biodegradation, and rejection of micropollutants during membrane filtration. The removals of BPA, BHT, and DEHP were found improve using enriched nitrifying sludge in comparison non-enriched condition. In contrast, DEHP was mainly adsorbed on the sludge surface and subsequently rejection by membrane filtration. The biotoxicity of treated water was reduced during MBR treatment as revealed by acute toxicity and genotoxicity tests.

Field of Study: Environmental Management Student's Signature .....
Academic Year: 2012 Advisor's Signature .....
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LIST OF ABBREVIATIONS

PAEs	Phthalic acid esters
PAHs	polycyclic aromatic hydrocarbons
AOB	ammonia oxidizing bacteria
An	Anthracene
Nap	Naphthalene
ATU	Allylthiourea
BHT	4-Methyl-2,6-di-tert-butylphenol
BPA	Bisphenol A
DEHP	Bis(2-ethylhexyl)phthalate
DOP	Di-n-octyl phthalate
BBP	Benzyl butyl Phthalate
DEP	Diethylphthalate
DnBP	Di-n-butylphthalate
DO	dissolved oxygen
FISH	fluorescence in situ hybridization
MBR	membrane bioreactor
mgL^{-1}	milligram per liter
μgL^{-1}	microgram per liter
MLSS	mixed-liquor suspended solid
mM	millimolar
ng	nanogram
NOB	nitrite oxidizing bacteria
SVI	sludge volume index
VSS	volatile suspended solid
WWTP	wastewater treatment plant
wt	weight

CHAPTER I

INTRODUCTION

1.1 Backgrounds

Municipal solid waste usually contains hazardous substances originated from household/industrial chemicals such as paints, vehicle maintenance products, mercury-containing waste, batteries, personal care products, and pharmaceuticals products. Hazardous substances in household waste are not strictly controlled under hazardous waste regulations in many countries including the U.S. (Slack *et al.*, 2005), and these substances does affect landfill leachate composition. Landfill leachate constitutes complex mixture and containing large amount of trace organic contaminants. While most of trace organic contaminants have not been regulated, there is an urgent need to remove them from landfill leachate during treatment in order to reduce environmental contamination. For young leachate, biological techniques can yield a reasonable removal of COD, NH_3-N, and heavy metals. On the other hands, physico-chemical treatment has found to be suitable as a refining step for biologically treated stabilized leachate (less biodegradable).

The pollutants in landfill leachate can be divided into four groups as follows; dissolved organic matter, inorganic macro components, heavy metal, and xenobiotic organic compounds (XOCs). Xenobiotic organic compounds usually present in relatively low concentrations (usually less than 1 mgL^{-1} of individual compounds). These compounds include plasticizer, phenolics, pesticides, aliphatic and aromatic hydrocarbons, pharmaceuticals, polyaromatic hydrocarbons, chlorinated/non-chlorinated hydrocarbons, alkylphenol ethoxylates, and alkyl phosphates. Many countries reported that these compounds were detected at a higher concentration in leachate from municipal solid waste landfill sites. (Paxéus, 2000; Kjeldsen *et al*., 2002; Baun *et al*., 2004).

The membrane bioreactors (MBRs) technology seems to be a good alternative for wastewater with high organic and nutrient loadings. Some researchers demonstrated that the complicated molecular structure cannot be removed using conventional treatment method but however can be removed by MBRs (Kimura *et al.*, 2005). Two-stage MBR utilizing inclined-plate separator in first stage anoxic reactor followed by second stage aerobic submerged MBR was developed for the treatment of wastewater containing both carbonaceous and nitrogenous pollutants at satisfactorily and stable efficiency (Xing *et al.*, 2006). This system was also applied for the treatment of organic and nitrogen compounds in partially stabilized leachate. Several advantages over conventional treatment methods include high treatment efficiency, low operating cost, and operational simplicity without excess sludge withdrawal (Chiemchaisri *et al.*, 2009).

Several studies indicated that the MBRs had potential to remove endocrine disrupting chemicals (EDCs), but the removal mechanism of EDCs was still not clear due to the different physical and chemical properties of EDCs. The removal of toxic organic contaminants by MBRs technology was widely applied in the treatment of landfill leachate. MBRs were capable of removing phenolic compounds such as bisphenol A (BPA), and nonylphenol (Wintgens *et al.* 2003; Chen *et al.*, 2008; XU *et al.* 2008). Some researchers found that the amount of pharmaceutical active compounds (PhACs) such as sulfamethoxazole, propyphenazone, vitamin C-synthesis, diacetone sorbose (DAS), and diacetone alpha-keto-gulonic acid (DAG) were decreased by MBRs (Nghiem *et al.*, 2009; Clara *et al.*, 2005). Tran *et al.* (2009) suggested that the removal efficiency of pharmaceuticals compounds in biological wastewater treatment not only depend on the chemical characteristics of target pharmaceuticals but also relies on the microbial species especially nitrifiers as well. Consequently, enrichment of nitrifier microorganisms is significant factor on the biodegradation of xenobiotic organic compounds. However, the recalcitrant compound removal in landfill leachate by MBR is still not clear and requires further investigation.

1.2 Objectives

The objectives of this study are:

1.2.1 To investigate the performance of two-stage membrane bioreactor focused on phenolic compounds and phthalic acid esters (PAEs) removal, simultaneously with carbonaceous and nitrogenous substances removal from municipal landfill leachate.

1.2.2 To evaluate the mechanisms responsible for removing phenolic compounds and phthalic acid esters (PAEs) in membrane bioreactor.

1.2.3 To evaluate biosafety of treated water using bioassay techniques.

1.3 Scope of the Study

1.3.1 The MBR experiments were conducted in a pilot scale at Nonthaburi solid waste disposal site, Thailand and a laboratory scale at Department of Environmental Engineering, Kasetsart University. The MBRs were operated at ambient temperature without sludge wastage except for sampling purposes.

1.3.2 Landfill leachate used in the experiment were collected from Nonthaburi solid waste disposal site in Thailand. MBR influent leachate, a mixture of fresh and stabilized leachate, obtained on-site from garbage trucks and a leachate storage pond was prepared at approximately 1:10 mixing ratio (v/v).

1.3.3 In pilot-scale MBR experiment, ten major micro-pollutants found in landfill leachate are investigated. They were naphthalene (Nap), anthracene (An), 4-Methyl-2,6-di-tert-butylphenol (BHT), Bisphenol A (BPA), Dimethylphthalate (DMP), Diethylphthalate (DEP), Di-n-butylphthalate (DnBP), Benzyl butyl Phthalate (BBP), Bis (2-ethylhexyl) phthalate (DEHP), and Di-n-octyl phthalate (DOP). For laboratory-scale experiment, the study focused on BPA, BHT, and DEHP at higher

concentrations of 1,000 μgL^{-1} by adding supplement BPA, BHT, and DEHP into the original leachate samples.

1.3.4 Organism species used in the bio-toxicity experiment are Nile Tilapia (*Oreochromis niloticus*) and Common Carp (*Cyprinus carpio*).

1.4 Expected Outcomes

1.4.1 The performance of two-stage membrane bioreactor focused on phenolic compounds and phthalic acid esters (PAEs), simultaneously with carbonaceous and nitrogenous substances removal in municipal solid waste landfill leachate are revealed.

1.4.2 The mechanisms of membrane bioreactor sludge and their activities affecting phenolic compounds and phthalic acid esters (PAEs) removal are understood.

1.4.3 Biosafety of treated water using bioassay technique could be verified using local fish species.

1.5 Structure of the Dissertation

This dissertation consists of the following chapters.

Chapter 1 contains an introduction of this research.

Chapter 2 contains the review of literature relating to the fundamental and basic knowledge of landfill leachate and the treatment of landfill leachate.

Chapter 3 describes all of the experimental set-up used in this research.

Chapter 4 presents the performance of pilot-scale and laboratory-scale MBR.

Chapter 5 presents the mechanisms of phenolic compounds, and phthalic acid esters removal in MBR.

Chapter 6 presents the bio-toxicity study of treated water from the MBR.

Chapter 7 presents the conclusion obtained from the study.

CHAPTER II

BACKGROUND AND LITERATURE REVIEW

2.1 Landfill Leachate

2.1.1 Formation of Landfill Leachate

After the initial period of waste placement in a landfill, microbial processes proceed under anoxic conditions. Hydrolytic and fermentation processes are solubilize the waste components during the acid fermentation phase producing organic acids, alcohols, ammonia, carbon dioxide and other low molecular weight compounds. This process occurs at low pH (typically around at pH 5) and is increased by the presence of moisture in the landfill. The methane fermentation stage occurs after several months.

Methanogenic leachate is neutral pH and contains moderate organic compounds that are not easily degradable and are fermented to yield methane, carbon dioxide and other gaseous end products (Harmsen, 1983). Stabilization of landfill waste proceeds in five sequential and distinct phases. The rate and characteristics of leachate produced and biogas generated from a landfill vary from one phase to another and reflect the processing taking place inside the landfill. The phases of leachate formation are in these following five steps (Figure 2.1).

Initial adjustment phase (Phase I) - Moisture accumulation takes place to support an active microbial community for biochemical decomposition after placement of solid waste within landfills.

Transition phase (Phase II) - Moisture content exceeds field capacity of the waste and leachate is formed. Transformation from an aerobic to anaerobic environment creates reducing condition. And as a result, the primary electron

acceptor shifts from oxygen to nitrates and sulfates with the displacement of oxygen into carbon dioxide. Concentration of COD and volatile organic acids (VOA) becomes significant by the end of this phase.

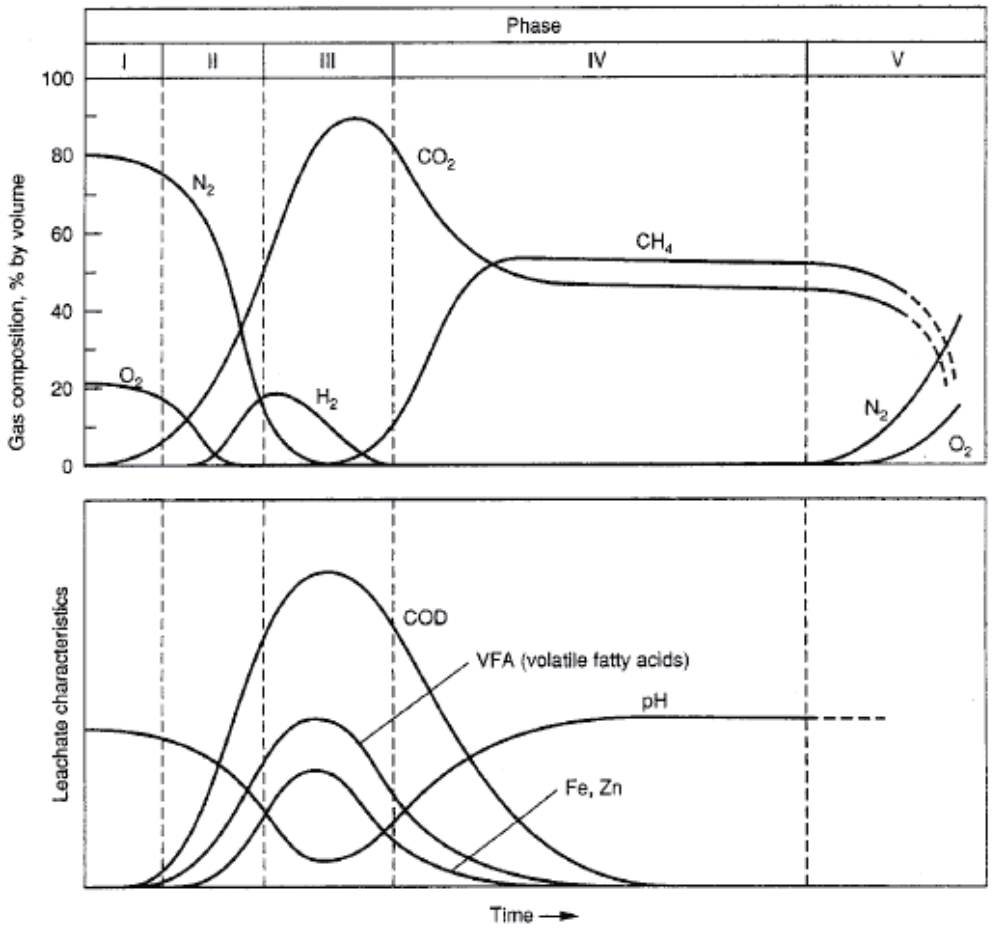


Figure 2.1 Generalized phases in generation landfill gases (I-initial adjustment, II-transition phase; III-acid phase; IV-methane fermentation; V-maturation phase) (Tchobanoglous *et al*., 1993).

Acid formation phase (Phase III) - In this phase, solubilization of solid waste, followed by the microbial conversion of biodegradable organic content, results in the production of intermediate VOAs at high concentrations. pH value was decreased with a concomitant mobilization and possible complexation of metal species. Biomass

growth associated with acidogenic bacteria and rapid consumption of substrate and nutrients takes place.

Methane fermentation phase (Phase IV) - Methanogenic bacteria consumes intermediate acids and converts into methane and carbondioxide. Increment in pH are controlled by the bicarbonate buffering system, takes place, which supports the growth of methanogenic bacteria. As nutrients continue to be consumed, complexation and precipitation of heavy metal proceed. The organic strength of the leachate decreases with the increase in gas production.

Maturation phase (Phase V) - In this phase, gas production drops and biological activity shifts to relative dormancy. As a result, leachate strength remains constant and at much lower concentration than earlier phases. More microbial resistant organic materials may be slowly converted with a possible production of humic-like substances capable of complexing with heavy metal, and remobilizing them.

2.1.2 Characteristics of Landfill Leachate

The landfill leachate has fluctuating composition of organic, inorganic and heavy metal components with time and it is difficult to treat. The main factors affecting the quality of leachate are landfill age, quality and quantity of solid waste, biological and chemical processes occurring in the landfill, and amount of precipitation and percolation. Leachate contains high strength of organic matter and ammonia discharged is simulated algae growth through nutrient enrichment, deplete dissolved oxygen, and cause toxic effects in the surrounding water environment. Landfill design and operation have a major impact and influence on the leachate generation.

Basic factors influencing the leachate generation are annual precipitation, runoff, infiltration, evaporation, transpiration, freezing, mean ambient temperature, waste composition, waste density, initial moisture content and depth of the landfill.

The quantities of leachate depend on rainwater percolation through wastes, biochemical processes in waste's cells, the inherent water content of wastes and its degree of compaction into the landfill tip.

The production is generally greater whenever the waste is less compacted because the compaction reduces the filtration rate. The quantity of leachates can be calculated by considering water balance in the landfill. The water can be categorized into three groups: water entering the landfill, water used by chemical and biological reactions in the landfill and water leaving the landfill (Tchobanoglous *et al.*, 1993). The design of the landfill, the climate, the nature of the waste, and the operation of the landfill influence the water balance in the landfill (Lema *et al.*, 1988).

The information of leachate characteristics is necessary for the control of landfill function, the design, and the operating condition of leachate treatment systems. The characteristics of leachate were classified into four categories: physical characteristics, inorganic chemicals, organic chemicals, and toxicity. Moreover, most of the waste in municipal landfill in Asia (except Japan, Singapore) was organic waste about 60-90% and contains plastic about 3-18%. The composition of landfill leachate at a particular time depends on many factors, which include types and composition of waste, rate of water infiltration, landfill design, operation and age, method by which it was emplaced, moisture content, climate and degree of stabilization.

The variation of leachate composition may also result from environmental conditions at the time of sampling, during storage and precision of reported results may be affected to some extent by substances causing interference in standard analytical method (Robinson and Maris, 1985). For instance, landfill age has a significant effect on leachate composition especially organics and ammonia concentrations. Generally, leachate produced in younger landfills is characterized by the presence of substantial amounts of volatile acids, as a result of the acid phase of fermentation. In mature landfills, the majority of organics in leachate were humic and fulvic-like fraction. The organic concentration as COD of fresh leachate is above 5000 mgL^{-1} and nitrogen content is below 400 mg NL^{-1}. In contrast, the ammonia

concentration of stabilized leachate was high (> 400 mg NL^{-1}) and recalcitrant compounds and biodegradable organic fraction was low (BOD_5/COD = 0.1) as shown in Table 2.1. Moreover, fluctuation of other indexes such as phosphorus, chlorides, calcium, magnesium, sulfate, dissolved solids, heavy metals, and aromatic hydrocarbons (benzene, toluene, ethylbenzene and xylene) depended rather on the seasonal variations than the landfill ages (Kulikowska and Klimiuk, 2006).

Table 2.1 Landfill leachate classification vs. age and treatments. (modified from Héctor *et al.*, 2004)

	Landfill Leachate		
	Fresh	**Moderate**	**Stabilized**
Age (year)	<5	5-10	>10
pH	<6.5	6.5-7.5	>7.5
COD (gL^{-1})	>10	4-10	<4
BOD_5/COD	0.5-1	0.1-0.5	<0.1
TOC/COD	<0.3	0.3-0.5	>0.5
NH_3-N (mgL^{-1})	<400	N.A.	>400
Heavy metals (mgL^{-1})	>2	<2	<2
Organic compound	80% VFA	5-30% VFA+HA+FA	HA+FA
Degree of biodegradability	High	Medium	Low

N.A.= Not Applicable; VFA=Volatile fat Acids; HA=Humic acid; FA=Fulvic acids

Pollutants in landfill leachate can be divided into four groups as follows: dissolved organic matter, inorganic macro-components, heavy metal, and xenobiotic organic compounds (XOCs). Other compounds may be found in leachate from landfills including borate, sulfide, arsenate, selenate, barium, lithium, mercury, and cobalt. However, in general, these compounds are found in very low concentrations and are only of secondary importance. Leachate composition may also be characterized by different toxicological tests, which provide indirect information on the content of pollutants that may be harmful to organisms (Kjeldsen *et al*, 2002).

2.1.2.1 Dissolved Organic Matters

Dissolved organic matters are used to describe the content of dissolved organic matter in leachate; Total Organic Carbon (TOC), Chemical Oxygen Demand (COD), and Biochemical Oxygen Demand (BOD), volatile fatty acids (that accumulate during the acid phase of the waste stabilization) and including more refractory compound such as fulvic and humic-like compounds. Dissolved organic matter can affect leachate composition in relation to other constituents through the complexing properties of the high-molecular-weight component of the dissolved organic matter. At the most general level, a low BOD/COD ratio suggests a leachate with low concentrations of volatile fatty acids and relatively higher amounts of humic and fulvic-like compounds.

2.1.2.2 Inorganic Macrocomponents

The inorganic macro components in landfill leachate are calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}), potassium (K^{+}), ammonium (NH_4^{+}), iron (Fe^{2+}), manganese (Mn^{2+}), nitrite (NO_2^{-}), nitrate (NO_3^{-}), sulfate (SO_4^{2-}), hydrogen carbonate (HCO_3^{-}) and chloride (Cl^{-}).

The concentrations of some inorganic macrocomponents in leachate depend, as in the case of the dissolved organic matter, on the stabilization of the landfill. Table 2.2 shows the cations calcium, magnesium, iron, and manganese are lower in methanogenic phase leachate due to a higher pH (enhancing sorption and precipitation) and lower dissolved organic matter content, which may form complexes with the cations. Sulfate concentrations are also lower in the methanogenic phase due to microbial reduction of sulfate to sulfide.

2.1.2.3 Heavy Metals

Cadmium (Cd^{2+}), chromium (Cr^{3+}), copper (Cu^{2+}), lead (Pb^{2+}),nickel (Ni^{2+}), and zinc (Zn^{2+}) and other compounds found in landfill leachate; for example,

borate, sulfide, arsenate, selenate, barium, lithium, mercury, and cobalt. In general, these compounds are found in very low concentrations (Kjeldsen *et al*., 2002)

Table 2.2 Landfill leachate composition in terms of various parameter with different Acidogenic and Methanogenic phase (Ehrig, 1983).

Parameter	Unit	Acidogenic Phase		Methanogenic Phase		Average
		Average	Range	Average	Range	
pH		6.1	4.5-7.5	8	7.5-9	
BOD_5	mgL^{-1}	13,000	4,000-40,000	180	20-550	
COD	mgL^{-1}	22,000	6,000-60,000	3,000	500-4,500	
BOD_5/COD	mgL^{-1}	0.58		0.06		
Sulfate	mgL^{-1}	500	70-1,750	80	10-420	
Calcium	mgL^{-1}	1,200	10-2,500	60	20-600	
Magnesium	mgL^{-1}	470	50-1,150	180	40-350	
Iron	mgL^{-1}	780	20-2,100	15	3-280	
Manganese	mgL^{-1}	25	0.3-65	0.7	0.03-45	
Zinc	mgL^{-1}	5	0.1-120	0.6	0.03-4	
Ammonia-Nitrogen	mgL^{-1}					740
Chloride	mgL^{-1}					2,120
Potassium	mgL^{-1}					1,085
Sodium	mgL^{-1}					1,340
Total phosphorus	mgL^{-1}					6
Cadmium	mgL^{-1}					0.005
Chromium	mgL^{-1}					0.28
Cobalt	mgL^{-1}					0.05
Copper	mgL^{-1}					0.065
Lead	mgL^{-1}					0.09
Nikel	mgL^{-1}					0.17

2.1.2.4 Xenobiotic Organic Compounds (XOCs)

Xenobiotic organic compounds found in landfill leachate are plasticizer, phenolics, pesticides, aliphatic and aromatic hydrocarbons, pharmaceuticals, polyaromatic hydrocarbons, chlorinated/non-chlorinated

hydrocarbons, alkylphenol ethoxylates, and alkyl phosphates (Paxéus, 2000; Kjeldsen *et al*., 2002; Baun *et al*., 2004). XOCs originating from household or industrial chemicals present in relatively low concentrations (usually less than 1 mgL^{-1} of individual compounds).

The most frequently found XOCs are the monoaromatic hydrocarbons (benzene, toluene, ethylbenzene, and xylenes) and halogenated hydrocarbons such as tetrachloroethylene and trichloroethylene. However, in general, these compounds have been found in low concentration. Sulfonates include some of the surfactants used in laundry detergents and shower soaps. Riediker *et al.* (2000) analyzed for four Swiss landfills for sulfonates. The results showed that benzenesulfonates (*p*-toluenesulfonate) and naphthalenesulfonates (naphtalene-1-sulfonate, naphtalene-2-sulfonate, naphtalene-1,5-disulfonate, naphtalene-1,6-disulfonate, naphtalene-2,7-disulfonate, and 2-aminonaphtalene-2,7-disulfonate) were present in landfill leachate, at a concentration in range of a few μgL^{-1} up to 11 mgL^{-1}.

Phthalate plasticizers are also micro-pollutants of concern; the conventional treatment of landfill leachate is not able to eliminate them. In this way, plasticizers are persist in the environment and contaminate superficial and groundwater. The most frequently observed phthalates are di-(2-hexylethyl)phthalate (DEHP), di-ethyl-phthalate (DEP), di-n-butyl-phthalate (DBP), and butyl-benzyl-phthalate (BBP) (Ejlertsson *et al., 1999).* Table 2.3 shows the various compounds were found in landfill leachate at low concentrations in ranged from ngL^{-1} to μgL^{-1} levels.

Table 2.3 Xenobiotic organic compounds (XOCs) concentration range in landfill leachate.

XOCs Concentration range	Concentration range (μgL^{-1})
Aromatic	
Benzene	0.2-1630
Toluene	1-12300
Xylenes	0.8-3500
Phenol	
Phenol	0.6-1200
Ethylphenols	<300
Cresols	1-2100
Bisphenol A	200-240
Alkylphenols	
Nonylphenol	6.3-7
Nonylphenolmonocarboxylate	0.5-3
Halogenated hydrocarbons	
Chlorobenzene	0.1-110
1,1,1-Trichloroethane	0.01-3810
Trans-1,2-Dichloroethylene	1.6-6582
Phthalates	
Diethyl phthalate	0.1-660
Di-(2-ethylhexyl) phthalate	0.6-235.9
Di-n-butylphthalate	0.1-70
Phthalic acid	2-14,000
Aromatic sulfonates	
Naphtlene-1-sulfonate	506-616
Naphtalene-2-sulfonate	1143-1188
Naphtalene-1,6-disulfonate	366-397
Naphtalene-2,7-disulfonate	129-145
2-aminonapphtalene-4,8-disulfonate	73-109
p-toluenesulfonate	704-1084

2.1.3 Biotoxicity Testing

Traditional risk assessment of landfill leachate, based on the presence of specific compounds, such as ammonia, metals or organic compounds identified in the leachate, ignores possible effects of interactions between chemicals in complex mixtures. Biotests, on the other hand, integrate the biological effects of all leachate components. The assessment of toxicity in landfill leachate has been done by using different living organisms. Table 2.4 shows example of biological species are used in acute toxicity tests for leachate toxicity. Fish, crustaceans, and luminescent bacteria are most frequently used methods. Table 2.5 shows the several studies of genotoxicity using comet assay in biological species in landfill leachate assessment. The chronic effects of landfill leachates have not received much attention, but of the different long-term effects genotoxicity has been studied in some details.

Table 2.4 Biological species used in acute toxicity tests for leachate toxicity assessment.

Species Test	LC $(EC)_{50}$ [%]	References
Vibrio *fisheri* (luminescent bacteria)	EC50-48h =11.3 – 15	Silva *et al.,* 2004
	EC50-48h = 1.3 – 6.1	Baun *et al.,* 2004
Artemia salina (Water flea)	LC50-48 h= 11.9 – 25.6	Silva *et al.,* 2004
	LC50-48h = 39.93	Olivero-Verbel *et al.,* 2008
Daphnia similis (Water flea)	EC50-48h = 2.0 - 2.3	Silva *et al.,* 2004
*Brachydanio rerio (*Zebra fish*)*	LC50-48 h= 2.2	Silva *et al.,* 2004
	LC50-48h = 2.2 – 5.7	Sisinno *et al.* 2000
*Sarotherodon mossambicus (*Tilapia*)*	LC50-96h = 1.4 - 12	Wong et al., 1989
*Oryzias latipes (*Medaka fish*)*	LC50-48h = 19.2 - 50	Osaki *et al.* 2006
Clarias Gariepinus	LC50-96h = 36.6	Oshode *et al.,* 2008
*Cyprinus Carpio (*Common Carp*)*	LC50-96h = 1.13-3.82	Jaffar *et al,* 2009

Table 2.5 The several studies of genotoxicity using comet assay.

Species Test	Observation	References
Carassius auratus (Gold fish)	Erythrocytes from peripheral blood and gill cells	Deguchi *et al.*,2007
Triticum aestivum	Meristematic cells from the roots	Guangke *et al.*,2008
A.Cepa		Bortolotto *et al.*, 2009
G. Brasiliensis	Meristematic cells from the roots Erythrocytes from peripheral blood	Bortolotto *et al.*, 2009

2.2 Treatment of Landfill Leachate

2.2.1 Conventional and Advanced Leachate Treatment Process

The conventional landfill leachate treatments were classified into three major groups: (a) leachate transfer: recycling and combined treatment with domestic sewage; (b) biodegradation: aerobic and anaerobic processes; and (c) chemical and physical methods: chemical oxidation, adsorption, chemical precipitation, coagulation/flocculation, sedimentation/flotation, and air stripping as shown in Table 2.6. The conventional biological treatment process and physico-chemical process are considered as the most appropriate technologies for manipulation and management of high strength effluents like landfill leachates. The treatment of fresh leachate, biological techniques can yield a reasonable treatment performance with respect to COD, NH_3-N and heavy metals. In contrast, the treatment of stabilized leachate, physico-chemical processes have been found to be an appropriate process in order to organic recalcitrant substances removal. The integrated chemical–physical–biological process ameliorates the drawbacks of individual processes contributing to a higher efficiency of the overall treatment process. However, the continuous hardening of the discharge standards in most countries and the landfill ages with more stabilized landfill leachates, the conventional treatment processes (biological or physico-chemical) are not sufficient anymore to reach the level of purification required to fully reduce the negative impact of landfill leachates on the environment. It implies that

new treatment alternatives species must be proposed. Therefore, more effective treatments based on membrane technology has emerged as a viable treatment alternative to comply and pending water quality regulations in most countries.

Table 2.6 The treatment efficiency vs. ages of landfill leachate (Renoua *et al*., 2008).

Process	**Ages of landfill leachate**			**Residues**
	Fresh	Moderate	Stabilized	
Transfer				
Combined treatment with domestic sewage	Good	Fair	Poor	Excess biomass
Recycling	Good	Fair	Poor	-
Lagooning	Good	Fair	Poor	Sludge
Physico-chemical				
Coagulation/flocculation	Poor	Fair	Fair	Sludge
Chemical precipitation	Poor	Fair	Poor	Sludge
Adsorption	Poor	Fair	Good	-
Oxidation	Poor	Fair	Fair	Residual O_3
Stripping	Poor	Fair	Fair	Air-NH_3 mixture
Biological				
Aerobic processes	Good	Fair	Poor	Excess biomass
Anarobic processes	Good	Fair	Poor	Excess biomass
Membrane bioreactor	Good	Fair	Fair	Excess biomass
Membrane filtration				
Ultrafiltration	Fair	Fair	Fair	Concentrate
Nanofiltration	Good	Good	Good	Concentrate
Reverse osmosis	Good	Good	Good	Concentrate

2.2.2 Microorganisms in Leachate Treatment System

Biological treatment process has been applied to treatment landfill leachate because of its high treatment efficiency and economical. The species of microorganisms in wastewater treatment system depends on the composition of wastewater, process design and operational. Moreover, different from physical

treatment and chemical treatment, biological treatment depends on the metabolism of microbial communities to remove organic and inorganic substances, or transform to nontoxic forms. Several functional groups of microorganisms in the MBR and activated sludge system are presented in Table 2.7.

Table 2.7 The commonly reported microorganisms in MBR and activated sludge system (Nielsen *et al.*, 2009).

Functional group	populations
Floc forming bacteria (saprophytes): primarily facultative heterotrophs, soil, and aquatic genera	*Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Arthrobacter, Zooglea, Acinetobacter, Citromonas, Bacillus*
Nitrifying bacteria: ammonia oxidizing bacteria (AOB), and nitrite oxidizing bacteria (NOB)	*Nitrosomonas, Nitrobacter, Nitrospirillum*
Predators: protozoa, rotifers, and nematodes	*Vorticella, Aspicidica, Paramedium*
Nuisance bacteria and eucaryotes: bulking, foaming, and overgrazing	*Nocardia, Microthrix, Sphaerotilus*, fungi, snails
Specialty populations	Phosphate accumulating organisms (PAO), algae (lagoons)
Other	Viruses (bacteriophage), yeast, pathogens (*Campylobacter, E. coli, Salmonella, Giardia, Cryptosporidium*)

In both an MBR and activated sludge system, the dominant group of autotrophic bacteria has been shown to be β-subclass Proteobacteria; all characterized ammonia oxidizers (i.e. nitrifiers) belong to this group. These bacteria are dominant in an MBR which higher proportion of other bacteria. The long sludge age condition was influence to the Proteobacteria-β population in the system. *Nitrosomonas spp*. and *Nitrosospira spp*. are the autotrophic ammonia-oxidising bacteria found in activated sludge, and *Nitrobacter spp*., and *Nitrospira spp*. are the nitrite-oxidizing bacteria, and it is thus between these groups that the nitrification process is carried out. The ammonia-oxidizing bacteria are MBR system specific. Nitrifying bacteria are known to be slow-growing bacteria. The long sludge retention time is available to an MBR system which highly advantageous for nitrification process (Judd, 2011).

Nitrification is a key process of nitrogen removal in wastewater treatment system. Nitrogen in leachate occurs in many forms such as organic nitrogen (protein and urea) and ammonia nitrogen. The removal of nitrogen can be removed by two processes that include assimilation, and nitrification-denitrification. Microorganisms assimilate ammonia nitrogen, and can be transformed in cell biomass. For nitrification-denitrification, the nitrogen removal can be classified into two-steps. In the first step, the nitrification process is performed by a group of autotrophic microorganisms. The principal mechanism for the nitrogen removal takes place by two reactions under aerobic condition, one is ammonia oxidized to nitrite by *Nitrosomonas spp.* and the other is nitrite to nitrate by *Nitrobacter spp*. In second step, the denitrification occurs under anoxic condition which nitrate converted to nitrogen gas. The nitrogen transformations in biological treatment process are illustrated in Figure 2.2 (Metcaft and Eddy, 2003). The biological leachate treatment processes can be achieved with combined advanced treatment process that effective for removing organic pollutants.

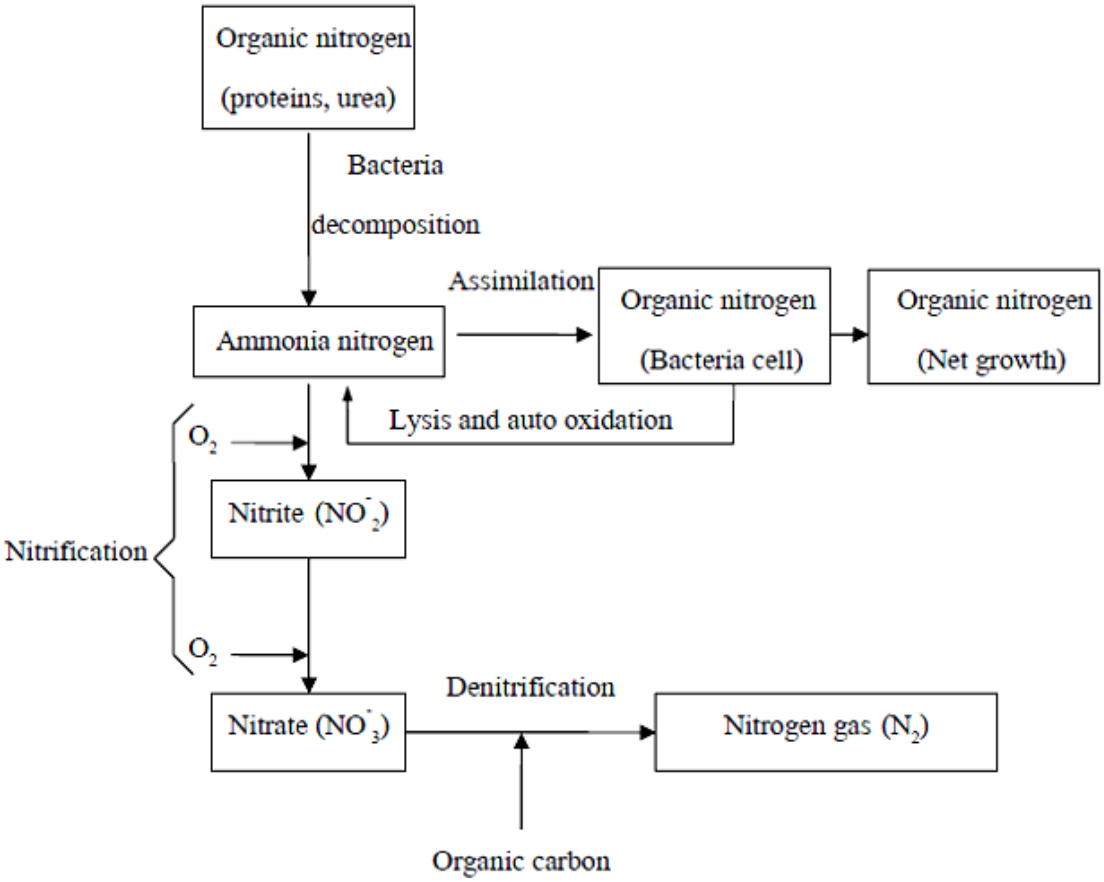


Figure 2.2 Nitrogen transformations in biological treatment process (Metcaft and Eddy, 2003).

2.2.3 Membrane Technology Applied to Wastewater Treatment

Membrane is defined as a thin film separation of two or more components from a fluid flow. The advantages of membrane technology include continuous separation, low energy consumption, easy combination with other existing technique, easy up-scaling, and no additives used. The membrane filtration is classified into four narrower ranges based on particle size as follows: microfiltration, ultrafiltration, nanofiltration, and reverse osmosis.

Microfiltration is the coarsest size of the membrane filtration classes. The microfiltration is applied to separate suspended particles from dissolved substances.

Microfiltration membranes are classified by pore diameter cut-off (PDCO) which has the diameter of the particle in the range of 0.1 to 10 μm (Cheryan, 1998).

Ultrafiltration is used for separation of large macromolecules such as proteins, starches, and all types of microorganism. Ultrafiltration membranes are classified by molecular weight cut-off (MWCO) which is defined as the molecular weight of the smallest molecules. Ultrafiltration covers particle and molecular weight in range of 1,000 to 500,000 daltons (Cheryan, 1998).

Nanofiltration membrane retains solute molecules ranging from 100 to 1,000 daltons in molecular weight. The membranes are classified by molecular weight cut-off like ultrafiltration membranes or by percentage sodium chloride rejection like reverse osmosis membranes. Nanofiltration can remove the contaminants as small as 0.001 μm (Taylor and Jacobs, 1996).

Reverse osmosis involves the tightest membranes which are capable of separating even the smallest solute molecules or particles with diameter of as small as 0.0001 μm (Taylor and Jacobs, 1996). Reverse osmosis membranes are classified by percentage rejection of sodium chloride in an aqueous solution under specified conditions, and range from 95 to 99.5%.

Membranes can be manufactured by a wide variety of materials which include inorganic and organic membranes. The inorganic membranes have better chemical, mechanical and thermal stabilities, but contain the disadvantages of being very fragile and are more expensive than the organic membranes. The organic membranes are widely used in water and wastewater applications because they are more flexible and can be put into a compact module with very high surface area. The organic membranes can be made from cellulose, and all synthetic polymers which have relatively good chemical, mechanical and thermal stability tendencies, and also provide the membranes with better antifouling properties through the use of hydrophilic polymers (Cheryan, 1998).

The membrane can be devided into two operation filtration such as dead-end filtration and cross-flow filtration. The filtration of coarse particles down to several micrometers is achieved by the conventional dead-end filtration. Particles retained by the filter in dead-end filtration build up with time as a cake layer resulting in an increase of resistance to filtration. This requires frequent cleaning or replacement of filters. For cross-flow filtration, a fluid (feed) stream runs tangential to a membrane, establishing a pressure differential across the membrane. These causes some of the particles to pass through the membrane. Remaining particles continue to flow across the membrane. In contrast to the dead-end filtration, the use of tangential flow prevents thicker particles from building up cake layer by a high velocity gradient near the membrane surface, which assists in reducing the fouling and polarization effects.

2.2.4 Membrane Bioreactor Systems (MBRs)

MBRs combine biological treatment ptocess and membrane to effectively reduce pollutants in wastewaters, and are similar to convention activated sludge systems (CASs) with the exception that the biomass (i.e., microorganisms) which is responsible for removing the pollutants of concern are retained within the bioreactor component of the system using membranes rather than secondary clarifiers. The early designs of MBRs simply replaced the secondary clarifier of CASs with an external membrane. However, most MBRs are now designed with the membrane submerged within the bioreactor component of the system.

The treatment performances of external and submerged MBRs are similar; however, the capital and operating cost for submerged membrane systems are typically much lower than those for external systems, and comparable to those for CASs (Jefferson *et al.,* 2000). The typical MBR configuration for biological nutrient removal is shown in Figure 2.3. A summary of the advantages and disadvantages of MBRs system is presented in Table 2.8.

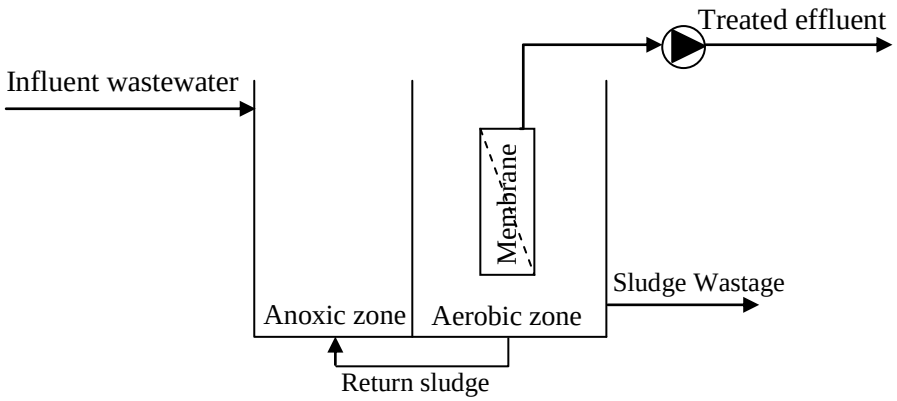


Figure 2.3 Typical MBR configuration for biological nutrient removal.

The bioreactor components of MBRs are designed to remove biologically degradable contaminants. The easily biodegradable substrates have a high maximum rate of substrate consumption and are therefore rapidly consumed. Less biodegradable substrates can also be consumed rapidly by maintaining a high biomass concentration in the bioreactor.

Table 2.8 Summary of the advantages and disadvantages of MBRs system.

Advantages	Disadvantages
- Produces high-quality effluent with reuse potential (i.e., The final effluent does not contain suspended matter)	- Long-term history of operation is not available
- System can be configured for enhanced nutrient removal	- Membrane configurations are not standardized and most are proprietary
- Process performance not affected by variations in influent load and quality	- Membranes typically must be replaced every 7 to 10 years
- Process performance not affected by settling characteristics of biomass	- Membrane replacements relatively expensive
- Relatively small footprint (i.e., primary clarifiers are typically not required)	- Pilot testing required to design full-scale system
- Relatively low sludge production	
- Relatively easy to automate (i.e., reduced system complexity and improved operability)	

For MBRs, the membrane component of the system retains the biomass within the reactor. Since membrane can retain virtually all of the biomass, relatively high biomass concentrations can be achieved in MBRs, resulting in relatively high substrate consumption rates, and therefore, relatively small bioreactor volume. Membrane biological processes can be applied to the treatment of municipal industrial wastewaters. MBRs used for water de-pollution are based on the association of the bioreactor in which a culture of microorganisms degrades the polluting compound, and a membrane filtration separator.

Their main advantage is the ability to keep all biomasses in the bioreactor, thus removing all suspended solids from the treated water and disinfecting is according to the membrane cut-off threshold. Separation of HRT and SRT means a better control of biological activity. The MBR systems have been operated in long SRT (5-50 days) with high MLSS in the reactor and low F/M ratio (Visvanathan *et al*., 2000). The MBRs have greater nitrification potential. Typical biomass concentrations in MBRs, measured as mixed liquor suspended solids (MLSS), are ranged from 8 to 12 gL^{-1}. At biomass concentrations greater than approximately 12 gL^{-1}, oxygen transfered in the bioreactor component of the system is limiting and inhibiting the growth of the aerobic biomass. High biomass concentrations can also negatively affect the permeate flux through the membrane component of MBRs. However, bioreactors can also be designed to promote the growth of biomass that is capable of removing such as nutrients nitrogen (Metcalf and Eddy, 2003).

The main propose of MBRs is to improve the efficiency of the biological process step such that high-quality effluent is obtained. To optimize the MBR process, many parameters have to be considered. These include solid concentrations, sludge age, and hydraulic retention time (HRT) in the biological step as well as the rate of permeate flux, material costs and the energy cost of the membrane separation (Visvanathan *et al.*, 2000). The treatment and disposal of the sludge wastage also need to be concerned. These are the following factors affecting performance of the MBR system including transmembrane pressure, crossflow velocity, effects of aeration on flux, membrane fouling, mode of operation, module arrangement, and viscosity.

Landfill leachate constitutes a very complex mixture, which may contain large amount of trace organic contaminants. While most of trace organic contaminants have not regulated, there is an urgent need to remove them during landfill leachate treatment in order to better protect the environment. Treating young leachate, biological techniques can yield a reasonable treatment performance with respect to COD, NH_3-N, and heavy metals. On the other hand, physio-chemical treatment has been found to be suitable as a refining step for biologically treated stabilization (less biodegradable), in order to remove organic refractory substances. In recent years, with the continuous hardening of the discharge standards in most countries and ageing of landfill sites with more stabilized leachates, convention treatments (biological or physico-chemical) are not sufficient to reach the level of purification needed to fully reduce negative impact of landfill leachate on the environment. The membrane bioreactors (MBRs) technology seems to be a good alternative for all wastewater with high organic and nutrient loadings. Moreover, the removal of trace organic contaminants by MBRs is widely applied in the treatment of landfill leachate. MBR system seemed to enhance removal of micro-pollutants with intermediate biodegradability. For easily degradable and intrinsically recalcitrant compounds, MBRs could not make a significant difference in terms of overall removal efficiencies. Lag phases reduction for trace organic compounds degradation and show a stronger memory effect, which implies that they may respond quicker to variable influent concentrations. Finally, MBR treatment turned out to be less sensitive to operational variables such as HRT and will hence show a higher robustness than conventional systems, also for micro-pollutant removal. Whether these observations are related to the higher sludge concentrations and more intense microbial interactions in MBRs is not clear and requires further investigation (De Wever *et al.*, 2007)

The organic material remaining in the effluent from MBRs consists mainly of soluble and relatively poorly biodegradable microbial products generated during treatment. The bioreactor component of MBRs is also capable of degrading some of the organic material that can foul membranes. As a result, the fouling of the membrane component of MBRs is less extensive than which occurs during direct membrane filtration of wastewater. In addition, MBRs are more robust than CASs and

can produce a consistently high-quality effluent even when the hydraulic or organic load to the system is variable. The extent of nitrogen and phosphorus removal that can be achieved using MBRs is typically comparable to that achieved using CASs, both with and without enhanced nutrient removal. However, some studies have reported higher nutrient removal efficiencies for MBRs, compared to CASs. The higher removal efficiencies that can be achieved with MBRs are in part due to the ability of the membrane component of these systems to retain virtually all particulate material, and associated nutrients, within the bioreactor. However, some studies have also suggested that the foulant layer that forms on membranes in MBRs can contribute directly to the removal of soluble nutrients.

The inclined-plate membrane bioreactor at zero excess sludge discharge was capable of removing wastewater contain both carbonaceous and nitrogenous pollutants at satisfactorily and high stable efficiency. The inclined-plates in this case were functioned as counterflow inducer instead of projection area enlarger or settling length reducer for particle sedimentation. Of paramount importance was that no excess sludge was discharged and huge amount of sludge disposal cost could be more competitive process towards the concept of sustainable development and cleaner production. As the result, at return sludge recycle ratio of 300% was reduced more than 70% total-nitrogen removal (Xing *et al.*, 2000). Similar to Nindee (2009), performed two-stage membrane bioreactor treated organic and nitrogen compounds in partially stabilized leachate. The characterization of bacterial population in two-stage membrane bioreactor showed in anoxic tank and aerobic tank. The dominated nitrogen transforming bacteria in anoxic and aerobic tank were ammonia oxidizing β-Proteobacteria. Several studies show that micro-pollutants in wastewater, and landfill leachate were removed by nitrifier cultures with ammonium oxidizing activity. The nitrification in the degradation of micro-pollutants was also dominant. The results suggest that nitrification can enhance the biotransformation of micro-pollutants. The effectiveness of MBRs in removing xenoestrogenic substances such as nonylphenol and bisphenol A from landfill leachate by more than 80% has been demonstrated by Wintgens *et al.* (2003). The removal of trace organic contaminants by submerged membrane bioreactors has also been shown by Nghiem *et al*. (2009).

Bisphenol A and sulfamethoxazole were selected in this study as model trace organics for the endocrine disrupting chemical (EDCs) and pharmacuetical active compounds (PhACs), respectively. Bisphenol A is a well know EDCs while Sulfamethoxazole is one of most frequently used antibiotics. Approximately 90% removal of Bisphenol A and 50% removal of Sulfamethoxazole were recorded and suggested that the removal efficiencies base on physiochemical properties of trace organic contaminants. For comparison, a conventional activated sludge reactor (CASR) was simultaneously tested using the same BPA sludge loading as the submerged MBRs without activated sludge discharge in order to reduce the secondary pollution. The result showed that MBRs could bear much higher volume loading than CASR and still achieve the same BPA removal efficiencies (Chen *et al.*, 2008). Yiping *et al.* (2008) measured the removal efficiencies of organic micro-pollutants in the treatment of landfill leachate by combined anaerobic MBRs and found to be as follows: 94% of organochlorine (OCP), > 77% of 4-nonylphenol (4-NP), > 59% of polycyclic aromatic hydrocarbons (PAHs). Other researchers found that both MBRs and conventional activated sludge plants could remove some pharmaceutical substances (Clara *et al.*, 2005) and some studies investigated the amount of pharmaceutical compounds that contain in landfill leachate such as propyphenazone, vitamin C-synthesis, diacetone sorbose (DAS), and diacetone alpha-keto-gulonic acid (DAG) and found that they could be reduced in the membrane bioreactor system. Several studies have reported that MBRs can effectively remove some trace organic contaminants of concern such as endocrine-disrupting compounds (EDCs), as well as pharmaceutical products and personal care products (PPCPs). Moreover, certain EDCs and PPCPs can be removed to a greater extent using MBRs than CASs. The removal of hydrophobic EDCs and PPCPs is believed to occur predominantly via the adsorption of these compounds onto biomass, and the subsequent retention of these compounds within the bioreactor component of the system for a long enough period of time to be degraded. Some studies also suggest that the foulant layer that forms on membrane surfaces also contributes to retaining EDCs and PCPPs in the bioreactor component of MBRs. Some EDCs and PCPPs can be removed using MBRs, and of the compounds that are removed, some are not consistently removed by all MBRs. It is likely that operating parameters, such as biomass concentration and fouling control

measures, affect the ability of MBRs to remove EDCs and PhACs. De Wever *et al*., (2007) stated that apolar compounds (hydrophobic compounds), sorption to the biomass and subsequent retention of the solids by the membranes will be a major removal mechanisms. For polar compounds (hydrophllic compounds), sorption will be limited and elimination can only be achieved through biodegradation. Theoretically, several operational conditions exist in MBRs, which are favor of enhanced biotransformation and mineralization of micro-pollutants. First, MBRs often operate at high sludge ages. In general, this allows adaptation of microorganisms, and potentially slow growing specialist bacteria in particular. It will be established a more diverse microbial community with boarder physiological capabilities. Second, higher biomass concentrations lead to intensification of biological processes and may increase the interaction between microorganisms and the chances of genetic information exchange. Third, of the higher biomass concentrations, the feed to microorganism (F/M) ratio is lower which could result in more complete mineralization.

Few papers report on micro-pollutant removal during MBR treatment. Some point to an improved removal efficiency compared to CAS treatment for nonylphenols and nonylphenol ethoxylates. De Wever *et al*. (2004) showed that not only the removal of easily degradable linear alkylbenzene sulfonates was slightly better, MBR effluents also contained lower amounts of the more recalcitrant sulfophenylcarboxylate metabolite. Other authors conclude that removal rates in MBR and CAS are comparable for selected pharmaceuticals, fragrances, endocrine disrupting compounds, naphthalene sulfonates and benzothiazole-2-sulfonate (Clara *et al*., 2005a). Increasing the sludge retention time (SRT) above 15 d was found to improve micro-pollutant removal in all biological processes. Hence, when MBR and CAS were operated at comparable SRT, no difference in micro-pollutant removal was detected (Clara *et al*., 2005b). It is clear from the above that literature on micro-pollutant removal by MBR is as yet limited and to some extent contradictory.

CHAPTER III

MATERIALS AND METHODS

There are three main experimental parts in this study consisting of pilot-scale inclined tube membrane bioreactor system (pilot-scale it-MBR), and laboratory-scale inclined tube membrane bioreactor system (laboratory-scale it-MBR), investigation mechanisms responsible for toxic organic compound removal, and bio-toxicity study as shown in Figure 3.1.

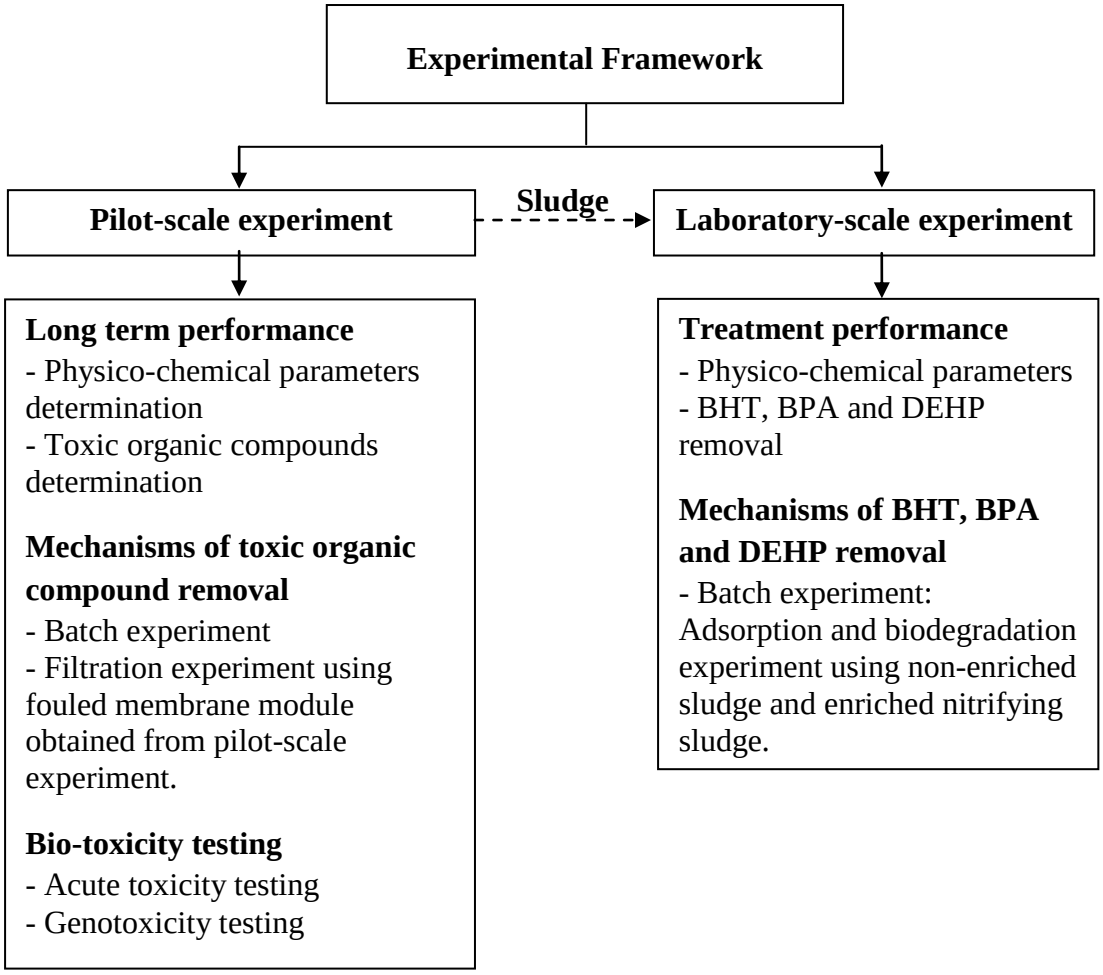


Figure 3.1 Experimental framework.

3.1 Experimental Set-up and Reactor Operation

3.1.1 Pilot-scale it-MBR System

A pilot-scale it-MBR unit with capacity of 2 m^3d^{-1} was installed at Nonthaburi solid waste disposal site in Thailand. The schematic diagram of the experimental system is shown in Figure 3.2. The anoxic reactor dimension is 1.0 m diameter and 2.0 m height with 1.25 m^3 working volume including approximately 0.25 m^3 of sludge storage zone. An inclined tube module (0.15 channel width 0.45 m. depth) was installed in the tank for sludge separation. In aerobic tank (1 m^3 working volume), a hollow fiber membrane module (Sterapore SUR^{TM}, Mitsubishi Rayon Engineering, Japan, PE, 0.4 μm pore size, 18 m^2 surface area) was used for solid liquid separation. Intermittent suction (10 min on and off) was performed to withdraw permeate from the membrane module at constant rate of 1 m^3d^{-1}. The aeration was continuously supplied to the aerobic reactor to maintain DO level at 3-4 mgL^{-1}. The system was initially operated as a single stage using aerobic tank to build up biomass during the first 100 days after which mixed liquor sludge from the aerobic tank was returned to the first tank to maintain MLSS concentration at 10,000 to 12,000 mgL^{-1} in order to control membrane fouling. Sludge wastage was not performed except for sludge sampling purposes (approx. 200 mL every 2 days interval). Hydraulic retention time (HRT) in both tanks was kept at 12 hours. The average membrane permeate flux was controlled at 0.11 $m^3m^{-2}d^{-1}$. The operating conditions are shown in Table 3.1.

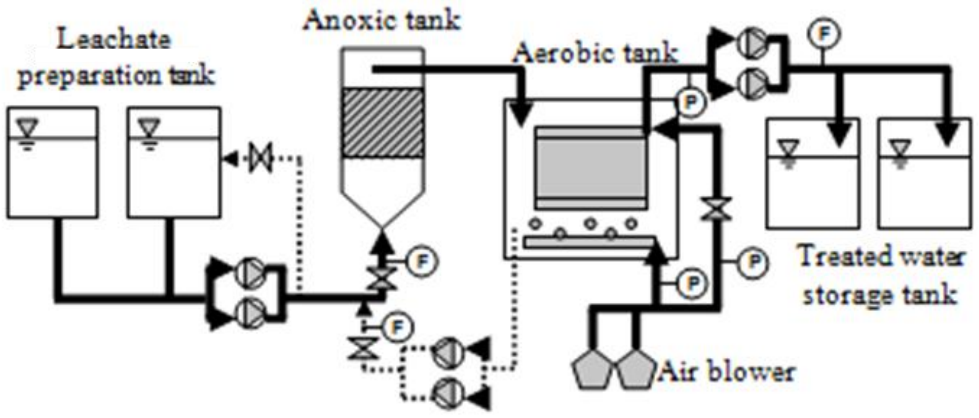


Figure 3.2 Schematic of two-stage it-MBR system.

Table 3.1 Operating condition of pilot-scale it-MBR at Nonthaburi disposal site.

Condition	Value
Volume of reactor	
- Anoxic reactor	1 m^3
- Aerobic reactor	1 m^3
HRT (anoxic + aerobic)	24 h
Flow rate	2 m^3d^{-1}
Permeate flux	0.11 $m^3m^{-2}d^{-1}$
DO level (aerobic reactor)	3-4 mgL^{-1}
Sludge recirculation	100 % of feed flow rate
Intermittent suction	10 min on / 10 min off
Membrane Specification	
- Model	Sterapore SUR334LB
- Material	Polyethylene (PE)
- Pore size	0.4 μm
- Surface area	18 m^2

3.1.2 Laboratory-scale it-MBR System

A laboratory-scale MBR unit with capacity of 60 Ld^{-1} was installed at Department of Environmental Engineering, Faculty of Engineering, Kasetsart University in Thailand. The schematic diagram of the experimental set up is illustrated in Figure 3.3. The MBR sludge was obtained from the pilot scale two-stage MBR system at Nonthaburi solid waste disposal site in Thailand. The anoxic reactor width is 30 cm, length is 30 cm, and height is 50 cm. An inclined tube module (2.5 cm. channel width 30 cm. depth) was installed in the tank for sludge separation. In aerobic tank (30 Liters working volume), a Sterapore SADF™ (SADF0790M mini module) PVDF hollow fiber membrane module supplied by Mitsubishi Rayon Engineering, Japan was submerged in aerobic reactor for solid liquid separation. A membrane had nominal pore size of 0.4 μm with total effective membrane surface area of 0.07 m^2 surface area was used for solid liquid separation. Intermittent suction

(5 min on and 1 min off) was performed to withdraw permeate from the membrane module at constant rate of 60 Ld^{-1}. The aeration was continuously supplied to the aerobic reactor to maintain DO level at 3-4 mgL^{-1}. The MLSS concentration in aerobic tank was maintained at 10,000 to 12,000 mgL^{-1} in order to control membrane fouling by sludge recirculation 100% from aerobic tank to anoxic tank. Sludge wastage was not performed except for sludge sampling purposes (approx. 12 mL every 2 days interval). Hydraulic retention time (HRT) in both tanks was kept at 12 hours. The average membrane permeate flux was controlled at 0.4286 $m^3m^{-2}d^{-1}$. The operating conditions are shown in Table 3.2.

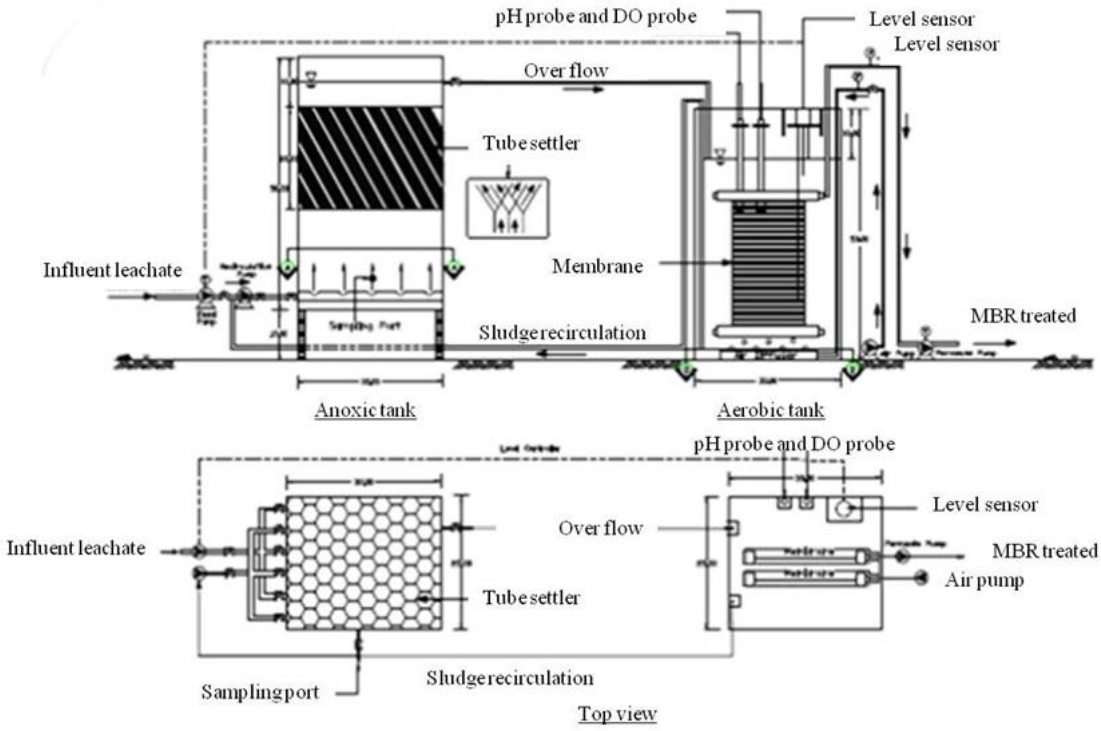


Figure 3.3 Schematic of laboratory scale it-MBR system.

Table 3.2 Operating condition of laboratory-scale it-MBR.

Condition	Value
Volume of reactor	
- Anoxic reactor	30 L
- Aerobic reactor	30 L
HRT (anoxic + aerobic)	24 h
Flow rate	60 Ld^{-1}
Permeate flux	0.4286 $m^3m^{-2}d^{-1}$
DO level	4-6 mgL^{-1}
Sludge recirculation	100 % of feed flow rate
Intermittent suction	5 min on / 1 min off
Membrane Specification	
- Model	SADF0790M
- Material	Polyvinylidene Fluoride (PVDF)
- Pore size	0.4 µm
- Surface area	0.07 m^2

3.2 MBR Influent Leachate Preparation

MBR influent leachate, a mixture of fresh and stabilized leachate, obtained on-site from garbage trucks and a leachate storage pond was prepared at approximately 1:10 mixing ratio (v/v). This preparation was performed to ensure that the organic concentrations were kept relatively constant and not significantly fluctuated during the experimental period. Along the operation period, raw and treated wastewater characteristics were regularly monitored.

3.3 Sample Preparation and Analytical Parameter

Leachate samples were kept inside glass containers and stored at a temperature of 4^{o}C. Prior to analysis, the wastewater samples were filtered through the glass microfiber filter (GF/C). Biomass concentration and characteristics in terms of mixed liquor suspended solids (MLSS), sludge volume index (SVI), particle size analysis (Mastersizer 2000E, Malvern, UK), and EPS production using lowry assay method (Lowry *et al.*, 1951), and phenol/sulfuric acid method (Dubois *et al.*, 1956) were occasionally determined. The characteristics of landfill leachate were performed according to Standard Methods for the Examination of Water and Wastewater (APHA, 1998). pH and electrical conductivity (EC) were analyzed using portable meters (Digicon). Organic compounds were determined in terms of biochemical oxygen demand (BOD_5) using a 5-day BOD test, chemical oxygen demand (COD) using closed dichromate reflux method. Suspended solids (SS) were determined by gravimetric method. Ammonia nitrogen (NH_3-N) and total Kjeldahl nitrogen (TKN) were analyzed by distillation and macro-kjeldahl methods. Table 3.3, and Table 3.4 show the chemical characteristics of leachate fed to the pilot-scale, and laboratory-scale two-stage MBR system, respectively.

Table 3.3 Chemical characteristics of leachate used in pilot-scale two-stage MBR system.

Parameter	**1st stage operation** (day 0-98)		**2nd stage operation** (day 100-300)	
	Range	Avg. (SD)	Range	Avg. (SD)
Temp(^{o}C)	27.2-29.5	28.2(0.7)	26.8-29.7	28.1(0.6)
pH	8.67-8.84	8.78(0.05)	8.53-8.85	8.66(0.16)
BOD(mgL^{-1})	3,020-3,600	3,322(179)	6450-7542	7,009(343)
COD(mgL^{-1})	6,160-7,318	6,928(328)	9000-9800	9,389(210)
SS(mgL^{-1})	1,135-1,456	1,243(107)	1120-1480	1,248(104)
TKN(mgL^{-1})	145-162	152(6)	201-240	229(11)
NH_4^+-N(mgL^{-1})	105-132	119(8)	138-174	164(9)
NO_2^--N(mgL^{-1})	0.001-0.008	0.005(0.002)	0.003-0.01	0.006(0.002)
NO_3^--N(mgL^{-1})	0.055-0.932	0.522(0.284)	1.12-2.02	1.632(0.235)
EC ($mS(cm)^{-1}$)	20.7-28.7	23.3(1.8)	23.0-26.9	26.3(1.0)

Table 3.4 Chemical characteristics of leachate used in laboratory-scale two-stage MBR system.

Parameter	MBR influent	
	Range	Avg. (SD)
Temp(^{o}C)	27.5-30.7	28.3(0.5)
pH	8.63-8.89	8.70(0.15)
BOD(mgL^{-1})	6,000-6,987	6,598(269)
COD(mgL^{-1})	9,000-9,846	9,273(237)
TOC(mgL^{-1})	2,000-2,882	2,476(241)
SS(mgL^{-1})	1,120-1,680	1,250(105)
TKN(mgL^{-1})	200-230	214(8)
NH_4^+-N(mgL^{-1})	112-128	119(4)
NO_3^--N(mgL^{-1})	1.215-2.148	1.632(0.235)
NO_2^--N(mgL^{-1})	0.010-0.125	0.054(0.002)
EC($mS(cm)^{-1}$)	23.0-30.1	27.8(1.5)

3.4 Determination of Toxic Organic Micro-pollutants and Their Removal Mechanisms

Priority toxic organic micropollutants in landfill leachate were naphthalene (Nap), anthracene (An), 4-Methyl-2,6-di-tert-butylphenol (BHT), Bisphenol A (BPA), Dimethylphthalate (DMP), Diethylphthalate (DEP), Di-n-butylphthalate (DnBP), Benzyl butyl Phthalate (BBP), Bis (2-ethylhexyl) phthalate (DEHP), and Di-n-octyl phthalate (DOP). Their concentrations were also found most in 10 μgL^{-1} level except BHT, which was found that higher concentrations (10 mgL^{-1} range). Ten toxic organic micro-pollutants that were found at this studied site can be classified into three groups as follows; PAHs, phenolic compounds, and PAEs. The chemical properties of those toxic organic micro-pollutants are shown in Table 3.5. The laboratory scale experiment focused on BPA, BHT, and DEHP at higher concentrations (adding BPA, BHT, and DEHP to 1,000 μgL^{-1}) for the study on biodegradation, adsorption, and filtration experiment.

Table 3.5 Chemical properties of selected phenolic compounds and phthalic acid esters.

Compound	Cas no.	Formula	MW	Bp (^{o}C)	Mp (^{o}C)
Naphthalene	91-20-3	$C_{10}H_8$	128.17	218	80-82
Anthracene	120-12-7	$C_{14}H_{10}$	178.23	340	215
4-Methyl-2,6-di-tert-butylphenol	128-37-0	$C_{15}H_{24}O$	220.35	265	69-73
Bisphenol A	80-05-7	$C_{15}H_{16}O_2$	228.29	220	158-159
Dimethylphthalate	131-11-3	$C_{10}H_{10}O_4$	194.18	2	282
Diethylphthalate	84-66-2	$C_{12}H_{14}O_4$	222.24	298-299	−3
Di-n-butylphthalate	84-74-2	$C_{16}H_{22}O_4$	278.34	340	-35
Benzyl butyl Phthalate	85-68-7	$C_{19}H_{20}O_4$	312.36	370	<-35
Bis(2- ethylhexyl)phthalate	117-81-7	$C_{24}H_{38}O_4$	390.56	386	-50
Di-n-octyl phthalate	117-84-0	$C_{24}H_{38}O_4$	390.56	380	-

MW = molecular weight, Bp = boiling point, Mp = melting point

3.4.1 Batch Experiment

Batch experiments were performed to investigate the micropollutants biodegradation by sludge taken from the pilot-scale MBR system, and laboratory scale MBR system at the end of the experimental period (300 days). Three batch reactors (300 mL stoppered conical flasks) were filled with 200 mL of MBR sludge and initial concentrations of BHT, BPA, and DEHP were set at 1,000 μgL^{-1}. The batch reactors were wrapped in aluminum foil to prevent possible photodegradation of EDCs and put on a shaker at 125 rpm. The samples were taken at constant time intervals (0–24 h) for the determination of BHT, BPA, and DEHP in dissolved and particulate forms. The concentrations of mixed liquor suspended solids (MLSS) in the experiments were controlled at 1,000 mgL^{-1}. All the experiments were performed at 22.0 ± 1.0 ^{o}C, pH was regularly determined and found to be 7.5 ± 0.2.

For the adsorption experiment, inactivated sludge was used for determining adsorption capacity of MBR sludge. The sludge samples obtained from the pilot-scale MBR were inactivated three times by pasteurization at 121 ^{o}C for 15 min in order to terminate microbial activities. Same procedures with those used in biodegradation experiment were performed using inactivated sludge and BHT, BPA and DEHP were analyzed in dissolved and particulate forms. The adsorption experiments were performed at 22.0 ± 1.0 ^{o}C and pH of 7.5 ± 0.2.

3.4.2 Biodegradation Test Using Enriched and Inhibited Nitrifying Sludge

The enrichment of nitrifying sludge was modified from Tran *et al.* (2009) and Liza *et al.* (2011). The MBR sludge was taken from laboratory scale it-MBR system. Nitrifying sludge was enriched in fill-and-draw operation with a 4d-cycle in a 2 L reactor at room temperature for more than 4 months. At the enrichment period, MBR influent leachate with ammonium was used as enrichment medium. The ammonium concentration was gradually increased from 100 to 300 $mgNH_4\text{-}NL^{-1}$. The pH in the reactor was controlled at 7.5–8.0 using $NaHCO_3$ 30 gL^{-1}. Air pump was used to aerate

the culture to maintain a dissolved oxygen level higher than 6.0 mgO_2L^{-1}. During the enrichment nitrifying sludge, the NH_4-N, NO_2-N, NO_3-N were measured. All batch experiments were implemented in triplicate.

Batch experiments were done in parallel with the operation of the laboratory-scale it-MBR system. The role of nitrification on BHT, BPA, DEHP degradation were also assessed by adding 20 mgL^{-1} of allyl-thiourea (ATU) to the mixed liquor. ATU is known to inhibit the activity of AOB, which is the first step in the nitrification process. The determination of the removal of BHT, BPA, DEHP in the reactor were due to biological activity, the similar batch experiment tested as earlier described were performed but the inactivated sludge was performed by pasteurization at 121 ^oC for 15 min (three times) in order to terminate microbial activities. The experiments with the full inhibition of biological activities were also carried out to distinguish pure adsorption onto sludge from biodegradation mechanism.

3.4.3 Filtration experiment

In the filtration experiment, retentions of BHT, BPA and DEHP by fouled and cleaned membranes were investigated. Three filtration conditions were examined, i.e. fouled membrane consisting of both cake and gel foulant layer, water cleaned membrane consisting of gel layer and chemical cleaned membrane. To prepare the fouled membrane for this experiment, 50 mg of sludge was filtrated through a 0.45 µm membrane (mixed cellulose ester, Advantech) to obtain cake and gel layer. After that the cake layer was removed by spraying water. Finally, membrane was soak in 0.3% NaOCl for 1 hour. The filtration on membrane fouling was determined by the target compound removal when filtered through each membrane specimen. The filtration experiment set-up is illustrated in Figure 3.4.

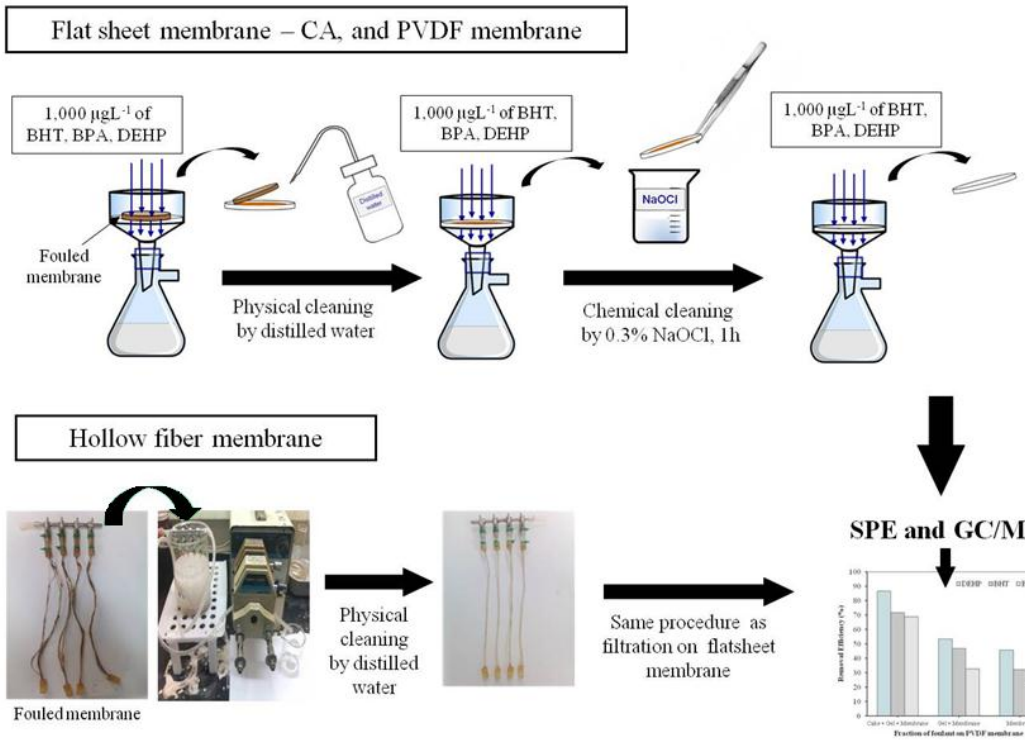


Figure 3.4 Membrane preparation for the filtration experiment.

3.4.4 Toxic Organic Micro-Pollutants Analyses

Solid phase extractions (SPE) were used to determination concentration of phenolic compounds and phthalic acid esters in landfill leachate sample. The organic micro-pollutants analytical method that used in this experiment was modified from the previous study (Threeparaksapan *et al.*, 2010). SPE were carried out using C18-Bond Elut® (Varian, Inc.), as SPE resin. Pre-packed columns holding 500 mg of SPE sorbent were cleaned by pure water and followed by solvent wash (methanol). Firstly, soluble leachate samples (typically 100 ml) were pumped through the C18-SPE columns at flow rate of 1 ml/min. The loaded columns were dried with clean air and then eluted by 2x3 ml of dichloromethane/methanol mixture (1:9, v/v), respectively). The volume was reduced to 0.5 ml by nitrogen evaporator/concentrator (ChanoVap, Amani, Thailand). The filter residue samples were sonicated for 1 h with 50 ml of methanol/dichloromethane mixture (1:4, v/v). The prepared sample used the same

conditions and SPE column from soluble sample extraction according to analytical organic micro-pollutants in soluble sample.

3.4.5 Gas Chromatography with Mass Spectrometer (GC/MS)

Gas chromatography with mass spectrometry (GCMS-QP2010 plus, Shimadzu, Japan) was used to determine concentration of ten toxic organic contaminants. The device was equipped with a 30 m RTX-35MS capillary, 0.25 mm I.D. with thickness of 0.50 µm. Helium was used as carrier gas. One µL of sample was injected automatically (splitless 1 min). The chromatographic separation process was performed by program which set column oven temperature from 60 to 175^{o}C at 6^{o}C/min, and then increased to 270^{o}C at 3^{o}C/min. The ions were produced by electron impact at 70 eV, ion source temperature at 200^{o}C, and interface temperature at 270^{o}C. The compounds were detected and quantified using the scan mode.

The quantification of BPA, BHT, and DEHP in batch experiment were performed with One µL of sample was injected automatically (split 1 min). The chromatographic separation process was performed by program which set column oven temperature from 150^{o}C held for 1 min and raised to 270^{o}C at 20^{o}C/min held for 7 min. The ions were produced by electron impact at 70 eV, ion source temperature at 200^{o}C, and interface temperature at 270^{o}C condition. The compounds were detected and quantified using the sim/scan mode.

The organic micro-pollutants in leachate samples were identified by comparison with GC/MS library (Wiley7) developed from standard substances. The detection limits for phenolic compounds and phthalic acid esters measurements were 1.0 µgL^{-1}.

3.5 Biotoxicity Determination

3.5.1 Acute Toxicity Testing

The fish species were conducted using Nile Tilapia (*Oreochromis niloticus*), and Common Carp (*Cyprinus carpio*) obtained from a local breeder and transported immediately to the laboratory in appropriately aerated plastic bags. In the laboratory, fish species were kept separately in 120 liters glass aquaria (0.40 m width, 0.75 m length, and 0.45 m depth) containing de-chlorinated tap water. They were acclimated for 14 days with continuous aeration and the water was renewed every 3 days. The photo-period was set at 12:12 hour (light:dark) condition during the entire experiment. Care is taken in order to keep the mortality rate less than 5% in the last 5 days before the experiments was started. In triplicate, 10 adult fishes were placed in water sample that are diluted to five dilutions, corresponding to 50-2,000 $mgCODL^{-1}$ and 2-12 $mgNH_3L^{-1}$. The exposure test was carried out at temperature room of $28.1^{o}C$ for 96 hour. The number of dead fish was recorded every 24 hour. In triplicate, non-exposed fish were observed in fresh water under same conditions as mentioned above as control experiment. The 96 hour LC_{50} for fish species and its 95% confident limits were calculated using probit transformation of the mortality data, and the relationship between pollutant concentrations, i.e. NH_3, and COD. The mortality ratio was developed based on experimental data. The program based on Finneys Probit Analysis method using SPSS (version 16.0) for Windows.

3.5.2 Genotoxicity Testing

The analyses of *C. carpio* and *O. niloticus* DNA strand breaks were performed which 10 fishes were exposed to 20 liters of water sample that are diluted at LC_{10} with dechlorinated tap water for 7 days under conditions of 12:12 hour light:dark. In triplicate, non-exposed fish are observed in fresh water under same conditions to be control experiment. Blood of fish are collected at 0, 7 days all of the experiment. Peripheral blood of fish was collected from a caudal vein using 1 ml heparinized

syringe. A 15 μl of blood is diluted with 1 ml of chilled phosphate-buffered saline (PBS). Slide preparation, 2.5 μl of diluted sample were mixed with 50 μL of PBS and 50 μl of 0.5% LMP agarose in micro-centrifuge tube at 37-40^{o}C and layered on comet slide, and after this layer were solidified at 4^{o}C. The slides were immersed in the alkalilysis buffer (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na_2EDTA, 10 M Tris HCl, pH 10, 10% DMSO, 1% Triton X-100) for 3-24 hour at 4^{o}C. Then, the slides were placed in alkaline electrophoresis buffer (10 N NaOH, 200 mM EDTA 5 ml, pH>13) for 10 min.

Electrophoresis was performed at 15 V, 250 mA for 25 min at 4^{o}C. The slides were then neutralized with neutralization buffer (tris-hydroxymethyl-aminomethane 48.5 g, pH 7.5) for 20 min. After that, slides were immerged in ethyl alcohol for 5 min and pure water for 10 min. It's dried by placement on hot plate at 50^{o}C. Finally, the cells were stained with 50 μL of SYBER safe^{TM} Green. Comet images are analyzed using a fluorescence microscope, 515 nm and barrier filter, 590 nm (magnification 10×) to determine the sufficient cell dispersed.

3.5.3 Analysis of Comet Assay

A total of 100 cells from each slide were randomly scored and analyzed using an image analysis system (Tritek Comet Score Freeware Version 1.5). The comet parameter, i.e. percent tail DNA (% tail DNA = 100 – head DNA) as determined by the software was used for quantification of DNA damage. Bivariate relationships between physicochemical parameters (COD, UIA, pH, and EC), and mortality of tested organisms using Pearson correlation and the association between LC_{50} and physicochemical parameters was performed using multiple linear regressions. For the statistical analysis, significance was evaluated at $P<0.01$ and $P<0.05$.

3.6 Microbial Community Analysis Using Fluorescence *In Situ* Hybridization

The microbial communities in sludge samples were evaluated by using FISH technique at the end of experimental period. For FISH technique, the method used in this study was described as following steps.

3.6.1 Preparations of Samples

MBR sludge and enriched nitrifying sludge were collected from laboratory scale it-MBR and enrichment reactor, respectively. After the collection, take 2 ml of mixed liquor from membrane bioreactor to 50 ml polyethylene tube. Centrifuged the sample at 4,000 rpm during 10 minutes at 4°C and discard the supernantant. The extraction was performed by addition 4 ml of 8% Paraformaldehyde/PBS (8% PFA) to sludge sample and resuspended the sample by shaking vigorously or vortex. After that, the fixation was proceeded by stored the sample over night at 4°C. Centrifuged at 4,000 rpm during 12 minutes at 4°C and discard the supernatant (Note: PFA is a carcinogenic substance, dispose off this appropriately). Addition 5 ml of 1x PBS and resuspended the sample by vortex (repeat washed with PBS for 3 times). Then, addional 4 ml of 1x PBS and mixed with 4 ml of 99.5% ethanol. Finally, resuspended the sample and stored the sample at -20°C until hybridizing reaction.

3.6.2 Fluorescence In Situ Hybridization (FISH)

The sample obtained from the preparation step was carried on by immobilization of 5μL fixative sample onto the gelatin coated slide and dehydration with various concentrations of ethanol (50%>80%>95%>50%>80%) during 3 minutes for each ethanol concentration. The oligonucleotide probes used in this study were commercially synthesized with reverse-phase cartridge purification (RP1) method and fluorescently 5’ labeled with fluorescein-isothiocyanate (FITC) dye (Sigma-aldich, Singapore). The hybridization conditions including the formamide

concentration in the hybridization buffer and NaCl concentration. In addition, for simultaneous hybridization with the probes which require different hybridization stringencies (Wagner *et al.,* 1996) as shown in Table 3.6. For hybridization step, 6 µL hybridization buffer and 1 µL probe were overlaid on the sample slides. The FISH assay was performed according to the protocol described by Amann *et al*. (1995) at 46 ^{o}C for 2.5 h in hybridization buffer (0.9 M NaCl, 20 mM tris-hydrochloride, 0.01% sodium dodecyl sulfate (SDS), formamide) containing 5 ng of probe μL^{-1} in incubator. A negative control (no probe) was included for every sample to observe autofluorescence. After hybrization, the slides were rinsed and immersed in pre-warmed washing buffer (20 mM tris-hydrochloride, 0.01% SDS, NaCl) at 48 ^{o}C for 10 min. After washing, the slides were rinsed briefly with DI water, air-dried, and mounted with a cover slip using a Prolong Gold anti-fade reagent. Coat the borders with enamel to avoid drying. The samples are ready for the microscope observation. The FISH samples were examined by fluorescence microscopy (BX51 microscope; DP71 camera, Olympus, Japan). Barried filters, UMW-B2, UMW-G2 and UMW-U2 were used to collected the excited fluorescence of the FITC-labeled probes, and the Rhodamine Red-labeled probes, DAPI blue-labeled probes respectively. The cellSens dimension imaging software version 1.4.1 was used as an image analysis tool to help in determining the relative area taken up by target cells complimentary to the specific probe compared to the area of cells complimentary to the EUB338 probe. The average area fraction was determined by evaluating at least nine representative microscopic fields.

Table 3.6 Oligonucleotide FISH probes used in this study.

Probe	Probe sequence (5'–3')	Target site (rRNA) and position	Specificity	%FA	NaCl (M)	Reference
EUB338	GCTGCCTCCCGTAGGAGT	16S (338–355)	Most bacteria	15	0.318	Amann *et al*. (1990)
Nso1225	CGC CAT TGT ATT ACG TGT GA	16S (1,224-1,243)	Ammonia oxidizing *β-Proteobacteria*	35	0.079	Mobarry *et al*. (1996)
NIT3	CCTGTGCTCCATGCTCCG	16S (1035–1048)	*Nitrobacter spp*.	40	0.056	Wagner *et al*. (1996)

CHAPTER IV

REMOVAL OF TOXIC ORGANIC MICROPOLLUTANTS IN PILOT SCALE AND LABORATORY SCALE MEMBRANE BIOREACTOR

The experimental results and their discussion are divided into two main parts; 4.1) Treatment performance of pilot scale two-stage MBR system; and 4.2) Treatment performance of laboratory-scale two-stage MBR system.

4.1 Treatment Performance of Pilot-scale Two-stage MBR System.

4.1.1 Organic and Nitrogen Removal in Pilot-scale Two-stage MBR.

During the reactor operation over 300 days, the chemical characteristics of leachate fed to the MBR system during the operation. During the 1st stage operation, average BOD and COD concentrations were 3,300 mgL^{-1} and 6,900 mgL^{-1} yielding BOD and COD loading rates of 3.3 and 6.9 $kgm^{-3}d^{-1}$. When anoxic tank was introduced in the 2nd stage operation, BOD and COD concentrations in leachate were increased to 7,000 mgL^{-1} and 9,400 mgL^{-1} resulting in the loading rates of 7.0 and 9.4 $kgm^{-3}d^{-1}$, respectively. Meanwhile, TKN loading rates were between 0.15-0.23 $kgm^{-3}d^{-1}$ in both stages of operation.

During the first stage operation, the aerobic reactor removed 85% of BOD and 81% of COD on average. Meanwhile, NH_4^+ and TKN removals were 83% and 29%, respectively. The treatment performance was found relatively stable over the entire operation period despite gradual change in sludge biomass. MLSS concentration in aerobic reactor was gradually increased from 7.4 gL^{-1} up to 15 gL^{-1} yielding a biomass increasing rate of 0.078 gd^{-1} in response to organic loading to the reactor as it was operated under minimum sludge wastage condition and observed

biomass yield of 0.03 gMLSS(gBOD)$^{-1}$ removed. At the end of start-up period (100th day), sludge in aerobic reactor was re-circulating back to anoxic reactor resulting in a drop in biomass concentration. MLVSS/MLSS ratio of biomass was kept between 0.47-0.55. Satisfactory treatment performance and biomass built-up in the system could be achieved during this short-term start-up period. When anoxic reactor was introduced in the second stage operation, BOD and COD removals were improved to 97% and 87% whereas NH_4^+ and TKN removals also increased to 91% and 67% respectively. Most of the removals were taken place in aerobic reactor as less than 15% removal was observed in the anoxic reactor. The performance of MBR in terms of organic (BOD, COD) and nitrogen (NH_4^+, TKN) removals are presented in Table 4.1. Corresponding biomass variations in the system are shown in Figure 4.1.

The biomass concentration in anoxic reactor increased from about 12.7 gL^{-1} to almost 20 gL^{-1} during 200 days of operation (0.037 gd^{-1} increasing rate, 50% of first stage operation) whereas the biomass concentration in aerobic reactor was kept constantly at about 12 gL^{-1} by the re-circulation operation. Observed biomass yield during this two-stage operation without sludge discharge was kept as low as 0.006 gMLSS(gBOD)$^{-1}$ removed, while MLVSS/MLSS ratio was maintained relatively constant as the 1st stage operation and gradually increased when the re-circulation of sludge was performed. During the entire operation period, biomass in the system had good settling properties as suggested by their SVI value of less than 60 mLg^{-1}. The particle size analyses (Figure 4.2) revealed that there was increasing trend of sludge particle size in the aeration tank of MBR during the first 200 days and possibly in association with an increase in biomass concentration. It was likely due to the agglomeration of particles in aerobic reactor especially when biomass re-circulation was introduced. Nevertheless, a decrease trend of modest floc size was observed as the operation period was prolonged. These changes in biomass characteristics could also affect the pollutant removal efficiencies in the system. Chiemchaisri and Yamamoto (2005) demonstrated that floc size in MBR played an important role in facilitating oxygen transfer for microbial activities along the MBR operation without sludge wastage by promoting nitrification in the system.

Table 4.1 Performance of pilot scale two-stage MBR during first and second stage operation.

Parameter	Effluent concentration (mgL^{-1})				Removal efficiencies (%)			
	Anoxic reactor		Aerobic reactor		Anoxic reactor		Total	
	Range	Avg. (SD)	Range	Avg. (SD)	Range	Avg.(SD)	Range	Avg.(SD)
1st stage operation (aerobic reactor only)								
BOD	-	-	300-650	490(114)	-	-	79-91	85(4)
COD	-	-	1,200-1,450	1,292(97)	-	-	78-83	81(2)
NH_4^+	-	-	15-25	20(3)	-	-	76-88	83(4)
TKN	-	-	98-120	107(8)	-	-	18-38	29(6)
2nd stage operation (anoxic-aerobic reactors)								
BOD	5,050-7,060	6,203(530)	110-670	222(159)	6-22	12(6)	90-98	97(2)
COD	6,410-8,910	8,093(686)	880-1,645	1,196(233)	8-31	14(7)	82-91	87(3)
NH_4^+	112-165	149(10)	3-26	15(8)	2-19	10(3)	85-98	91(5)
TKN	198-236	217(11)	45-120	76(23)	1-13	5(3)	45-81	67(11)

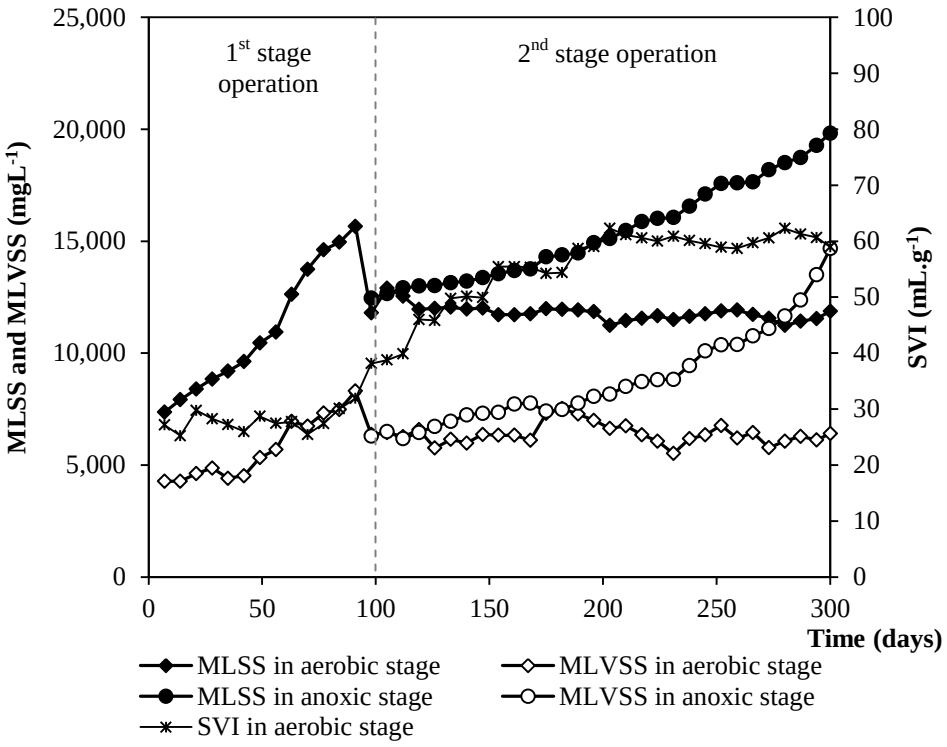


Figure 4.1 Biomass and Solid volume index in the pilot scale two stage MBR system during 1st and 2nd stage operation.

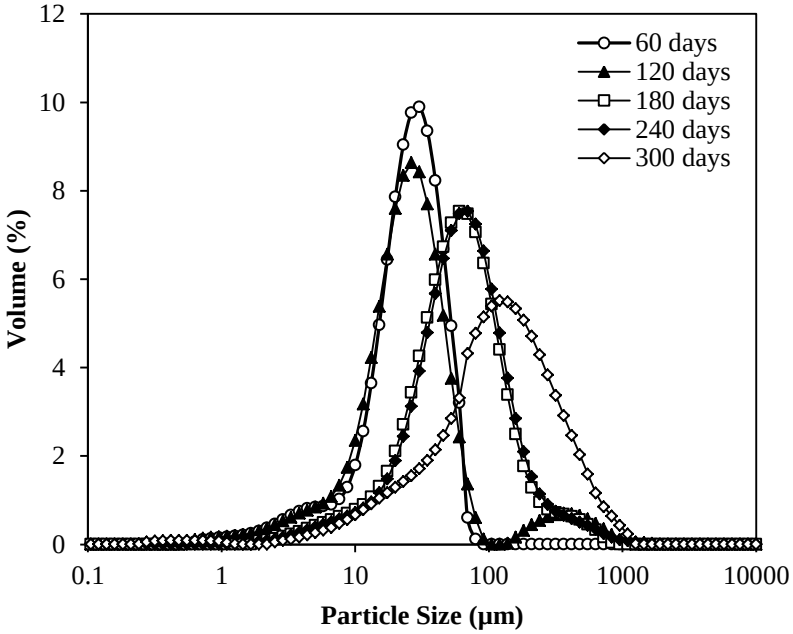


Figure 4.2 Particle size distribution in aerobic reactor during the pilot scale two stage MBR operation.

Our previous study on the characterization of biomass in anoxic and aerobic sludge revealed high fraction of nitrifying bacteria in two-stage membrane bioreactor treating municipal solid waste leachate (Chiemchaisri *et al*., 2011). An introduction of sludge re-circulation and increasing sludge age could affect the biomass properties and bacterial population in the system as well. While the introduction of sludge re-circulation from aerobic tank to anoxic tank may result in a reduction of strict aerobic organisms like nitrifying bacteria and promote the growth of facultative organisms, an increasing sludge age could also yield a higher percentage of slow growing nitrifying organisms in the aerobic MBR tank (Chiemchaisri *et al*., 2011). After the treatment, the effluent from aerobic reactors could either be reused on-site or further treated in the polishing unit or domestic wastewater treatment system to comply with more stringent regulations. Our previous investigation (Chiemchaisri *et al*., 2011) has demonstrated that the system operated at lower organic loading could be produced excellent effluent quality for direct non-potable reuse, i.e. washing of solid waste collection truck. Nevertheless, this study was aimed at development of compact treatment system which could apply directly to the treatment of high strength leachate while maintaining relatively short hydraulic retention time (24 hours) of the treatment in the system.

4.1.2 Toxic Organic Micro-pollutants Removals in Pilot-scale MBR Operation.

The analyses of organic micro-pollutants in leachate revealed the presence of following compounds in leachate, i.e. napthalene (Nap), anthracene (An), 4-methyl-2,6-di-tert-butylphenol (BHT), bisphenol A (BPA), dimethylphthalate (DMP), diethylphthalate (DEP), di-n-butylphthalate (DnBP), benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (DEHP), di-n-octyl phthalate (DOP). These compounds can be classified into three groups as polycyclic aromatic hydrocarbons (PAHs), phenolics compounds, and phthalic acid esters (PAEs). In previous researches, these compounds are reported as xenobiotic organic compounds (XOCs) commonly found in landfill leachate (Paxéus, 2000; Kjeldsen *et al*., 2002; Baun *et al*., 2004).

The concentrations of ten toxic organic micro-pollutants during the reactor operation over 300 days in influent and effluent from MBR are determined. The influent of Nap, An, BHT, BPA, DMP, DEP, DnBP, BBP, DEHP, and DOP concentrations in soluble form were 9.6±0.1 μgL^{-1}, 9.8±0.2 μgL^{-1}, 10,750±608 μgL^{-1}, 75.4±7.2 μgL^{-1}, 20.8±0.5 μgL^{-1}, 12.5±1.0 μgL^{-1}, 35.4±7.8 μgL^{-1}, 21.5±3.0 μgL^{-1}, 65.0±6.6 μgL^{-1}, and 8.0±0.2 μgL^{-1}. Their concentrations in soluble form were also found most in 10 μgL^{-1} level except BHT, which was found that higher concentrations (10 mgL^{-1} range). For the solid form, the MBR influent contained the concentration of Nap, An, BHT, DMP, DEP, DnBP, BBP, DEHP, and DOP concentrations were 9.1±0.1 μgL^{-1}, 8.9±0.2 μgL^{-1}, 735±164 μgL^{-1}, 9.7±0.1 μgL^{-1}, 10.8±0.1 μgL^{-1}, 9.6±0.3 μgL^{-1}, 20.7±2.3 μgL^{-1}, 440±45 μgL^{-1}, and 8.2±0.1 μgL^{-1}. BPA was not detected in solid form. The results are within the ranges generally observed in landfills (Kjeldsen *et al*., 2002). During the MBR operation, the observed removals of toxic organic compounds during the MBR treatment were as follows; Naphthalene (76%), Anthracene (74%), BHT (83%), BPA (96%), DMP (78%), DEP (81%), DnBP (87%), BBP (77%), DEHP (96%), and DOP (82%) as shown in Table 4.2. It was found that most of phenolic compounds (BHT and BPA) were mainly detected in soluble form and the biodegradation in MBRs contributed to more than 78% of their removals. On the other hand, DEHP which is one of PAEs used as plasticizer was mainly found in solid phase and could be eliminated about 30% by adsorption onto the sludge in the MBR by attachment onto small colloidal particles. Figure 4.3 shows the variation of soluble phenolic compounds and PAEs removal in MBR and biomass concentration. It was found that the removal efficiencies of all three compounds had an increasing trend along the operation period. Among them, BPA had the highest removal efficiencies, being completely removed after 100 days followed by BHT and DEHP respectively. Despite these biomass variations in the system, the removal efficiencies of those selected compounds were not adversely affected. This observation suggested that an improvement of BPA, BHT and DEHP removal was possibly beneficial from the enhanced biodegradation with increasing sludge retention time along the reactor operation.

Table 4.2 Removals of selected organic micro-pollutants in pilot scale two-stage MBR.

Compounds	MBR influent (μgL^{-1})		MBR effluent (μgL^{-1})	Removal Efficiency (%)	
	Soluble	Solid		Total	Soluble
PAHs					
Nap	9.6 (0.1)	9.1 (0.1)	4.5 (0.6)	76	53
An	9.8 (0.2)	8.9 (0.2)	4.9 (0.2)	74	50
Phenolics					
BHT	10,750	735 (164)	2,520 (172)	83	81
BPA	75.4 (7.2)	ND	14.6 (1.0)	96	96
PAEs					
DMP	20.8 (0.5)	9.7 (0.1)	7.2 (0.6)	78	67
DEP	12.5 (1.0)	10.8 (0.1)	4.4 (0.1)	81	65
DnBP	35.4 (7.8)	9.6 (0.3)	11.4 (0.2)	87	86
BBP	21.5 (3.0)	20.7 (2.3)	9.2 (1.4)	77	54
DEHP	65.0 (6.6)	440 (45)	22.8 (3.0)	96	66
DnOP	8.0 (0.2)	8.2 (0.1)	3.0 (0.2)	82	63

ND: Not Detected

* Average (SD) values, No. of samples = 12

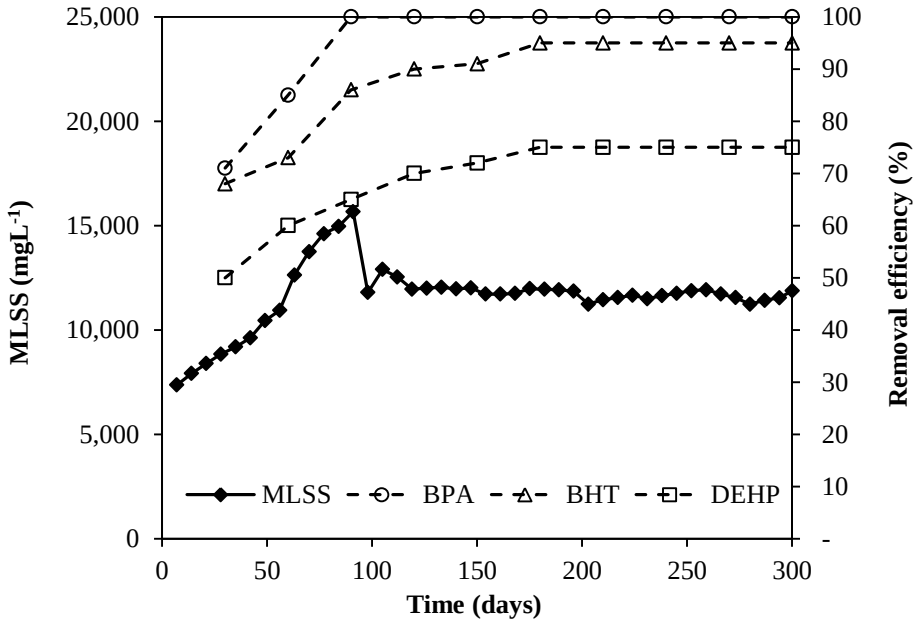


Figure 4.3 Soluble phenolic and PAEs removals and MLSS concentration with time in which system indicate that it is from pilot-scale MBR.

The observed concentrations and removal efficiencies of those organic micro-pollutants by two-stage MBR were summarized in Table 4.3. During the first stage operation, average removal efficiencies of most compounds were found relatively low with only 5 out of 10 compounds had higher removal efficiencies than 30%. DEHP and DOP were found to be removed at relatively higher degree (36-37%) while DMP was only removed by 9%. Depending on the form they presented in fed leachate, phenolic compounds such as BHT and BPA that were mainly detected in soluble form could be removed via biological activities in the MBR. Only the other hands, phthalates such as DEHP and DOP were presented in association with solid particles and their removals through adsorption and retention by membrane filtration could become predominant. During the second stage operation, the removals of micro-pollutants improved to 50-76%. DEHP and DOP were highly removed by about 75% whereas DMP and DEP could be removed at about 50%. For most compounds, the removals in anoxic reactor contributed to only minor fraction in the total removals ranging from 5-16%. As the biomass concentration in aerobic reactor during 2^{nd} stage operation was maintained at almost the same level as those during 1^{st} stage operation, the introduction of anoxic tank with sludge re-circulation or increasing sludge age in the system could be responsible for these improvements in micro-pollutant removals. Moreover, it should be noted that enhancement of micro-pollutant removals were taken place simultaneously with improved nitrification or TKN removal (29% in 1^{st} stage to 69% in 2^{nd} stage operations). It is therefore possibly that nitrifying organisms was also responsible for the removal of micro-pollutants through their co-metabolic pathways. Tran *et al*. (2009) reported that enrichment of nitrifier culture is significant factor on the biodegradation of XOCs in biological wastewater treatment system. Figure 4.4 shows the relationship between octanol-water partition coefficient (K_{ow}) of micro-pollutants and their removals during 1^{st} and 2^{nd} stage operations. It was clearly seen that the properties of compounds affected their removals in both stages of operation. The compound with highest K_{ow} value (DOP, 8.05) were removed by 37% and 76% during 1^{st} and 2^{nd} stage operations whereas DMP with K_{ow} of 1.53 were removed by only 9% and 50% in the same period.

Table 4.3 Concentrations of organic micro-pollutant and their removals during the reactor operation over 300 days.

Compound	1st stage operation			2nd stage operation				
	Inf.	Eff.	%R	Inf.	Eff_{anoxic}	%R	$Eff_{aerobic}$	%R
Nap	18.6(0.04)	14.4(0.8)	23	19.0(0.3)	16.7(0.5)	11	6.0(3.2)	68
An	18.6(0.1)	12.7(3.1)	32	18.5(0.1)	16.3(0.6)	12	5.6(2.4)	70
BHT	11489(373)	7678(1561)	33	11412(719)	9656(653)	16	2907(1360)	74
BPA	72.7(3.3)	61.4(5.6)	16	81.6(2.3)	64.7(4.9)	11	26.3(24.3)	67
DMP	30.3(0.3)	27.6(0.9)	9	31.1(0.4)	28.8(0.5)	5	15.6(4.2)	50
DEP	23.4(0.6)	20.6(0.9)	12	23.4(0.4)	21.9(0.4)	7	11.5(3.8)	51
DnBP	43.6(6.1)	32.9(2.9)	24	49.5(3.9)	38.7(5.2)	11	15.6(7.3)	68
BBP	39.7(2.3)	27.1(3.9)	32	41.1(2.2)	39.7(2.3)	15	11.6(5.6)	72
DEHP	513(31)	328(30)	36	488(21)	433(31)	16	122(52)	75
DOP	16.1(0.1)	10.2(1.0)	37	16.1(0.1)	13.6(0.5)	16	3.9(2.1)	76

ND: Not Detected

* Average (SD) values, No. of samples = 12

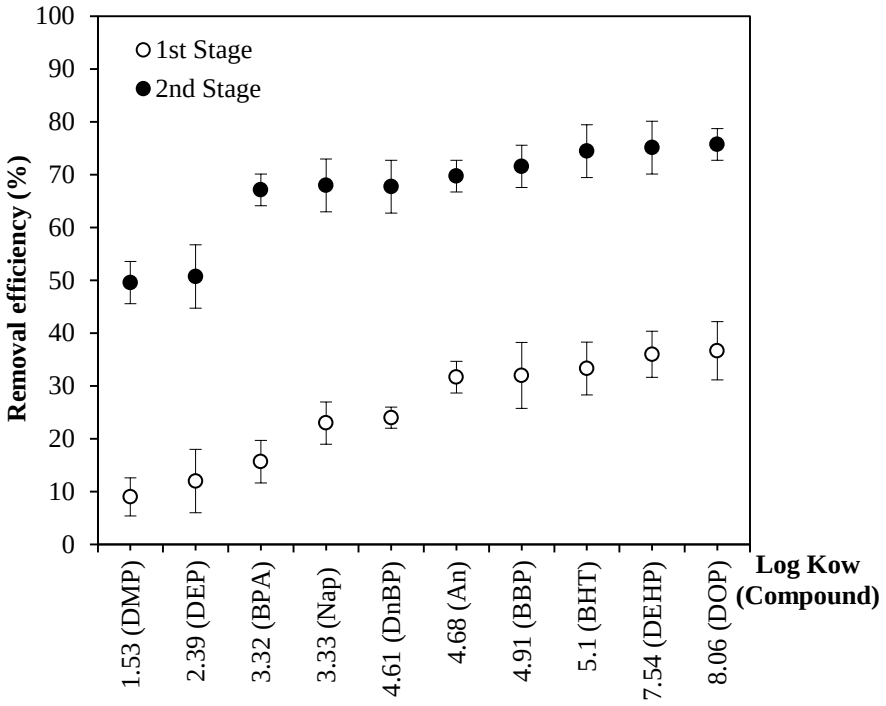


Figure 4.4 Relationship between log K_{ow} of organic micro-pollutants and their removals in pilot-scale two-stage MBR system during 1st and 2nd stage operation.

This observation indicates that adsorption of micro-pollutants onto sludge solid particles also played important role in their removals. Nevertheless, the differences in their removals between both stage operations were found to be larger than the effect of K_{ow}. Those differences were rather influenced by the effect of biological activities rather than adsorption as the biomass concentrations were maintained at the same level during both stages of operation. It was also noticed that the removal efficiencies of most compounds were maintained relatively constant (within±10%) during long term operation. In this study, our experimental results suggest the beneficial effect of maintaining high biomass concentration and long sludge age on the removal of micro-pollutants present in municipal solid waste leachate.

4.2 Treatment Performance of Laboratory-scale Two-stage MBR System.

4.2.1 Organic and Nitrogen Removal in Laboratory-scale MBR.

During the laboratory-scale two-stage MBR operation, average BOD and COD concentrations in landfill leachate were 6,598 mgL^{-1} and 9,273 mgL^{-1} yielding BOD and COD loading rates of 6.6 and 9.3 $kgm^{-3}d^{-1}$, respectively. Meanwhile, average TKN loading rates were between 0.20-0.23 $kg.(m^3.d)^{-1}$ in the operation period. The performance of laboratory scale MBR in terms of organic (BOD, COD) and nitrogen (NH_4^+, TKN) removals are presented in Table 4.4. Corresponding biomass variations in laboratory scale two-stage MBR system are shown in Figure 4.5. During the long term operation, the aerobic reactor removed 99% of BOD and 96% of COD on average. Meanwhile, NH_4^+ and TKN removals were 90% and 85%, respectively. The treatment performance was found relatively stable over the entire operation period despite gradual change in sludge biomass. MLSS concentration in aerobic reactor was gradually increased from 5.6 gL^{-1} to 10.7 gL^{-1}. MLVSS/MLSS ratio of biomass was kept between 0.48-0.58. Most of the removals were taken place in aerobic reactor as less than 20% removal was observed in the anoxic reactor.

Table 4.4 Performance of laboratory-scale two-stage MBR.

Parameter	Effluent concentration (mgL^{-1})				Removal efficiencies (%)			
	Anoxic reactor		Aerobic reactor		Anoxic reactor		Total	
	Range	Avg. (SD)	Range	Avg. (SD)	Range	Avg.(SD)	Range	Avg.(SD)
BOD	5,120-6,975	6,115(492)	60-112	67(18)	10-20	18(5)	96-100	99(3)
COD	6,125-8,850	7,990(328)	340-465	385(40)	12-19	15(2)	87-98	96(2)
TOC	2,050-2,630	2,520(205)	218-240	230(8)	9-17	11(3)	85-94	91(7)
NH_4^+	114-135	129(10)	3-26	12(5)	11-18	14(5)	88-98	90(5)
TKN	200-240	230(11)	32-58	33(12)	5-12	8(3)	72-90	85(3)

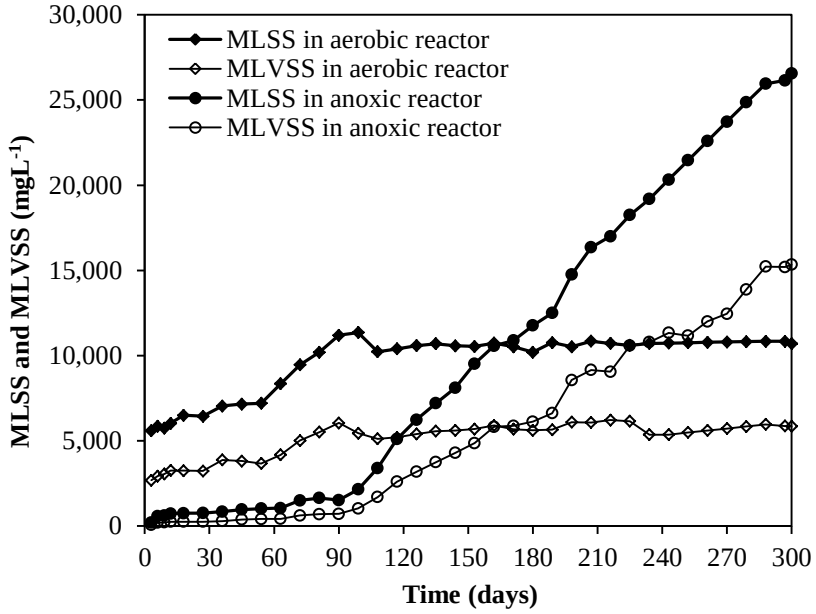


Figure 4.5 Biomass in the laboratory-scale MBR system.

The biomass concentration in anoxic reactor was raised to 27 gL^{-1} during 300 days of operation whereas the biomass concentration in aerobic reactor was kept constantly at about 11 gL^{-1} by the re-circulation operation. Particle size and size distribution are defined as the relative percentage by weight or number of each of the different size fractions of particulate matter (Figure 4.6). Particle sizing is carried out using a Malvern Mastersizer 2000E (Malvern, UK.) which is based on static laser light scattering. The Mastersizer software generates a volume weighed floc size distribution. In order to describe the mean particle size, the volume weighed average diameter which is also known as the mass mean diameter. Fresh MBR sludge sample is collected directly from the system. For analysis, fixing the obscuration level at 10-15% in the Mastersizer software controlled the sludge concentration. In order to promote nitrification activity, floc size in MBR played an important role in facilitating oxygen transfer for microbial activities in the MBR during operation without sludge discharge. An introduction of sludge re-circulation and increasing sludge age could affect the biomass properties and bacterial population in the system (Chiemchaisri and Yamamoto, 2005; Chiemchaisri *et al*., 2011).

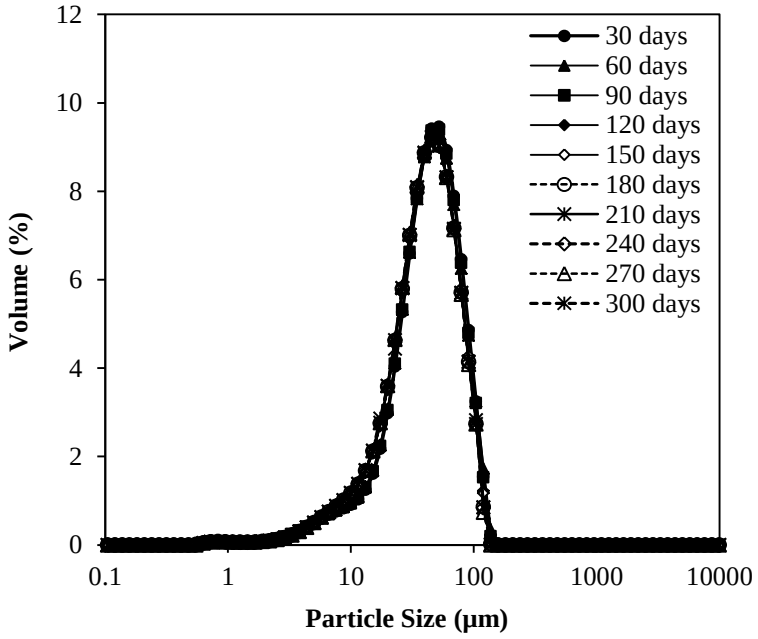


Figure 4.6 Particle size distribution in aerobic reactor during it-MBR laboratory scale operation (n=10).

EPS production in MBR sludge collected from laboratory-scale MBR at 300 days operation was analyzed in terms of soluble and bound EPS. In this study, EPS in term of protein was higher than carbohydrate and found in bound EPS more than soluble EPS similar to our previous investigation (Chiemchaisri *et al.,* 2011). Protein content of soluble EPS and bound EPS were found 39 mg/gVSS and 248 mg/gVSS, respectively. The ratio of protein to carbohydrate (P/C) of bound EPS and soluble EPS were found 23.16, and 37.20, respectively.

Wilén *et al.* (2003) suggested that EPS compositions, such as protein and carbohydrate, strongly influence the surface properties such as hydrophobicity and surface charge of sludge. This observation agreed that hydrophobic fraction of bound EPS was made up only proteins (Jorand *et al.,* 1998). Moreover, hydrophobicity of sludge capable to removed some of contaminants in landfill leachate by adsorption on sludge surface.

4.2.2 Removals of Selected Toxic Organic Micro-pollutants in Laboratory-scale Two-stage MBR.

The analyses of organic micro-pollutants in leachate revealed the presence compounds are 4-methyl-2,6-di-tert-butylphenol (BHT), bisphenol A (BPA), bis(2-ethylhexyl)phthalate (DEHP). Previous research reported that these compounds are xenobiotic organic compounds (XOCs) commonly found in landfill leachate (Paxéus, 2000; Kjeldsen *et al*., 2002; Baun *et al*., 2004). It was found that the removal efficiencies of all three compounds had an increasing trend along the operation period. Among them, DEHP had the highest removal efficiencies, followed by BHT, and BPA, respectively. Despite these biomass variations in the system, the removal efficiencies of those selected compounds were not adversely affected at the concentration of 1,000 μgL^{-1} each compound was added into the reactor. The removal efficiencies of those organic micro-pollutants by laboratory-scale two-stage MBR were summarized in Table 4.5. The results showed that anoxic reactor could remove BPA, BHT, and DEHP only 3%, 4%, and 8%, respectively, and the selected micro-pollutant removals were taken place mainly in aerobic reactor. The removal efficiencies of each compound were found 65%, 70%, 72% of BPA, BHT, and DEHP, respectively. Table 4.5, and Figure 4.7 show the relationship between octanol-water partition coefficient (K_{ow}) of three selected micro-pollutants and their removals during laboratory-scale two-stage MBR operation. DEHP with highest K_{ow} value (K_{ow} 7.54) was removed by 72% whereas BPA with K_{ow} of 3.32 was removed by 65% in the same period. It was clearly seen that the properties of compounds affected their removals during operation. De Wever *et al.,* 2007 suggested that the micro-pollutant removals probably due to retention of slow growing micropollutant degrading bacteria. On the other hand, high SRT were needed to achieve high sludge concentrations, typical for MBRs. For several polar pollutants, the degradation could not be linked to a change in operational parameters such as sludge concentration, sludge load, organic load, and other operational parameters. Moreover, this must have been the result of microbial adaptation. MBR treatment seemed to enhance removal of micro-pollutants with intermediate biodegradability. For easily degradable and intrinsically recalcitrant compounds, MBRs could not make a significant difference in

terms of overall removal efficiencies. Finally, MBR treatment turned out to be less sensitive to operational variables such as HRT and will hence show a higher robustness than conventional systems, also for micro-pollutant removal.

Table 4.5 Concentrations of selected organic micro-pollutant and their removal efficiency.

Compound	Initial concentration (μgL^{-1})	Concentration (μgL^{-1})			Removal efficiency (%)	
		Eff_{anoxic}	In MBR	$Eff_{aerobic}$	$\%R_{anoxic}$	$\%R_{aerobic}$
BPA	1,000	965(20)	615(31)	350(14)	3	65
BHT	1,000	952(25)	652(16)	300(8)	4	70
DEHP	1,000	940(13)	660(18)	280(12)	8	72

* Average (SD) values, No. of samples = 6

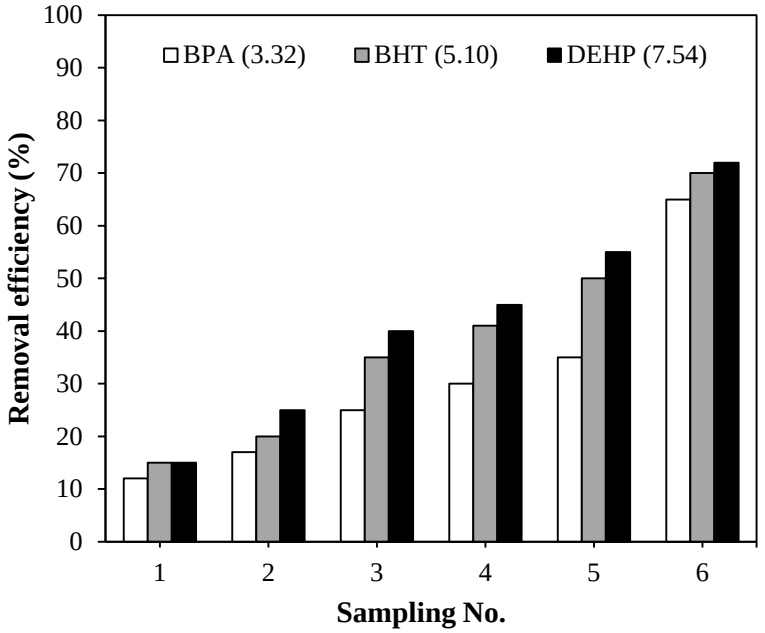


Figure 4.7 Relationship between log K_{ow} of organic micro-pollutants and BPA, BHT, and DEHP removals during laboratory-scale two-stage MBR operation.

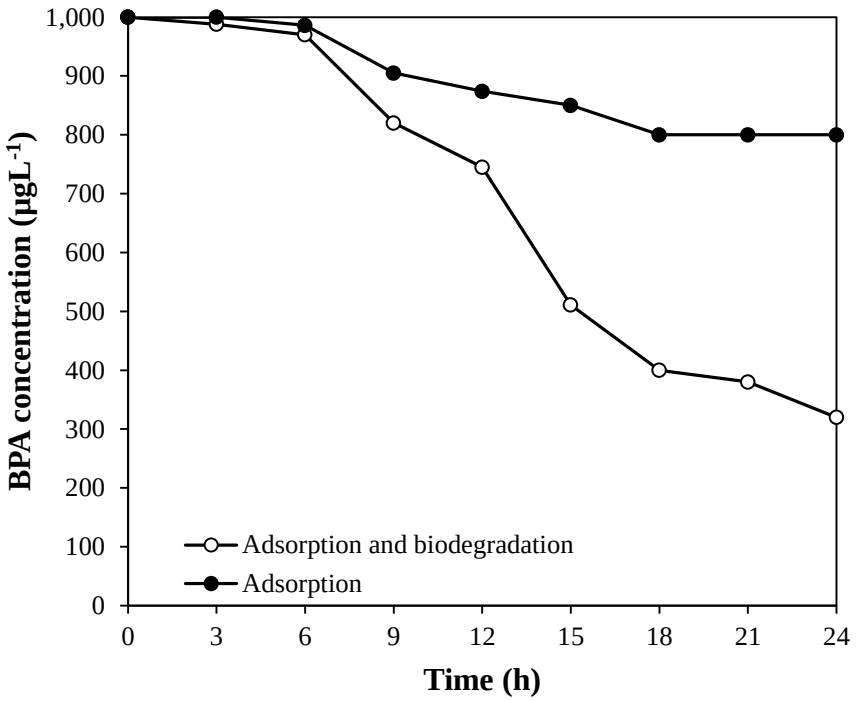
CHAPTER V

MECHANISMS OF PHENOLIC COMPOUNDS AND PHTHALIC ACID ESTERS REMOVAL IN MEMBRANE BIOREACTOR

The mechanisms of micro-pollutants removal from landfill leachate in MBR were investigated. The experiment were divided into three parts are as followed; 1) Batch study of BPA, BHT, and DEHP removals by MBR sludge; 2) Biodegradation of BPA, BHT, and DEHP by non-enrich and enriched nitrifying sludge; and 3) Removals of BPA, BHT, and DEHP through the foulants on membrane.

5.1 Batch Study of BPA, BHT, and DEHP Removals by MBR Sludge.

Batch study was performed to investigate the biodegradation of selected phenolic compounds and phthalic acid esters by sludge taken from the pilot-scale MBR at the end of the experimental period (300 days). The initial concentrations of BHT, BPA, and DEHP were controlled at 1,000 μgL^{-1}. The samples were taken at constant time intervals during 24 h period for the determination of BHT, BPA and DEHP concentrations in dissolved and particulate forms. For the adsorption experiment, inactivated sludge was used for determining adsorption capacity of MBR sludge. The sludge samples obtained from the pilot-scale MBR were inactivated three times by pasteurization at 121 ^{o}C for 15 min in order to terminate microbial activities. Same procedures with those used in biodegradation experiment were performed using inactivated sludge. BHT, BPA, and DEHP were analyzed in dissolve, and particulate forms. The concentrations of mixed liquor suspended solids (MLSS) in these batch experiments were controlled at 1,000 mgL^{-1}. Figure 5.1 and Figure 5.2 show the decrease of BPA, BHT, and DEHP concentrations in batch experiments using MBR sludge. The concentration of BPA and BHT were found much higher in the soluble phase. The trend of concentrations of BPA, and BHT were similar at 0 to 3 h.


1,000
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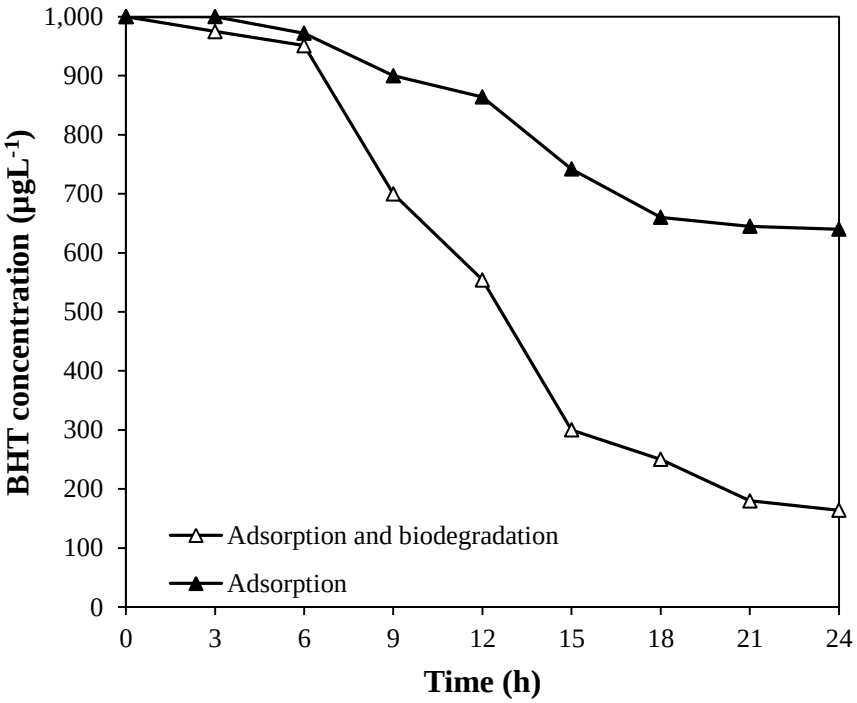
800
700
600
500
400
300
200
100
0
BPA concentration (µgL-1)
0
3
6
9
12
15
18
21
24
Time (h)
Adsorption and biodegradation
Adsorption

(a)


1,000
900
800
700
600
500
400
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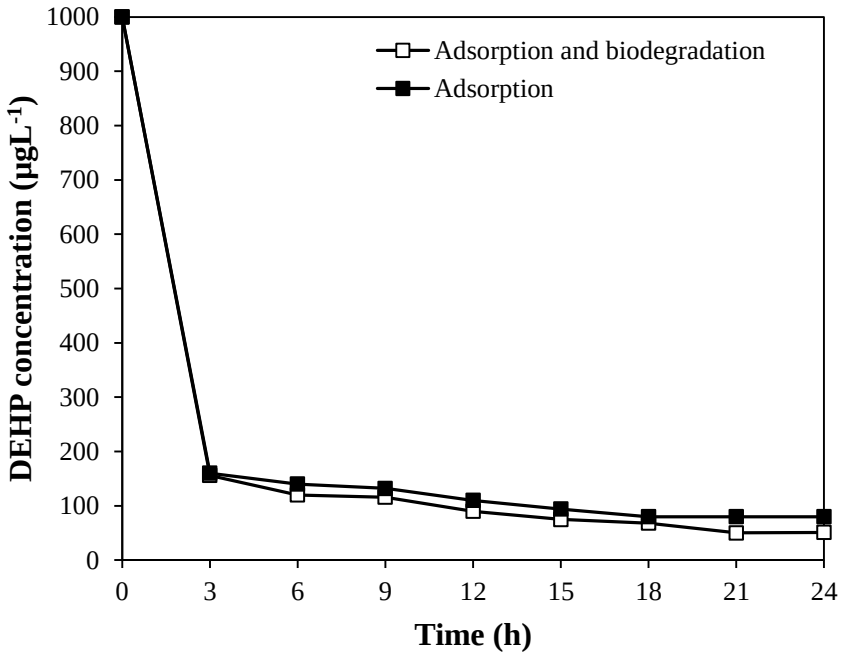
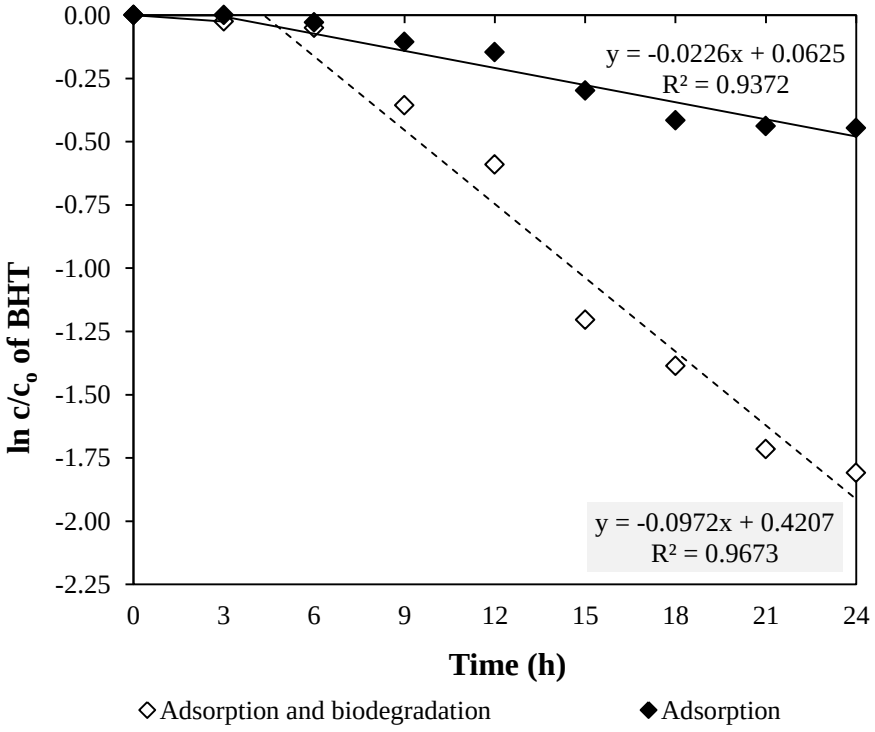
200
100
0
BHT concentration (µgL-1)
0
3
6
9
12
15
18
21
24
Time (h)
Adsorption and biodegradation
Adsorption

(b)

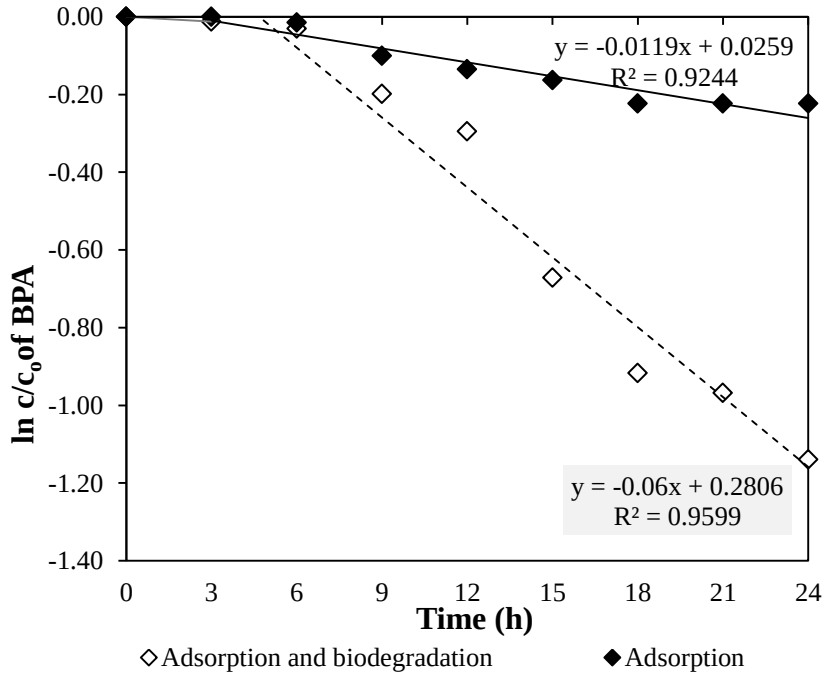


(c)

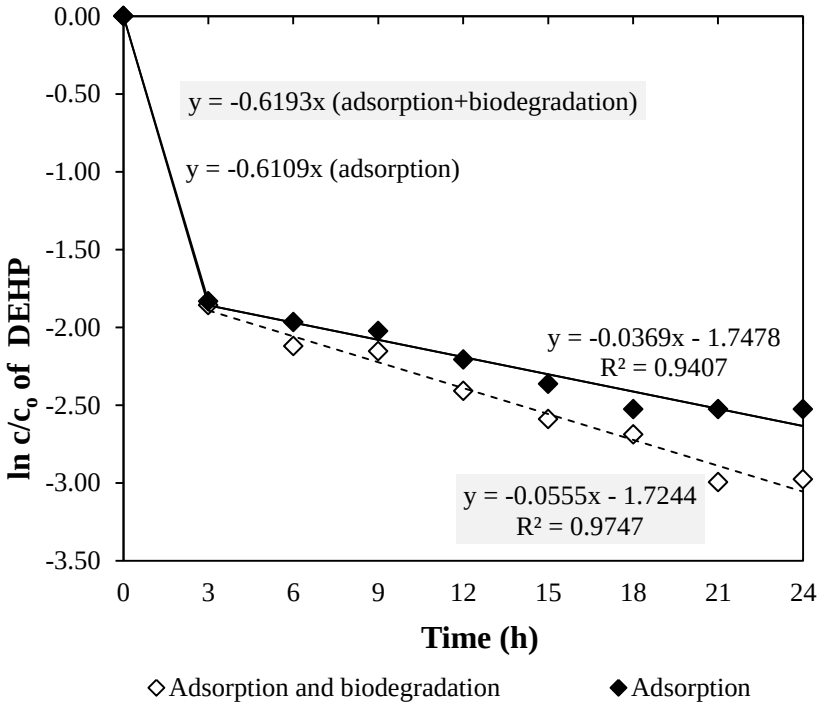
Figure 5.1 BPA (a), BHT (b), and DEHP (c) removals by MBR sludge



(a)



(b)



(c)

Figure 5.2 Removal rates of BHT (a), BPA (b), and DEHP (c) by MBR sludge.

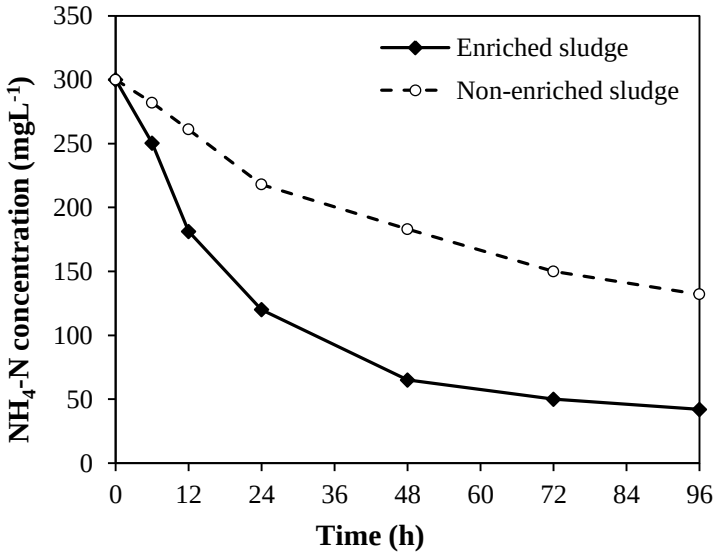
After 3 to 18 h, the concentration of BPA, and BHT were decreasing rapidly by both biodegradation and adsorption mechanisms. During 24 h of biodegradation, the concentrations of BPA and BHT were reduced by approximately 164, and 320 μgL^{-1}. It was found that biodegradation was the important mechanism for the removal of BPA and BHT which removal (adsorption+biodegradation) rates of 0.0481 h^{-1}, and 0.0746 h^{-1}, respectively. The removal efficiencies of BPA, and BHT through biodegradation mechanism were 80%, and 77%, respectively. The removal efficiencies of BPA, and BHT were found only 20%, and 23%, respectively through adsorption mechanism. On the other hand, the removal of DEHP was found higher in the solid phase. The biodegradation mechanism was not significant for DEHP reduction as it was removed mostly through adsorption mechanism. Trend of DEHP concentration was decreased rapidly at 0 to 3 h which adsorption rate of 0.0186 h^{-1}. After 3 h, DEHP concentration was gradually decreased but the DEHP concentration not changed much and the adsorption rate was found to be 0.0084 h^{-1}. During 24 h of adsorption, DEHP concentration was remained at 80 μgL^{-1} and the removal efficiency of DEHP through adsorption mechanism was 99%. It was found that most of phenolic compounds (BHT and BPA) were mainly removed by biodegradation mechanism and DEHP which is relatively hydrophobic compound (log K_{ow} = 7.54) and mainly found in solid phase was removed through adsorption onto the sludge in the MBR. Log K_{ow} value of BPA, and BHT were approximately 3.32, and 5.10, respectively. In general for compounds with log K_{ow} < 2.5, the adsorption to activated sludge is not contribute significantly to the removal of the organic micro-pollutants via excess sludge withdrawal. Between log K_{ow} of 2.5 to 4, moderate biosorption is expected whereas values higher than 4.0 are synonym to high adsorption potential. Previous study has shown that nitrifying sludge was associated to the removal of BPA in batch experiment within 24 h (Kim *et al.*, 2007). The fate of organic micro-pollutants during MBR treatment depends on physico-chemical properties of the compound, operational parameters (biomass concentration, sludge retention time, hydraulic retention time, temperature and pH) of wastewater to be treated. Nevertheless, biosorption and biodegradation processes are reported to be two of the most important removal (Cirja *et al*., 2008).

5.2 Biodegradation of BPA, BHT, and DEHP

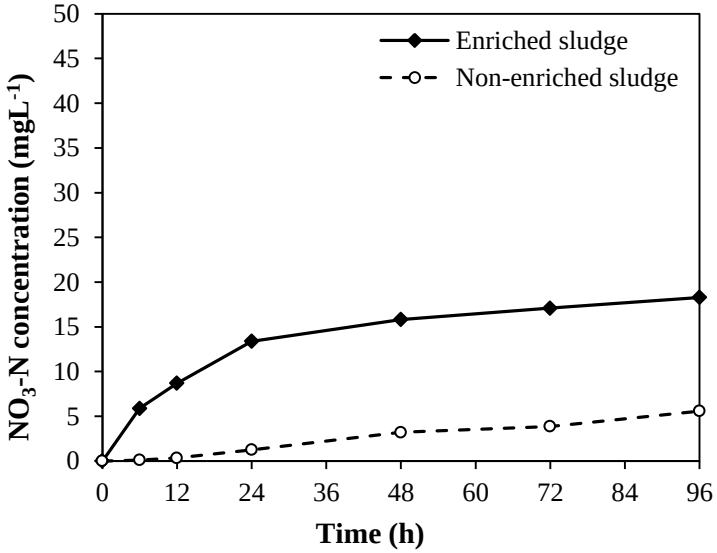
In order to investigate the role of nitrifying organisms in sludge on the biodegradation of BPA, BHT, and DEHP, batch experiment using non-enriched, and enriched nitrifying sludge, and sludge with inhibitors were performed. The MBR sludge was taken from laboratory scale it-MBR. Nitrifying sludge was enriched for more than 4 months. At the enrichment period, MBR influent leachate with ammonium was used as enrichment medium. The ammonium concentration was gradually increased from 100 to 300 mgNH_4-NL^{-1}. The nitrification process is performed by a group of autotrophic microorganisms. The principal mechanism for the nitrogen removal takes place by two reactions, one is ammonia oxidized to nitrite by *Nitrosomonas spp.* and the other is nitrite to nitrate by *Nitrobacter spp*.. The ammonium concentration was consumed during the enrichment nitrifying bacteria, and transformation to nitrate concentration as shown in Figure 5.3.

During 24 h of enrichment nitrifying sludge, the ammonium concentration was oxidised by *Nitrosomonas spp.* and nitrate concentration was produced by *Nitrobacter spp*.. The large difference in NH_3-N reduction (250 μgL^{-1}), and nitrate production during batch experiment was mainly due to N uptake for heterotrophic growth as the influent leachate contained initial BOD concentration of 5,000 mgL^{-1}.

Batch experiments were done in parallel with the operation of the laboratory-scale it-MBR system. The role of nitrification on BHT, BPA, DEHP degradation were also assessed by adding 20 mgL^{-1} of allyl-thiourea (ATU) to the mixed liquor. ATU is known to inhibit the activity of AOB, which is the first step in the nitrification process. The BPA, BHT, and DEHP removals in the reactor were due to biological activity, the similar batch experiment tested as earlier described were performed but the inactivated sludge was performed with the triplicate pasteurization at 121 ^oC for 15 min in order to terminate microbial activities. The experiments with the full inhibition of biological activities were also carried out to distinguish pure adsorption onto sludge from biodegradation mechanism.



(a)



(b)

Figure 5.3 Changes in NH_4-N (a), and NO_3-N (b) concentrations during enrichment of nitrifying in MBR sludge.

The enrichment of nitrifier culture is a significant factor on the biodegradation of XOCs in biological wastewater treatment system (Tran *et al*., 2009). It is therefore possibly that nitrifying organisms was also responsible for the removal of micro-pollutants through their co-metabolic pathways. In this part, the quantification of ammonia oxidizing bacteria and nitrite oxidizing bacteria of sludge from laboratory-scale two-stage MBR system, and sludge used in batch experiment were determined by FISH technique to find out the information supported about the microorganisms responsible in micro-pollutant removals. The percentage of *Ammonia oxidizing β Proteobacteria*, and *Nitrobacter spp.* in anoxic and MBR sludge were determined from the relative percentage of NSO1225, and NIT3 probe, respectively. The quantification of EUB338 probe was determined by specification for all bacteria. The percentages were determined by evaluating at least nine representative microscopic fields. The dominant bacteria use in this research was performed according to Chiemchaisri *et al.*, 2011.

The MBR sludge collected from laboratory-scale two-stage MBR system was used in batch experiment The enrichment of nitrifying sludge was performed by adding NH_4-N 100 to 300 mgL^{-1} as substrate. In the comparability with all bacteria, enriched nitrifying sludge that used in batch experiment found that the percentage of *Ammonia oxidizing β Proteobacteria*, and *Nitrobacter spp.* were higer than non-enrich nitrifying sludge in the percentage of 68.5%, and 23.1%, respectively. In laboratory-scale two-stage MBR system, the bacteria community in anoxic and aerobic reactors was performed using the FISH technique. The results reveal the similarity between the *Ammonia oxidizing β Proteobacteria*, and *Nitrobacter spp.* in both reactors.

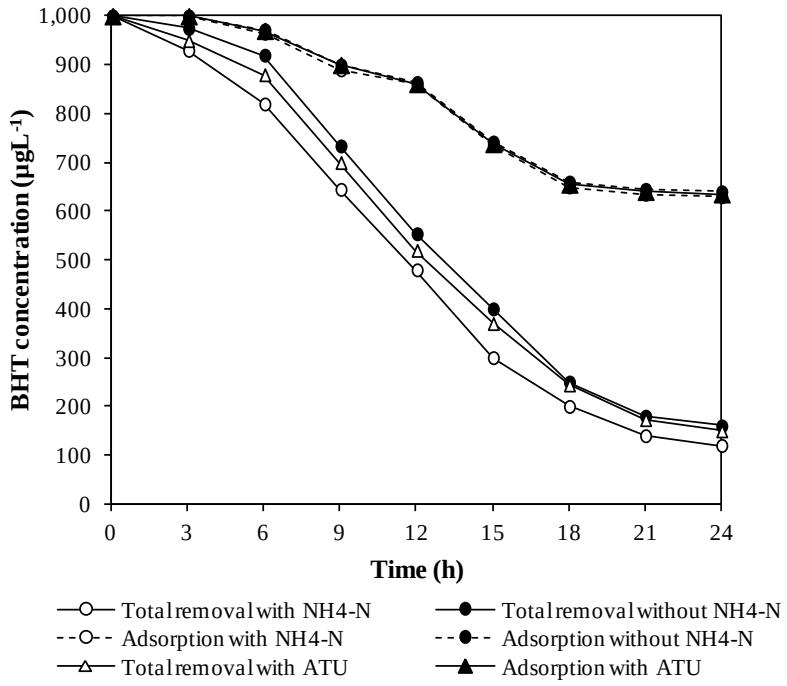
Comparatively, the higher percentage of MBR sludge, hybridized with NSO1225, specific for the detection of *Ammonia oxidizing β Proteobacteria*, and NIT3 with specific for the detection of *Nitrobacter spp.* were found in aerobic reactor. Table 5.1 presents the relative percentage of *Ammonia oxidizing β Proteobacteria*, and *Nitrobacter spp.* to the total microorganisms in sludge collected from laboratory-scale MBR, and enriched nitrifying sludge that used in batch experiment.

Table 5.1 The relative percentage of *Ammonia oxidizing β Proteobacteria*, and *Nitrobacter spp.* to the total microorganisms of sludge.

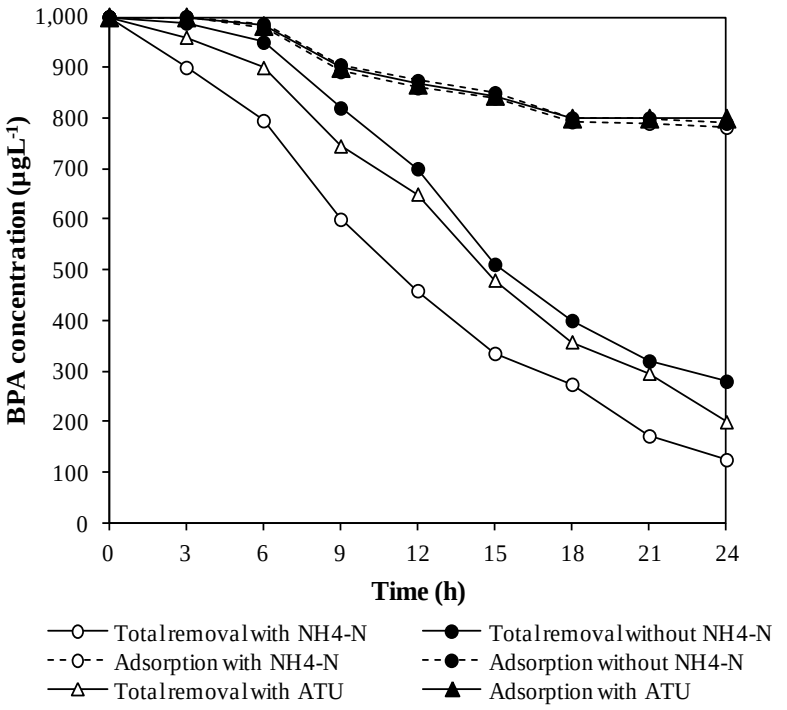
Bacterial group (probe)	**Laboratory-scale**		**Batch experiment**
	Anoxic sludge	**MBR sludge**	**Enriched nitrifying sludge**
Ammonia oxidizing β Proteobacteria (NSO1225)	3.98[a] (26.2[b])	14.54[a] (22.3[b])	21.80[a] (25.9[b])
Nitrobactor spp. (NIT3)	3.14[a] (9.1[b])	5.03[a] (9.5[b])	8.90[a] (14.2[b])

Remark %of DAPI[a], %among all bacteria[b]

Non-enrich, and enriched nitrifying sludge activity testing were performed to investigate the biodegradability of two selected phenolic compounds. The concentrations of mixed liquor suspended solids (MLSS) in these batch experiments were controlled at 1,000 mgL^{-1}. The initial concentrations of BHT, and BPA were set at 1,000 μgL^{-1}. Figure 5.4 to Figure 5.6 show the samples were taken at constant time intervals during 24 h period for the determination of BHT, and BPA concentrations in dissolved and particulate forms. For the adsorption experiment, inactivated sludge was used for determining adsorption capacity of enriched nitrifying sludge. The enriched nitrifying sludge samples were inactivated three times by pasteurization at 121 ^{o}C for 15 min in order to terminate microbial activities. Same procedures with those used in biodegradation experiment were performed using inactivated sludge. The concentration of BPA, and BHT removal in batch experiments using non-enrich, and enriched nitrifying sludge were decreasing rapidly via biodegradation mechanisms. During 24 h of biodegradation, the concentrations of BHT and BPA were reduced by approximately 120, and 125 μgL^{-1} of enriched nitrifying sludge with NH_4-N addition. For enriched nitrifying sludge without NH_4-N addition, the concentrations of BHT and BPA were reduced by approximately 160, and 280 μgL^{-1}. The concentrations of BHT and BPA were reduced by approximately 150, and 200 μgL^{-1} of enriched nitrifying sludge with ATU. Table 5.2 shows the removal rate, and of these compounds at 24 h time testing.

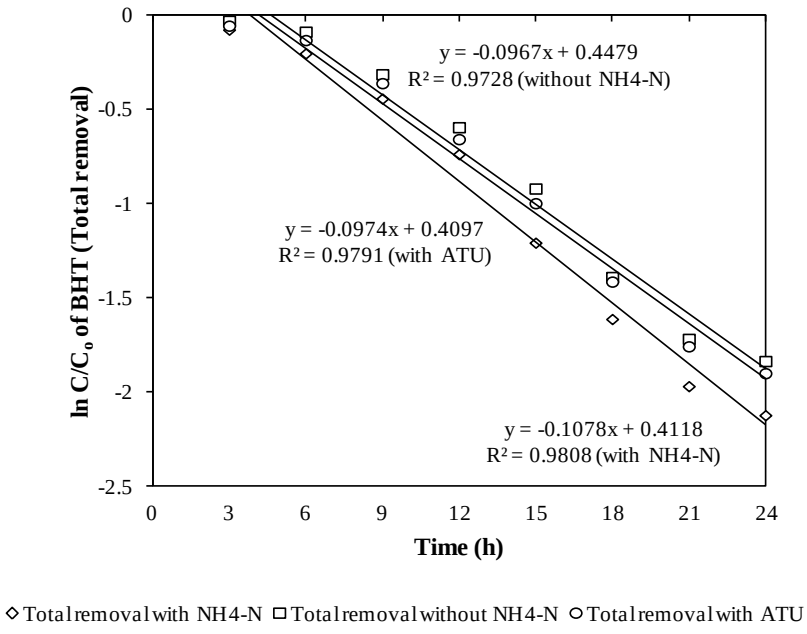


(a)

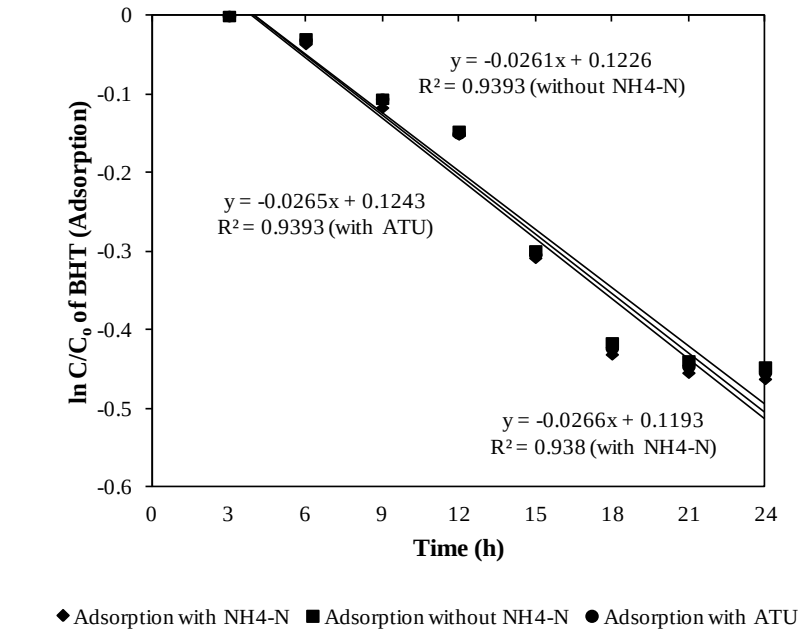


(b)

Figure 5.4 BHT (a), BPA (b) removals by enriched and inhibited sludge.

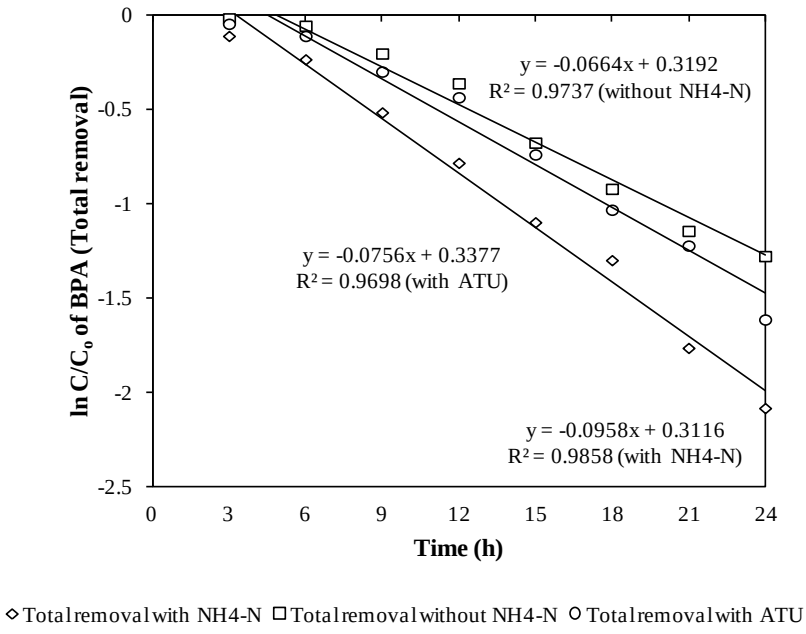


(a)

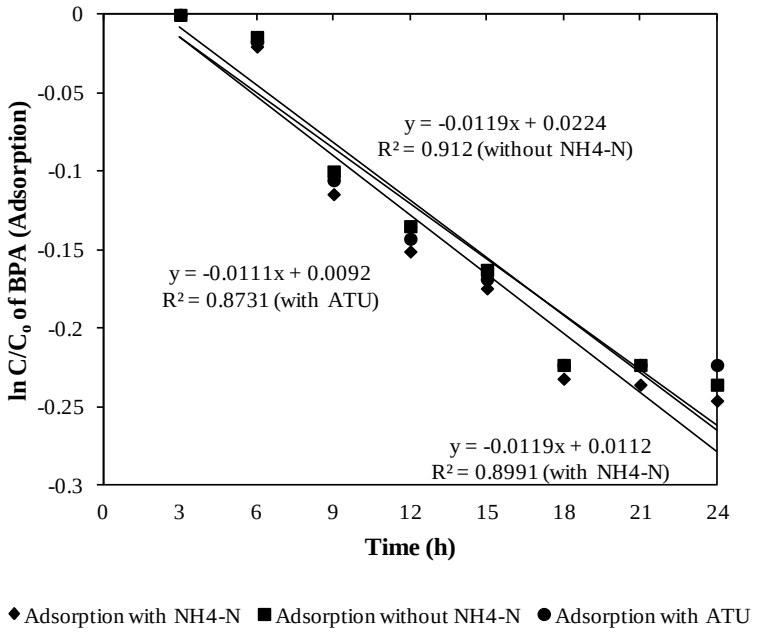


(b)

Figure 5.5 Total removal rate of BHT (a) and adsorption rate of BHT (b) using enriched and inhibited sludge.



(a)



(b)

Figure 5.6 Total removal rate of BPA (a) and adsorption rate of BPA (b) using enriched and inhibited sludge.

Table 5.2 Removal of BPA, and BHT by enriched nitrifying sludge with and without inhibitors at 24 h.

Enriched nitrifying sludge	Removal rates at 24 h				Removal efficiency (%)			
	Total removal		Adsorption		Adsorption		Biodegradation	
	BHT	BPA	BHT	BPA	BHT	BPA	BHT	BPA
without-NH_4-N	0.0967	0.0664	0.0261	0.0119	27	18	73	82
ATU	0.0974	0.0756	0.0265	0.0111	27	15	73	85
NH_4-N addition	0.1078	0.0958	0.0266	0.0119	25	12	75	88

The removal (adsorption+biodegradation) rates of BHT by enriched nitrifying sludge with NH_4-N addition, enriched nitrifying sludge without NH_4-N, and enriched nitrifying with ATU were 0.1078 h^{-1}, 0.0967 h^{-1}, and 0.0974 h^{-1}, respectively. The removal (adsorption+biodegradation) rates of BPA by enriched nitrifying sludge with NH_4-N addition, enriched nitrifying sludge without NH_4-N, and enriched nitrifying with ATU were 0.0958 h^{-1}, 0.0756 h^{-1}, and 0.0664 h^{-1}, respectively. The removal efficiencies of BHT through biodegradation mechanism by enriched nitrifying sludge with NH_4-N addition, enriched nitrifying sludge without NH_4-N, and enriched nitrifying with ATU were more than 73%, respectively.

The removal efficiencies of BPA through biodegradation mechanism by enriched nitrifying sludge with NH_4-N addition, enriched nitrifying sludge without NH_4-N, and enriched nitrifying with ATU were 88%, 82%, and 85%, respectively. The removal rates of their co-metabilc pathways on BHT, and BPA removal were found 0.0106 h^{-1}, and 0.0294 h^{-1}, respectively. For the removal rates of nitrifying bacteria on these compounds were found only 0.0100 h^{-1}. The results shown that, not only nitrifying bacteria degrading phenolic compounds but also the heterotrophic organisms also help to removed phenolic compounds in MBR operated under long sludge age condition.

5.3 Removals of BPA, BHT, and DEHP Through Foulants on Membrane.

5.3.1 BPA, BHT, and DEHP Removal Through Foulants on Flat Sheet Membrane.

In order to provide further information concerning the organic micro-pollutants removal mechanisms in MBR, their removal through fouled membrane filtration was also investigated. The initial concentration of selected target compounds were performed at 1,000 μgL^{-1}. The effluent concentrations of BPA, BHT, and DEHP were 311.1 μgL^{-1}, 65.9 μgL^{-1}, and 242.2 μgL^{-1} by foulants on cellulose acetate membrane consist of both cake and gel foulant layer. For the retention by gel layer, the effluent concentrations of BPA, BHT, and DEHP were 672.5 μgL^{-1}, 110.3 μgL^{-1}, 463.3 μgL^{-1}. In the case of the effluent concentrations of BPA, BHT, and DEHP that removed by cellulose acetate membrane layer were 777.9 μgL^{-1}, 268.5 μgL^{-1}, 769.9 μgL^{-1}.

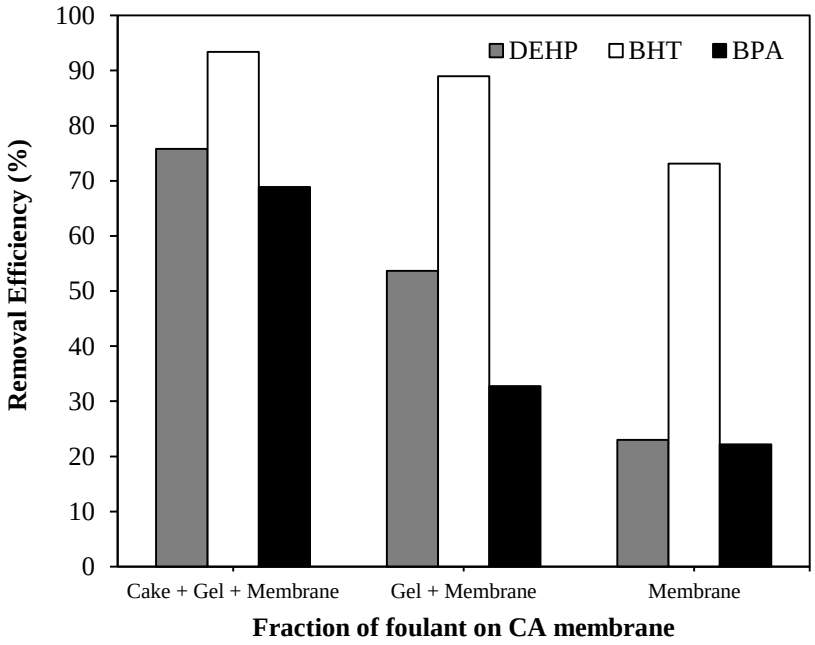
The removal efficiencies of BPA, BHT, and DEHP were found 68.89%, 93.41%, and 75.78% by foulants on cellulose acetate membrane consist of both cake and gel foulant layer. For the retention by gel layer, the BPA, BHT, and DEHP concentrations were removed 32.75%, 88.97%, and 56.37%, respectively. In the case of cellulose acetate membrane retention, the removal of BPA, BHT, and DEHP were 22.21%, 73.15%, and 23.01%, respectively.

The filtration experiments on polyvinylidene fluoride membrane were performed with the initial concentration of selected target compounds were 1,000 μgL^{-1}. The effluent concentrations of BPA, BHT, and DEHP were 388.7 μgL^{-1}, 284.2 μgL^{-1}, and 132.6 μgL^{-1} by foulants on polyvinylidene fluoride membrane consist of both cake and gel foulant layer. For the retention by gel layer, the effluent concentrations of BPA, BHT, and DEHP were 578.1 μgL^{-1}, 529.9 μgL^{-1}, and 465.0 μgL^{-1}. In the case of the effluent concentrations of BPA, BHT, and DEHP that removed by polyvinylidene fluoride membrane layer were 718.2 μgL^{-1}, 678.4 μgL^{-1}, and 542.6 μgL^{-1}.

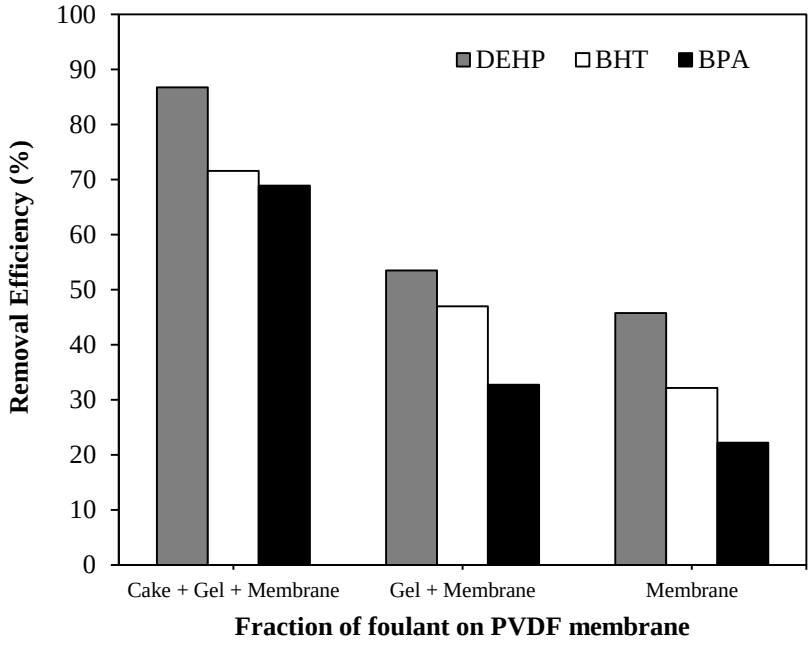
The removal efficiencies of BPA, BHT, and DEHP were 61.13%, 71.58%, and 86.74% by foulants on polyvinylidene fluoride membrane consist of both cake and gel foulant layer. For the retention by gel layer, the BPA, BHT, and DEHP concentrations were removed 42.19%, 47.01%, and 53.50%, respectively. In the case of polyvinylidene fluoride membrane retention, the removal of BPA, BHT, and DEHP were 28.18%, 32.16%, and 45.74%, respectively. Table 5.3, and Figure 5.7 show the removal of target compounds through foulants on flat sheet membrane.

Table 5.3 Removals of target compounds through foulants on flat sheet membrane.

Fraction	Initial concentration (μgL^{-1})	Final concentration (μgL^{-1})			Removal efficiency (%)		
		BPA	BHT	DEHP	BPA	BHT	DEHP
Cellulose acetate (CA) membrane							
Cake + Gel + Membrane	1,000	311.1	65.9	242.2	68.89	93.41	75.78
Gel + Membrane	1,000	672.5	110.3	463.3	32.75	88.97	56.37
Membrane	1,000	777.9	268.5	769.9	22.21	73.15	23.01
Polyvinylidene Fluoride (PVDF) membrane							
Cake + Gel + Membrane	1,000	388.7	284.2	132.6	61.13	71.58	86.74
Gel + Membrane	1,000	578.1	529.9	465.0	42.19	47.01	53.50
Membrane	1,000	718.2	678.4	542.6	28.18	32.16	45.74



(a)



(b)

Figure 5.7 Removal of target compounds through foulants on flat sheet membrane cellulose acetate membrane (a), and Polyvinylidene fluoride membrane (b).

5.3.2 BPA, BHT, and DEHP removal through foulants on hollow fiber membrane

The removal of target compounds through foulants on hollow fiber membrane were shown in Table 5.4, and Figure 5.8. The initial concentration of selected target compounds were performed at 1,000 μgL^{-1}. The effluent concentrations of BPA, BHT, and DEHP were 314.0 μgL^{-1}, 298.1 μgL^{-1}, and 242.1 μgL^{-1} by fouled membrane consist of both cake and gel foulant layer. For the retention by gel layer, the effluent concentrations of BPA, BHT, and DEHP were 546.8 μgL^{-1}, 502.1 μgL^{-1}, and 473.8 μgL^{-1}.

In the case of the effluent concentrations of BPA, BHT, and DEHP that removed by membrane layer were 665.5 μgL^{-1}, 615.0 μgL^{-1}, and 519.2 μgL^{-1}. The removal efficiencies of BPA, BHT, and DEHP were found 68.61%, 70.19%, and 75.79% by fouled membrane consist of both cake and gel foulant layer. For the retention by gel layer, the BPA, BHT, and DEHP concentrations were removed 45.32%, 49.79%, and 52.62%, respectively. In the case of membrane retention, the removal of BPA, BHT, and DEHP were 33.45%, 38.50%, and 48.08%, respectively. These results show that the presence of foulants increased the retention of the target compounds on membrane.

Table 5.4 Removals of target compounds by HF fouled and cleaned membrane.

Fraction	Initial concentration (μgL^{-1})	Final concentration (μgL^{-1})			Removal efficiency (%)		
		BPA	BHT	DEHP	BPA	BHT	DEHP
Cake + Gel + Membrane	1,000	314.0	298.1	242.1	68.61	70.19	75.79
Gel + Membrane	1,000	546.8	502.1	473.8	45.32	49.79	52.62
Membrane	1,000	665.5	615.0	519.2	33.45	38.50	48.08

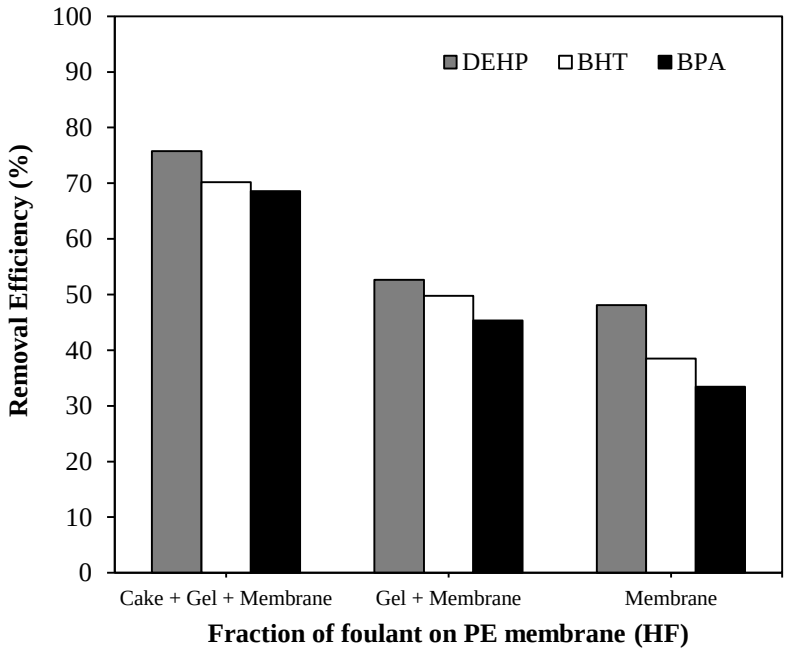


Figure 5.8 Removal of target compounds through foulants on hollow membrane.

Nghiem and Hawkes (2007) suggested that the biodegradation, adsorption, and rejection by foulant are three main mechanisms which can influence organic micro-pollutants retention in fouled membranes including enhanced concentration polarization, pore blocking and adsorption to the fouling layer. Nevertheless, it is highly variable and dependent on properties of the organic micro-pollutants such as electrical charge and molecular weight as well as organic substance properties and membrane materials.

The two-stage MBR system was applied to treatment landfill leachate. In order to provide further information concerning the organic micro-pollutants removal mechanisms in MBR, biodegradation, adsorption, and rejection by fouled membrane were investigated (Figure 5.9). The initial concentration of selected target compounds were performed at 1,000 μgL^{-1}. The results shown that BPA, and BHT were removed under biodegradation condition. In contrast, DEHP was adsorbed on the sludge surface, and very low biodegradation. Moreover, foulant on membrane layer was associated to removed the BPA, BHT, and DEHP concentration remaining in aerobic tank.

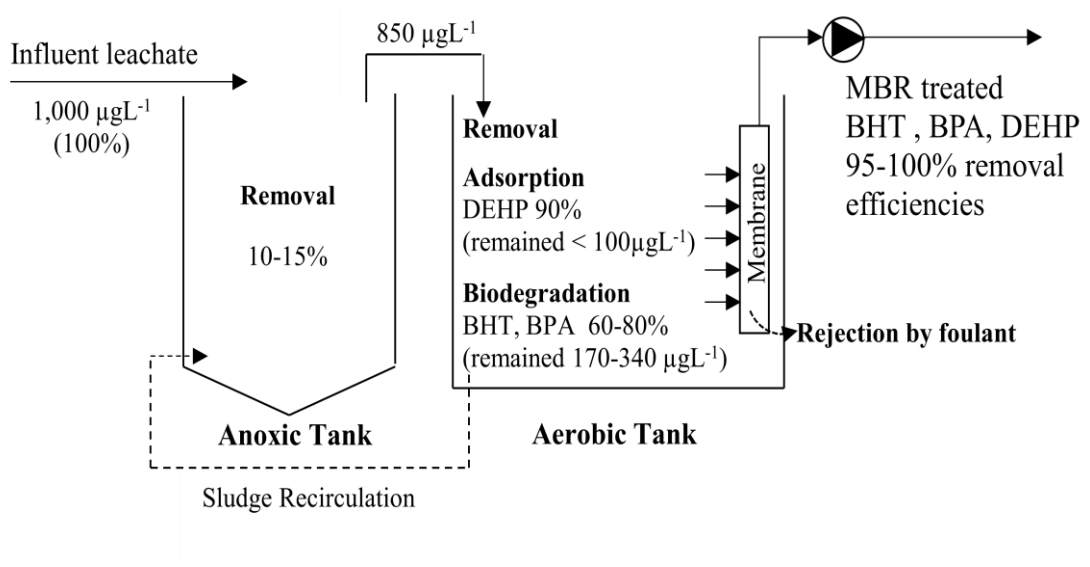


Figure 5.9 Overall mechanisms of BPA, BHT, and DEHP removal in MBR.

CHAPTER VI

BIO-TOXICITY REDUCTION OF LEACHATE DURING MBR TREATMENT

The lechate bio-toxicity reduction during MBR treatment system was studied in term of acute toxicity and chronic toxicity on local fish species are Nile Tilapia (*Oreochromis niloticus*), and Common Carp (*Cyprinus carpio*). The bio-toxicity testing was conducted using acute toxicity with the mortality of fish species, and geno-toxicity testing with DNA strand breaks by comet assay technique.

6.1 Acute Toxicity Determination (LC_{50})

The lethal concentrations (LC_{50}) of selected fish species exposed to MBR influent leachate and MBR treated leachate. Table 6.1 shows that LC_{50} for raw leachate were 1.94% (v/v), and 2.02% (v/v) for *O. niloticus* and *C. carpio* treated water whereas they were 19.54% (v/v), and 17.57% (v/v) for treated water respectively. This experiment shown that the 96 hour LC_{50} of ammonia causing acute toxicity on *C. carpio* and *O. niloticus* were found to be 2.3-2.6 mgL^{-1}, and 2.2-2.5 mgL^{-1}, respectively. The effect of COD and NH_3 concentration in leachate on mortality of living organisms are shown in Figure 6.1.

After the treatment, higher LC_{50} values were obtained from the test using treated water. For COD, LC_{50} of untreated and treated leachate in case of *O. niloticus* were found to be 187 mgL^{-1} and 225 $mgL^{-1}$and *C. carpio* were found to be 202 mgL^{-1} and 194 mgL^{-1} whereas those for NH_3, *O. niloticus* were found to be 2.3 mgL^{-1} and 4.9 mgL^{-1} and *C. carpio* were found to be 2.4 mgL^{-1} and 4.4 mgL^{-1}, respectively. The changes of *O.* niloticus and *C. carpio* were accounted for COD 16.89% and 3.96%, NH_3 53.06% and 45.45% of initial LD_{50} in raw leachate.

Table 6.1 LC_{50} values of MBR influent and MBR treated leachate on tested species.

Fish species	LC_{50}* (%concentration)	Corresponding pollutant concentrations*	
		COD(mgL^{-1})	NH_3-N(mgL^{-1})
MBR influent			
O. niloticus	1.94 (1.81-2.09)	187 (173-201)	2.3 (2.2-2.5)
C. carpio	2.02 (1.88-2.18)	194 (180-209)	2.4 (2.3-2.6)
MBR treated			
O. niloticus	19.54 (18.47-20.66)	225 (212-238)	4.9 (4.6-5.2)
C. carpio	17.57 (16.41-18.80)	202 (189-216)	4.4 (4.1-4.7)

* Average (range) values, no. of samples = 33.

These observations may imply that there was a combined toxic effect between ammonia and other organic compounds present in leachate. Therefore, the removal of some toxic organic compounds during biological treatment using MBR helped reducing the bio-toxicity of leachate in this study. This result shows a significant reduction in bio-toxicity of leachate after two-stage MBR system, similar to that reported in the previous literature (Theepharaksapan *et al.*, 2011). Some differences in bio-toxicity effect were observed among the tested species. Previous research shown that the evaluation acute toxicity of landfill leachate from three different landfill in Malaysia to Common Carp (*Cyprinus carpio)* and reported 96 hour LC_{50} values of 1.1–3.82 % (v/v). The 96 hour LC_{50} for municipal landfill on fingerlings of *Clarias Gariepinus* was 36.6% (v/v). The 48 hour LC_{50} for leachates of ten sampling from municipal solid wastes landfill on *Artemia franciscana* were 3.2% and 39.3% (Olivero *et al.,* 2008).

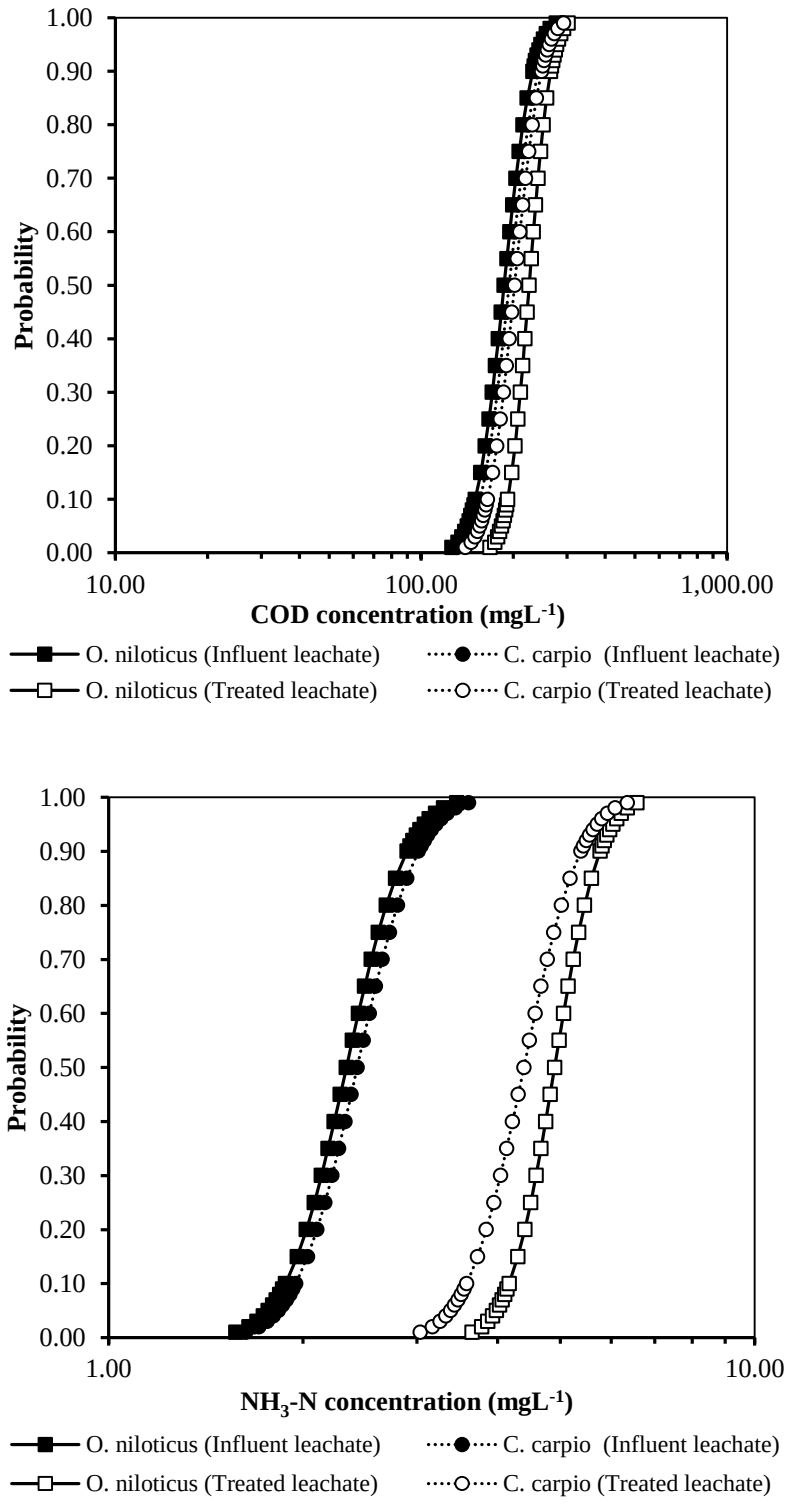


Figure 6.1 Effect of COD and NH_3-N concentration in leachate on mortality of living organisms.

6.2 Relationship Between Acute Toxicity and Chemical Parameters

This experiment indicated the correlation coefficient and significant differences between mortality of tested organisms and physicochemical parameters which were UIA, COD, pH, and EC. Table 6.2 shows the correlation coefficient between mortality, and COD concentration values of *O. niloticus*, and *C. carpio* were 0.708, and 0.808 respectively, whereas UIA values were 0.509, and 0.572 respectively. The significant levels, and COD concentration values of *O. niloticus*, and *C. carpio* were found to be 0.000, and 0.000 respectively, whereas un-ionized ammonia concentration values were 0.002, and 0.000 respectively. The result indicated that mortality of *O. niloticus*, and *C. carpio* was significant positive correlated ($P<0.01$) with ammonia and COD. Further parameter such as conductivity was found correlated with mortality of *O. niloticus*, and *C. carpio* as 0.256, and 0.307, respectively. The significant levels were 0.000, and 0.001, respectively. A negative correlation were found as mortality and pH values of *O. niloticus*, and *C. carpio* with the value of -0.132 (0.001), -0.138 (0.000). This result suggests that unionized ammonia have a direct relationship to toxicity, it is increase the sensitivity of *C. carpio*, and *O. niloticus* respectively. In term of organic matter, COD concentration increased the sensitivity of *O. niloticus*. The ammonia was the main cause of the toxicity measured in the bio-toxicity determination. The toxicity in landfill leachate depends on several factors that may influence leachate toxicity (Clement *et al.*, 1993). Kjeldsen *et al.* (2002) stated that the geno-toxicity test found that organic compounds in leachate may cause the mutagenic activity.

Table 6.2 Correlation between acute toxicity and physicochemical parameters.

	Mortality		COD	UIA	pH	EC
	O.niloticus	*C.carpio*				
Mortality of O.niloticus	**1**					
	(0.000)					
Mortality of C.carpio	0.988*	**1**				
	(0.000)	(0.000)				
COD	0.708*	0.808*	**1**			
	(0.000)	(0.000)	(0.000)			
UIA	0.509*	0.572*	0.879*	**1**		
	(0.002)	(0.000)	(0.000)	(0.000)		
pH	-0.132*	-0.138*	0.240*	0.854*	**1**	
	(0.001)	(0.000)	(0.000)	(0.000)	(0.000)	
EC	0.256*	0.307*	0.652*	0.849*	0.105*	**1**
	(0.000)	(0.001)	(0.000)	(0.000)	(0.001)	(0.000)

UIA = unionized-ammonia , *Correlation is significant at the 0.01 level, no. of samples = 33

6.3 Geno-toxicity Evaluation (Level of DNA Damage)

The comet assay was utilized as biomarker of the genotoxic potential of the raw and treated leachate, which was diluted using degree of acute toxic level at LC_{10}, on fish species. Figure 6.2 shows the DNA damage appearances of comet in peripheral erythrocytes of *O. niloticus* and *C. carpio* after exposure in MBR influent leachate and MBR treated leachate. Level of DNA damage was analyzed using image analysis on 100 cells per sample. After period exposure, DNA damage in blood cells showed the reversible, with a reduction of percentage of DNA damage compared 7^{th} exposure days as shown as Figure 6.3. This type of damage is possibly reversible, which has been observed in environmental monitoring studies that after a recuperation period under non-polluted conditions in the laboratory, reflecting the reversibility and non-persistence of such damage.

The DNA strand breaks, particularly as measured by the comet assay, act as a biomarker of mutagenicity in fish and other aquatic species. They also emphasized that this approach should be combined with the use of other biomarker. The sensitivity of tested species, the result shows that the %DNA damage values of *O. niloticus* were higher than %DNA damage of *C. carpio*, demonstrating that *O. niloticus* was considerably more sensitive. This difference can be caused by different food web of tested fish.

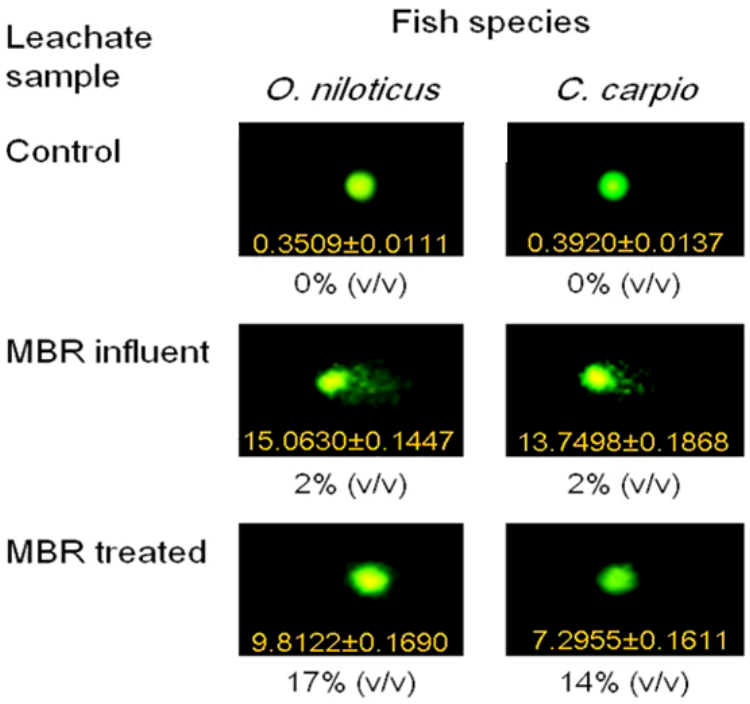


Figure 6.2 DNA damage appearances of comet in peripheral erythrocytes of fish species (*O.niloticus* and *C.carpio*) as a result of MBR influent leachate and MBR treated leachate at LC_{10}.

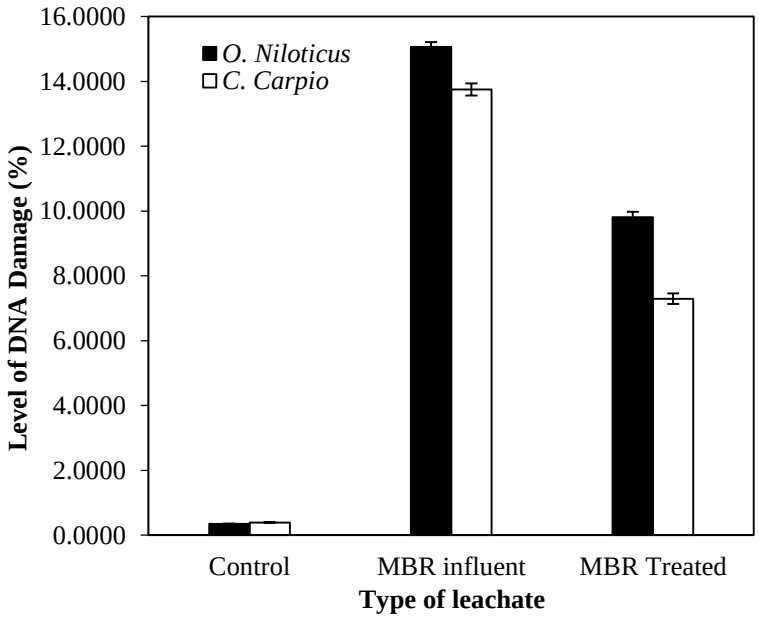


Figure 6.3 Level of DNA damage at 7 days of MBR influent leachate and MBR treated leachate (%) on fish species.

6.4 Relationship Between DNA Damage, and Chemical Pollutants.

The significant differences between DNA damage at 7th with chemical pollutant concentrations including; COD, UIA, pH, and EC. It is possible that DNA damage and these parameter may not correlate at this level of significant or this size of sample (n=300) as shown in Table 6.3. The long-term effects mutagenicity/geno-toxicity on fish species were investigated. For the comparisons with previous research (Baun *et al.*, 2004) which conducted with leachate collected from ten Danish landfill, it was found that the leachate could cause mutagenic effect after its pre-concentration, and the authors suggested that XOCs in leachate would possibly cause this mutagenic activity. Base on multiple geno-toxicity tests of leachate from municipal solid waste landfills, it is suggested that landfill leachate may contain a large variety of organic compounds that are acutely and chronically toxic, and these leachate toxicity remains largely unknown.

Table 6.3 Correlation between DNA damage and physicochemical parameters.

	DNA Damage at 7 days		COD	UIA	pH	EC
	O.niloticus	*C.carpio*				
DNA Damage of *O.niloticus*	**1**					
	(0.000)					
DNA Damage of *C.carpio*	0.978*	**1**				
	(0.000)	(0.000)				
COD	0.706*	0.751*	**1**			
	(0.000)	(0.000)	(0.000)			
UIA	0.809**	0.872**	-0.009*	**1**		
	(0.018)	(0.012)	(0.000)	(0.000)		
pH	0.071**	0. 146**	0.245**	0.720**	**1**	
	(0.021)	(0.037)	(0.020)	(0.020)	(0.000)	
EC	0.358**	0.306**	-0.167**	0.155**	-0.178**	**1**
	(0.015)	(0.020)	(0.041)	(0.019)	(0.032)	(0.000)

UIA = unionized-ammonia, no. of samples = 300.

*Correlation is significant at the 0.01 level, **Correlation is significant at the 0.05 level

CHAPTER VII

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

Membrane bioreactor was applied to treat phenolic compounds and phthalic acid esters in municipal solid waste leachate. Over long term operation of 300 days without sludge wastage along the operation period, the micro-pollutants removal efficiencies were 77-96% depending on hydrophobic property of the compounds. BPA, and BHT concentration were decreased through the biodegradation mechanism. DEHP concentration was mainly removed through adsorption on to sludge surface. The removal efficiency of micropollutants are depends on their log K_{OW} as well as their speciation behaviour. The effect of biological activities was responsible for the removal of micro-pollutants especially at high sludge age condition. Moreover, long sludge age could affect biomass activities and promote organic micro-pollutants removals in MBR. Moreover, the factors affecting to the removal emerging micro-pollutants during MBR treatment depends on physico-chemical properties of the compounds and operational parameters, i.e. biomass concentration, sludge retention time. Membrane bioreactor treatment seemed to enhance removal of micro-pollutants with intermediate biodegradability. This research provided a perspective on application of membrane bioreactor to remove low concentrations of organic micro-pollutants in landfill leachate under long sludge retention time and high biomass concentration. The enriched nitrifying sludge were enhanced BPA, BHT removals under biodegradation condition compared with non-enriched sludge. In contrast, DEHP removal was adsorbed on the sludge surface and the filtration mechanisms played a major role. Moreover, the presence of foulants increased the retention of the target compounds on membrane. In laboratory scale experiment, the removals of BPA, BHT, and DEHP were 65%, 70%, 72%, respectively at initial concentration of 1,000 $\mu g.L^{-1}$.

The results shown that anoxic reactor could remove BPA, BHT, and DEHP only 3%, 4%, and 8%, respectively, and the selected micro-pollutant removals were took place in aerobic reactor. The removal efficiencies of each compound were found 65%, 70%, 72% of BPA, BHT, and DEHP, respectively. The removal mechanisms can be classified into three parts such as biodegradation, adsorption, and filtration mechanisms. The removals of phenolic compounds were found through microbial biodegradation. The enrichment of nitrifier culture is a significant factor on the biodegradation of XOCs in biological wastewater treatment system. The enriched nitrifying sludge were enhanced BPA, BHT removals under biodegradation condition compared with non-enriched sludge. The removal efficiencies of BPA through biodegradation mechanism by enriched nitrifying sludge with NH_4-N addition, enriched nitrifying sludge without NH_4-N, and enriched nitrifying with ATU were 88%, 82%, and 85%, respectively. The removal rates of their co-metabilc pathways on BHT, and BPA removal were found 0.0106 h^{-1}, and 0.0294 h^{-1}, respectively. For the removal rates of nitrifying bacteria on these compounds were found only 0.0100 h^{-1}. The results shown that, not only nitrifying bacteria degrading phenolic compounds but also the heterotrophic organisms also help to removed phenolic compounds in MBR operated under long sludge age condition. In contrast, DEHP removal was adsorbed on the sludge surface and the filtration mechanisms played a major role. The lechate bio-toxicity reduction during MBR treatment system was studied in term of aute toxicity and chronic toxicity on local fish species are *Oreochromis niloticus* (*O. niloticus*), and *Cyprinus carpio* (*C. carpio*). The results suggested that UIA have a direct relationship to toxicity, it is increase the sensitivity of *C. carpio* and *O. niloticus* respectively. The comet assay was utilized as biomarker of the genotoxic potential of the raw and treated leachate. As the result of level of DNA damage, the sensitivity of tested species, the result shows that the %DNA damage values of *O. niloticus* were higher than %DNA damage of *C. carpio* and demonstrated that *O. niloticus* was considerably more sensitive. This difference can be caused by different food web of tested fish. This research provided a significant reduction in bio-toxicity of leachate after two-stage membrane bioreactor system.

7.2 Recommendations

The following studies are recommended for further studies.

7.2.1 Investigating the role of EPS and particle size on micropollutant removals by the MBR sludge on various micro-pollutants found in landfill leachate according to long sludge age could affect biomass activities and promote organic micro-pollutants removals in MBR.

7.2.2 Identifying heterotrophic organisms capable of degrading micropollutants in MBR operated under long sludge age condition according to the result of biodegradation mechanism by nitrifying sludge and inhibitory addition. The heterotrophic organisms influence to phenolic compounds removal in MBR operated under long sludge age condition.

7.2.3 Investigating biodegradation of micro-pollutants in biofilm or foulant layer formed on the membrane surface according the result shown that foulant on membrane layer could rejected hydrophobic compounds.

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APPENDICES

APPENDIX A

Experimental set-up

A.1 Pilot scale it-MBR

Figure A.1 Configuration of pilot-scale it-MBR at Nonthaburi disposal site, Thailand.

(a)

(b)

Figure A.2 Configuration of media were used in Pilot scale it-MBR: Membrane module (a), and Incline tube module (b).

A.2 Lab scale it-MBR

Figure A.3 Configuration of Laboratory-scale it-MBR at Kasetsart University.

(a)

(b)

Figure A.4 Configuration of media were used in Lab scale it-MBR: Membrane module (a), and Incline tube module (b).

APPENDIX B

Micropollutant Properties

B.1 Chemical properties of ten priority micropollutants found in landfill leachate

Table B.1 Chemical properties of ten priority micropollutants found in municipal landfill leachate in this study.

Class	Compound	Cas No.	Formula	MW	Bp (^{o}C)	Mp (^{o}C)
PAHs	Naphthalene	91-20-3	$C_{10}H_8$	128.17	218	80-82
	Anthracene	120-12-7	$C_{14}H_{10}$	178.23	340	215
Phenolics	4-Methyl-2,6-di-tert-butylphenol (BHT)	128-37-0	$C_{15}H_{24}O$	220.35	265	69-73
	Bisphenol A (BPA)	80-05-7	$C_{15}H_{16}O_2$	228.29	220	158-159
PAEs	Dimethylphthalate	131-11-3	$C_{10}H_{10}O_4$	194.18	2	282
	Diethylphthalate	84-66-2	$C_{12}H_{14}O_4$	222.24	298-299	−3
	Di-n-butylphthalate	84-74-2	$C_{16}H_{22}O_4$	278.34	340	-35
	Benzyl butyl Phthalate	85-68-7	$C_{19}H_{20}O_4$	312.36	370	<-35
	Bis(2- ethylhexyl)phthalate	117-81-7	$C_{24}H_{38}O_4$	390.56	386	-50
	Di-n-octyl phthalate	117-84-0	$C_{24}H_{38}O_4$	390.56	380	-

MW = molecular weight, Bp = boiling point, Mp = melting point

B.2 Mass spectrum of ten priority micropollutants by GCMS (Wiley library).

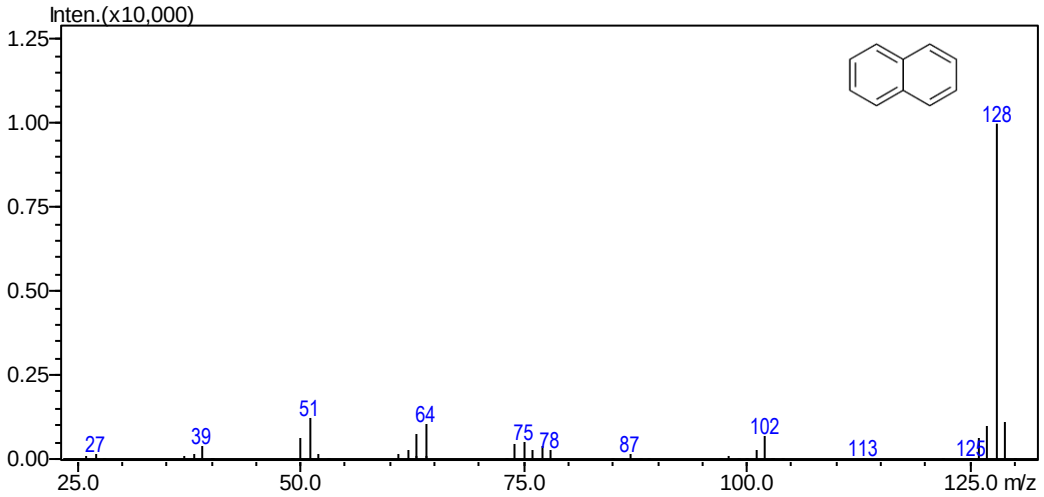


Figure B.1 Mass Spectrum of Naphthalene (Cas No. 91-20-3).

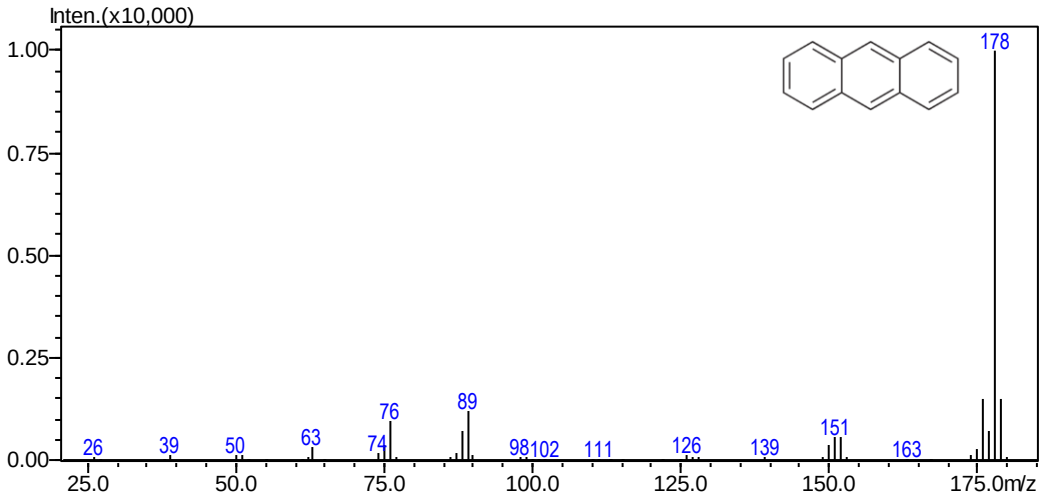


Figure B.2 Mass Spectrum of Anthracene (Cas No. 120-12-7).

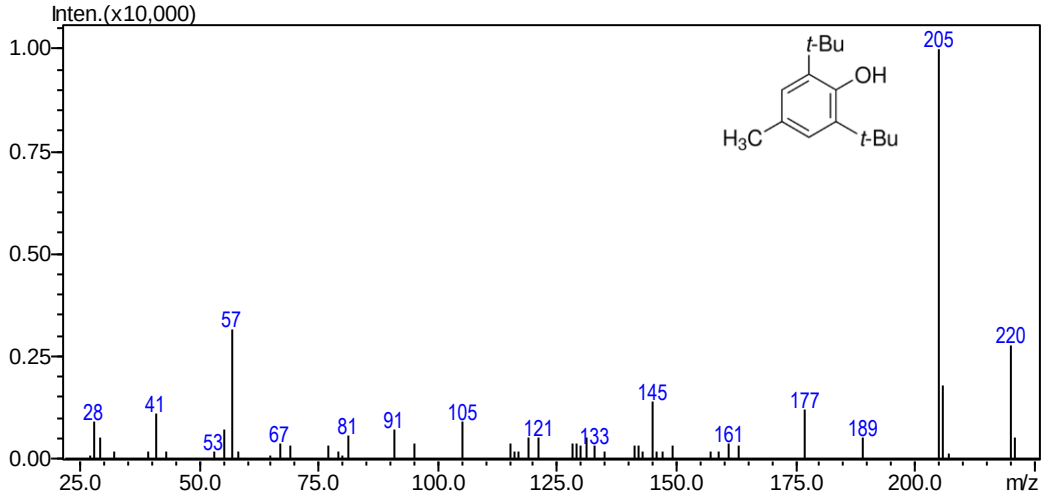


Figure B.3 Mass Spectrum of 4-Methyl-2,6-di-tert-butylphenol (Cas No. 128-37-0).

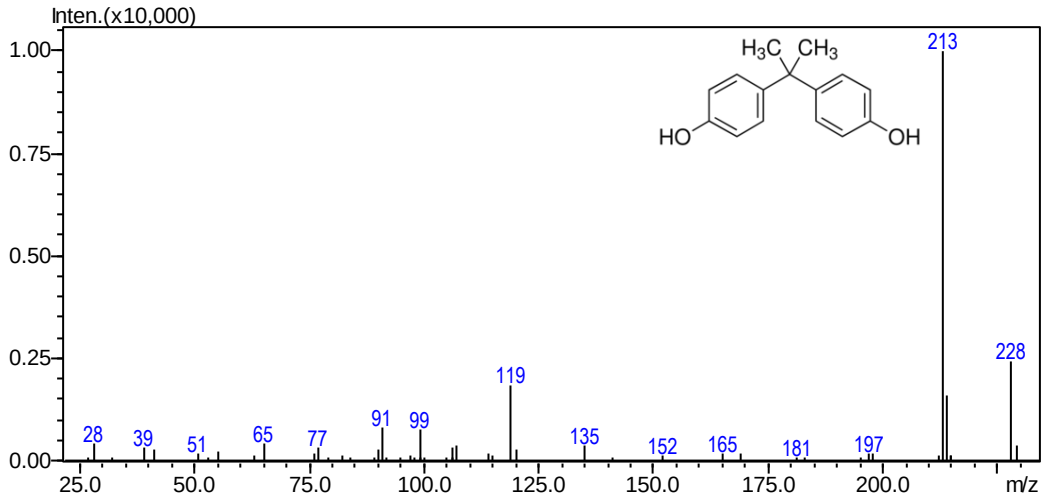


Figure B.4 Mass Spectrum of Bisphenol A (Cas No. 80-05-7).

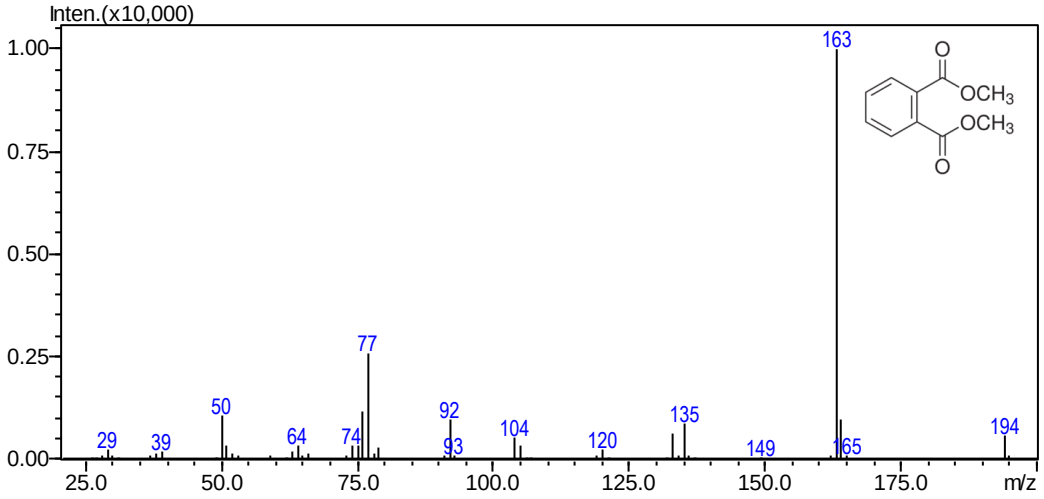


Figure B.5 Mass Spectrum of Dimethylphthalate (Cas No. 131-11-3).

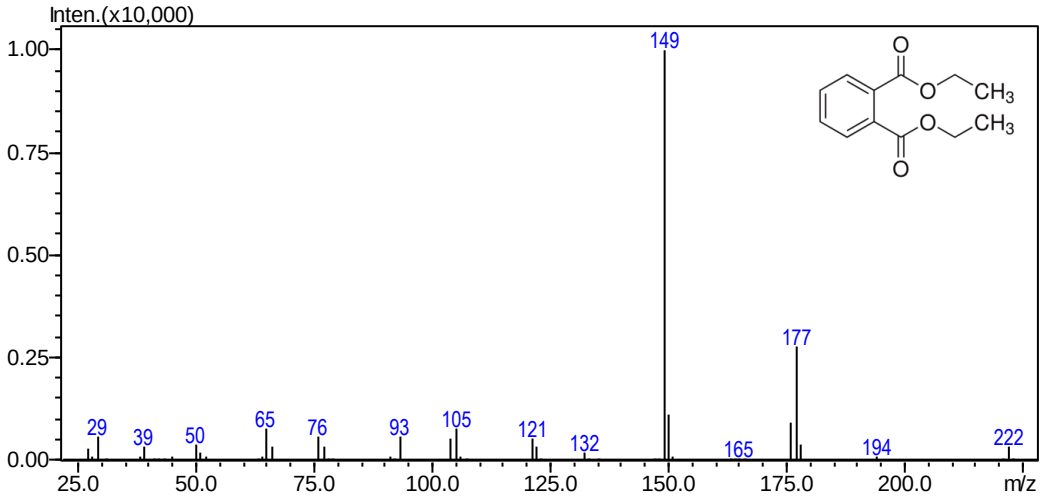


Figure B.6 Mass Spectrum of Diethylphthalate (Cas No. 84-66-2).

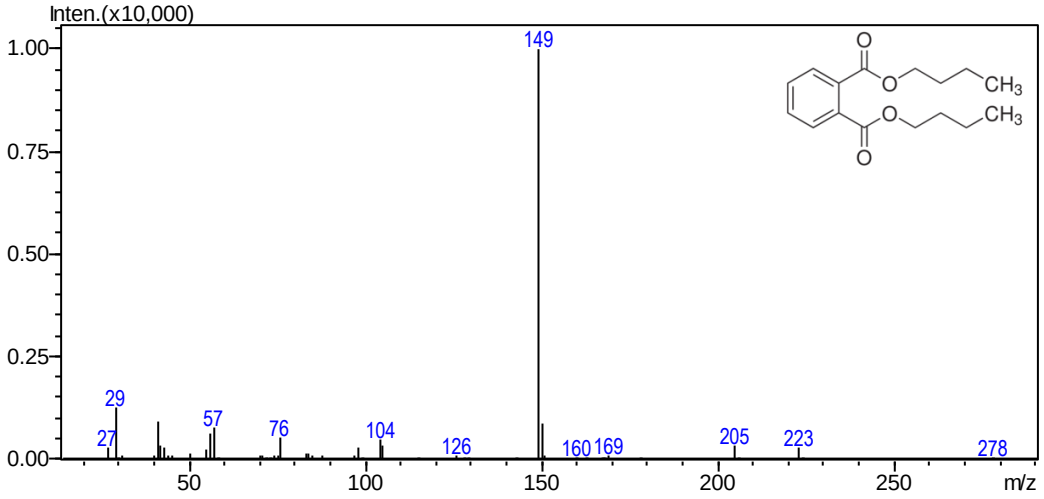


Figure B.7 Mass Spectrum of Di-n-butylphthalate (Cas No. 84-74-2).

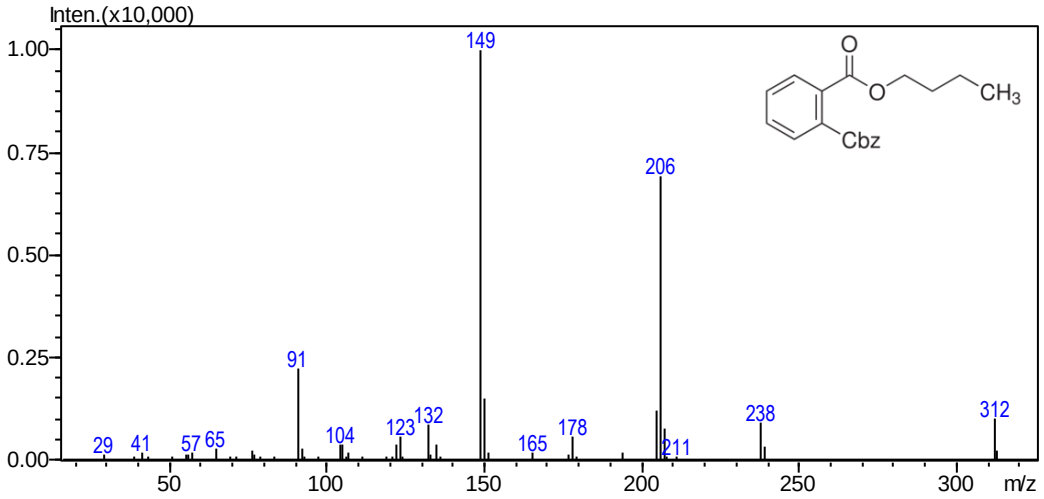


Figure B.8 Mass Spectrum of Benzyl butyl Phthalate (Cas No. 85-68-7).

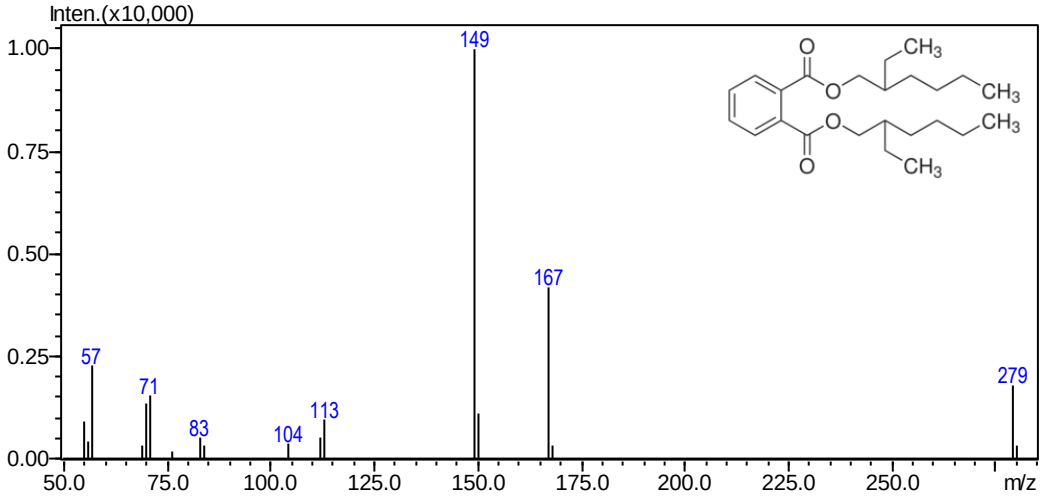


Figure B.9 Mass Spectrum of Bis(2-ethylhexyl)phthalate (Cas No. 117-81-7).

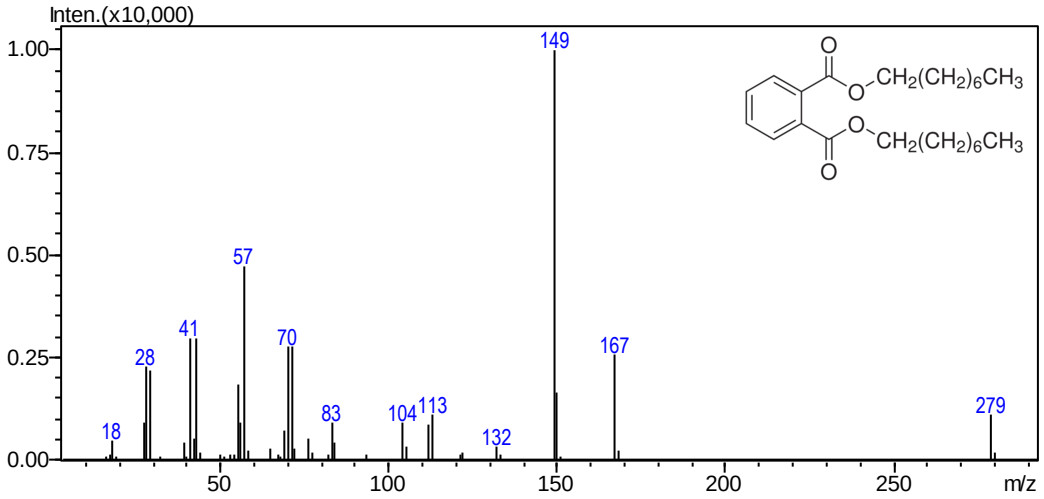
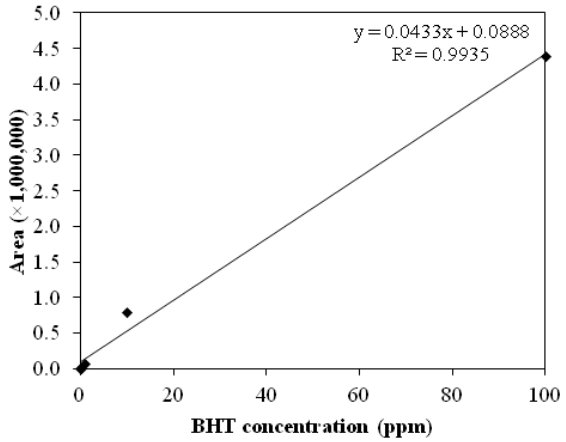
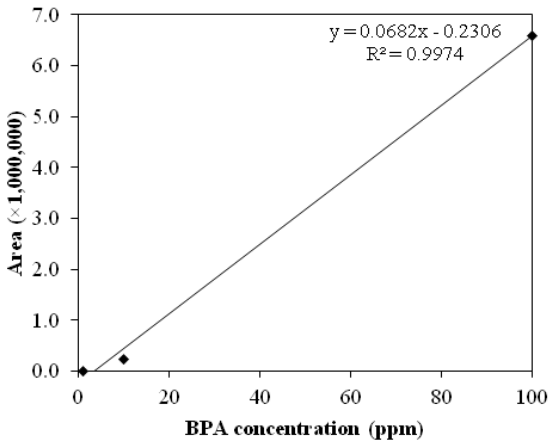


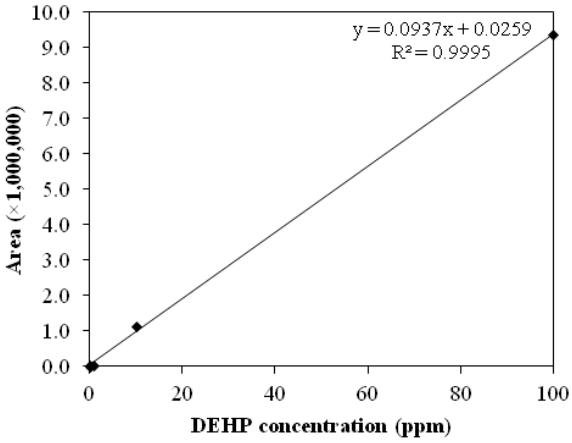
Figure B.10 Mass Spectrum of Di-n-octyl phthalate (Cas No. 117-84-0).



(a)



(b)

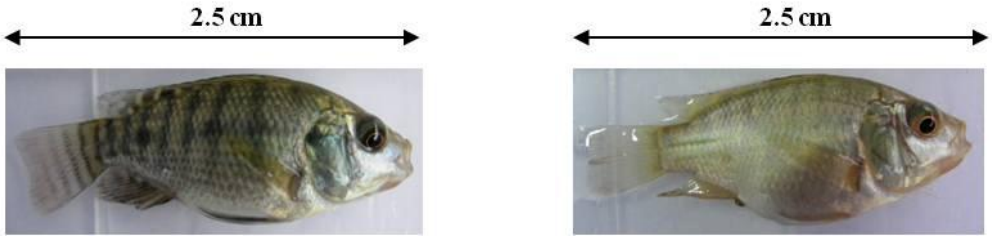


(c)

Figure B.11 Calibration curve of BHT (a), BHT (b), and DEHP (c).

APPENDIX C

Biotoxicity testing



(a) (b)

Figure C.1 Test species Nile Tilapia (a) Common Carp (b).

Figure C.2 Configuration of Aquarium glass for bio-toxicity testing.

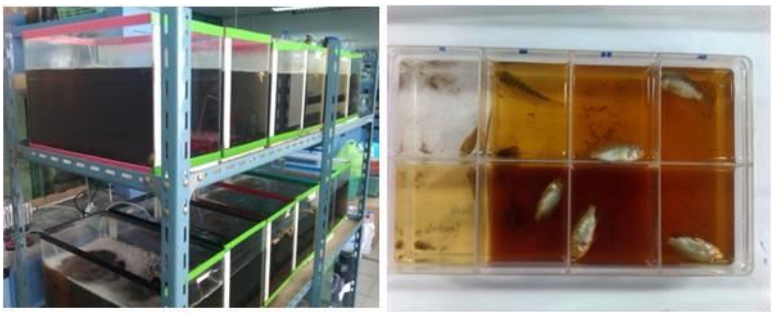


Figure C.3 Configuration of bio-toxicity testing experiment.

APPENDIX D

Experimental Data

D.1 Treatment Performance

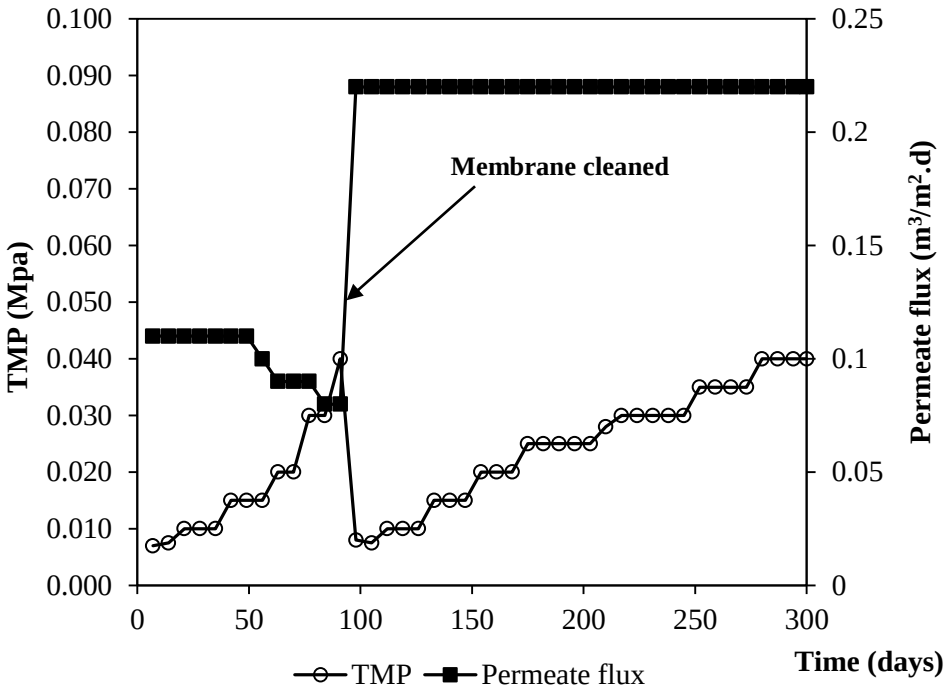


Figure D 1.1 TMP and Permeat flux in pilot scale MBR.

Table D 1.1 Biomass in aerobic tank (Pilot-scale MBR).

Time (days)	MLSS (mgL^{-1})	SV30 (mLL^{-1})	SVI (mLg^{-1})	MLVSS (mgL^{-1})	MLVSS/MLSS (ratio)
7	7,365	200	27	4,272	0.58
14	7,920	200	25	4,277	0.54
21	8,396	250	30	4,618	0.55
28	8,842	250	28	4,863	0.55
35	9,190	250	27	4,411	0.48
42	9,620	250	26	4,521	0.47
49	10,450	300	29	5,330	0.51
56	10,936	300	27	5,687	0.52
63	12,625	350	28	6,944	0.55
70	13,748	350	25	6,737	0.49
77	14,610	400	27	7,320	0.50
84	14,955	450	30	7,478	0.50
91	15,667	500	32	8,304	0.53
98	11,796	450	38	6,370	0.54
105	12,900	500	39	6,450	0.50
112	12,540	500	40	6,270	0.50
119	11,955	550	46	6,575	0.55
126	12,000	550	46	5,760	0.48
133	12,050	600	50	6,146	0.51
140	11,965	600	50	5,983	0.50
147	12,016	600	50	6,368	0.53
154	11,720	650	55	6,329	0.54
161	11,720	650	55	6,329	0.54
168	11,750	650	55	6,110	0.52
175	11,980	650	54	7,308	0.61
182	11,950	650	54	7,529	0.63
189	11,925	700	59	7,274	0.61
196	11,855	700	59	6,994	0.59
203	11,240	700	62	6,632	0.59
210	11,450	700	61	6,756	0.59
217	11,552	700	61	6,354	0.55
224	11,670	700	60	6,068	0.52
231	11,500	700	61	5,520	0.48
238	11,645	700	60	6,172	0.53
245	11,756	700	60	6,348	0.54
252	11,880	700	59	6,772	0.57
259	11,925	700	59	6,201	0.52
266	11,726	700	60	6,449	0.55
273	11,550	700	61	5,775	0.50
280	11,240	700	62	6,070	0.54
287	11,420	700	61	6,281	0.55
294	11,542	700	61	6,117	0.53
300	11,870	700	59	6,410	0.54

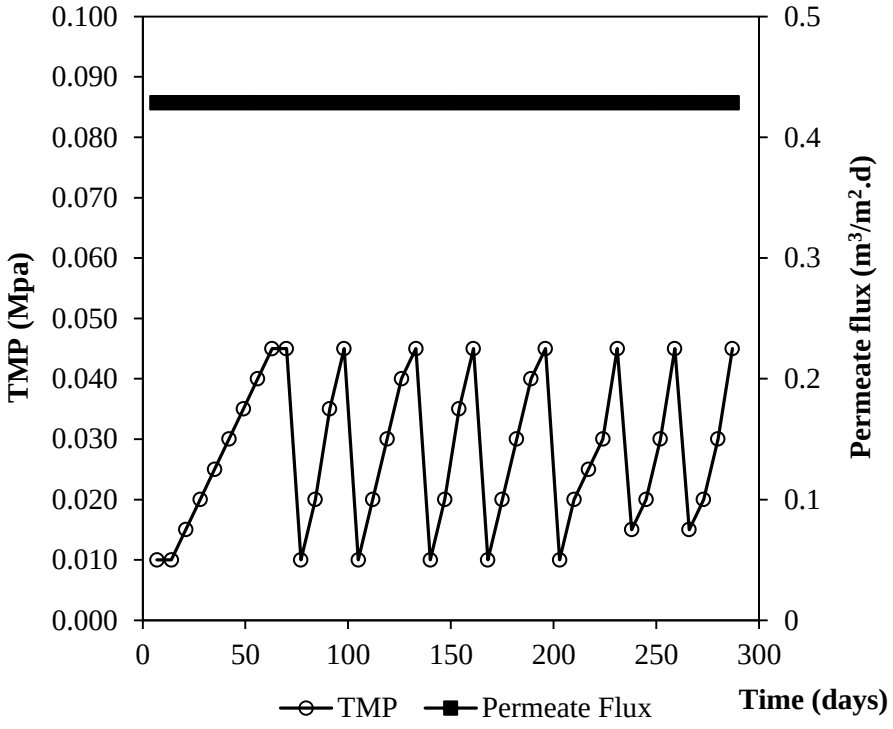


Figure D 1.2 TMP and Permeat flux in laboratory scale MBR.

Table D 1.2 Biomass in aerobic tank (Laboratory-scale MBR).

Time (days)	MLSS (mgL^{-1})	SV30 (mLL^{-1})	SVI (mLg^{-1})	MLVSS (mgL^{-1})	MLVSS/MLSS (ratio)
3	5,600	350	63	2,688	0.48
6	5,855	350	60	2,928	0.50
9	5,750	380	66	3,048	0.53
12	6,030	380	63	3,256	0.54
18	6,500	450	69	3,250	0.50
27	6,445	500	78	3,224	0.50
36	7,040	500	71	3,872	0.55
45	7,154	550	77	3,812	0.53
54	7,200	580	81	3,672	0.51
63	8,350	600	72	4,175	0.50
72	9,452	660	70	5,010	0.53
81	10,200	680	67	5,508	0.54
90	11,190	700	63	6,045	0.54
99	11,350	700	62	5,448	0.48
108	10,230	700	68	5,115	0.50
117	10,400	700	67	5,200	0.50
126	10,580	720	68	5,400	0.51
135	10,700	750	70	5,564	0.52
144	10,576	760	72	5,605	0.53
153	10,540	800	76	5,692	0.54
162	10,735	800	75	5,900	0.55
171	10,520	850	81	5,680	0.54
180	10,200	850	83	5,610	0.55
189	10,765	880	82	5,656	0.53
198	10,516	900	86	6,098	0.58
207	10,850	950	88	6,076	0.56
216	10,720	980	91	6,216	0.58
225	10,600	980	92	6,150	0.58
234	10,718	980	91	5,359	0.50
243	10,738	980	91	5,364	0.50
252	10,752	980	91	5,488	0.51
261	10,778	980	91	5,605	0.52
270	10,799	980	91	5,720	0.53
279	10,819	980	91	5,840	0.54
288	10,839	980	90	5,960	0.55
297	10,840	980	90	5,872	0.54
300	10,700	980	92	5,845	0.55

Table D 1.3 BOD, and COD removal in MBR system

	BOD(mg/L)			COD(mg/L)		
Day	Influent	1st stage	2nd stage	Influent	1st stage	2nd stage
7	3,045		650	6,500		1,400
14	3,250		630	6,940		1,380
21	3,020		600	6,160		1,320
28	3,140		500	6,760		1,450
35	3,550		530	7,318		1,440
42	3,300		545	7,230		1,300
49	3,600		530	7,250		1,250
56	3,500		500	7,180		1,200
63	3,280		450	7,000		1,200
70	3,390		480	7,110		1,250
77	3,410		330	6,890		1,200
84	3,320		320	6,740		1,200
91	3,380		300	6,980		1,200
Avg	3,322		490	6,928		1,292
SD	179		114	328		97
Avg(SD)	3,322(179)		490(114)	6,928(328)		1,292(97)
98	6,550	6,100	670	9,306	8,401	1,600
105	6,580	6,100	640	9,000	8,055	1,645
112	6,750	6,260	600	9,200	8,250	1,510
119	7,450	6,930	450	9,800	8,910	1,500
126	6,550	5,996	330	9,350	8,461	1,500
133	7,500	6,988	315	9,400	8,430	1,420
140	6,700	6,200	230	9,000	8,020	1,415
147	6,850	6,316	210	9,600	8,605	1,388

Table D 1.3 BOD, and COD removal in MBR system (continued)

	BOD(mg/L)			COD(mg/L)		
Day	Influent	1st stage	2nd stage	Influent	1st stage	2nd stage
154	7,000	6,486	200	9,600	8,535	1,361
161	7,542	7,060	200	9,400	8,457	1,334
168	6,500	6,044	195	9,320	8,240	1,307
175	7,500	7,010	186	9,450	8,473	1,280
182	7,455	7,009	184	9,320	8,260	1,235
189	7,300	6,814	150	9,400	8,465	1,226
196	6,700	6,220	150	9,630	8,722	1,190
203	6,560	6,062	174	9,300	8,263	1,152
210	7,300	6,866	150	9,000	8,030	1,145
217	7,200	6,687	147	9,250	8,530	1,118
224	7,000	6,150	145	9,600	8,750	1,091
231	7,245	6,265	145	9,620	8,470	1,064
238	6,750	5,620	135	9,600	8,413	1,037
245	7,310	5,930	125	9,525	8,344	1,010
252	7,100	5,860	125	9,340	8,166	985
259	7,345	5,960	125	9,620	8,400	919
266	7,100	5,650	110	9,400	7,600	973
273	6,870	5,420	120	9,500	7,133	935
280	6,450	5,050	118	9,000	6,540	895
287	6,950	5,550	115	9,280	6,899	880
294	7,015	5,700	110	9,300	6,410	880
300	7,160	5,780	110	9,550	6,550	880
Avg	7,009	6,203	222	9,389	8,093	1,196
SD	343	530	159	210	686	233
Avg±SD	7,009(343)	6,203(530)	222(159)	9,389(210)	8,093(686)	1,196(233)

Table D 1.4 TKN, and NH_3-N removal in MBR system.

	TKN(mg/L)			NH_3-N(mg/L)		
Day	Influent	1st stage	2nd stage	Influent	1st stage	2nd stage
7	157		118	114		24
14	146		120	120		25
21	151		115	112		22
28	145		110	120		20
35	162		100	118		21
42	150		110	132		20
49	147		100	122		16
56	155		115	105		25
63	160		110	117		20
70	146		100	116		18
77	148		100	113		15
84	150		98	132		16
91	155		100	125		18
Avg	152		107	119		20
SD	6		8	8		3
Avg(SD)	152(6)		107(8)	119(8)		20(3)
98	227	220	120	172	160	22
105	218	212	120	174	165	21
112	202	200	110	166	150	24
119	201	198	100	158	140	22
126	240	235	100	170	150	26
133	217	210	97	168	155	20
140	207	200	95	165	152	23
147	240	236	93	150	142	18

Table D 1.4 TKN, and NH_3-N removal in MBR system (continued).

	TKN(mg/L)			NH_3-N(mg/L)		
Day	Influent	1st stage	2nd stage	Influent	1st stage	2nd stage
154	225	220	90	164	150	19
161	235	215	90	168	150	23
168	231	208	90	174	155	20
175	228	216	88	174	158	25
182	231	220	86	158	140	21
189	239	230	85	169	150	18
196	232	224	81	150	138	20
203	228	210	67	160	142	24
210	240	225	65	170	150	22
217	240	228	65	172	155	18
224	230	205	62	148	132	12
231	240	226	60	138	112	10
238	232	222	60	153	140	9
245	233	214	58	172	150	7
252	240	228	56	165	152	5
259	229	200	55	159	146	5
266	218	210	51	170	150	8
273	221	200	50	172	160	4
280	233	222	47	170	150	3
287	240	235	45	174	148	3
294	235	230	45	158	155	3
300	238	214	45	170	160	3
Avg	229	217	76	164	149	15
SD	11	11	23	9	10	8
Avg±SD	229(11)	217(11)	76(23)	164(9)	149(10)	15(8)

Table 1.5 Micro-pollutants removal in MBR (1st stage operation).

Compound	Inf (µg/L)		Eff(µg/L)	
	Range	**Avg. (SD)**	**Range**	**Avg. (SD)**
1st stage operation				
PAHs				
Nap	18.60-18.67	18.63(0.04)	13.39-14.93	14.35(0.84)
An	18.47-18.64	18.56(0.09)	9.66-15.85	12.69(3.10)
Phenolics				
BHT	11,132.71-11,877.03	11488.58(373.23)	5,900-8,821.13	7,678.46(1,560.64)
BPA	70.12-76.33	72.67(3.25)	56.10-67.17	61.36(5.55)
PAEs				
DMP	30.03-30.55	30.33(0.27)	26.78-28.53	27.60(0.88)
DEP	22.94-24.02	23.42(0.55)	19.70-21.57	20.59(0.94)
DnBP	39.49-50.66	43.61(6.13)	29.70-35.46	32.91(2.94)
BBP	37.62-42.13	39.70(2.28)	22.95-30.76	27.08(3.93)
DEHP	479.51-539.59	512.55(30.49)	297.3-357.80	328.08(30.27)
DOP	16.05-16.25	16.12(0.11)	9.32-11.21	10.21(0.95)
2nd stage operation				
PAHs				
Nap	18.54-19.49	18.98(0.33)	2.92-12.05	6.03(3.19)
An	18.23-18.71	18.49(0.14)	3.14-9.12	5.60(2.44)
Phenolics				
BHT	10,352.28-12,445.70	11,411.71(718.83)	1,759.89-5,462.56	2,906.99(1,359.97)
BPA	78.65-85.05	81.55(2.27)	ND-62.89	26.30(24.34)
PAEs				
DMP	30.42-31.65	31.11(0.41)	11.08-21.30	15.64(4.20)
DEP	22.65-23.90	23.35(0.36)	7.01-16.49	11.50(3.81)
DnBP	41.72-54.09	49.48(3.92)	6.49-27.08	15.55(7.26)
BBP	36.98-44.50	41.13(2.21)	6.26-19.23	11.60(5.55)
DEHP	467.68-530.84	488.05(20.70)	71.12-196.43	121.82(52.40)
DOP	16.05-16.34	16.12(0.10)	1.61-7.51	3.92(2.13)

D.2 Acute Toxicity Testing

Table D 2.1 Confidence limits for COD.

Probability	*O. niloticus*, 95% Confidence Limits for COD		*C. carpio*, 95% Confidence Limits for COD	
	MBR influent	MBR treated	MBR influent	MBR treated
0.01	126.000	168.055	130.474	139.656
0.02	131.939	173.945	136.683	145.833
0.03	135.851	177.789	140.774	149.893
0.04	138.870	180.736	143.933	153.022
0.05	141.375	183.169	146.554	155.615
0.06	143.543	185.266	148.823	157.856
0.07	145.472	187.125	150.841	159.848
0.08	147.220	188.804	152.671	161.653
0.09	148.828	190.345	154.355	163.312
0.10	150.325	191.775	155.921	164.855
0.15	156.680	197.808	162.577	171.397
0.20	161.923	202.738	168.069	176.781
0.25	166.560	207.065	172.929	181.535
0.30	170.838	211.030	177.412	185.913
0.35	174.900	214.772	181.671	190.064
0.40	178.843	218.384	185.806	194.089
0.45	182.743	221.936	189.896	198.064
0.50	186.664	225.488	194.010	202.055
0.55	190.669	229.098	198.212	206.127
0.60	194.827	232.824	202.576	210.348
0.65	199.220	236.740	207.187	214.802
0.70	203.956	240.937	212.160	219.599
0.75	209.194	245.550	217.660	224.894
0.80	215.185	250.792	223.953	230.942

Table D 2.1 Confidence limits for COD (continnued).

Probability	*O. niloticus*, 95% Confidence Limits for COD		*C. carpio*, 95% Confidence Limits for COD	
	MBR influent	MBR treated	MBR influent	MBR treated
0.85	222.386	257.042	231.519	238.197
0.90	231.788	265.129	241.402	247.650
0.91	234.118	267.120	243.852	249.989
0.92	236.676	269.300	246.541	252.555
0.93	239.521	271.717	249.532	255.406
0.94	242.738	274.443	252.916	258.630
0.95	246.461	277.585	256.832	262.355
0.96	250.907	281.322	261.509	266.801
0.97	256.484	285.986	267.377	272.370
0.98	264.089	292.305	275.381	279.953
0.99	276.536	302.550	288.485	292.335

Table D 2.2 Confidence limits for NH_3.

Probability	*O. niloticus* , 95% Confidence Limits for NH_3		*C. carpio*, 95% Confidence Limits for NH_3	
	MBR influent	MBR treated	MBR influent	MBR treated
0.01	1.575	3.653	1.631	3.036
0.02	1.649	3.781	1.709	3.170
0.03	1.698	3.865	1.760	3.259
0.04	1.736	3.929	1.799	3.327
0.05	1.767	3.982	1.832	3.383
0.06	1.794	4.028	1.860	3.432
0.07	1.818	4.068	1.886	3.475
0.08	1.840	4.104	1.908	3.514
0.09	1.860	4.138	1.929	3.550
0.10	1.879	4.169	1.949	3.584
0.15	1.959	4.300	2.032	3.726
0.20	2.024	4.407	2.101	3.843
0.25	2.082	4.501	2.162	3.946
0.30	2.135	4.588	2.218	4.042
0.35	2.186	4.669	2.271	4.132
0.40	2.236	4.747	2.323	4.219
0.45	2.284	4.825	2.374	4.306
0.50	2.333	4.902	2.425	4.393
0.55	2.383	4.980	2.478	4.481
0.60	2.435	5.061	2.532	4.573
0.65	2.490	5.147	2.590	4.670
0.70	2.549	5.238	2.652	4.774
0.75	2.615	5.338	2.721	4.889
0.80	2.690	5.452	2.799	5.020
0.85	2.780	5.588	2.894	5.178
0.90	2.897	5.764	3.018	5.384
0.91	2.926	5.807	3.048	5.435
0.92	2.958	5.854	3.082	5.490
0.93	2.994	5.907	3.119	5.552
0.94	3.034	5.966	3.161	5.622

Table D 2.2 Confidence limits for NH_3 (continued).

Probability	*O. niloticus* , 95% Confidence Limits for NH_3		*C. carpio*, 95% Confidence Limits for NH_3	
	MBR influent	MBR treated	MBR influent	MBR treated
0.95	3.081	6.034	3.210	5.703
0.96	3.136	6.116	3.269	5.800
0.97	3.206	6.217	3.342	5.921
0.98	3.301	6.354	3.442	6.086
0.99	3.457	6.577	3.606	6.355

Table D 2.3 Appearance of DNA damage on *O. niloticus*.

Cell no.	*O. niloticus* , Level of DNA damage		
	Control	MBR influent	MBR treated
1	0.00128	0.38833	0.12916
2	0.00161	0.34525	0.06409
3	0.00119	0.09524	0.01530
4	0.00148	0.31680	0.02134
5	0.00161	0.25468	0.00117
6	0.00154	0.40728	0.00174
7	0.00062	0.36805	0.00098
8	0.00187	0.25259	0.00133
9	0.00280	0.35778	0.00101
10	0.00181	0.27543	0.00313
11	0.00095	0.26948	0.00182
12	0.00108	0.12440	0.00375
13	0.00092	0.20000	0.07983
14	0.00101	0.41779	0.03672
15	0.00265	0.38524	0.09001
16	0.00055	0.38825	0.00669
17	0.00075	0.12790	0.06840
18	0.00087	0.26326	0.00202
19	0.00061	0.22577	0.00136
20	0.00076	0.15543	0.00092
21	0.00123	0.06247	0.00159
22	0.00176	0.32449	0.00189
23	0.00112	0.40196	0.00157
24	0.00095	0.27372	0.00119
25	0.00111	0.09839	0.00096
26	0.00076	0.19179	0.00170
27	0.00045	0.29847	0.00123
28	0.00073	0.28094	0.00132
29	0.00089	0.31429	0.00217
30	0.00108	0.20255	0.00278

Table D 2.3 Appearance of DNA damage on *O. niloticus* (continued).

Cell no.	*O. niloticus* , Level of DNA damage		
	Control	MBR influent	MBR treated
31	0.00216	0.24885	0.00154
32	0.00079	0.32410	0.00167
33	0.00190	0.34878	0.00154
34	0.00128	0.49009	0.00181
35	0.00251	0.15190	0.00000
36	0.00095	0.12841	0.00132
37	0.00099	0.30158	0.00189
38	0.00176	0.06671	0.00119
39	0.00327	0.29750	0.00123
40	0.00102	0.24136	0.00161
41	0.00082	0.10991	0.00095
42	0.00101	0.14746	0.00108
43	0.00093	0.23347	0.00182
44	0.00077	0.08666	0.00154
45	0.00812	0.10303	0.00090
46	0.00057	0.33131	0.00128
47	0.00082	0.24315	0.00108
48	0.00985	0.00075	0.00132
49	0.08224	0.39548	0.00079
50	0.00062	0.11317	0.00125
51	0.00081	0.01679	0.00052
52	0.00186	0.08099	0.00059
53	0.00052	0.04069	0.00057
54	0.00104	0.03027	0.00044
55	0.00104	0.06306	0.00036
56	0.01080	0.21231	0.09709
57	0.04010	0.00058	0.00061
58	0.03020	0.27917	0.49650
59	0.00104	0.38157	0.00092
60	0.00089	0.18945	0.00084

Table D 2.3 Appearance of DNA damage on *O. niloticus* (continued).

Cell no.	*O. niloticus* , Level of DNA damage		
	Control	MBR influent	MBR treated
61	0.00143	0.17073	0.09594
62	0.00092	0.55607	0.01891
63	0.00164	0.11853	0.09965
64	0.00183	0.15650	0.03401
65	0.00115	0.20728	0.13179
66	0.00125	0.03145	0.09147
67	0.00087	0.11881	0.20628
68	0.00105	0.03611	0.72672
69	0.00075	0.07762	0.70307
70	0.00052	0.17899	0.57295
71	0.00057	0.00060	0.27009
72	0.00058	0.00072	0.59753
73	0.00090	0.32856	0.29083
74	0.00051	0.18076	0.21071
75	0.00112	0.18843	0.66362
76	0.00123	0.00130	0.21885
77	0.00175	0.00055	0.23823
78	0.00115	0.02001	0.00253
79	0.00133	0.40339	0.18995
80	0.00068	0.00047	0.05777
81	0.00049	0.00051	0.31888
82	0.00060	0.32468	0.22633
83	0.00087	0.00095	0.40110
84	0.00095	0.05846	0.21478
85	0.00063	0.00111	0.13600
86	0.00125	0.00031	0.00073
87	0.00118	0.00240	0.00142
88	0.00082	0.00097	0.28697
89	0.00083	0.01549	0.05948
90	0.00090	0.02244	0.12104

Table D 2.3 Appearance of DNA damage on *O. niloticus* (continued).

Cell no.	*O. niloticus* , Level of DNA damage		
	Control	MBR influent	MBR treated
91	0.00142	0.00054	0.02528
92	0.06033	0.36468	0.14366
93	0.00091	0.00042	0.32652
94	0.00181	0.32142	0.06146
95	0.00149	0.00042	0.00035
96	0.00174	0.00046	0.00942
97	0.00198	0.00040	0.13899
98	0.00133	0.22423	0.25358
99	0.00189	0.00494	0.46400
100	0.00154	0.00073	0.15899
Avg.	0.35088	15.06303	9.81218
SD	0.01107	0.14470	0.16901

Table D 2.4 Appearance of DNA damage on *C. carpio*.

Cell no.	*C. carpio*, Level of DNA damage		
	Control	MBR influent	MBR treated
1	0.00128	0.36352	0.04397
2	0.00161	0.51615	0.01596
3	0.00119	0.50230	0.06675
4	0.00148	0.27112	0.03431
5	0.00161	0.25367	0.21433
6	0.00154	0.00765	0.12954
7	0.01274	0.00014	0.05457
8	0.01476	0.00018	0.00098
9	0.00280	0.17423	0.00095
10	0.00181	0.17027	0.00070
11	0.00095	0.14583	0.00077
12	0.00108	0.01466	0.25806
13	0.00092	0.01324	0.00084
14	0.00101	0.11310	0.00098
15	0.00265	0.02517	0.00073
16	0.00055	0.10479	0.00138
17	0.00075	0.02798	0.00174
18	0.00087	0.30114	0.00123
19	0.00061	0.01466	0.57211
20	0.00076	0.01324	0.57680
21	0.00123	0.09350	0.52335
22	0.00176	0.04285	0.62742
23	0.00112	0.10479	0.29071
24	0.00095	0.02798	0.44293
25	0.00111	0.00016	0.40336
26	0.00076	0.00018	0.44912
27	0.00045	0.00015	0.64804
28	0.00073	0.01181	0.21520
29	0.00089	0.00020	0.13565
30	0.00108	0.00015	0.18800

Table D 2.4 Appearance of DNA damage on *C. Carpio* (continued).

Cell no.	*C. Carpio*, Level of DNA damage		
	Control	MBR influent	MBR treated
31	0.00216	0.00014	0.47732
32	0.00079	0.00020	0.44934
33	0.00190	0.00008	0.00063
34	0.00128	0.00832	0.00072
35	0.00251	0.00017	0.00034
36	0.00095	0.00149	0.00031
37	0.00099	0.00018	0.00041
38	0.00176	0.00014	0.00037
39	0.00327	0.00018	0.00047
40	0.00102	0.00011	0.00058
41	0.00082	0.00030	0.00036
42	0.00101	0.00031	0.00041
43	0.00093	0.05264	0.00026
44	0.00077	0.00011	0.00060
45	0.00812	0.00013	0.00047
46	0.00057	0.00025	0.00037
47	0.00082	0.00013	0.00028
48	0.00985	0.00968	0.00019
49	0.11146	0.00012	0.00037
50	0.01348	0.00014	0.00026
51	0.00081	0.00013	0.00033
52	0.00186	0.00027	0.00026
53	0.00517	0.00016	0.00047
54	0.06608	0.00023	0.00033
55	0.01679	0.00033	0.00085
56	0.01364	0.00013	0.00034
57	0.04608	0.13237	0.00044
58	0.00089	0.00019	0.00039
59	0.00074	0.00028	0.00052
60	0.00108	0.01679	0.00063

Table D 2.4 Appearance of DNA damage on *C. Carpio* (continued).

Cell no.	*C. Carpio*, Level of DNA damage		
	Control	MBR influent	MBR treated
61	0.00102	0.00034	0.00029
62	0.00117	0.00016	0.00021
63	0.00079	0.00020	0.00025
64	0.00085	0.41461	0.00038
65	0.00099	0.58970	0.00027
66	0.00066	0.42355	0.00036
67	0.00347	0.09162	0.00024
68	0.00119	0.36183	0.00017
69	0.00162	0.19585	0.00027
70	0.00137	0.09655	0.00052
71	0.00160	0.39770	0.01177
72	0.00084	0.31357	0.10283
73	0.00207	0.51252	0.03356
74	0.00139	0.03252	0.13278
75	0.00070	0.49606	0.08916
76	0.00108	0.16760	0.02046
77	0.00128	0.58251	0.19866
78	0.00167	0.38658	0.15463
79	0.00182	0.36217	0.11779
80	0.00108	0.30172	0.07487
81	0.00160	0.39212	0.00050
82	0.00313	0.40219	0.06757
83	0.00051	0.52521	0.00063
84	0.00227	0.16122	0.00069
85	0.00198	0.23951	0.00055
86	0.00099	0.58502	0.00048
87	0.00142	0.31818	0.00044
88	0.00098	0.54094	0.00049
89	0.00069	0.52736	0.00049
90	0.00175	0.20288	0.00069

Table D 2.4 Appearance of DNA damage on *C. Carpio* (continued).

Cell no.	*C. Carpio*, Level of DNA damage		
	Control	MBR influent	MBR treated
91	0.00098	0.40134	0.00052
92	0.00058	0.18455	0.00089
93	0.00073	0.00023	0.00048
94	0.00071	0.00014	0.00038
95	0.00082	0.00011	0.00063
96	0.00155	0.00015	0.00042
97	0.00174	0.00026	0.00054
98	0.00123	0.00022	0.00032
99	0.00115	0.00015	0.00084
100	0.00095	0.00012	0.00079
Avg.	0.39203	13.74977	7.29554
SD	0.01365	0.18682	0.16114

D.3 Removal Mechanisms

Table D 3.1 Removal rates of BPA, BHT, and DEHP by MBR sludge.

Time (h)	ln c/c_o of total removal			ln c/c_o of adsorption		
	BHT	BPA	DEHP	BHT	BPA	DEHP
0	0	0	0	0	0	0
3	-0.02532	-0.01207	-1.8579	0	0	-1.83258
6	-0.05024	-0.03046	-2.12026	-0.0284	-0.0141	-1.96611
9	-0.35667	-0.19845	-2.15417	-0.10536	-0.09982	-2.02495
12	-0.59059	-0.29437	-2.40795	-0.14618	-0.13467	-2.20727
15	-1.20397	-0.67139	-2.59027	-0.29841	-0.16252	-2.36446
18	-1.38629	-0.91629	-2.68825	-0.41552	-0.22314	-2.52573
21	-1.7148	-0.96758	-2.99573	-0.4385	-0.22314	-2.52573
24	-1.80789	-1.13943	-2.97593	-0.44629	-0.22314	-2.52573

Table D 3.2 Total removal rates of BPA, BHT by enriched nitrifying sludge.

Time (h)	ln c/c_o of total removal (24h)					
	with NH_4-N		without NH_4-N		with ATU	
	BHT	BPA	BHT	BPA	BHT	BPA
0	0	0	0	0	0	0
3	-0.07257	-0.10536	-0.02532	-0.01207	-0.05129	-0.04082
6	-0.19845	-0.22941	-0.08338	-0.05129	-0.12783	-0.10536
9	-0.4385	-0.51083	-0.30925	-0.19845	-0.35667	-0.29437
12	-0.73397	-0.77871	-0.59059	-0.35667	-0.65393	-0.43078
15	-1.20397	-1.09362	-0.91629	-0.67139	-0.99425	-0.73397
18	-1.60944	-1.29463	-1.38629	-0.91629	-1.41059	-1.02722
21	-1.96611	-1.76026	-1.7148	-1.13943	-1.75446	-1.2174
24	-2.12026	-2.07944	-1.83258	-1.27297	-1.89712	-1.60944

Table D 3.3 Removal rates of BPA, BHT by enriched nitrifying sludge.

Time (h)	ln c/c_o of adsorption (24h)					
	with NH_4-N		without NH_4-N		with ATU	
	BHT	BPA	BHT	BPA	BHT	BPA
0	0	0	0	0	0	0
3	0	0	0	0	0	0
6	-0.03563	-0.0202	-0.0284	-0.0141	-0.03046	-0.01715
9	-0.11653	-0.11429	-0.10536	-0.09982	-0.10536	-0.10536
12	-0.15082	-0.15082	-0.14618	-0.13467	-0.14966	-0.14272
15	-0.30788	-0.17435	-0.29841	-0.16252	-0.30381	-0.16842
18	-0.43078	-0.23193	-0.41552	-0.22314	-0.42312	-0.22314
21	-0.45413	-0.23572	-0.4385	-0.22314	-0.44629	-0.22314
24	-0.46204	-0.2459	-0.44629	-0.23572	-0.45413	-0.22314

Table D 3.4 Removal efficiencies of laboratory scale MBR

Compounds	Time (no.)					
	1	2	3	4	5	6
BPA (3.32)	12	17	25	30	35	65
BHT (5.10)	15	20	35	41	50	70
DEHP (7.54)	15	25	40	45	55	72

BIOGRAPHY

Miss Varinthorn Boonyaroj was born on October 11, 1982 in Bangkok, Thailand. She received her bachelor degree in Environmental Health from Burapha University and later, pursued the master degree study in Environmental Engineering at Kasetsart University. Then she pursued her Ph. D study in International Environmental Management Program of NCE-EHWM, Chulalongkorn University, from 2008 to 2013.

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