AEROBIC SUBMERGED MEMBRANE BIOREACTOR FOR SPENT CAUSTIC WASTEWATER TREATMENT

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ABSTRACT

Spent caustic (SC) is one of the petroleum industry wastewater that is toxic and hazardous to living things and environment. The aim of this study is to treat SC by aerobic submerged membrane bioreactor (ASMBR) using microfiltration (MF) hollow fibre membrane to improve the quality effluent that subsequently reduces the membrane fouling. At the beginning, the new operation parameters were identified namely mixed liquor suspended solid (MLSS) and solid retention time (SRT) for this system. MLSS was designed from 5 to 9 g L^{-1} and SRT from 20 to 80 days. Since membrane was used, membrane fouling remains a problem for MBR. Hence, biofouling reducers (BFRs) consisting of powdered activated carbon (PAC), zeolite (ZEO) and eggshell (ES) were added during the operation into ASMBR and eggshell is the new biofouling reducer in MBR area. Furthermore, the capability of ASMBR was continued by increasing the organic loading (OL) from 2 to 4 gCOD L^{-1}. The effluent quality, microbial products trend, and long-term trans-membrane pressure (TMP) performance were observed in all BFR experiments in ASMBR. Meanwhile, a dominant bacteria strain has been identified in ASMBR where it was implicated in treating spent caustic by using biochemical and molecular methods. Finally, this study developed a respirometric analysis by Activated Sludge Model No. 1 (ASM1) to calibrate design parameters that describe the degradation process in ASMBR. The models require characterisation of SC wastewater using chemical oxygen demand (COD) fractionation. Thus, the model was completed by observing COD effluent model trend from validation process. As a result, a good ASMBR was found to be the one operated at MLSS 5 g L^{-1} at SRT 40 days with less microbial products, good quality effluent and low membrane fouling rate. The average percentage removal showed 99% sulphide removal and more than 94% of COD removal during steady state operation. By adding PAC, higher reduction of the fouling rate (92%) and enhanced the removal performance were observed with 69.1% efficiency as compared with other BFRs. The sequences of amplified DNA fragment show 99% similarity with 16S rRNA sequence of *Bacillus thuringiesis Bt407* and *Carnobacterium maltaromaticum LMA28*. The COD fractionation shows inert particulate COD (X_i) (1.8 - 2.3 g L^{-1}) dominating in SC wastewater. The accumulation of X_i in ASMBR is correlated to hasten membrane fouling rate. From model simulation, BFR was proven to increase the growth rate of biomass with maximum specific growth rate (μ_{maxH}) in the range of 0.177 to 0.2 d^{-1} as BFRs were added.

ABSTRAK

Sisa kaustik (SC) adalah salah satu daripada air sisa industri petroleum yang toksik dan berbahaya kepada hidupan dan alam sekitar. Kajian ini dijalankan bertujuan untuk mengolah sisa kaustik menerusi kaedah bioreaktor membran terendam aerob (ASMBR) menggunakan penapisan mikro (MF) membran gentian beronggga untuk meningkatkan efluen kualiti dan mengurangkan kotoran membran. Pada mulanya, parameter operasi baru telah dikenalpasti iaitu campuran cecair pepejal terampai (MLSS) dan masa tahanan enapcemar (SRT) untuk sistem ini. MLSS ditetapkan dari 5 ke 9 g L^{-1} dan SRT dari 20 ke 80 hari. Apabila membran digunakan, kotoran membran masih menjadi masalah untuk MBR. Oleh itu, pengurang *bio*-kotoran (BFRs) yang diperbuat daripada serbuk karbon teraktif (PAC), zeolit (ZEO) dan kulit telur (ES) telah ditambah ke dalam ASMBR dan kulit telur adalah pengurang *bio*-kotoran yang baru bagi MBR. Tambahan pula, keupayaan ASMBR diteruskan dengan meningkatkan muatan organik (OL) dari 2 ke 4 gCOD L^{-1}. Kualiti efluen, perkembangan produk mikrob, dan prestasi tekanan *trans*-membran (TMP) untuk jangka masa panjang dikaji dalam semua eksperimen BFR terhadap sistem ASMBR. Sementara itu jenis bakteria dominan telah dikenalpasti di dalam ASMBR yang terlibat dalam mengolah sisa kaustik menggunakan kaedah biokimia dan molekul. Akhir sekali, kajian ini menggunakan penganalisaan respirometrik menerusi Model Enapcemar Teraktif No. 1 (ASM1) untuk menentukur parameter bagi menerangkan proses degradasi dalam ASMBR. Model ini memerlukan perincian air sisa kaustik (SC) melalui pecahan permintaan oksigen kimia (COD). Oleh itu, model ini lengkap dengan memerhatikan perkembangan model COD efluen dari proses pengesahan. Hasilnya, ASMBR beroperasi pada nilai optimum MLSS 5 g L^{-1} pada SRT 40 hari dengan produk mikrob berkurangan, efluen yang berkualiti dan kadar kotoran rendah. Peratusan purata penyingkiran menunjukkan 99% penyingkiran sulfida dan lebih 94% penyingkiran COD semasa operasi dalam keadaan tetap. Dengan menambah PAC, pengurangan yang lebih tinggi untuk kadar kotoran membran (92%) dan peningkatan prestasi penyingkiran telah dilihat dengan kecekapan 69.1% berbanding dengan BFR lain. Jujukan-jujukan yang telah diperkembangkan daripada serpihan DNA menunjukkan bahawa persamaan 99% pada 16S rRNA bakteria *Bacillus thuringiesis Bt407* dan *Carnobacterium maltaromaticum LMA28.* Pemecahan COD menunjukkan COD lengai zarah (X_i) (1.8 – 2.3 g L^{-1}) mendominasi dalam SC air sisa. Pengumpulan X_i dalam ASMBR dikaitkan dengan kepantasan kadar kotoran membran. Menerusi model simulasi, BFR terbukti dapat meningkatkan kadar pertumbuhan biojisim dengan kadar pertumbuhan maksimum tertentu (μ_{maxH}) di dalam julat dari 0.177 ke 0.2 d^{-1} apabila BFRs ditambah.

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η	-	Dynamic Viscosity of the Permeate
μ	-	Viscosity
A	-	Adenine
AFLP	-	Amplified Fragment Length Polymorphism
AnSMBR	-	Anaerobic Submerged Bioreactor
APHA	-	American Public Health Association
ASM	-	Activated Sludge Model
ASMBR	-	Aerobic Submerged Bioreactor
ASMBR-BFR0	-	Control ASMBR (Without BFR) (OL1)
ASMBR-BFRZEO	-	ASMBR-BFR Zeolite (OL1)
ASMBR-BFRES	-	ASMBR-BFR Egg Shell (OL1)
ASMBR-BFR1PAC	-	ASMBR-BFR Powdered Activated Carbon (OL1)
ASMBR-BFR2PAC	-	ASMBR-BFR Powdered Activated Carbon (OL2)
ASMBR-BFR3PAC	-	ASMBR-BFR Powdered Activated Carbon (OL3)
ASM1	-	Activated Sludge Model No. 1
ASM2	-	Activated Sludge Model No. 2
ASM2d	-	Activated Sludge Model No. 2d

ASM3	-	Activated Sludge Model No. 3
b_H	-	Decay Coefficient for Heterotrophic Biomass
BAP	-	Biomass-associated Product
BFR	-	Biofouling Reducer
BOD	-	Biological Oxygen Demand
C	-	Cytosine
C_0	-	Initial adsorbate concentration
C_e	-	Adsorbate equilibrium concentration after adsorption
CAS	-	Conventional Activated Sludge
CDU	-	Continuous Distillation Unit
Cell COD	-	Total COD – Soluble COD
CFMF	-	Cross-flow Microfiltration
CIA	-	Central Intelligence Agency
CO	-	Carbon Monoxide
COD	-	Chemical Oxygen Demand
C_t	-	Oxygen Concentration at Time
C_s	-	Saturation Oxygen Concentration
CSTR	-	Continuous Stirred Tank Reactor
CT	-	Capillary Tube
DEMF	-	Dead-end Microfiltration
DNA	-	Deoxyribo Nucleic Acid
DO	-	Dissolved Oxygen
DOE	-	Department of Environment
ED	-	Electrodialysis
Na_2EDTA	-	Disodium salt dihydrate
ES	-	Egg shell

EPA	-	Environmental Protection Agency
EPS	-	Extracellular Polymeric Sucstance
ERIC-PCR	-	Enterobacterial Repetitive Intergenic Consensus PCR
FC	-	Pleated Filter Cartridge
FCC	-	Fluidize Catalytic Cracker
FESEM-EDX	-	Field Emission Scanning Electron Microscope-Energy Dispersed X-ray
FISH	-	Fluorescence in situ Hybridisation
F/M	-	Food per Microorganism Ratio
FS	-	Flat Sheet
G	-	Guanine
g	-	G force
GAC	-	Granular Activated Carbon
HF	-	Hollow Fibre
H_2O_2	-	Hydrogen Peroxide
H_2SO_4	-	Sulfuric acid
HRT	-	Hydraulic Retention Time
iMBR	-	Immersed Membrane Bioreactor
J	-	Flux
J_c	-	Critical Flux
K	-	Permeability (LMH/ΔkPa)
K_{La}	-	Oxygen Mass Transfer Coefficient
$K_{O,A}$	-	Oxygen autotrophic half-saturation coefficient
$K_{O,H}$	-	Oxygen heterotrophic half-saturation coefficient
K_s	-	Haft saturation constant
kPa	-	kilo Pascal

LMH	-	Liter per Meter square per Hour
LPG	-	Liquefied Petroleum Gas
M	-	Molar
MBR	-	Membrane Bioreactor
MF	-	Microfiltration
MFR	-	Membrane Fouling Reducer
MLSS	-	Mixed Liquor Suspended Solid
MLVSS	-	Mixed Liquor Volatile Suspended Solid
MT	-	Multi-tubular
NaOH	-	Sodium Hydroxide
NF	-	Nanofiltration
OED	-	Optimal Experimental Design
OL	-	Organic Loading
OLR	-	Organic Loading Rate
OSHA	-	Occupational Safety Health Administration
OUR	-	Oxygen uptake rate
P	-	Pressure
PAC	-	Particulate Activated Carbon
P_{ave}/TMP_{ave}	-	Pressure Average
PCR	-	Polyerase Chain Reaction
PCR-RFLP		PCR-Restriction Fragment Length Polymorphism
PE	-	Polyethylene
PEG	-	Polyethylene Glycol
PES	-	Polyethylsulphone
PP	-	Polypropyle
PVDF	-	Polyvinylidene Difluoride
Q_{per}	-	Permeate floerate

q_e or $\frac{x}{m}$	-	Adsorbent
UAP	-	Substrate Utilization-associated product
UF	-	Ultrafiltration
RAPD-PCR		Random Amplified Polymorphic DNA-PCR
RIS	-	Resistance in Series
R_m	-	Membrane resistance
RNA	-	Ribonucleic Acid
RO	-	Reverse Osmosis
RPM	-	Revolutions per Minute
RT-PCR	-	Real Time PCR
R_{tot}	-	Total Resistance
S	-	Sulfur
S^{2-}	-	Sulfide
SC	-	Spent Caustic
SCOD	-	Soluble COD
Si	-	Silica
S_i	-	Inert Soluble COD
sMBR	-	Side-stream Membrane Bioreactor
SMP	-	Soluble Microbial Product
SO_4^{-2}	-	Sulfate
SRT	-	Sludge Retention Time/ Solid Retention Time
S_s	-	Readily Biodegradable COD
SSC	-	Synthetic Spent Caustic
SW	-	Scheduled Waste
T	-	Temperature
TCOD	-	Total COD

t_{fil}	-	Filtration time
TEA	-	Tri-acetate
t_{rel}	-	Relaxation time
TMP	-	Trans-Membrane Pressure
TN	-	Total Nitrogen
TOC	-	Total Organic Carbon
TP	-	Total Phosphorus
TSS	-	Total Suspended Solid
μ_{maxH}		Maximum specific Autotrophic Growth Rate
μ_H	-	Heterotrophic Growth Rate
μ_{maxH}	-	Maximum specific Heterotrophic Growth Rate
U	-	Uracil
UF	-	Ultrafiltration
USEPA	-	United State Environmental Protection Agency
UV	-	Ultravoilet
V	-	Volume
VSS	-	Volatile Suspended Solid
WAO	-	Wet Air Oxidation
X_i	-	Inert Particulate COD
X_s	-	Slowly Biodegradable COD
Y	-	Yield
Y_H	-	Heterotrophic Yield
Y_{obs}	-	Observed Yield
ZEO	-	Zeolite

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Petroleum is one of the sources of fossil fuel energy in Malaysia. As human population grows, most of the countries that produce petroleum earn much income because of high worldwide demand. According to government of Malaysia Energy Information Administration, Malaysia has six refineries with total capacity of 88, 218 m^3d^{-1} and the three largest refineries include Shell Port Dickson Refinery, Petronas Melaka-I and Petronas Melaka-II with 24,645 m^3d^{-1}, 14,760 m^3d^{-1} and 20,034 m^3d^{-1} respectively (Malaysia, 2010). With the increasing capacity of petroleum production, the amount of pollution to the environment could also increase. Water pollution is one of the pollution problems that needs more attention in order to sustain clean water for the future.

Spent caustic contains noxious properties such as high organic and inorganic sulphur compounds, high residual alkalinity and other contaminants such as phenolic, cresylic and naphthenic acids. These different compounds contained in spent caustic wastewater depend on different product streamlines. It has been classified as hazardous waste by Government of Malaysia Environmental Quality Act (Scheduled Waste) Regulation 25 (EQA, 1974). There are various existing treatment methods of spent caustic such as wet air oxidation (WAO), oxidation by hydrogen peroxide (H_2O_2), deep well injection disposal and incineration in a way to achieve the best water discharge. Some smaller industries that produce small amounts of spent caustic tend to send or sell the spent caustic for recovery and reuse to other industries like pulp and paper mill. Nevertheless, for the industries that have their own treatment plant, they have to bear the high cost of start-up and maintenance in order to achieve the best removal of wastewater.

Some researchers have investigated the studies of biological treatment of spent caustic. The treatment includes either aerobic or anaerobic conditions to complete the oxidation process. Specific bacteria such as haloalkaliphilic sulphide oxidizing bacteria, autotrophic sulphidic oxidizer and sulphide-oxidizing bacterium have been cultured and inoculated before they could be used for treating high strength spent caustic wastewater especially in high sulphide medium (Graaff *et al.*, 2012; Kolhatkar and Sublette, 1996; Rajganesh and Sublette, 1995).

Membrane bioreactor (MBR) is one of the processes that have proved capable of treating various types of wastewater. Most often MBR is used to treat low and medium strength wastewater and a lot of studies have been done on treating municipal and domestic wastewater. Lack of studies presented the ability of MBR in treating high strength wastewater as secondary treatment. Meanwhile, spent caustic treatment often involve physical and chemical treatment for oxidation such as wet air oxidation or hydrogen peroxide before dilution with other streams line prior to entering conventional activated sludge treatment (CAS). Settleability always becomes a big problem in conventional activated sludge clarifier due to foaming or

bulking of activated sludge. With the presence of the membrane, it changes the part of clarifier and eliminate settleability problem.

In order to minimize biofouling problem, several parameters like permeability, flux, pressure (TMP) and resistance are considered. In addition, biomass behaviour need to be controlled especially mixed liquor suspended solid (MLSS) concentration and solid retention time (SRT) since they are closely related in producing biomass products such as soluble microbial product (SMP) and extracellular polymeric substance (EPS) with tendencies to settle on membrane surface and pore blocking formed (Laspidou and Rittmann, 2002). Studies on membrane fouling are still on going but several studies have been carried out on methods used to reduce fouling problems. These include physical (backwash and relaxation), chemical (normally use sodium hypochlorite) or combination of physical and chemical cleaning.

Basically, physical cleaning only removes the coarse solid or cake on the surface of the membrane, while chemical cleaning removes the flocs caused by physical and biological fouling effects. The frequent use of chemical cleaning will change the characteristic of the membrane and reduce its performance. Therefore, biofouling reducer (BFR) is applied to prolong the use of membrane. In this study, powder activated carbon (PAC), zeolite (ZEO) and powdered eggshell (ES) was selected as BFR to minimize the membrane fouling and extend permeate performance. Besides, high volumetric organic loading of toxic compound will increase biological activities that lead to increase in the production of SMP and EPS and tend to accumulate on membrane surface. Hence BFR is used to reduce and stabilize the performance due to its adsorption characteristic (Mutamim *et al.*, 2012a). Dominant bacteria that are capable of degrading compounds in spent caustic (SC) wastewater were identified. Furthermore, Activated Sludge Model No. 1 (ASM1) approach was used to calibrate, optimize and characterize the behaviour of every process to establish the scientific link for each process (Salmiati *et al.*, 2010).

1.2 Problem Statement

Caustic solution used to remove unwanted contaminant in petroleum refinery and petrochemical processes and produce spent solution can be classified as sulphidic, phenolic or naphthenic spent caustic depending on composition of hydrocarbon streams or processes. Hence, the fluctuation in SC quality for different refineries and petrochemicals causes the reuse and recovery companies to have operational problems in SC processes. Apart from that, handling and transportation cost for reuse and recovery are not economically reliable nowadays. SC wastewater is high-strength industrial wastewater with biodegradability below acceptable limit and is very hazardous. In some petroleum industries, SC has been treated by neutralization with acid or flue gas but contaminants are not fully oxidized (Kemmer, 2010). Oxidization by physical and chemical treatment give incomplete oxidation of organic and inorganic SC wastewater that require further treatment (Berne and Cordonnier, 1995).

Figure 1.1 (refer to page 6) shows the report by researchers on existing physio-chemical treatments and biological treatment for SC. Physio-chemical treatments like wet air oxidation (WAO), fenton, electrocoagulation and incineration by applying high pressure and temperature are quite expensive. Besides, these treatments also create secondary pollution problems by adding some chemicals such as hydrogen peroxide in fenton process where it is formed by incomplete oxidation of sulphide to thiosulphide. Additionally, the chemical storage is related to safety measures. Deep well injection treatment of SC also tends to increase soil pollution.

Biological treatment is needed to complete the oxidation process since it is safer and cheaper because it operates at low temperature and pressure. However, there are certain ranges of concentration or small amount of SC wastewater that can go for conventional biological treatment. Thus, there is bulking or foaming of sludge and low efficiency for settleability due to filamentous growth (Thiothrix). Furthermore, it will affect the removal efficiency if the shock pollution loading

occurred or large basin is needed to get a good removal result (Ng and Hermanowicz, 2005). However, there is limited research recorded on biological treatment of SC wastewater.

Most of the previous studies focused more on the application of single genus namely *Thiobacillus* in batch reactors. In studying SC by using *Thiobacillus,* there is the need to maintain temperature at 30°C and to use pure oxygen to maintain the bacteria in complex processes and this requires high hydraulic retention time (HRT) (effect low production rate) for maximum oxidation (Graaff *et al.*, 2011; Kolhatkar and Sublette, 1996). A study by Graaff *et al.* (2011) and (2012) showed the system takes 3.5 days for maximum oxidation efficiency of 85% in gas-lift reactor that consist complex processes (condensation, abiotic bubble and biological processes). In a study by Park *et al.* (2009), two anoxic reactors, three aerobic reactors and clarifier had been used to treat low COD concentration (80 - 254 mg L^{-1}). It is a complex process with sludge recycled in anoxic and aerobic reactors. Two conditions of sludge media (anoxic and aerobic) to get more nitrogen removal also need to be controlled. The main problems encountered when clarifier is used are settleability and large footprint.

The novelty of this study is the configuration of the ASMBR that was used to treat SSC wastewater. The advantages of using ASMBR are small footprint, less energy consumption and no issues with sludge settleability. ASMBR has the potential to grow a robust bacteria in bulking sludge such as filamentous bacteria that known as a good bacteria in toxic compound removal (Judd, 2006). In conventional activated sludge treatment, the presence of high filamentaous bacteria causes bulking sludge and poor settleability. Moreover, the aim is to treat high strength SSC wastewater that contains high organic and inorganic contaminants such as sulphide and phenol which are known to be very toxic to the environment. MF hollow fiber membrane was used due to its low cost, compact and low water hold-up but easily fouled (Malak, 1999). The aim is to prolong the use of the MF membrane by using various BFRs and varying the loading rate of COD and to ensure the effluent meet standard requirement. The dominant bacteria in the reactor was capable of removing

contaminants in SSC was identified. In addition, the fouling effect caused by MLSS concentration and SRT for the process were identified as optimum conditions for this system as reviewed in Chapter 2 (Biomass Behaviour and Fouling Mitigation). In treating SSC, the suitable concentration of MLSS must be identified to improve degradation and at the same time reduce membrane fouling (Bottino *et al.*, 2009; Melin *et al.*, 2006). Meanwhile, SRT correlated with formation of microbial products by controlling F/M ratio. According to Judd, (2006), high SRT create starvation condition (low F/M ratio) that reduce the microbial production as discussed in Chapter 2 (Biomass Behaviour and Fouling Mitigation). However, both parameters hasten the membrane fouling when operated too high (Bottino *et al.*, 2009; Jiang *et al.*, 2008; Judd, 2006). Hence, COD fractions for SSC wastewater for each process were identified before it could be applied in ASM1 to obtain design parameters by adjusting model coefficients to match the result of respirometric experiment (Damayanti *et al.*, 2010; Salmiati, 2008; Salmiati *et al.*, 2009).

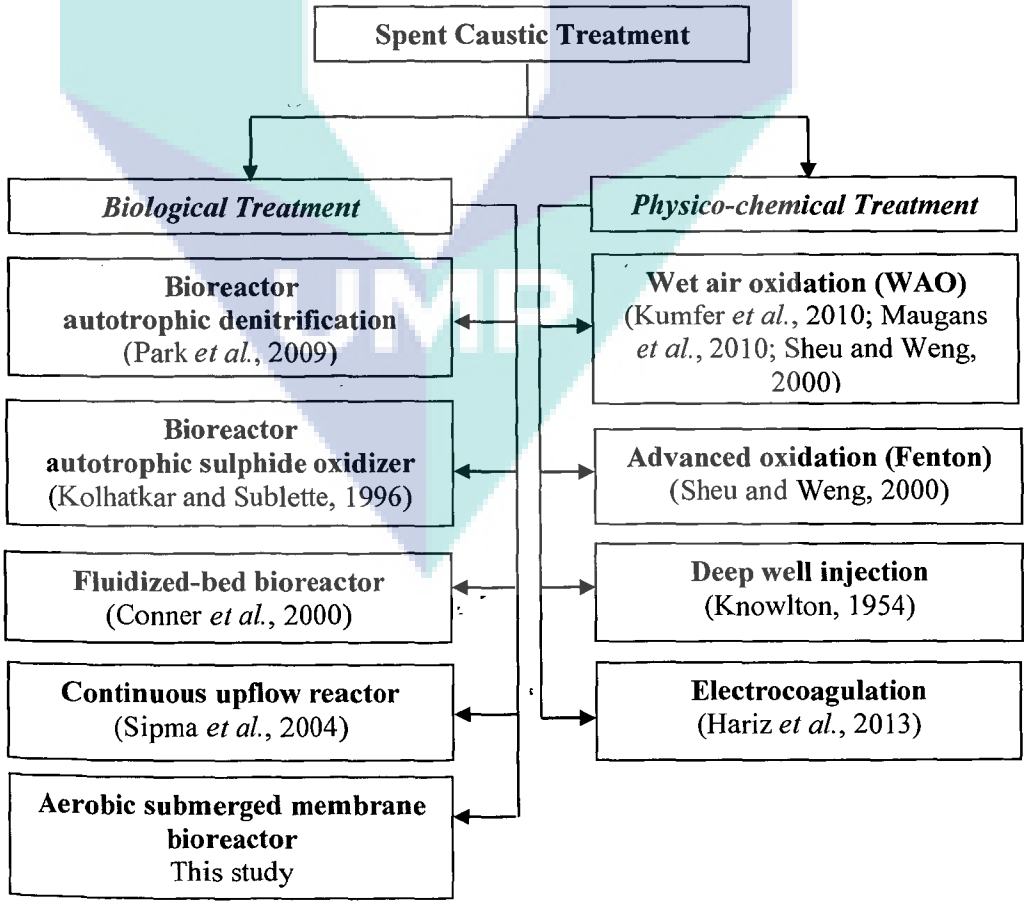


Figure 1.1 The reported spent caustic treatment

1.3 Aim and Objectives

The aim can be achieved by the following specific objectives:

i) To determine the optimum condition for the ASMBR operation in treating synthetic spent caustic (SSC) namely MLSS concentrations and SRTs;

ii) To assess the effects of different types of BFRs and COD loading on the fouling trend of ASMBR;

iii) To identify dominant strain of bacteria in mixed culture in ASMBR that implicated in treating spent caustic;

iv) To simulate COD fractions and process variables in ASMBR process at various BFRs and COD loading using ASM1.

1.4 Scope of Study

The scope of this study is as follows:

i) The 4L lab-scale ASMBR using MF hollow fiber membrane was set-up to treat SSC wastewater. This set-up was completed with pressure gauge, peristaltic pump, water level meter, air flowmeter, air pump, air diffuser, pH meter, DO meter and pressure data logger (online with computer to log DO and pressure reading);

ii) The synthetic spent caustic (SSC) was used to get less fluctuation in nutrients value. The stability of SSC was analysed on the COD

change trend for several days in nature, aeration reactor and after membrane filtration conditions without the existence of biomass;

iii) The study of MLSS concentrations of 5, 7 and 9 g MLSS L^{-1} and SRT parameters of 20, 40 and 80 days to get optimum condition for ASMBR operation. These two parameters directly affect biological viability in the reactor as reviewed in Chapter 2 (Biomass Behaviour and Fouling Mitigation). MLSS and SRT were designed based on the COD removal, biomass growth performance and low microbial products for high strength wastewater based on previous study as stated in Chapter 3 (MLSS concentration and SRT). The optimum performance condition was analysed based on organic and nutrient removal performance and membrane fouling (resistance in series (RIS), critical flux, TMP trend and SMP and EPS);

iv) Since membrane fouling became a major problem in MBR, the study continued with three types of BFRs (as reviewed in Chapter 2; Fouling Mitigation) to enhance the performance of ASMBR in reducing the effect of fouling. Initially, the concentration of BFRs were identified by adsorption batch process. The optimum BFRs concentration from adsorption process were applied in ASMBR;

v) The study continued with optimum performance of ASMBR-BFR in various COD loading of ≈2000 - 4000 mgCODL^{-1} of SSC wastewater. In ASMBR-BFRs with OL1 to OL3, experiments were analysed in organic and nutrient removal performance and membrane fouling (TMP trend and SMP and EPS);

vi) In ASMBR-BFRs, experiments were analysed in organic and nutrient removal performance, membrane fouling (critical flux, TMP trend and SMP and EPS) and biomass and membrane fouling morphologies (microscopic analyser and FESEM);

vii) The analysis of microbial population with biochemical and molecular identification methods to identify the dominant bacteria in ASMBR system;

viii) The study of COD fractions and process variables using respirometric analysis of spent caustic in ASMBR for various BFR and various COD loading in treating SSC wastewater purposely is to determine the design parameters for better understanding of the system. COD fractions are fitted in to ASIM 4004 for model calibration. Model calibration used in this study is to obtain designed parameters (μ_{maxH}, μ_{maxA}, b_H, K_s, $K_{O,H}$, $K_{O,A}$) that match the result from respirometric experiment by adjusting model coefficients and validate COD effluent model.

1.5 Significance of Study

This study intended to explore the capability of ASMBR performance with and without enhancement by BFR to treat spent caustic wastewater of various COD loading rates. The significance of this study can be summarized as:

i) The novelty of this study is ASMBR configuration with the use of MF hollow fiber membrane in treating SSC wastewater;

ii) Since this ASMBR is novelty in treating SSC, the identification of optimum operating condition is important and it is based on organic and nutrients removal and membrane fouling trend. The different range of MLSS concentration and SRT for high strength wastewater were selected as parameter controller due to their major effect on ASMBR operation;

iii) The innovative of this study continued by applying various BFRs into ASMBR in treating SSC wastewater purposely to enhance the organic and nutrient removal and reduce membrane fouling. In this study, the application of powdered egg shell has good contaminants adsorption as new BFR in this system is considered original besides other BFR (PAC and ZEO) since eggshell is capable of adsorbing, cheaper and readily available;

iv) This originality of study also applies to various COD loading rates of SSC wastewater in ASMBR objectively to challenge capability of ASMBR;

v) Identification of dominant bacteria that grows is a new study and it is able to degrade SSC contaminants in ASMBR system;

vi) Newness of this study is also to obtain the COD fractions needed to accomplish modelling to identify variable coefficients using ASM1 and it is applied for ASMBR with and without BFR and various COD loadings.

1.6 Thesis Organization

As a guide, this thesis consists of six chapters. The summary of the chapters are as follows:

Chapter 2 explained the characteristics of the spent caustic and its effect on human and environment. It also explained the system used, aerobic submerged membrane bioreactor (ASMBR) in a way to treat spent caustic and also provides background information about the types of MBR. It contained the parameters to be considered during the process operation, methods, characteristics and factors

affecting the performance of the system. The chapter also explained the theoretical data collection and selective methods for sample analysis. It also discussed the theoretical method using Activated Sludge Model No. 1 (ASM1).

Chapter 3 focused on the best method in conducting ASMBR and methods used in preparing the sample. It showed the setup of lab-scale plant and its procedure and steps to be taken before, during and after the experiment and data collection is being run. Analysis included FESEM as image analyser for membranes and BFRs before and after use, to analyse organic and nutrients removal and microbial test to identify the type of dominant bacteria in the reactor. Methods of COD fractionations and stoichiometric and kinetic coefficients were elaborated using ASM1.

Chapter 4 discussed MLSS concentration and SRT result. MLSS concentrations and SRT discussed the performance of organic and nutrient removal, TMP trend and change of RIS data. SMP and EPS data were also collected for MLSS concentration and SRT study. This chapter also discussed the various BFRs and COD loading on organic and nutrients removal, TMP performing and SMP and EPS. Morphology of biomass and membrane were observed and discussed.

Chapter 5 discussed the dominant microbial identifications. Respirometric characteristics for COD fractionations and stoichiometric and kinetic coefficient for different BFRs and COD loading in ASMBR were also discussed in this chapter. The processes were characterized on COD fractions and stoichiometric and kinetic coefficients for various BFRs and COD loading using ASM1.

Chapter 6 showed the conclusion of the result from the experiment. The conclusion was based on whether the objectives were achieved or not. It also summarized the process of research including problem solving, suitability of the methods and possibility of future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Petroleum in one of the energy from fossil fuel sources that most countries depending on. The sulphur content in crude oil varies between 0.1 to 8% (w/w) (Müllera *et al.*, 2012). During refining process, volatile sulphur compounds are removed by adsorption with activated carbon, caustic scrubbing and amine treating (Sipma *et al.*, 2004). Alternatively, volatile sulphur compounds are treated by activated carbon through gas adsorption and caustic scrubbing by caustic soda (Alnaizy, 2008). The product from scrubbing includes spent caustic that can be categorised into three types: sulphidic spent caustic, phenolic spent caustic and naphthenic spent caustic.

** Part of this chapter has been published in Desalination 305 (2012) 1-11 section (2.4.3) and Chemical Engineering Journal 225(2013) 109-119 section (2.5, 2.5.1-2.5.5)*

Caustic soda is able to reduce odour and colour. The solution is purposely to improve organic acid like naphthenics acids and phenols and sulphur compounds such as hydrogen sulphide and mercaptans during oil refining and petrochemical processing. It is in the form of strong aqueous solution and is diluted according to requirements. This solution can be reactive when contacted with organic and inorganic chemicals and can cause explosion or fire. When reacted with metals, it releases flammable hydrogen gas.

Spent caustic is one of petroleum industries wastes. It originates from sodium hydroxide, soda caustic or potassium hydroxide which removes undesired sulphur during the process. It is difficult to estimate the characteristic of this wastewater due to high noxious properties, for instance sulphides and mercaptans and according to the Environmental Quality Act 1974, these components are listed in schedule and need proper treatment before it can be discharged to the environment (EQA, 1974). Spent caustic from petroleum refinery has a different composition and usually the quantity is smaller than petrochemical and there are three types of spent caustic which are sulphidic, phenolic and naphthenic spent caustic (Berne and Cordonnier, 1995).

Small volume of spent caustic has been disposed into deep well or sold to outside buyers like pulp and paper mill operators. However, some of spent caustic have been treated by neutralization and oxidation processes to acceptable limits before it can be discharged into general effluent or open systems (Sipma *et al.*, 2004). This treatment is more difficult and very costly due to the high volume wastewater.

Membrane technology is not new in our technology. It was used long time ago as separator with its unique behaviour. With combination of membrane technology and biological treatment, a separation field system is created and referred to as membrane bioreactor system.

2.2 Petroleum Refinery Process

Historically, the refinery process began from the 1860s near Oil Creek, Pennsylvania. In Malaysia, crude from Tapis industry is almost good enough for direct use in diesel engines but need to be routed to refineries for separation into higher value components (Robinson, 2006). Crude petroleum can be classified into several fractions:

i) Natural and also known as casing head - Gasoline, natural gas, liquid petroleum gas, LPG.

ii) Light Distillation - Motor gasoline, solvent naphtha, jet fuel, kerosene, light heating oil

iii) Intermediate Distillation - Heavy fuel oil, diesel oil, gas oil.

iv) Heavy Distillation - Heavy mineral oil (medicinal), heavy flotation oil, lubricating oil, waxes (candles, sealing, paper treating, insulating)

v) Residue - Lubricating oil, fuel oil, petrolatum, road oil, asphalts and coke.

Basically the process is developed to separate the crude oil into fractions and this happens because every compound has different boiling point and volatility. However simple distillation cannot be done because high temperature is needed to separate the compounds. The components with the same boiling point and volatility need other sources to be injected, like pressure. It is because every component has its own partial pressure and principally depends on the vapour pressure of the component in a pure state. These processes consist of distillation processes, thermal cracking processes, catalytic processes, reforming processes and treatment processes (Robinson, 2006; Speight, 2011).

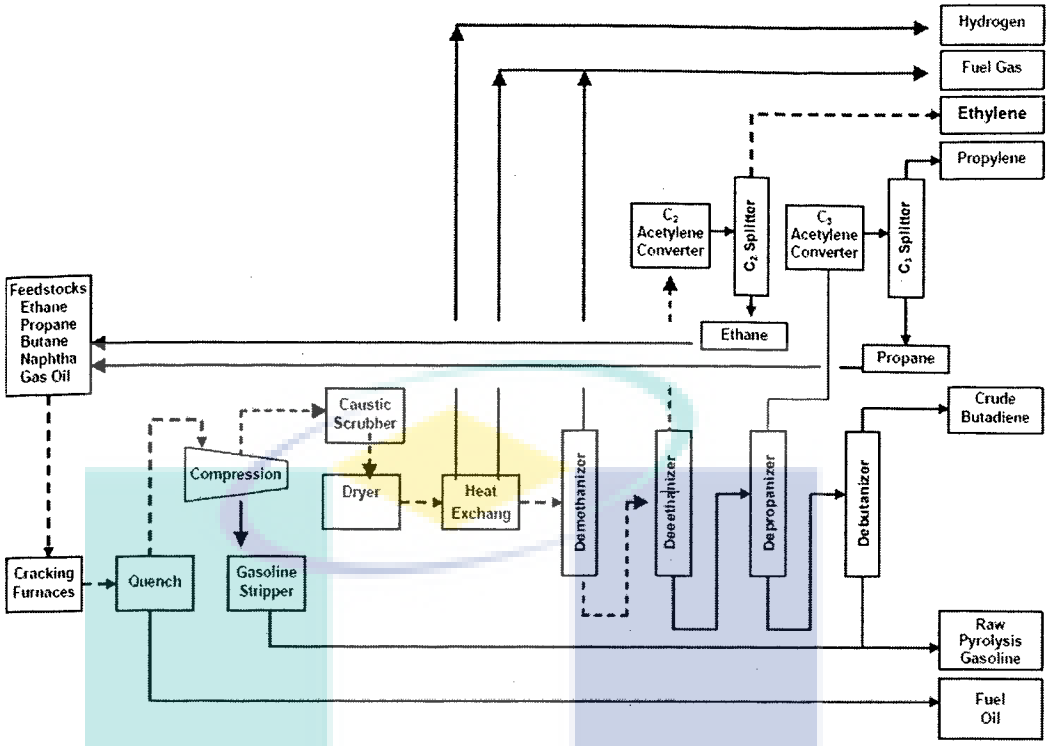


Figure 2.1 Ethylene process (American Chemictry Council, 2004)

2.4.1 Caustic Soda

Jones (2006) stated that caustic soda solution is purposely to absorb hydrogen sulphide or light mercaptans during oil refining process from light petroleum products. It is in the form of strong aqueous solution and is diluted according to requirements. This solution can be reactive when in contact with organic and inorganic chemicals and can cause explosion or fire. When reacted with metal, it releases flammable hydrogen gas. Table 2.1 shows the characteristics of caustic soda (Jones, 2006).

Table 2.1: Characteristics of Caustic Soda (Jones, 2006)

Characteristic	Description
Molecular weight	40
Melting point	12°C (50% solution; freezing point), 62°C (70-73% solution)
Boiling point	140°C (50% solution)
Specific gravity	1.53 (50% solution), 2 (15.5°C; 70-73% solution)
Solubility in water	Soluble in all proportions
Solubility in other liquid	Soluble in all proportions in ethanol, methanol and glycerol
pH value	12 (0.05% solution); 13 (0.5% solution); 14 (5% solution)

Caustic soda, also known as sodium hydroxide (NaOH), lye or sodium hydrate is caustic compounds which react with organic matter. Caustic soda is available commercially in various white forms and is also forms solutions of various concentrations in water because of its solubility, alcohol and glycerine and rapidly absorbs carbon dioxide and moisture from air. Caustic soda can be prepared by the reaction of sodium carbonate (soda) in concentrated solution form with calcium hydroxide (slaked lime). In the industries, it can be produced through electrolysis method of brine with 25% aqueous sodium chloride. The functions are to neutralise the acids, hydrolysis, condensation and saponification besides replacement of other groups in organic compounds of hydroxyl ions.

Caustic soda is widely used in petroleum refining process as a remover of odour, colour, stability, carbon residue and other properties of the oil. Contaminants include organic compounds containing sulphur, nitrogen, oxygen, dissolved metals and organic salts. Soluble salts dissolved in emulsified water react with caustic soda. It is used to neutralize and to extract acidic materials that may occur naturally in crude oil, acidic reaction products that may be produced by various chemical treating processes and acidic materials formed during thermal and catalytic cracking such as H_2S, phenolics and organic acids (Wong and Hung, 2004). Emission of hydrogen

sulphide is a big problem and is by a method called caustic scrubbing (Bosch *et al.*, 2009).

2.4.2 Spent Caustic

Generally spent caustic wastewater can be categorized as spent sulphidic caustic, phenolic spent caustic and naphthenic spent caustic depending on compositions of hydrocarbon streams and processes. Table 2.2 shows the compositions of the three types of SC (Veerabhadraiah *et al.*, 2011).

Table 2.2: The components for different types of spent caustic (Alnaizy, 2008; Veerabhadraiah *et al.*, 2011).

Component	Sulphidic	Phenolic	Naphthenic
Naoh, wt%	2-10	1-15	1-4
Sulphide, wt%	0.5-4	0-1	0-0.1
Mercaptide, wt%	0.1-4	0-4	0-0.5
Cresylic acids, wt%	-	10-25	0.3
Naphthenic acids, wt%		-	2-15
Carbonate as CO, wt%	0-4	0-0.5	-
pH	13-14	12-14	12-14

In La Pampilla Refinery in Peru, spent caustic comes from the fluidized catalytic cracker and continuous distillation units. Once it becomes spent, it will has to be disposed in a proper way because spent caustic is hazardous, odorous and corrosive. The mechanism of scrubbing or washing process related to hydrogen sulphide's reaction with soda caustic to form unstable hydrosulphide ion and water is shown below. Unstable hydrosulphide ion will attach with any counter ion, for instance sodium, Na^+, usually at a pH of 9 – 12 and form sulphide-loaded alkaline

solution. This process also produces mercaptans and other unstable contaminants. Equations 2.1 to 2.5 are reactions that happen at the washing or scrubbing stage (Bosch *et al.*, 2009; Rajganesh *et al.*, 1995).

$$H_2S + 2NaOH \rightarrow Na_2S + 2H_2O \quad (2.1)$$

$$Na_2S + H_2S \rightarrow 2NaHS \quad (2.2)$$

$$RSH + NaOH \rightarrow NaSR + H_2O \quad (2.3)$$

$$2NaHS + 2O_2 \rightarrow Na_2S_2O_3 + H_2O \quad (2.4)$$

$$2RSNa + 1/2O_2 + H_2O \rightarrow 2NaOH + RSSR \quad (2.5)$$

During alkylation process, sulphuric acid is used as catalyst and caustic soda is used to wash (neutralize) the hydrocarbon stream before going to the fractionating section. Figure 2.2 shows the process of alkylation which produces spent caustic (Kemmer, 2010).

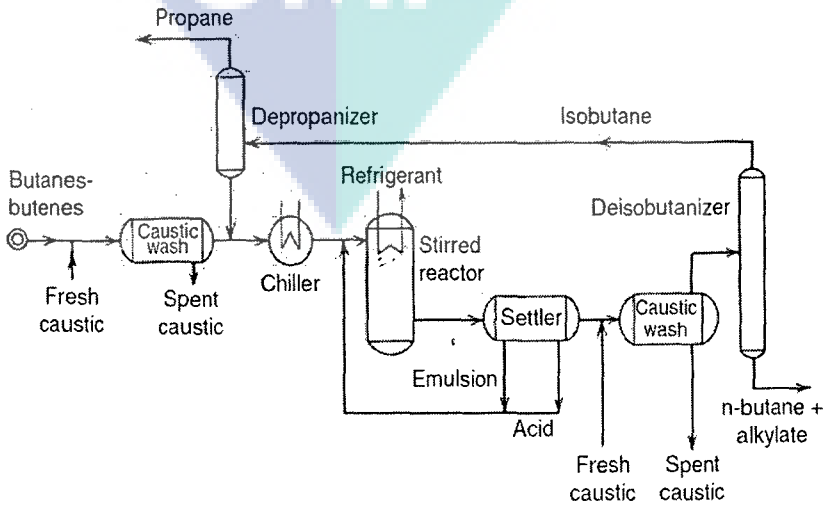


Figure 2.2 Alkylation process using caustic (Kemmer, 2010).

At the Miguel Hidalgo Refinery, spent caustic is produced from the desulphurization process of crude oil. The process consists of two stages: prewashing stage where the fuel is mixed with 6% caustic soda solution to remove H_2S and is converted to sodium thiosulphate ($Na_2S_2O_3$) and the second stage is the sweetening stage where the catalytic oxidation consists of 19% caustic soda solution and hot air oxidized mercaptans to disulphide (Olmos *et al.*, 2004). Figure 2.3 shows the Refineria de Petroleos de Manguinhos located in Brazil. This spent caustic comes from gasoline sweetening, gasoline and LPG prewashing and from gasoline and LPG mercaptans extraction (Carlos and Maugans, 2000).

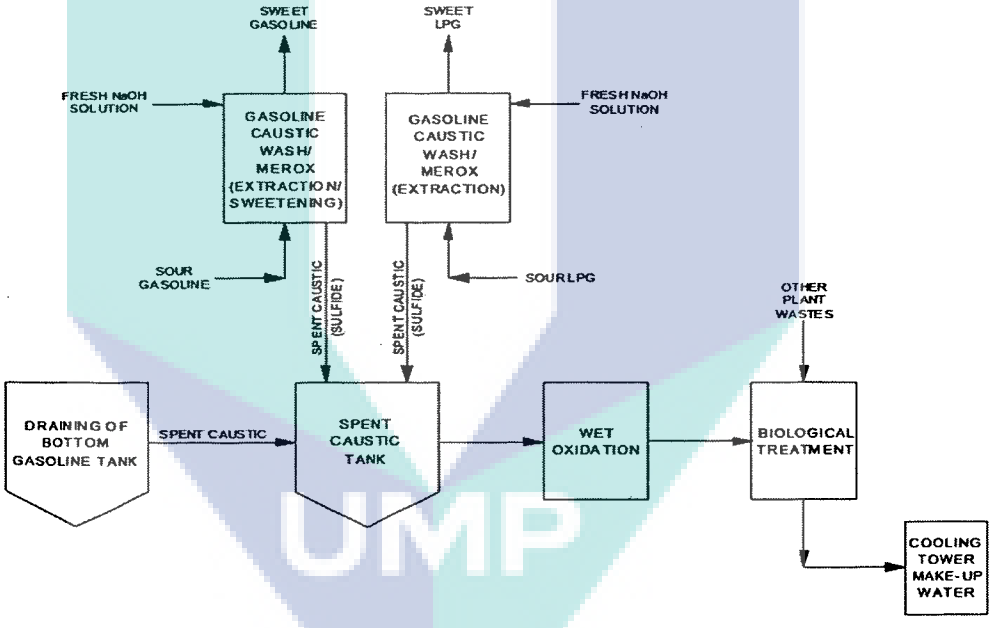


Figure 2.3 Refineria de Petroleos de Manguinhos (Carlos and Maugans, 2000)

Figure 2.4 shows the caustic scrubber from ethylene process. Caustic scrubber is to remove hydrogen sulphide (H_2S) and carbon dioxide (CO_2) from ethylene gas and producing spent caustic at the end of this process. The spent caustic also contains sulphides and mercaptans (Mamrosh *et al.*, 2008).

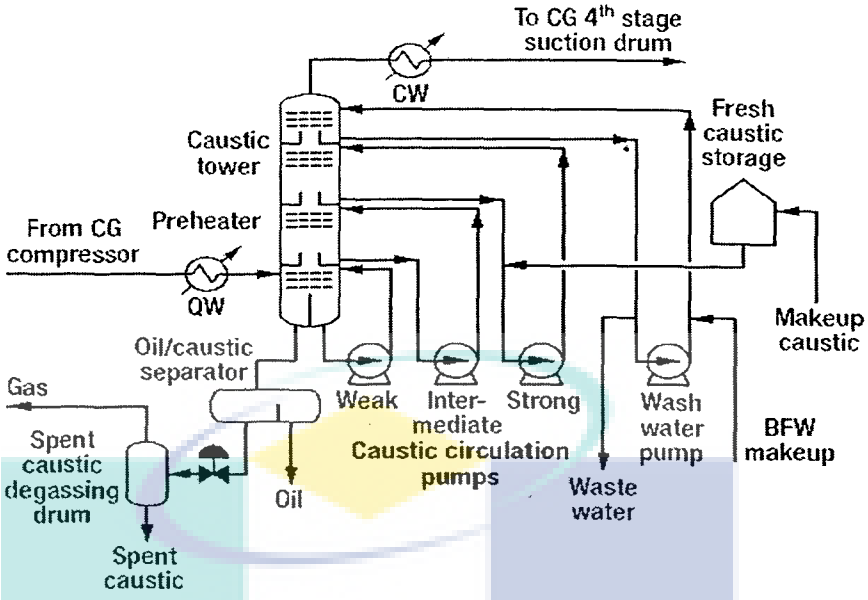


Figure 2.4 Caustic scrubber process (Maugans *et al.*, 2010)

2.4.3 Characteristics of Spent Caustic

High strength industrial wastewater is difficult to define. It can be different from food industries and chemical industries. Summer (2003) stated that high strength wastewater is defined as the wastewater that contains fats, oil and grease or other organic or inorganic compounds in great amount according to the types of sources that take part (Summer, 2003). Basically it is called high strength because the components in the wastewater are in huge amount; for instance, high amount of COD (Robinson, 2001), ammonia, suspended solid or heavy metal (Hogye, 2003) and sometimes shock loading will happen. According to Mara and Horan (2003), the standard for substances for instance phenol, toluene, sulphides, cyanides and heavy metal should be set at very low limit, otherwise excessive amount will lead to oxygen depletion in water, besides being inhibiting and toxic to the biomass due to the disruption of enzyme and protein cells. However, some heavy metals are micronutrients and are required in small amount for organisms' metabolic function (Mara and Horan, 2003).

Wastewater strength can be levelled through the biodegradability characteristic of wastewater. BOD_5 is measured based on the quantity of oxygen that will be required to biologically stabilize the organic matter present, besides to measure the efficiency of some treatment processes in 5 days. COD is to measure oxygen equivalent to the material in wastewater including organic and inorganic materials that can be oxidized chemically in 2 hours. General industries seldom use BOD test as daily analysis due to the long period of time required to get the result and a pre-treatment is needed when dealing with toxic waste; otherwise COD test is usually used. BOD corresponds with COD to measure the 'hardness' of wastewater or biodegradability and usually it is done in preliminary operation. Strength of wastewater can be based on the biodegradable and non-biodegradable elements contained in the wastewater. High ratio of BOD_5/COD is shown as readily biodegradable, otherwise low ratio BOD_5/COD indicates as slowly biodegraded or contains a part of non-biodegradable or toxic elements. Furthermore, to treat wastewater with low BOD_5/COD ratio, the slow biomass acclimatization may be required for stabilization (Metcalf and Eddy, 2004). Durai and Rajasimman (2011) stated that 0.3 BOD_5/COD ratio for tannery wastewater is low as compared with domestic wastewater ratio, 0.5 because it contains BOD_5 inhibitors (Durai and Rajasimman, 2011).

Samudro and Mangkoedihardjo (2010) stated that biodegradability is a measurement of allowable level of organic matter that can be used as indicator to know the level of wastewater (Samudro and Mangkoedihardjo, 2010). This ratio describes the biodegradability level of materials in which organic matter containing wastewater is readily broken down into the environment. Besides that, this ratio shows the level allowable of organic matter to degrade by biomass (Samudro and Mangkoedihardjo, 2010). Generally, BOD_5/COD 0.5 is considered as readily biodegradable or easily treatable (Metcalf and Eddy, 2004). Kumfer *et al.* (2010) for example, showed biodegradability greater than 0.5 for spent caustic wastewater after treatment by using wet air oxidation (WAO). If the ratio value is less than 0.5, the wastewater needs to have physical or chemical treatment before a biological treatment takes place (Kumfer *et al.*, 2010; Samudro and Mangkoedihardjo, 2010). Table 2.3 shows the characteristics of high strength wastewater for different

industries. The biodegradability ratio for textile industries and tannery is low as compared with food industries due to high content of 'hard' BOD_5/COD.

Wastewater that is originated from industries is considered as high strength wastewater. Industries prefer to use COD because it covers organic and inorganic substances and also to get instant result. There is no specific range to differentiate between low, medium and high strength of wastewater in the industries. In areas of biodegradable wastewater for instance, COD is deemed as low strength level when it is less than 1000 mg L^{-1} (Ganesh *et al.*, 2007). For example, even though petrochemical has 1000 mg L^{-1} COD, it is considered high strength level but for food industries, 1000 mg L^{-1} COD is considered as medium strength. This is because chemical industries contains 'hard' COD or high contain non-biodegradable compound (S_i and X_i), for example heavy metal as compared to food industries which normally contain high content of biodegradable compound (S_s and X_s), for instance nitrogen or phosphorus elements (Robinson, 2001).

A study that was carried out by United Nations (Nations, 2003) has shown that pipelines and equipment in industrial sectors such as cooling water, boiler water, process water and irrigation and maintenance and landscape face a big problem when in contact with high strength wastewater. It can cause clogging, corrosion, scaling, biological growth and foaming in any systems. It can also happen especially when the high strength wastewater is discharged into the environment that clogs the soil. Usually, treated wastewater would be discharged into the environment, or some of the industries would reuse treated water or sell it to other industries. The treated water needs to meet the standard that has been stated in the environment regulation; for instance in Malaysia, the industries need to comply with the Environmental Quality Act 1974 where parameters limiting the effluent in Third Schedule are provided, otherwise fines will be charged (EQA, 1974).

Table 2.3: Characteristics of High Strength Wastewater for Different Industries

Industry	COD ($g\ L^{-1}$)	BOD ($g\ L^{-1}$)	COD /BOD	NH_4-N ($g\ L^{-1}$)	TSS ($g\ L^{-1}$)	PO_4^{3-} ($g\ L^{-1}$)	Phenol ($g\ L^{-1}$)
Tannery (Artiga *et al.*, 2005)	2	-	-	-	-	-	-
Tannery (Robinson, 2001)	16	5	0.313	0.45	-	-	-
Textile (Badani *et al.*, 2005)	6	0.7	0.117	0.02	-	0.12	-
Textile (Brik *et al.*, 2006)	4	0.5	0.125	0.0048	-	2	-
Dyeing (Feng *et al.*, 2010)	1.3	0.25	0.192	0.1	0.2	-	-
Textile (Yigit *et al.*, 2009)	1.5	0.5	0.333	0.05	0.14	7	-
Wheat Starch (Sutton, 2003)	35	16	0.457	-	13.3	-	-
Dairy (Robinson, 2001)	3.5	2.2	0.629	0.12	-	-	-
Beverage (Robinson, 2001)	1.8	1	0.556	-	-	-	-
Palm Oil (Yuniarto *et al.*, 2008)	67	34	0.507	0.5	24	-	-
Pet Food (Acharya *et al.*, 2006)	21	1	0.476	0.11	54	0.2	-
Dairy Product (Katayon *et al.*, 2004)	0.88	0.68	0.773	-	2.48	-	-
Phenolic (Viero *et al.*, 2008b)	0.797	-	-	0.131	-	-	0.0373
Pharmaceutical (Chang *et al.*, 2008)	6.3	3.225	0.51	-	-	-	-

As mention before, spent caustic is high strength industrial wastewater that needs to be treated before it can be discharge to the environment. Before spent caustic can be treated, their characteristics need to be identified depending on the type of feedstock, cracking severity, sulphur content in the feed and caustic utilization (Kumfer *et al.*, 2010). Below is the general characteristic of spent caustic and Table 2.4 shows the raw spent caustic from different industries.

i) Sulphides and mercaptans have very strong odours. According to OSHA (Jeffress, 1970), these compounds are classified as very toxic and can be corrosive to plant. Besides, spent caustic is acid neutralized which causes hydrogen sulphide and mercaptans gases to be released.

ii) Spent caustic wastewater is high with phenols. Concentrations as low as 400 mg L^{-1} have been shown to inhibit the removal of COD, ammonia and phosphorous and have bad impact in settling of sludge during treatment, especially in biological treatment processes (Kumfer *et al.*, 2010).

iii) Physical characteristics of spent caustic generally have pungent smelling reddish liquid appearance, strongly basic in the range of pH 13-14, corrosive agent by sodium hydroxide and acid or base strength within 2-10% (API, 2009).

iv) Sulphide is known as an inhibitor to trans-membrane protein in bacteria especially cytochrome oxidase by transferring electron from cytochrome to oxygen to form water (Klok *et al.*, 2012).

Table 2.4: Untreated Spent Caustic from different industries.

Parameter	(Kumfer *et al.*, 2010)	(Byun *et al.*, 2006)	(Kolhatkar and Sublette, 1996)
COD (mg/L)	62,700	30,000	26,700
BOD_5 (mg/L)	7,260	-	-
BOD_5/COD	0.29	-	-
Sulphides, S^{2-} (mg/L)	17,800	16,700	5800
Mercaptans (mg/L)	9,880	-	-
Sulphates, SO_4^{2-}	<275	-	-
Phenol (mg/L)	-	29.6	2,800
pH	-	12.7	13

2.4.4 Law and Regulation

Malaysia and world concern about pollution as it is in the level of serious condition, an action needs to be taken. One of the actions taken is by forming the rule and regulations that need to be complied by all industries and parties that are involved in producing any wastes or effluents. Environmental Protection Agency (EPA) established the Pollution Prevention Act of 1990 in strategy for pollution prevention and develop source reduction model. The main objective where the owners are responsible to collect all data manufacturing facilities and report annually on source reduction and recycling activities and authorizes by EPA.

The Clean Water Act is focusing on pollution control programs such as wastewater standards for industry and Environmental Protection Agency (EPA) has full implementation authority. Marine Protection, Research and Sanctuaries Act gave authority to the EPA to protect oceans from any dumping that can pollute the marine. Fines can be imposed for illegal dumping (Robinson, 2006). The EPA review shows the production, handling and recycling of spent sulphidic caustic, for instance, under its Hazardous Waste Management System (EPA 1998). In attempt to achieve the "fishable" and "swimmable" water, the total maximum daily load (TMDL) programme has been established in US. Section 303(d) of the Clean Water Act (CWA) (1972) requires the establishment of a TMDL for all impaired waters (US). The TMDL addresses each pollutant or pollutant class and control techniques based on both point and non-point sources, although most of the emphasis seems to be on non-point controls. Thus, recycling water process applied to improving the quality of point discharges (Judd 2006).

Malaysia has also planned and implemented its environmental protection management policy and activities in the control of the industrial wastewater discharge adopted in the US. Regarding to Laws of Malaysia, Environmental Quality Act 1974 have stated in section 7 under Environmental Quality (Sewage and Industrial Effluents) Regulation 2005, the effluent discharged into any inland waters

shall be analysed in accordance with nineteenth edition of the methods specified in Second Schedule or else in accordance with such other methods of analysis agreed by Director General of Environmental Quality. In section 8 under the same regulation shows the parameter limits of effluents which are allowed to be discharged into inland waters (any reservoirs above the low water line or intake water) that need to fulfil in all industries except for palm oil and rubber industries.

There are two standards that need to fulfil Standard A, for any inland water within the catchment areas specified in the Forth Schedule otherwise comply with Standard B. Table 2.5 shows some parameters from Third Schedule (EQA, 1974). Rivers are polluted from point and non-point sources and limited data are available on the long-term performance of industrial wastewater treatments that would be impossible to evaluate the contribution of wastewater on water pollution. For industrial effluent point source, event mean concentration (EMC) (represents the flow-weight pollutant concentration for any storm event) for COD and BOD are 120 mg L^{-1} and 20 mg L^{-1} (Mamun and Zainudin, 2013)

Table 2.5: Parameter Limits of Effluent of Standards A and B (EQA, 1974)

Parameter	Unit	Standard A	Standard B
(i)Temperature	°C	40	40
(ii)pH	-	6.0-9.0	5.5-9.0
(iii)BOD_5 at 20°C	mg/L	20	50
(iv)COD	mg/L	120	200
(v)Suspended solid	mg/L	50	100
(vi)Sulphide (S^{2-})	mg/L	0.50	0.50
(vii)Phenol	mg/L	0.001	1

2.4.5 Existing Spent Caustic Treatment

Spent caustic generated by refineries and petrochemical can be sent off-site to a commercial recovery operation for beneficial reuse, injected into deep wells for disposal, incinerated or managed on-site. It can also be sold because it has commercial value after going through some additional processes, for instance, sodium sulphide (Jones, 2006). By injecting the spent caustic into deep wells for disposal, it can pollute the underground water if it continues for a long time (American Petroleum Institute, 2009).

In order to have a good environment management, incinerators are used to treat any waste, but they require lots of power consumption and are not economical. For small refineries and petrochemicals which produce small amounts of spent caustic, the practice is to send the waste to other industries for recovery and reuse. Apart from reusing or recycling the caustics, they can also separate the other substances in spent caustic and sell or use them in other processes. Generally most of the petroleum industries producing spent caustic use conventional treatment. The treatments consist of neutralization, oxidation by physical-chemical treatment before it is discharged into the general basin for conventional biological treatment (Berne and Cordonnier, 1995). Table 2.6 shows the existing spent caustic treatment.

Table 2.6: Advantages and disadvantages of existing treatment

Treatment	Advantage	Disadvantage
Wet air oxidation (Carlos and Maugans, 2000; Ellis *et al.*, 1994)	• Efficient removal	• High start-up and operational costs • Operating at high temperature and pressure
Fenton (Sheu and Weng, 2000)	• Easy treatment	• High H_2O_2 consumption

		• High chemical cost • Incomplete oxidation
Deep well injection (American Petroleum Institute, 2009)	• null	• Environmental impact
Biological (Graaff, 2012)	• Efficient removal • Low temperature and pressure	• Lab scale • Specific bacteria need to be maintained

(a) Physicochemical Treatments

Neutralization is the chemical treatment for desulphurization. However it is difficult to reach SO_4^{2-} state and major $S_2O_3^{2-}$ formed. During neutralization, the factors required to achieve optimum performance are the type of reactor, retention time and type of acid. Wet air oxidation (WAO) is frequently used in petroleum industries. However, the process needs high pressure and temperature to oxidize the high loading pollutants. WAO is known as pre-treatment process and the water discharged is collected in equalization tank before entering biological treatment (Carlos and Maugans, 2000). Table 2.7 shows the performance of WAO in treating spent caustic. Even though this treatment is resource recovery, it is not effective enough in treating spent caustic and need high pressure, catalysts, energy consumption (high temperature) and oxygen demand (Heimbuch and Wilhelmi, 1985). Unfortunately 65% to 85% removal of COD can be achieved but not all streams can be treated by WAO. Additionally, this treatment is costly for chemicals used and produces sulphur dioxide (SO_2) that is very toxic (Janssen *et al.*, 2000). Another method is liquid incineration that is too expensive.

Table 2.7: WAO Performance in treating of Spent Caustic

Parameter	Phillips Petroleum Company, Sweeny Texas (135°C) (Ellis *et al.*, 1994)		Rafinaria de Petroleos de Manguinhos, Rio de Janeiro, Brazil (Carlos and Maugans, 2000)		National Petroleum Refiners Association Conference Phoenix, AZ (Kumfer *et al.*, 2010)		Respol YPF Refinery in La Pampilla, Peru (Maugans *et al.*, 2007)	
	Influent	**Effluent**	**Influent**	**Effluent**	**Influent**	**Effluent**	**Influent**	**Effluent**
COD ($g\ L^{-1}$)	-	-	72,000	15,000	73,900	7,200	73,000	6,300
BOD (mg/L)	-	-	-	-	-	4220	-	-
Phenol (mg/L)	-	-	1,700	3	6,110	0.36	6,500	36
Sulphides (mg/L)	4,031	<2	2,700	<1	8,040	<1	8,500	<1
Mercaptans (mg/L)	-	-	2,800	2	1,800	<30	1,500	<30
Thiosulphate, $S_2O_3^{2-}$(mg/L)	<224	959	-	-	-	-	1,500	<30
Sulphites, SO_3^{2-} (mg/L)	<64	204	-	-	170	<2	100	<2
Sulphates, SO_4^{2-} (mg/L)	<55	2,940	-	-	24,000	-	-	-
pH	-	-	-	-	13.3	8.9	13.2	8.9

Fenton is the one of the advanced treatment that used H_2O_2 as oxidation agent. However, incomplete oxidation often to happen from dissolved sulphide to thiosulphate ($S_2O_3^{2-}$) and reduces 70 to 80% of COD. Storage handling of H_2O_2 needs to be considered if safety becomes first priority. Besides, by mix ferrous iron and H_2O_2 under specific condition, created reactive hydroxyl or peroxide radical which become inhibitor to bacteria or living things (Veerabhadraiah *et al.*, 2011).

(b) Biological Treatment

The concept of biological treatment is actually to biodegrade pollutants into acceptable products. Normally biological treatment is to convert dissolved, suspended and colloidal organic into form that is more settleable. Besides removing nutrient, for instance, nitrogen and phosphorus, it is also to remove organic or inorganic matter from the wastewater so that it is sufficient enough to be discharged to the environment (Metcalf and Eddy, 2004). Biological treatment is one of the spent caustic treatments and is an inexpensive form of treatment. Rajganesh *et al.*, (1995) has found that biological treatment can oxidize sulphides to sulphate by using sulphur-oxidizing bacteria called *Thiobacillus denitrificans* (Rajganesh *et al.*, 1995).

Furthermore, the completeness of sulphides oxidation to sulphate is where it can dramatically reduce the pH of wastewater. Janssen *et al.* (2000) came out with the new bacteria which is adaptable with mercaptans called *Methylophaga sulfidovorans* (Janssen *et al.*, 2000). This treatment has the disadvantage that it needs a large aerobic reactor containing sulphide-oxidizing bacteria for conversion of sulphides to elemental sulphur and sulphate. Spent caustic has very high pH. Bosch (2008) has shown that bacteria from genus *Thioalkalivibrio* (halo-alkaliphilic bacteria) which can stand high pH (~10) can be used to treat this spent caustic. Normally these bacteria can be found in hypersaline soda lake (Bosch *et al.*, 2008). The reaction below shows the conversion of sulphide to elemental sulphur and sulphate. The reaction in Equation 2.6 reduces the pH whereby that of Equation 2.7 increases the pH. When oxygen is low, more sulphur will be formed and vice versa (Janssen, 2011). It is crucial to derive and maintain the condition for specific bacteria for long operation. Nevertheless, variation in pollution loading reduces the effectiveness of the treatment and requires a proper framework to achieve optimum condition of the systems.

Sulphide → Sulphate

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \qquad (2.6)$$

Sulphide → Sulphur

$$HS^- + 0.5O_2 \rightarrow S^o + OH^- \quad (2.7)$$

2.4.6 Effect of Spent Caustic to Human and Environment

Hydrogen sulphide in crude oil is an acutely toxic gas at low part per billion (ppb) concentrations and for longer exposure can cause death. It can easily be dissolved in caustic solution (sodium hydroxide) because of its solubility at high pH due 10 to 12 and also can be released as a gas easily when pH is lowered.

According to the information and characteristics that have been discussed before, they show spent caustic is a highly heterogeneous group material depending on crude oil source and crude oil process involved (API, 2009). Besides, because it exists in aqueous form at low pressure, the possibility to volatile and spread to the water and soil is minimal.

As we know, spent caustic is highly variable, corrosive, aqueous mixtures and exists in liquid phase depending on the environment condition. It is also known as high ecotoxicity to marine life according to pH and corrosively characteristic. For spent caustic typically have pH greater than 12 and sulphide concentration exceeding 2 to 3 wt% and other contaminants. Due to its noxious properties, spent caustic have amount of free sodium hydroxide, 5 to 18 % that clearly show this waste is base solution. It is also highly corrosive to human tissue due to their extreme in pH. Spent caustic also affect pipes corrosive because of sulphur element that oxidize and forming inorganic sulphur (H_2SO_4) (Bitton, 2005).

2.5 Membrane Bioreactor (MBR)

The research on MBR has declined due to the difficulties in obtaining the membrane and the high capital and maintenance costs of the system. In the 1990s, submerged MBR was commercialized and it was found to have low operational cost (Le-Clech *et al.*, 2006) when compared with other types of MBR. In MBR, biological processes play major roles than filtration processes (Widjaja *et al.*, 2010) where particulates in wastewater are converted into end products before filtration is carried out by the membrane.

MBR is also known as an alternative for conventional activated sludge (CAS) treatment where clarifier is removed and substituted with membrane to overcome settleability problem when undesired biomass is formed. MBR produces high performance in treating water besides having small footprints compared to conventional activated sludge where clarifiers are eliminated. It also delivers high quality effluent (Chang *et al.*, 2006), is good in removing organic and inorganic contaminants, capable of resisting high organic loading (Zhang, 2009) and generates less sludge (Le-Clech *et al.*, 2006). With all the advantages of MBR, some industries install the MBR to minimize the cost of water by reusing the treated water for other processes. For instance, the treated water can be used for industrial sanitary and landscape purposes. High-quality treated water from MBR is reused for heat integration and processing by ensuring the treated water have small amounts of contaminants to prevent the breakdown of sensitive equipment or pipes (Radjenovic *et al.*, 2008). Table 2.8 is the general advantages and disadvantages of MBR (Mutamim *et al.*, 2012a; Mutamim *et al.*, 2013b).

Table 2.8: Advantages and disadvantages of membrane bioreactor

Advantage	Disadvantage
• A variety of industries can be applied the MBR. • Normally the quality of the water effluent is uniform. • A little or almost no chemical consuming. • The area consuming for equipment setup is small (small foot print) • Less energy consuming (compared with thermal treatment process, e.g. wet air oxidation) • No issues on sludge setteability.	• High maintenance and operation cost due to high cost of membrane • Limitation of pH, temperature, pressure and also corrosive chemicals towards membrane and biomass • Biofouling of the membrane

2.5.1 Overview of MBR

In conventional activated sludge (CAS) treatment, large clarifying basins are needed to make sure the flocs are completely settled. High power for diffuser is used in aeration basin to make sure the nutrients are totally converted to the end products. The difference when using MBR is that there are no more settling processes needed and the area used for clarifier can be eliminated besides acting as a separator (Ng and Kim, 2007).

The basic view of MBR configuration is important before the MBR modification for enhancement is made. Figure 2.5 shows the basic schematic diagram of MBR configuration. Figure 2.5 (a) shows a side-stream or external membrane module while Figure 2.5 (b) shows an immersed membrane bioreactor

(iMBR) or submerged membrane bioreactor (sMBR) module (Judd, 2006; Ng and Kim, 2007). For sMBR system, the feed wastewater is directly in contact with biomass. Wastewater and biomass are both pumped through the recirculation loop consisting of membranes. The concentrated sludge is recycled back to the reactor while the water effluent is discharged. The idea of separating the membrane and bioreactor is to ease the membrane maintenance but it will increase the operational cost due to recirculation loop installation (Frederickson, 2005).

The iMBR system has less operational cost because there is no recirculation loop compared to the sMBR system and a biological process occurs around the membrane in iMBR. Both iMBR and sMBR need to pump out the excess sludge to maintain sludge age. The mode of membrane transportation could be pressure driven or vacuum driven. Radjenovic *et al.* (2008) stated that pressure-driven filtration is used in sMBR and vacuum-driven is used for iMBR, which operates in dead-end mode (Radjenovic *et al.*, 2008). The air bubbles are supplied to both systems for aeration besides scouring, especially for the immersed system to reduce membrane fouling in cross-flow effect across the membrane surface (Chang *et al.*, 2006; Sombatsompop, 2007). There are also aerobic and anaerobic MBRs where oxygen acts as an important medium for microbial growth in the aerobic process whilst anaerobic operation is done without oxygen. Anaerobic MBR is less efficient in removing COD and takes a long time for start-up. Usually, anaerobic treatment is used for treating high strength wastewater at low temperature that is suitable for microbial growth. Moreover, it is difficult to adjust low temperature for the waste feed and it causes high fouling compared to aerobic systems at low flux (Metcalf and Eddy, 2004).

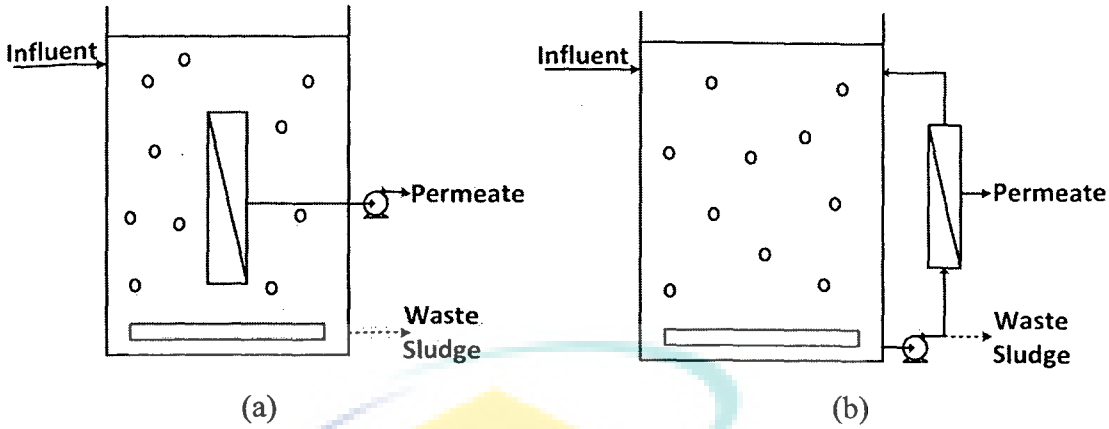


Figure 2.5 Basic schematic of MBR. (a) Immersed MBR, and (b) side-stream MBR (Judd, 2006).

2.5.2 Membrane Behaviour

The importance of studying membrane behaviour is to select good quality membrane in treating high strength industrial wastewater. High strength wastewater consists of diverse contaminants that could possibly corrode the membrane and lead to operational failure. The efficiency of the membrane also depends on the size of pores, types of materials, types of wastewater to be treated, solubility and retention time. Retention is observed due to the MLSS concentration change between the retentate (a part of solution that cannot cross over the membrane) and permeate (solution after filtration).

Permeability, flux, pressure (TMP) and resistance are the parameters that also need to be considered. Permeability is flux per pressure (J/dP) or LMH/kPa. Flux (LMH) is the flows of permeate per unit of membrane (component accessibility to the membrane) and it is related to hydraulic resistance, thickness of the membrane or cake layer and driven force. Driving force is the gradient of membrane potential area (unit area of the membrane) of mass transport and involves the pressure and concentration of particles. The mass transport mechanism for the membrane depends

on the structure and materials of the membrane. Membrane structure plays an important role in transport mechanism whether the structure is parallel or in series. Diffusion and solubility of the component are related to the kinetic ability of mass transport for membrane. For the membrane itself, pore-size membrane participates in kinetic mass transport (Koltuniewicz and Drioli, 2008).

The types of membranes used are different depending on the size of contaminants contacting during the treatment process. Basically contaminants with particle size of 100–1000 nm use microfiltration (MF) for removing suspended particles, ultrafiltration (UF) for particle size of 5–100 nm (bacteria and virus), and nanofiltration, (NF) for particles with size 1–5 nm for dissolved particles. In treating high strength industrial wastewater with shock loading of matter, microfiltration is chosen among the others in order to prolong membrane usage. MF used pressure as driving force to filter suspended particles and most of permeate which escape as permeate are dissolved solid and water. Most of the treatment plants use MF or UF instead of NF with regard to fouling and cost factors (Judd, 2006; Le-Clech *et al.*, 2006; Radjenovic *et al.*, 2008; Sombatsompop, 2007).

Two types of materials are used to construct membranes: polymeric and ceramic. Ceramic membrane is usually used for industrial wastewaters and has a good performance in filtration compared to polymer due to its high chemical resistance, is inert and easy to clean (Ciora and Liu, 2003; Hofs *et al.*, 2011; Jin *et al.*, 2010). Chemical stability does not only depend on the materials used but also on the size of the pore where it reduces the stability of the membrane when the structure is too fine. Ceramic also has higher hydrophilic ability due to the water contact angle. However, the main setback of ceramic membrane is its high cost of fabrication and it is easily breakable (Hofs *et al.*, 2011).

However, in recent membrane technology development, polymers have been used commercially in the form of PVDF, PES, PE and PP because of good physical and chemical resistance. Polymer membrane (porous membrane) has its own

weaknesses where it can foul easily because of its hydrophobic characteristic. The hydrophobic membrane is used because the pore size can easily be fabricated. PE is more quickly fouled compared to PVDF and PP (Le-Clech *et al.*, 2006). MF with PES material is more hydrophilic and mostly used in water filtration and high oxidant tolerance with pH 2-13 with very good cleanability. Hydrophobic membrane weakness can be improved by coating the membrane with hydrophilic polymer (Hanif, 2008).

Membrane configurations also play an important part since every configuration has its own advantages and disadvantages based on the cost, capability to withstand turbulence and back-flushing (normally suitable for HF membrane) (Judd, 2006). Large amounts of HF membranes make a bundle and the ends of the fibers are sealed in epoxy block connected with outside of the housing and can work under pressure and vacuum. Spiral-wound configuration is mostly used for NF and RO process with membranes is wound around the perforated tube through which permeates goes out. FS module configuration with separators and support membranes with the pieces of sheets membrane are clamped onto a plate. HF and FS modules are mostly submerged directly in mixed liquor with permeate drawn through the membranes using vacuum pump (Radjenovic *et al.*, 2008).

In membrane application, there are two types of membrane operations: dead-end and cross-flow operations (Figure 2.6 (a) and (b)). Both are pressure driven (TMP) with the dead ends fitted perpendicularly to the membrane surface. The solids from the feed that are greater than pore size are easier to deposit on the membrane surface. Most dead-end processes are batch processes (Mhurchu, 2008). In the cross-flow type, liquid flows parallel to a filter surface and suspended particles are transported across membrane surface by permeate flow due to pressure drop. Cross-flow filtration can reduce formation of cake layer on the surface of the membrane (Judd, 2006; Mhurchu, 2008).

Critical flux is an important parameter that needs to be considered during MBR operation. It is a value of flux that exists as irreversible deposit. Critical flux occur when flux start to decline and fouling start to observe. This can be achieved in clean water filtration (Field *et al.*, 1995). In other thought, critical flux represents the turning point between constant and non-constant permeability (Le-Clech *et al.*, 2003b). Critical flux for MBR happens when a thick biocake layer forms on the membrane and irreversible thus the biocake layer can be removed by chemical cleaning (Le-Clech *et al.*, 2003b).

From critical flux, the suitable flux for the operation can be defined based on the TMP sustainability. There is no standard method to find the critical flux due to difficulties during reporting data. One practical method that can be used, which is flux-step method, is shown in Figure 2.7. This method is relevant for short-term critical flux operation and not relevant for long-term operation. There are two concepts of flux, strong and weak. In the strong concept the flux obtained during sub-critical phase is equal to clean water flux but this concept is not relevant with MBR due to high sludge found in the reactor. In the weak concept, the flux is obtained during operational start-up and is maintained for a period of time but is not necessarily equal to clean water flux (Le-Clech *et al.*, 2003b). The highest flux can be determined when the flux is increased and there is no TMP increment or less permeates. It is shown when fouling is about to happen.

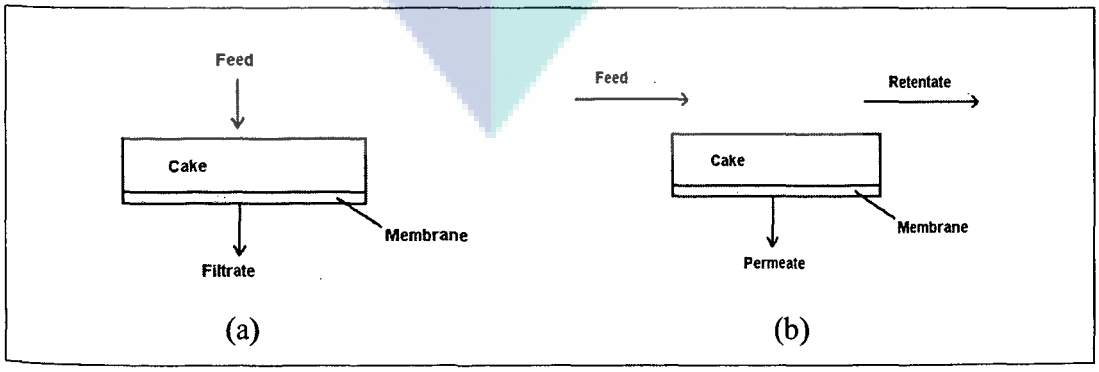


Figure 2.6 (a) Dead-end filtration (b) Cross-flow filtration (Mhurchu, 2008)

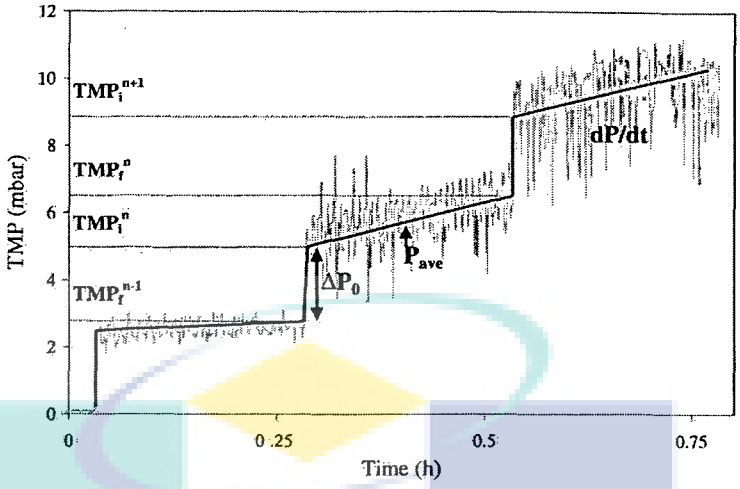


Figure 2.7 Flux-step method (Le-Clech *et al.*, 2003a)

The relationship between the fouling rate (dP/dt), permeability (J/dP) and flux has been observed. The critical flux is the intersection between permeability and fouling rate line (Ghengesh, 2011) with a typical one shown in Figure 2.8. Xu and Gao (2010) shows the natural flux mode method to identify critical flux at Figure 2.9 with the listing of critical flux identification method shown in Table 2.9 (Xu and Gao, 2010).

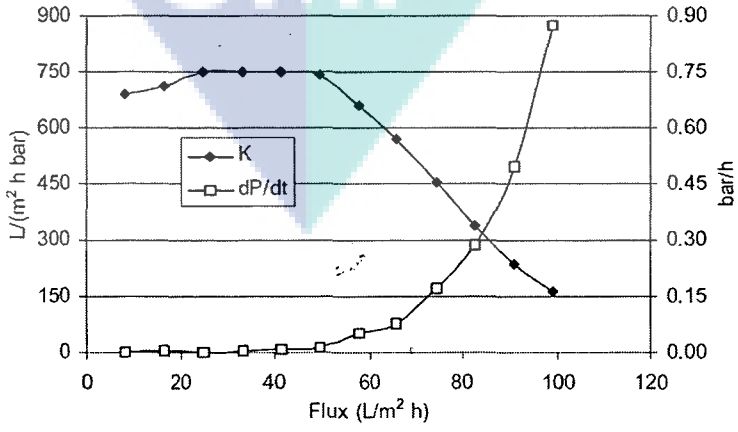


Figure 2.8 Relationship between permeability, fouling rate and flux to critical flux determination (Pollice *et al.*, 2005)

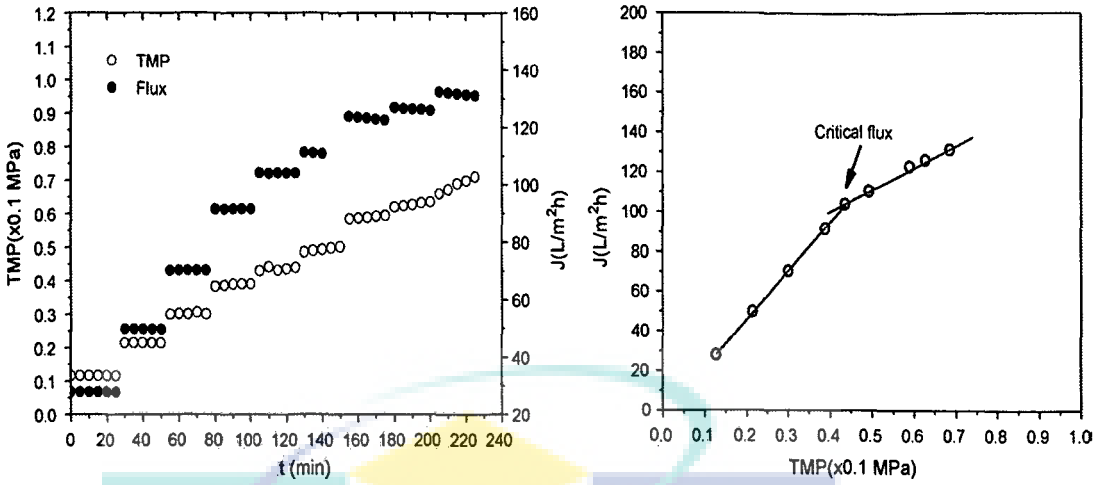


Figure 2.9 Natural flux mode method (Xu and Gao, 2010)

Table 2.9: Criteria for critical flux identification.

Reference	Criteria of critical flux
Le-Clech *et al.*, (2003b)	dP/dT = 0 (strong concept) dP/dt < 0.1 mbar min^{-1}
Bottino *et al.*, (2009) and Damayanti *et al.*, (2011)	dP/dt ≥ 0.5 mbar min^{-1}
Xu *et al.*, (2010)	Natural flux mode method (Flux versus TMP)
Le-Clech *et al.*, (2003) and Pollice *et al.*, (2005)	Mean permeability versus flux

2.5.3 Biomass Behaviour

Biomass in activated sludge from industries is heterogeneous with the basic nutrients such as glucose, nitrogen and phosphorus. The domination of biomass can

occur through the acclimation process and this depends on the major constituent of feed wastewater. CAS has been used for a long time but with respect to high strength wastewater, this method is noted to cope with the high content of organic loading and inorganic matter because of low biodegradability and inhibition and in some cases it can destroy the microbes because of shock loading of matters (Lin and Ho, 1996). This is because microbes take a long time to biodegrade the inorganic matters and need high concentration of biomass (MLSS) to ensure all the organics are totally biodegraded.

Basically, SRT is operated coupled with HRT for CAS. SRT is the solids or flocs growth in different sizes that need to be retained in the plant before it settles down for a period of time while HRT is the time taken for the organic matters to pass through the plant. This means that CAS relies on both to ensure the flocs are really settled before going for other treatments (Judd, 2006; Mutamim *et al.*, 2012a). Similarly, CAS needs biomass with fast growth rate and flocs formation species. If the biomass has low growth rate, it will lead to washing-out together with the excess sludge because of the shortage of SRT. Hence, production of sludge and F/M ratio is high and will end up with high excess sludge for disposal which will increase the total cost of wastewater treatment by about 50–60% (Radjenovic *et al.*, 2008).

To get the best performance in treating high strength wastewater, the MLSS must be high enough to increase the process of degradation. One of the treatment problems with MBR is that the increased MLSS hastens the membrane fouling due to high suspended solids (Bottino *et al.*, 2009; Melin *et al.*, 2006). During acclimation, long biomass adaptation is needed to degrade complex pollutants in high strength industrial wastewater and achieve high quality effluent (Viero and Anna, 2008a). On the other hand, in MBR system, SRT and HRT do not rely on each other because MBR is more on the membrane filtration rather than settling by gravity and this system does not consider the flocs growth but still maintains the minimum sludge production with low F/M ratio (less substrate is presented per unit of biomass) (Ng and Kim, 2007; Radjenovic *et al.*, 2008) and retaining the biomass in the reactor and sludge age. SRT control MLSS concentration and directly related

to the membrane biofouling that effect biomass viability in MBR (Benedek and Côté, 2003; Judd, 2006; Noor *et al.*, 2002; Widjaja *et al.*, 2010).

Besides that, the formation of flocs makes it easier to filter. However, if F/M is too low, the biomass in the activated sludge could not grow well (Widjaja *et al.*, 2010), or else if MBR has very high MLSS it will lead to clogging, low efficiency of aeration and will need a large bioreactor (increase the initial capital cost) (Radjenovic *et al.*, 2008). Low HRT will increase organic loading rate with reactor volume reduction and reduced performance of MBR whereas for a high HRT, MBR has a good performance (Fallah *et al.*, 2010; Jianga *et al.*, 2008; Viero and Anna, 2008a). Diverse pollutants and complex components leading to slow biodegradation have led to the use of MBR in treating industrial wastewater. HRT is closely related with quality of effluent water and does not influence nutrient removal in treating high readily biodegradable pollutants. However, in industry, wastewater contains plenty of slowly biodegradable pollutants and small variation on HRT affects the nutrients removal efficiency to achieve high quality effluent (Viero and Anna, 2008a). Dominguez *et al.* (2012) reported that biomass growth was closely related to OLR with the steady state OL rate for MF MBR and UF MBR being 0.15 kg COD $kgMLVSS^{-1}$ d^{-1}, where the biomass growth rate was 5–8 times faster at higher OL rate (Domínguez *et al.*, 2012).

Other studies showed the correlation between SRT (control MLSS concentration in reactor based on sludge discharge) and formation of SMP and EPS. Increased SRT will decrease SMP and EPS whereby the biomass will stay longer in the reactor and prolong the biological degradation process whereas for lower SRT, it will increase the level of SMP and fouling (Jianga *et al.*, 2008; Liang *et al.*, 2007; Masse *et al.*, 2006). Other studies showed reverse results (Masse *et al.*, 2006). High SRT also create starvation condition (low F/M ratio) that can reduce SMP formation, good for nitrification and less sludge production (Judd, 2006). Nevertheless, if SRT takes too long, it tends to foul the membrane with the accumulation of matters and high sludge viscosity (Jianga *et al.*, 2008). From previous studies, recorded low SMP and EPS production and low fouling potential occurred at SRT more than 20

days (Broeck et al., 2012; Chang *et al.,* 2006; Judd 2006; Liang *et al.,* 2007; Masse *et al.,* 2006).

In anaerobic membrane bioreactor, HRT and SRT are independent and also produce methane as a by-product and odour (Judd, 2006) whereas they do not use any aeration process and energy saving. In addition, methane can be collected for energy generation (Judd, 2008). There are several advantages of applying high SRT in MBR which include (i) slow biomass growth responsible for the biodegradable of organic and in organic pollutants; (ii) higher MLSS can be operated in MBR that induces starvation condition to achieve good quality of effluent; and (iii) high SRT create low F/M ratio that reduces SMP production and lead to lower membrane fouling (Broeck *et al.*, 2012; Judd, 2006).

2.5.4 MBR Limitation and Mitigation

Judd (2006) stated that cost is a major constraint in MBR technology in the 1990s because of high cost of membrane that led to increase in maintenance and operational costs. Membrane cost covers replacing of severe membrane fouling or corrupted membrane and membrane cleaning processes during maintenance (Judd, 2006; Le-Clech *et al.*, 2006).

(a) Membrane Fouling

Fouling is a major factor that needs consideration when it comes to membrane. When dealing with high strength wastewater containing high load of contaminants, it will lead to high clogging of the membrane due to the membrane characteristics, biomass and operating conditions. Factors that influence membrane

fouling during MBR operation covers membrane (membrane configuration, material, hydrophobicity, porosity, pore size), biomass (MLSS, EPS, SMP, floc structure and size, dissolved matter) and operating condition (MBR configuration, cross-flow velocity, aeration, HRT, SRT, TMP) (Huang *et al.*, 2001). Fouling can be monitored through TMP and flux changes. Originally, flux-step method is the correlation between TMP and flux at a time interval of 15 minutes (Judd, 2006) but the time interval can vary. When flux increases, TMP also increases and hence, more wastewater can be separated until the TMP levels off when the flux continues to increase. Decreasing phase shows that membranes have a high resistance and need cleaning before they become fouled which can lead to membrane damage.

Fouling is the physicochemical interaction between the biofluid and membrane to form a cake layer and the adsorption of the dissolved particles into membrane pores leading to flux decline. If a physical cleaning takes place, it is classified as reversible fouling. Irreversible fouling is due to the adsorption of the particles into the membrane and blocking the pore (Huang *et al.*, 2001). Figure 2.10 shows the mechanisms of fouling dependence on particle size to the pore diameter (Judd, 2006). The formation of cake that is inevitable on the membrane surface becomes one of the factors that lead to membrane fouling. In a general system, side streams of MBR have higher fouling tendency than submerged MBR. This is because the side stream of MBR needs high energy of pumping that produces high flux that will lead to repeating the fouling compared to submerged MBR (Judd, 2006).

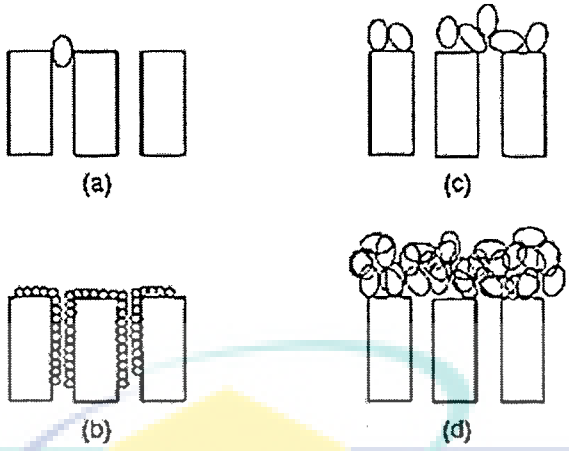


Figure 2.10 Fouling mechanisms (Judd, 2006) (a) complete blocking (b) standard blocking (c) intermediate blocking (d) cake filtration

Organic fouling in MBR is caused by deposition of small size of biopolymers such as proteins and polysaccharides on the surface of the membrane. These depositions are more difficult to remove than large particles like sludge flocs. Deposition of inorganic elements (Ca, Mg, Al, Si, etc.) detected on the surface of the membrane may lead to severe inorganic fouling. Wang *et al.* (2007) showed the severe inorganic fouling that happened at high alkalinity of activated sludge (Wang *et al.*, 2007). There is less research information on inorganic fouling especially in treating high strength industrial wastewater since they are more concerned with fouling caused by biomass and biopolymer.

Both EPS and SMP which are bound and in soluble form can lead to membrane fouling. EPS is located outside the cell surface and SMP is the organic compound that is released from substrate metabolism (substrate utilization-associated products, UAP) or biomass decay (biomass-associated products, BAP). Both consist of protein, polysaccharides, nucleic acids, lipid, humic acids, etc. The correlation between EPS and SMP with membrane is critically difficult. From Laspidou and Rittmann (2002), unified theory has found that EPS and SMP overlapped each other or else cells use electrons from the electron-donor substrate to build active biomass,

and produce bound EPS and UAP at the same time and in proportion to substrate utilization (Laspidou and Rittmann, 2002).

Bound EPS are hydrolysed to BAP, while active biomass undergoes endogenous decay to form residual dead cells. Finally, UAP and BAP, being biodegradable, are utilised by active biomass as recycled electron-donors substrates (Laspidou and Rittmann, 2002). In addition, some SMP can be adsorbed by biomass flocs to become bound EPS (Meng *et al.*, 2009). Wang *et al.* (2010) reported that two different sludge characteristics gave different fouling status. Excess growth filaments controlled by low DO concentration gave better filtration when compared with normal sludge (high DO concentration) due to large particle sludge distribution, lower hydrophobic contents in SMP and special fouling layer formed by filamentous bacteria (Wang *et al.*, 2010).

EPS is closely related with specific cake resistance, where an increase in EPS leads to increase in specific cake resistance (Ahmed *et al.*, 2007). Filamentous bacteria create bulking problem and at the same time lead to producing high EPS concentration rather than normal bacteria while SMP tend to accumulate in MBR or deposit into membrane pores (Meng *et al.*, 2009). Equation 2.8 shows the relationship between TMP and flux from the fundamental of Darcy's Law. This became a benchmark for measuring the resistance of membrane, driving force for each unit membrane area and fouling and the time for cleaning the membrane (Judd, 2006).

$$J = \mathrm{TMP}/(\eta . R_{tot}) \qquad (2.8)$$

Hai *et al.* (2005) in their study of textile industry stated that the mechanism of fouling occurs because of the formation of layer of cake fungi and sticky starch. FS membrane was vulnerable to internal pore blocking but HF happened due to the presence of cake layer in the latter case (Hai *et al.*, 2005). Badani *et al.* (2005) stated

that membrane fouling depends on the extent of sheer stress imposed on microbial flocs (Badani *et al.*, 2005). Manser *et al.* (2005) reported that flocs size from the MBR was ten times smaller than CAS. Since there was no selection for settleable flocs in MBR system, the biomass had no physical inducement to build large flocs that could lead to membrane fouling (Manser *et al.*, 2005).

Chang *et al.* (2008) reported the results from SEM to EDX spectra analysis towards the membrane before and after fouling, where cake layer deposited on the membrane surface was compared with the inner surface by biomass physiological properties (EPS and SMP). Inorganic elements (Mg, Ca, Cu, Rb, Pt and Al) were detected at the inner and outer surfaces of the membrane (Chang *et al.*, 2008). Amiri *et al.* (2010) stated that by increasing heavy metal concentration, permeability is reduced due to high formation of EPS (Amiri *et al.*, 2010). Vireo *et al.* (2008b) noted that SMP content might be considered an indicator of the fouling level since increase in SMP concentrations in MBR tend to reduce the permeability of the membrane (Viero *et al.*, 2008b). Dominguez *et al.* (2012) stated that high membrane fouling occurs during low sludge age or low MLSS concentration due to high solubility of EPS (Domínguez *et al.*, 2012).

(b) Fouling Mitigation

Operational parameters become a part of limitation in MBR. Therefore, the operational parameter needs to be wisely controlled to minimise its fouling effect. SRT is an important operating parameter that influences MBR performance especially in the control of fouling problem. A long SRT normally improves filtration performance and reduces EPS and SMP production by creating starvation conditions (Judd, 2006). A too long SRT leads to severe fouling due to high MLSS accumulation or old sludge (filamentous) production. Similarly if the SRT is too short, there will be reduced performance of MBR due to low biomass. High F/M ratio can also increase EPS concentration because of high food utilization by biomass (Meng and Yang, 2007).

Besides operational control, the membrane cleaning need to be done when the flux is slightly dropped (filterability reduction) and TMP increases drastically. There are three types of membrane cleaning – physical, chemical and combination of physical and chemical. Physical cleaning includes backwashing (suitable only for HF), and where the effluent is pumped in the reverse direction but it is not suitable for FS membrane. Membrane brushing is also a method of physical cleaning that could be applied in situ for FS membrane. It is a quick process but is less effective than chemical cleaning. Relaxation is the intermittent cessation of permeation for flux recovery if the membrane is submerged and scoured with air when permeation stopped.

The combination of relax/permeate and back flush/permeate can reduce chemical cleaning and prolong membrane life (Zsirai *et al.*, 2012). Basically, physical cleaning only removes the coarse solid or cake on the surface of the membrane, while chemical cleaning removes the flocs. It can also remove strong matters that stick on the membranes surface. It needs to put under consideration that the energy consumption for physical cleaning and almost 30% of permeate (effluent) is used for back washing. Blocher *et al.* (2002) stated that the purpose of chemical cleaning, besides fouling elimination, is also for membrane disinfection (Blocher *et al.*, 2002). For industrial purpose, in situ cleaning is usually performed if the fouling is not severe otherwise ex-situ cleaning takes place. Most of the studies showed that the first chemical used for membrane cleaning was sodium hypochlorite (NaOCl) (Blocher *et al.*, 2002; Hai *et al.*, 2005; Yejian *et al.*, 2008; Yigit *et al.*, 2009). Broeck *et al.* (2012) applied relax/filtrate cycle at influent to municipal wastewater to prolong the membrane life and reduce the cycle of chemical cleaning (Broeck *et al.*, 2012).

Hai *et al.* (2005) noted that when the flux per unit pressure dropped, the cleaning process was recovered by in situ membrane brushing because air bubbles from diffuser could not fully scrub the fungi off from the membrane. The worst thing was that the air bubble diffuser pushed the fungi towards the membrane. The ex situ cleaning and sludge withdrawal was carried out when the flux per unit

pressure was almost zero. Table 2.10 shows the value of TMP after the membrane was cleaned by water and chemical (Hai *et al.*, 2005). Katayon *et al.* (2004) reported that with diffuser at the bottom of the reactor, the membrane configuration with horizontally placement minimised the membrane fouling when compared with the vertical one (Katayon *et al.*, 2004).

Yigit *et al.* (2009) reported that during operation, backwashing routine was taken as 15 s per 10 min of permeate production. The first chemical back pulse when the TMP was 60 kPa was by sodium hypochlorite. However the ex situ cleaning (sodium hypochlorite with hydrochloric acid) was applied when irreversible fouling took place (Yigit *et al.*, 2009). Membrane configuration also plays an important part in reducing fouling. Katayon *et al.* (2004) reported that horizontal membrane configuration produced slow permeate with declining flux when compared with the vertical one (Katayon *et al.*, 2004). Generally, chemical cleaning is applied at every 7–14 days and the maximum allowable rate of pressure change is 0.6 bar/week (0.06 kPa/week) (Zsirai *et al.*, 2012).

Table 2.10: TMP value (kPa) after water and chemical cleaning (flux = 0.3) (Hai *et al.*, 2005)

	Hollow Fibre	Flat-sheet
Initial	6	7
Fouling	65	86
Cleaning:		
Water	10	86
Chemical (NaOCl)	6	7

Regular cleaning of the membrane shortens its life and membrane change is needed when the membrane can no longer be used. Therefore activated carbon (AC) is applied in MBR as a biofouling reducer (BFR) to prolong membrane life. AC has the ability to adsorb organic and other pollutants besides becoming a scrubber for membrane. Small sized and small pores of AC will have more surface area that

increases the adsorption velocity. Between the granular and powdered AC, the powdered version has a higher adsorption capacity (Yuniarto *et al.*, 2008) and it is able to remove low molecular weight organic rather than granules because it has a higher surface area.

PAC (nonpolar) is widely used for adsorption of organic compounds and is also able to remove pharmaceutical and toxic compounds without generating other toxic products (Utrilla *et al.*, 2013). PAC also has the tendency to be used as adsorbent and flocculent at the same time (Specchia and Gianetto, 1984). ZEO is capable of removing nutrients by physical adsorption (at low temperature) and chemical adsorption (at high temperature). In physical adsorption, it has the same mechanism as others porous materials while ion exchange are more selective (Wen *et al.*, 2006). Polar zeolite can normally be identified in nature while nonpolar zeolite (siliceous) is identified by dealumination syntheses. Zeolite consists of SiO_2 (63.4%), CaO (4.1%), FeO_3 (1.9%), MgO (1.1%) and others (29.5%). Eggshell (ES) contains calcium carbonate and protein that has proven to remove contaminants like heavy metal and phenolic and lignin compounds through adsorption processes and is economical due to its ready availability. Besides, the eggshell's porous nature has made it an attractive material to use as adsorbent. Moreover, ES has tendencies to flocculate in water (Amu *et al.*, 2005; Bhaumk *et al.*, 2012; Carvalho *et al.*, 2011; Ehrampoush *et al.*, 2011; Koumanova *et al.*, 2002; Zulfikar *et al.*, 2013).

PAC can also be a medium for bacterial sticking and growth. As a result, biological activity would increase by sustaining the PAC in a reactor. Widjaja *et al.* (2010) used a set-back flushing method for 10 min to increase the performance of MBR in treating shock loading of a toxic compound (Widjaja *et al.*, 2010). Biological degradation with added PAC gives better results compared with non-PAC because of their characteristic to adsorb organic matters. Besides that, PAC can treat COD in shock loading by increasing its quantity when it will stabilize the shock loading performance (Widjaja *et al.*, 2010).

Yuniarto *et al.* (2008) showed that by adding PAC, the performance of MBR increased up to 3.8% in removing high strength palm oil wastewater (Yuniarto *et al.*, 2008). Damayanti *et al.* (2011) showed the performance of three types of BFRs in removing SMP in palm oil mill effluent (POME) treatment. PAC gave good performance followed by Moringa oliefera (Mo) and zeolite (ZEO). PAC existed as cationic polymers with a surface area of 3000–4000 m^2g^{-1}, compared to ZEO and Mo whose surface areas ranged from 600 to 800 m^2g^{-1} and 713 to 744 m^2g^{-1}, respectively. PAC enhanced the flux three times lower than no-BFR and successfully formed flocs by charging the neutralization mechanism from organic and inorganic components and enlarging the floc to build up porosity in the cake layer (Damayanti *et al.*, 2011). Adsorbents and coagulants also have the ability to reduce the SMP where the SMP tends to be entrapped in biofloc (Meng and Yang, 2007).

Lee *et al.* (2007) reported that the membrane fouling reducer, MFR, flocculated the activated sludge to reduce the cake layer on the surface of the membrane. The result showed that in order to achieve 30 kPa of TPM, a small amount of MFR was needed effectively in the removal of high contaminants in wastewater. MFR from cationic polymer acted as a positive charge and when it was added into the reactor, it adsorbed the negative charges from the microbial flocs and changed into a neutral charge. The neutralized sludge floc then attracted each other to form large flocs by a charged neutralization mechanism which is also called flocculation process. On the other hand, in high concentration of MFR over the optimum concentration, the surface turned to positive charge and deflocculation began by a mechanism of electrostatic repulsion (Lee *et al.*, 2007). More study on flocculation, coagulation and adsorption with respect to fouling mitigation is required especially in the industrial sector to reduce the cost of membrane maintenance.

2.5.5 MBR Application in Industrial Wastewater Treatment

During MBR operation, there are different operating conditions depending on the level of constituents of high strength wastewater. The operating conditions cover the sludge behaviours (e.g. MLSS, DO, SRT and HRT) and membrane behaviours (e.g. membrane configuration and pore size). Table 2.11 shows the operational parameters according to the types of industries. Textile industries have low biodegradability compared to food industries due to the slow biodegradable organic or toxic matters present (Durai and Rajasimman, 2011). Food industries are known as high strength organic wastewater and the level of biodegradability is high due to the high content of readily biodegradable or organic matters (Katsou *et al.*, 2011).

Table 2.11: Operational parameters according the types of industries

	Textile (Yigit *et al.*, 2009)	Palm Oil (Yuniarto *et al.*, 2008)	Phenolic (Viero *et al.*, 2008b)	Pharmaceutical (Chang *et al.*, 2008)	Heavy Metal (Katsou *et al.*, 2011)
Application	Pilot	Laboratory	Laboratory	Pilot	Laboratory
Reactor Volume (L)	230	20	4.4	10000	210
Reactor Type	Aerobic; iMBR	Aerobic; iMBR	Aerobic; iMBR	Aerobic; iMBR	Aerobic; iMBR
Membrane	HF	FS, 1 module	HF	HF, 4220 strands	HF, PVDF
Membrane Surface Area (m^2)	-	0.1	0.00278	12.1 m^2/g	0.93
Pore Size (μm)	0.04	0.4	0.15	0.1	0.04
Flux ($Lm^{-2}h^{-1}$)	20	10	15-17	0.384-1.536	22.3
MLSS (gL^{-1})	13.9	5±	10	6-17	5.84-10.33
DO (mgL^{-1})	-	8±	-	-	-
HRT (day)	0.58	0.8±	10±	1	10.3
SRT (day)	25	-	-	>40	15
COD Removal (%)	97	94±	67	96	508-535
Colour Removal (%)	98	-	-	-	-
TSS Removal (%)	99	-	-	-	226-267
TN Removal (%)	78	-	-	-	44-53
Phenol Removal (%)	-	-	98	-	-

2.6 Microbial Process and Description

Energy is needed by microbe in the degradation process (catabolic pathway) and it comes from three sources: sunlight, organic compounds and inorganic

compounds. Aerobic microbes (heterotrophs) degrade organic compounds to carbon dioxide and water by consuming the oxygen available. Aerobic process occurs when organic compounds as electron donor transfer the electron-to-electron accepter (oxygen respiration).

The bacterial cells consist of inclusion for storage food, volutin granules and sulphur granules. Food is stored in the form of glycogen and starch. Sulphur-oxidizing bacteria or chemolithotrophic bacteria includes filamentous bacteria (*Beggiatoa* and *Thiothrix)* and non-filamentous bacteria (*Thobacillus, Thiospirillopsis* and *Thiovulum*) oxidize sulphides to produce energy. From oxidation of sulphide, element sulphur (S^o) is produced and stored in cytoplasm in the form of granular (Gerardi, 2006). The problem with these bacteria is low settleability but good in BOD removal and floc formation. At lower F/M ratio, the growth of sulphur-oxidizing bacteria increases. The sludge which contains filaments has light weight and colour (light brown) and do not settle.

Tang *et al.* 2009 stated that in aerobic condition, for the first step, sulphide is oxidized in producing sulphite by transferring six electrons to the electron acceptor (oxygen). There are two pathways of oxidation of sulphite to sulphate. First, sulphites oxidizes enzymes and directly transfer electrons to cytochrome to form adenosine triphosphate (ATP) as a coenzyme that is used to transport energy. The second pathway is reversal of adenosine phosphosulphate reductase activities which produces a large energy phosphate bond where adenosine monophosphate (AMP) converts to adenosine diphosphate (ADP). Here, thiosulphate acts as electron donor and splits into the element sulphur and sulphite and finally, both elements are oxidized to sulphate (Tang *et al.*, 2009). At the end, living cells use sulphates as nutrient (food).

Element sulphur especially sulphate is an important element in microorganisms to build up the component of amino acids and enzymes. Sulphide can be known as organic or inorganic compound. High contents of element sulphur

in wastewater are very poisonous to living cells. However there are several types of microbes available to mineralize the organic sulphur compounds either in aerobic or anaerobic treatment. Bitton (2005) stated that in anaerobic treatment, most of organic compounds (sulphate) are degraded to inorganic sulphur compounds (sulphides or mercaptans) which are very odorous and toxic (Bitton, 2005).

As a conclusion of sulphur cycle, oxidation process is by chemolithothrophic bacteria which have the capability to generate energy, play important role to convert sulphide to sulphate. Sulphide (S^{2-}) is degraded by aerobic bacteria before oxidized to element sulphur (S^0) and sulphate (SO_4^{2-}) as can be seen in Figure 2.7 and the reaction can be seen in Equation 2.9 and 2.10 below. Sulphide acts as electron donor to the bacteria and converts to sulphur and sulphate whereby oxygen acts as electron acceptor. In other situation, when the equation is not balanced, the accumulation of intermediates for instance sulphur, iron sulphide and hydrogen sulphide is produced (Tang *et al.*, 2009). Plant or other aerobic bacteria use sulphate as their sulphur nutrient and it releases very little sulphide because aerobic bacteria do not store sulphide in their cells.

$$S^{2-} + 1/2O_2 + 2H_2 \rightarrow S^0 + H_2O + \text{energy} \quad (2.9)$$

$$S^0 + 3/2O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ + \text{energy} \quad (2.10)$$

2.6.1 Bacteria Identification

It is important to identify important bacteria in certain treatment or process. Bacterial identification is concerned with the technique of getting accurate and definitive bacteria. It is important to understand bacterial nomenclature for easy

understanding. The nomenclature is divided into two: genus, a species where it represents the strains with high degree of overall similarities that differ from other strains and genus represents the groups of similar species. In addition the bacteria can be classified through the cell wall structure, cell membrane structure and DNA base composition (Tang and Stratton, 2006).

Few methods and techniques have been used to identify the bacteria and it includes microbiological, biochemical and molecular methods. Two types of identification approach are phenotypic (biochemical testing numerical analysis and cellular fatty acid) and genotypic (DNA-DNA hybridization, analysis of G+C content and 16S rRNA gene sequencing). Phenotypic is the physical appearance and biochemical characteristics while genotypic is a set of gene in bacteria. Reliable and rapid identification methods are needed for correct diagnosis and to reduce time consumed. The methods basically rely on several perspectives including technical, time, cost and regulatory (Spiegelman *et al.*, 2005). Depending on only one method reduce the accuracy and leads to misidentification (Janda and Abbott, 2002). Therefore, the crucial part is to know the capability of each method to discriminate among strains and their sensitivity in order to know the amount of species or strains in certain processes (Benito, 2005).

However every method has its own limitations and no test provides 100% accuracy result. Bacteria properties can be unstable at times and depend on the environmental changes such as temperature, pH level and substrate growth. The problem with commercial databases is unlisted identification technique applied during data collection. The problem of 16SrRNA gene in Gen Bank or other databases depend extensively on the bacteria strain and cause of misidentification. To reduce this limitation, identification can be made in both phenotypic and genotypic approach but it is time-consuming and expensive (Janda and Abbott, 2002). Table 2.12 describe the bacteria identification method.

Table 2.12: Bacteria identification method description

Method	Description
Traditional (Benito, 2005; Janda and Abbott, 2002)	- Scope work include cell counting, selective growth and microscopic examination - Identify general characteristics of bacteria community and some cases narrow down the part of community. - Study on morphology and physiological differences among species. - Need cultivation and pure cultures (time consuming) - Some database are limited, low accuracy identification - Gram staining, growth characteristics, antibiogram, biochemical technique
Molecular (Benito, 2005; Janda and Abbott, 2002; Keer and Birch, 2003)	- Rapid, specificity and sensitivity analysis in identify genus and species. - Purposely to identify an isolated bacteria up to species level - In ecological study, direct viable count is used to determine viability of environmental bacteria. - Routine identification - Sequencing DNA to 16rRNA - Species level technique: PCR-RFLP, Fluorescence in situ Hybridisation (FISH), Real Time PCR (RT-PCR) - Strain level technique: Random Amplified Polymorphic DNA-PCR (RAPD-PCR), Amplified Fragment Length Polymorphism (AFLP), Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR)

In case of CAS and MBR, Munz *et al.* (2008) states small flocs sludge is created in MBR rather than CAS and it reduces the diffusivity of substrate transport into the flocs. Besides, low semi saturation constant for oxygen have been reported. FISH analysis has been used to identify bacteria in MBR and CAS for tannery wastewater treatment and shows the change in characteristics of microbial community (Munz *et al.*, 2008). A study by Goa *et al.* (2014) showed the different microbial community structure in bio-cake form in aerobic sludge during fouling

process. The form of microbial chain community might be the cause of variation of metabolites and it may be become the primary cause of membrane fouling (Gao *et al.*, 2014).

2.7 Activated Sludge Model

Activated sludge model (ASM) basically describe the biological performance of activated sludge of wastewater treatment processes. The calibration of models is to estimate the model parameters to fit in certain set of data obtained from wastewater treatment processes. There are three types of models according to scope of analysis (Jiang *et al.*, 2005). ASM1 is the biological treatment model specifically to model organic COD and ammonium removal. From this model, oxygen demand and sludge production can be predicted. ASM2 is the advanced form of ASM1 where modelling of nitrogen and phosphorus removal is included. ASM2d is modelling of denitrification and phosphorus under anoxic condition. ASM3 is also known as respiration model where all biomass loss and energy requirement are captured together with biological nitrogen removal (Damayanti *et al.*, 2010; Ng and Kim, 2007). Damayanti *et al.* (2010) used ASM1 to identify oxygen transfer coefficient (K_{La}), COD fraction and heterotrophic yield (Y_H) in modelling POME treatment (Damayanti *et al.*, 2010).

2.7.1 Wastewater Characteristics and Coefficients for ASM

The steady state ASM calibration are the most sensitive parameters and liable to long term prediction and full-scale wastewater treatment plant (Kose, 2006). Dynamic calibration usually applied to short term prediction and detailed data is

needed more than in steady state calibration and it gives more reliable estimation (Ke-Jun *et al.*, 2004; Kose, 2006). A combination of DO concentration of dynamic calibration with response of sensitivity analysis was used to calibrate steady state model (Ke-Jun *et al.*, 2004). The OUR profile can be generated by using respirometer (DO probe) and all analysis was done according Standard Method (Jiang *et al.*, 2005).

Figure 2.11 shows the illustration of ASM1 model for heterotrophy on the carbon-based process. Basically for carbon-based, three main processes are involved in heterotrophy ASM1 which are growth of biomass, decay of biomass and hydrolysis (Salmiati, 2008). In industry, steady state calibration is applied and weather becomes an important data that need to be considered. Hence, it will have effect during high COD loading to the sludge wastage (Petersen *et al.*, 2002).

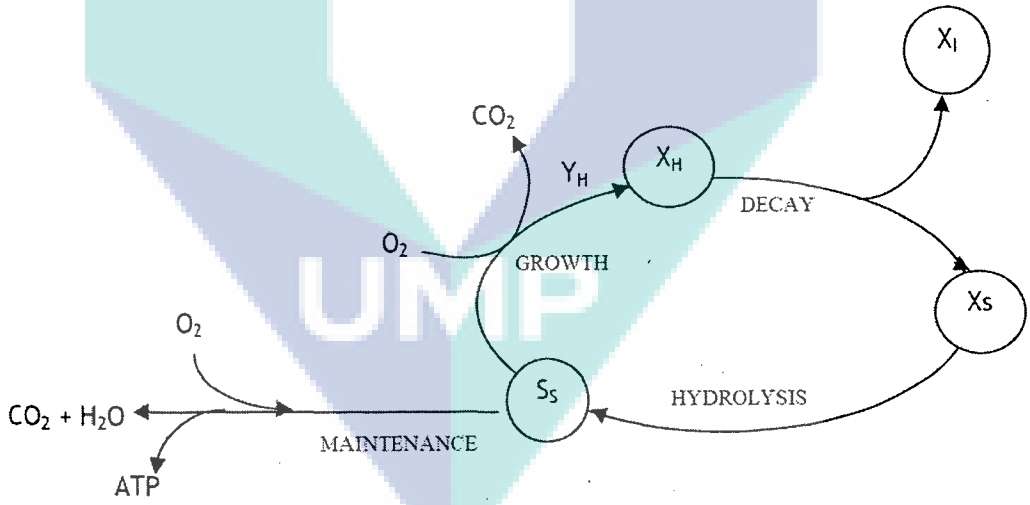


Figure 2.11 Heterotrophy COD flow of ASM1 (Canudas, 2005)

(a) Oxygen Transfer Coefficient (K_{La})

Oxygen is required in aerobic heterotrophic growth process. In ASM1, K_La is the mass transfer coefficient that is used to measure the gas to aqueous absorption

in biological aerobic treatment. It becomes an indicator for measuring specification aerator operated in the system in transferring oxygen. For gas-aqueous absorption, it is important to know oxygen saturation (C_S) for distilled water at standard condition (1 atm) (Ramalho, 1977). Equation 2.11 shows the mass balance approach for oxygen mass transfer.

$$\text{Accumulation} = \text{in} - \text{out} + \text{increase due to adsorption} \quad (2.11)$$

At equilibrium point in and out represent the net transport (Ferrer, 2007). In dynamic processes, Equation 2.12 can be simplified as (Damayanti *et al.*, 2010);

$$dC/dt = K_{La} (C_s - C_t) \quad (2.12)$$

(b) Stoichiometric and Kinetic Coefficients

In activated sludge, heterotrophic and autotrophic bacteria consume organic and inorganic compounds in wastewater and produce energy and microbial products. Heterotrophic or carbonaceous bacteria are dominant in the system and mainly utilise organic carbon compounds rather than inorganic ones (Davies, 2005). Autotrophic biomass uses the energy from synthesis of inorganic compounds to synthesise organic compounds and become a minor process in the system (Davies, 2005; Petersen *et al.*, 2003). Both need the presence of oxygen as electron acceptor to produce energy.

Heterotrophic yield (Y_H) indicates the COD fraction converted to cell mass and involves S_s and X_s since energy and electron acceptor are proportional to COD utilization. Hence, oxygen is consumed to convert to substrate equivalents (Petersen, B. *et al.*, 2003). Equations 2.13 and 2.14 show the calculations for the determination

of Y_H (Damayanti *et al.*, 2010; Henze *et al.*, 2000; Salmiati *et al.*, 2010). Heterotrophic growth rate (μ_H) and decay rate (b_H) may produce an identical net growth rate but will increase the oxygen demand and speed up the substrate cycling (Jeppsson, 1996). Heterotrophic decay rate can be principally characterized as the actual biomass similar to the maximum specific heterotrophic growth rate (μ_{maxH}) (Petersen *et al.*, 2003).

$$\text{Cell COD} = \text{TCOD} - \text{SCOD} \quad (2.13)$$

$$Y_H = \Delta\text{cell COD}/\Delta\text{soluble COD} \quad (2.14)$$

(c) COD fractionation

COD fraction of wastewater can be characterized by either physico-chemical or biological method. In ASM1, COD fraction is categorized into readily biodegradable (S_s), slowly biodegradable (X_s) and inert suspended organic matter (X_i and S_i) as shown in Figure 2.12. Table 2.13 shows the COD fraction of wastewater and Table 2.14 shows reported COD fractions in different wastewaters case study. Equation 2.15 shows the balance of influent wastewater (Henze *et al.*, 2000).

$$\text{Total COD} = S_i + S_s + X_s + X_i \quad (2.15)$$

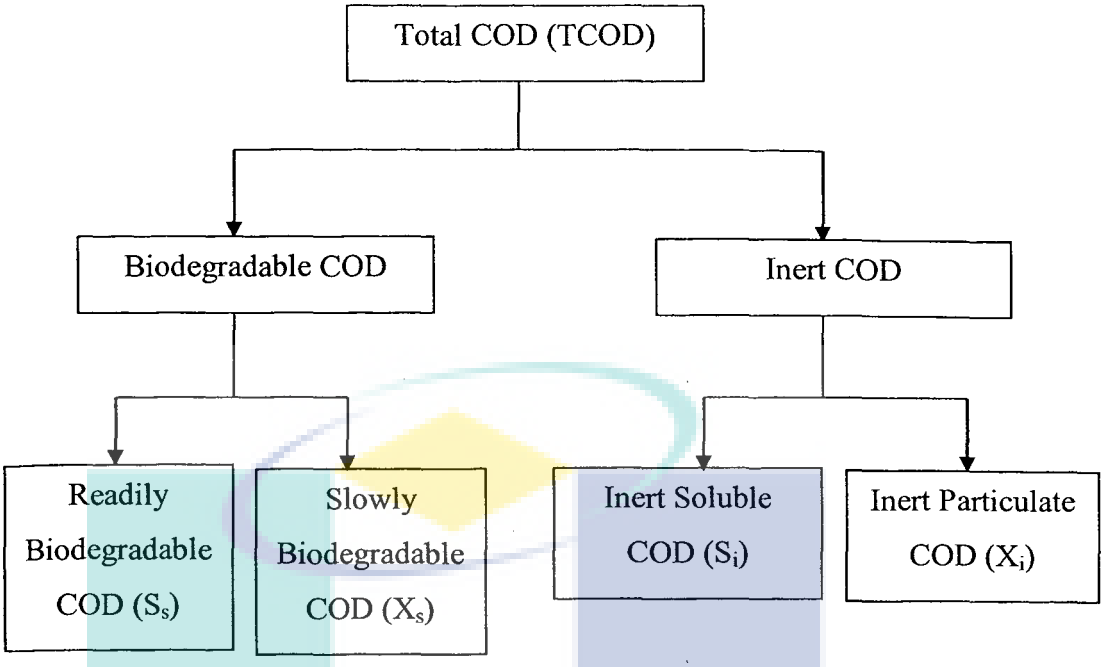


Figure 2.12 COD fractions of influent wastewater

Table 2.13: COD fractionation of wastewaters.

Parameter (mg L^{-1})	**Description**
Readily biodegradable COD (S_s)	COD fraction of soluble COD (SCOD) excludes the soluble inert organic matter (S_I). This fraction is easily remove during treatment (Davies, 2005) and directly related to microbial growth (Orhon *et al.*, 1996).
Slowly biodegradable COD (X_s)	Complex compound or colloidal that need to be hydrolysed into simple compound before it can be utilized by bacteria and can be part of readily biodegradable COD (Çıg˘gın *et al.*, 2011; Henze *et al.*, 2000).
Inert soluble COD (S_i)	The COD fraction with same concentration that enters and leaves the system (Henze *et al.*, 2000).
Inert particulate COD (X_i)	Inert suspended in influent wastewater or produced by decay and traps in the system or remove from the system via sludge wastage (Henze *et al.*, 2000; Petersen *et al.*, 2003).

Table 2.14: COD fractionation for different wastewater case study

Parameter	Municipal (Beck *et al.*, 2005)		Municipal (Petersen *et al.*, 2002)	Domestic (Fall *et al.*, 2012)	Industry (Keskitalo and Leiviskä, 2010)
	Grape Harvest Period	Normal period (dry weather)	40% households 60% industries	Receives 160 industries discharge	Pulp and paper mill
TCOD	2500	-	5607	2503	1167
BOD_5/COD	0.44	-	-	0.24	0.22
S_S	85%	31%	16%	0.8	30.9%
X_S	9.4%	50%	22%	36.8%	35.2%
S_I	1.2%	15%	12%	39.4%	26.1%
X_I	5%	4%	50%	23%	7.9%

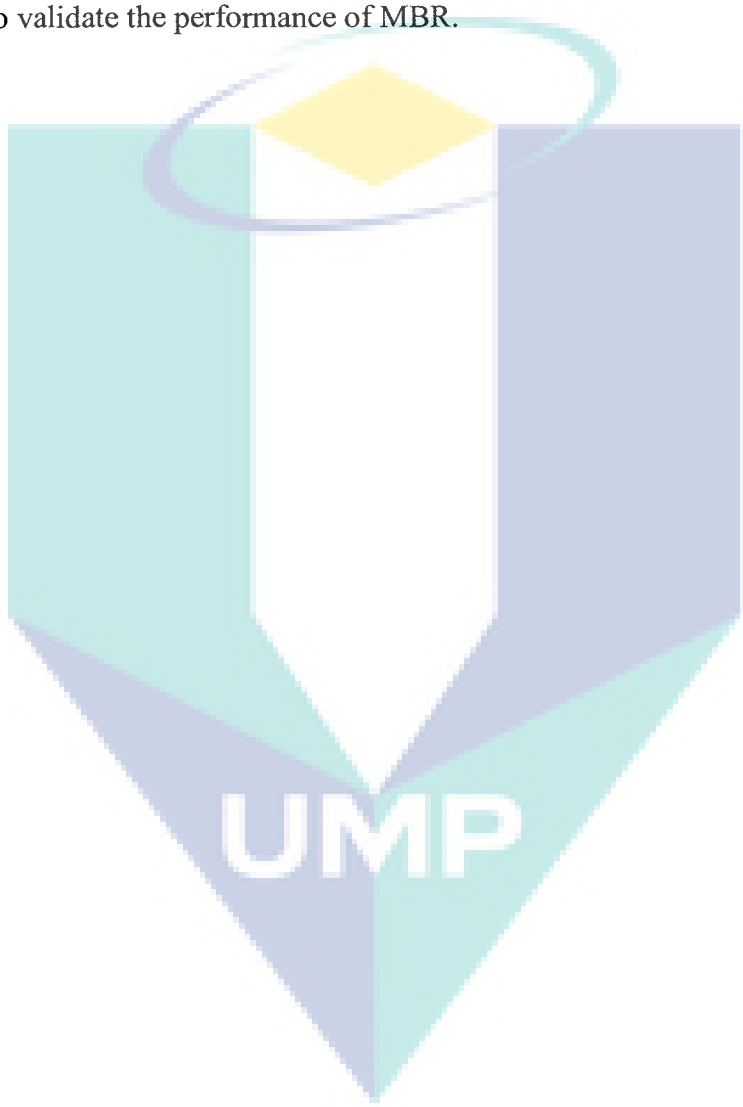
2.8 Conclusion

This chapter has summarised the origin and characteristics of spent caustic wastewater besides the existing treatment. Spent caustic is known as high strength industrial wastewater that is toxic and hazardous to the plant and environment. Generally, raw spent caustic treated through oxidation process like wet air oxidation process drops to a level which is acceptable for biological treatment.

MBR is one of wastewater treatment technologies that is able to overcome the problem of conventional biological treatment. However, the major limitation is membrane biofouling and mitigation can prolong the use of the membrane. Operational parameters that influence membrane biofouling and performance of

MBR include MLSS concentration, SRT, HRT, critical flux and organic loading which are different for every type of wastewater and MBR.

Besides the bacteria identification, traditional and molecular techniques can be used to know the cause of biofouling from bacteria perspective. In addition ASM is used to validate the performance of MBR.



CHAPTER 3

METHODOLOGY

3.1 Introduction

The study approach for synthetic spent caustic (SSC) treatment is by using aerobic submerged membrane bioreactor. Since this system operates using membrane, fouling affect is the main concern besides the nutrients removal performance. Optimum sludge retention time (SRT) and mixed liquor suspended solid (MLSS) concentration were identified as important parameters for operating ASMBR by analysing fouling effect. Hence, various types of BFR were applied to overcome the fouling problem.

The experiment was continued by looking at the performance of ASMBR at various COD loading using BFR with less fouling effect. Since this experiment used a mix of biomass collected from the conventional activated sludge plant, bacteria identification was the important stage in analysing ASMBR performance. Finally, the stoichiometric and kinetic coefficients and COD characteristics for this system were obtained using Activated Sludge Model No. 1 (ASM1) to study the behaviour of every process.

3.2 Outline of Study

This study consists of several stages as shown in Figure 3.1. Initially, SSC wastewater was characterized according to oxidation by natural, aeration and filtration processes purposely to observe effect of removal at every step of the processes without biomass presence and to study the stability of wastewater for several days. This characterization looked at the COD removal trend.

The study continued to obtain optimum parameters namely MLSS and SRT. At this stage, organic and nutrient removal and membrane fouling trends were analysed. In organic and nutrient removal, COD, sulphide and sulphate changes were recorded. Membrane fouling analysis consists of critical flux (MLSS concentration), resistance-in-series (RIS), TMP performance, microbial productions.

The major problem of using ASMBR is membrane fouling and how to prolong operational lifespan. BFR is one of the methods to enhance the ASMBR performance and at the same time reduce membrane fouling. At this stage, PAC, ES and ZEO were used as BFRs and the concentration of BFRs at the beginning was identified base on adsorption performance. All BFRs were analysed on adsorption performance (COD, protein and carbohydrate removal) since all BFRs are known as adsorbents. As the optimum concentration of BFRs identified, research continued by adding BFRs into ASMBR. Again, organic and nutrient removal and membrane fouling were analysed. The analysis of optimum condition (a good result in removal and TMP performance) in ASMBR was also performed at various COD loadings. Same analyses were done for various COD loadings in ASMBR-BFR.

The morphology of membrane fouling was investigated. Mixed culture morphologies in ASMBR-BFRs were analysed to give an overview of activities occurring in the reactor. The research identified the dominant bacteria strain in ASMBR. In all ASMBR runs, the same mixed population was grown since the same

wastewater was used and same condition had been applied. At this stage, biochemical and molecular methods were applied to ensure the accuracy of the analysis.

Overall, ASMBR system was characterized using ASM1 for COD fractions and variable coefficients. This modelling was purposely to design parameters and to observe the causes and limitations that may influence the ASMBR system operation. It reduces the limitation (time and cost) faced by researchers and engineers to explore chances of finding solution to upgrade the system. Hence, organic compounds removal (COD removal) was the main concern in this modelling since SSC wastewater consists of high organic matters as the main components in the wastewater (organic sulphide, phenol and sodium bicarbonate) and does not counter the influence of nutrient removal. COD fraction purposely to characterise wastewater either biodegradable or non-biodegradable. Moreover, DO of the system was identified. DO concentration, COD fraction and data from operation system were fit in ASM1 and calibrated. This model completed by observing COD effluent trend from the validation process for operational prediction.

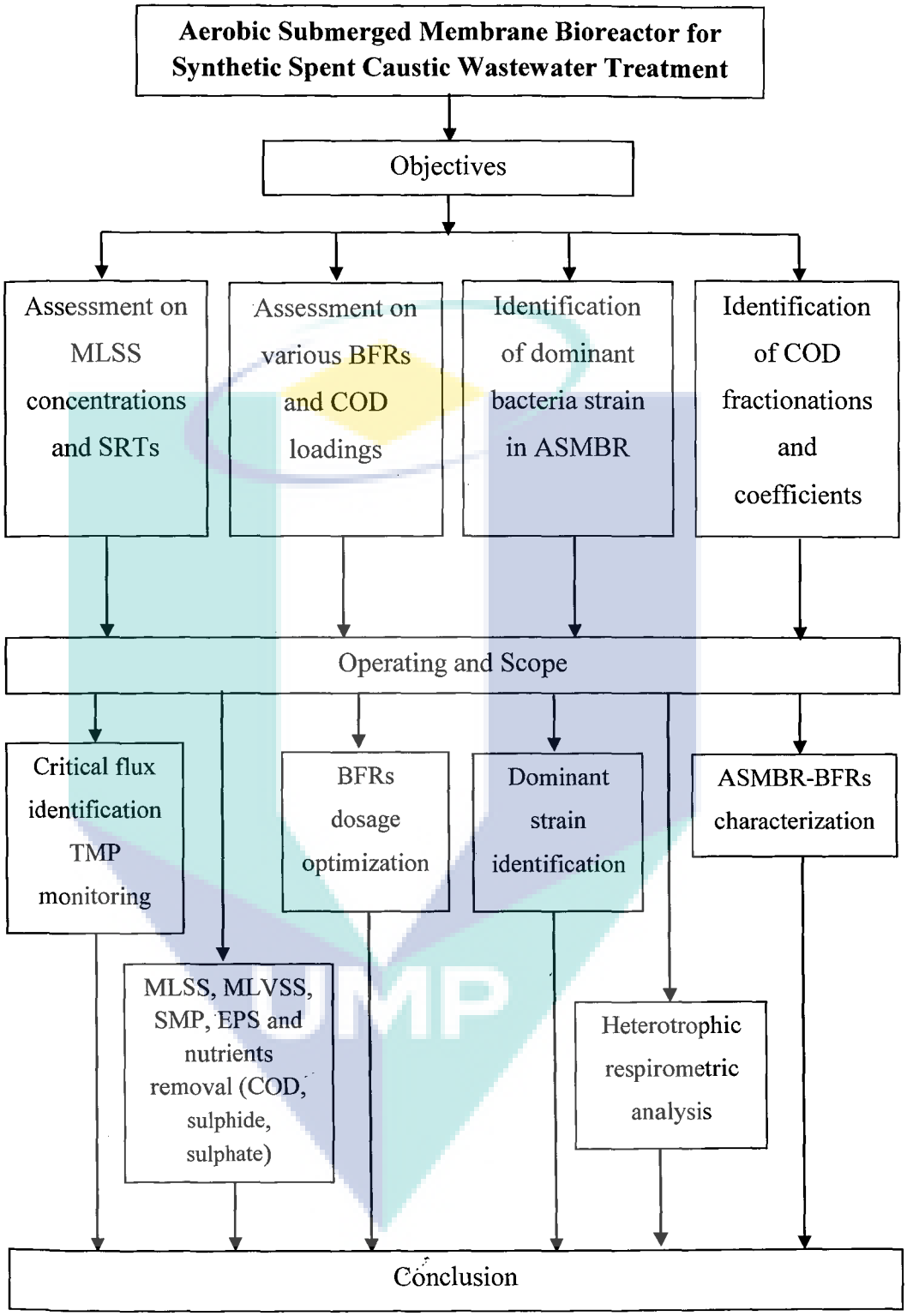


Figure 3.1 The outline of the study

3.2 Laboratory Scale ASMBR Set-up

The laboratory scale ASMBR is shown in Figures 3.2 and 3.3. The membranes were customised by Advanced Membrane Technology Research Centre (AMTEC), Universiti Teknologi Malaysia according the specification given in Table 3.1. MF hollow fibre membrane was chosen because it gave a low water hold-up besides low cost compact design and back-flushable (Malak, 1999). Submerged membrane bioreactor was chosen due to (i) less operational cost (no recirculation loop); and (ii) reduced membrane fouling (scouring by bubble aeration). The experiments were carried out at room temperature to 25±1°C. MF hollow fibre membrane was vertically submerged into 4L bioreactor and flowed outside-in mode. Air flowrate supply was controlled at 5 L min^{-1} with DO between 2 to 8 mgO_2 L^{-1} at the bottom of membrane unit for oxygen supply to biomass and to create a turbulence that helps in reducing membrane fouling.

The feed entering the bioreactor was controlled by water leveller, thus the feed and effluent had the same flow rate. The effluent was drawn from the membrane by peristaltic pump and pH of mixed liquor was maintained at 7. Equation 3.1 shows the influent flow rate when applying filter/relax (5minutes/1minute) cycle to prolong the membrane life (Broeck *et al.*, 2012). During the experiment, the fouling membranes were removed by backflush (backflush flux double with the filtration flux) at duration of 60 seconds for reversible fouling otherwise chemical cleaning took place by using 0.5% ppm NaOCl (fouling membranes were soaked into the chemical for 24 hours) for irreversible fouling.

$$Q_{per} = \frac{J.A.t_{fil}}{t_{fil} + t_{rel}} \qquad (3.1)$$

where Q_{per} is the permeate flowrate (L h^{-1}), J is the membrane flux, A is the membrane area (m^2), t_{fil} is the filtration time (minute) and t_{rel} is the relaxation time (minute). Every experiment was conducted with new membranes.

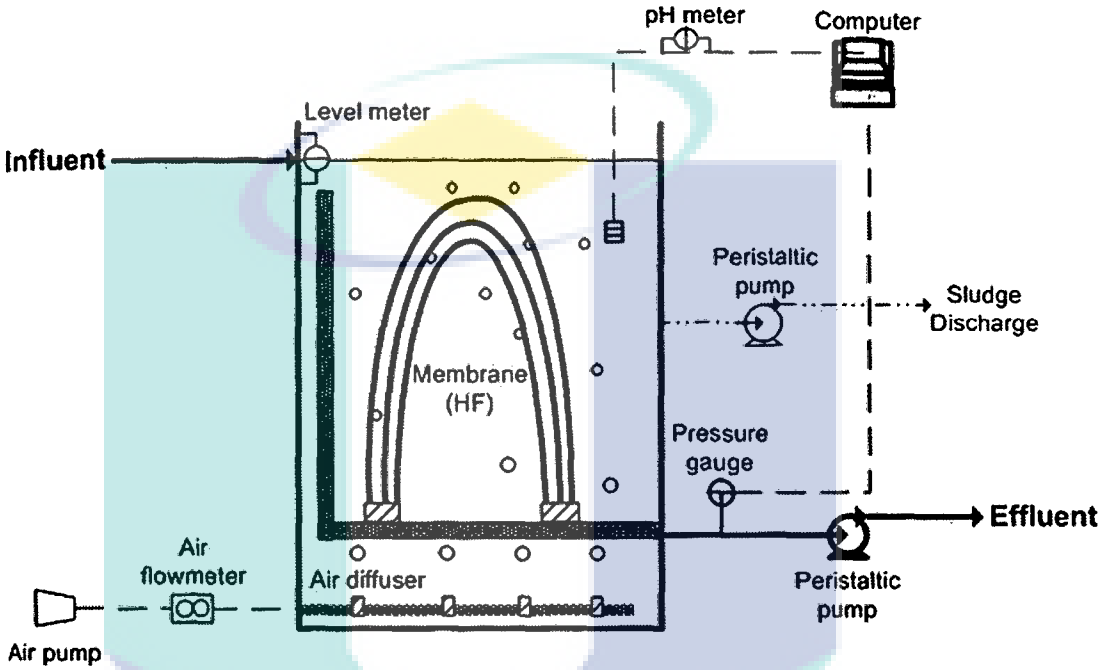


Figure 3.2 Schematic flow of ASMBR

Figure 3.3 4L ASMBR with U-shaped hollow fibre membrane

Table 3.1: Membrane specification for ASMBR

Characteristic	Description
Membrane material	Polyethersulphone
Membrane configuration	U-shaped hollow fibre
Membrane coating	Polyethylene glycol
Hydrophobicity	Hydrophilic
Maximum filtration	70±5kPa
Pore size, Ø	0.02 – 0.2 µm (MF)
Length	0.44±0.002 m/strand
Outer surface area	0.00165±0.0001 m^2/strand
Total outer surface area per module, A	0.075±0.02m^2/module
Outer diameter	0.9±0.02mm
Inner diameter	0.55±0.01mm
Number of fibres per module	50
pH tolerance	2-13
Membrane manufacturer	Universiti Teknologi Malaysia (UTM)

3.2.1 Synthetic Spent Caustic (SSC)

A study by Sipma *et al.* (2004) showed that the results for synthetic spent caustic (SSC) wastewater and actual spent caustic are very promising. Synthetic wastewater was used in this study to avoid the fluctuation of any chemical, for instance, heavy metal or toxic matter (Sipma *et al.*, 2004). However care should be taken scale-up when treating actual wastewater since in reality, spent caustic may contain a wide range of toxic compounds. Table 3.2 shows the ingredient for SCC wastewater solution. Thus, synthetic wastewater was chosen due to it being known and its stable composition. It was designed to mimic actual SC wastewater for biological treatment (Sipma *et al.*, 2004) and the main components were increased to challenge the capability of ASMBR.

Sodium sulphide (Na_2S) acted as energy source, sodium hydrogen carbonate ($NaHCO_3$) as carbon source and phenol as common non-sulphur component and as aromatic compound in synthetic spent caustic. Major nutrients and trace elements essential for bacteria growth were introduced to act as minor compounds in actual wastewater (Graaff *et al.*, 2011; Kleinjan *et al.*, 2005; Klok *et al.*, 2012; Kumfer *et al.*, 2010; Lohwacharin and Annachhatre, 2010; Sipma *et al.*, 2004; Spencer, 2007). The SSC was neutralized at a pH of 7 and in this study, sulphuric acid (H_2SO_4) was used due to its being less corrosive than hydrochloric acid (HCl) (Berne and Cordonnier, 1995).

Table 3.2: Synthetic spent caustic wastewater (Kleinjan, *et al.*, 2005; Lohwacharin and Annachhatre, 2010; Sipma *et al.*, 2004)

Components	**OL1**	**OL2**	**OL3**
Main Component ($g\ L^{-1}$)			
Na_2S	0.8	1.6	2.4
$NaHCO_3$	0.5	1.0	1.5
Phenol	0.7	1.4	2.1
Major nutrient	10 mL L^{-1}	10 mL L^{-1}	10 mL L^{-1}
Major Nutrient ($g\ L^{-1}$)			
NH_4Cl		0.4	
KH_2PO_4		0.2	
$MgSO_4.6H_2O$		0.1	
Trace Element		10 ml L^{-1}	
Trace Element ($g\ L^{-1}$)			
$CaCl_2$		5.54	
Na_2EDTA		50	
$ZnSO_4.7H_2O$		2.2	
$MnCl_2.4H_2O$		5.06	
$FeSO_4.7H_2O$		4.99	
$(NH_4)_6Mo_7O_{24}.4H_2O$		1.1	
$CoCl_2.6H_2O$		1.61	
$CuSO_4.5H_2O$		15.1	

3.2.2 Sludge Biomass Acclimation Process with SSC

Sludge biomass was collected from TITAN Chemicals, Pasir Gudang, Johor, Malaysia which is the nearest petrochemical plant to UTM. The sludge was seeded with SSC wastewater in a semi-batch column before it was transferred into ASMBR. The food to biomass ratio (F/M) was maintained at 0.5 – 1.2 kg COD. kg $MLSS^{-1}.d^{-1}$ according to biological treatment specification (Metcalf and Eddy, 2004; Spellman, 2003). The MLSS productions was observed until COD removal achieved 80%.

3.2.3 ASMBR Critical Flux

In the preliminary study, critical flux became the turning point between constant and non-constant permeability or reversible and irreversible fouling and it was identified by applying flux-step method (Le-Clech *et al.*, 2003b). This method was applied by logging the relative TMP when flux is increased step-by-step. In flux step method, one step constant was applied for 15 minutes while the corresponding TMP was recorded for every 1 minute. The highest flux can be determined when the flux is increased and there is no TMP increment or less permeates. Equations 3.2 to 3.5 are used to define the fouling performance for each flux-step (Le-Clech *et al.*, 2003a).

Initial TMP increase: $\Delta P_0 = TMP_i^n - TMP_f^{n-1}$ (3.2)

Rate of TMP increase: $\frac{dP}{dt} = \frac{TMP_f^n - TMP_i^n}{t_f^n - t_i^n}$ (3.3)

Average TMP: $\frac{TMP_f^n + TMP_i^n}{2}$ (3.4)

Permeability of the system: $K = \frac{J}{P_{ave}}$ (3.5)

3.2.4 MLSS Concentration and SRT

MLSS concentration is the parameter that nearly relates to the ASMBR operation affecting membrane fouling and nutrients removal. Designed criteria of MLSS concentration based on pre-experiment and started from 5, 7 and 9 g L^{-1} since the minimum of MLSS concentration with 80% COD removal are at 5 g L^{-1} during acclimatisation stage and SMBR limited to an MLSS concentration of 10 g L^{-1} (Judd 2006). The concentration was increased gradually until flux reading does not give significant result (less production or permeate). All MLSS concentrations run without sludge discharge or almost SRT infinity (sludge taken only for sampling). According to Damayanti *et al.* (2011), MLSS 5 g L^{-1} able to remove more than 80% of organic and TN.

Meanwhile, SRT's behaviour closely relates to suspended accumulation in MBR affecting membrane fouling. Since HRT and SRT are independent, the HRT measures the retention time for compounds or nutrients to pass through the reactor and related to flux of this system. The SRT measures wasted solid and according to Judd (2006), high strength wastewater needs longer SRT and three times more than SRT conventional activated sludge plant (~8 – 15 days). In this study, SRT was designed based on the strength of wastewater and previous studies shows lower SMP and EPS produced and lower fouling potential at SRT more than 20 days (Broeck et al., 2012; Chang *et al.*, 2006; Judd 2006; Liang *et al.*, 2007;). In this study, Equations 3.6 and 3.7 show the HRT and SRT uncorrelated in MBR process. V is volume of reactor (L), Q_w is sludge wastage rate (L day^{-1}) and Q_e is effluent flowrate (L day^{-1}) and Q_{in} is influent flowrate (L day^{-1}) of MBR and Figure 3.4 illustrate the black-box of MBR. It is reported that by changing the SRT, more sludge is produced

and there is increase in MLSS concentration (Judd, 2006). Hence, SRT experiment was designed at 20, 40 and 80 days to observe the performance in low, medium and high SRT.

$$HRT = \frac{V}{Q_e} \tag{3.6}$$

$$SRT = \frac{V}{Q_w} \tag{3.7}$$

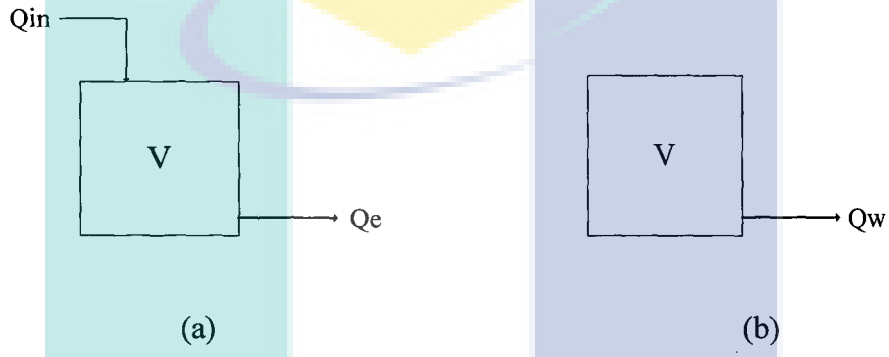


Figure 3.4 MBR black-box for; (a) HRT and (b) SRT.

3.2.5 Resistance-In-Series (RIS)

Equation 3.8 shows the relationship between TMP and flux from the fundamental of Darcy's law. This became a benchmark for measuring the resistance of membrane, fouling, driving force for each unit of membrane area and the time for cleaning the membrane (Judd, 2006; Mutamim *et al.*, 2012a; Mutamim *et al.*, 2013b). Equation 3.9 is a RIS model with simple model to describe membrane fouling mechanisms (Jifeng *et al.*, 2008; Mutamim, *et al.*, 2012a; Mutamim, *et al.*, 2013b). R_{tot} and R_m were obtained by using the fundamentals of Darcy's law as shown in Equation 3.8 which correlate with the TMP to the permeate flux (J) (Chang and Kim, 2005). R_m was measured by filtered deionized water through new membrane. R_{tot} was measured for TMP at the end of the experiment. By using

resistance series model as shown in Equation (3.9), the sludge resistance R_s could be calculated.

$$R = \Delta P \mu^{-1} J^{1} \quad (3.8)$$

$$R_{tot} = R_m + R_s \quad (3.9)$$

where μ is the viscosity of permeate (Pa.s), ΔP is a differential pressure across the membrane (Pa), J is flux (L m^{-2} s^{-1}) and R is resistance (m^{-1}). R_{tot} is the total filtration resistance, R_m is the clean membrane resistance by deionized water permeability, R_s is the sludge filtration that normally includes R_c as cake resistance (external) and R_f as fouling resistance (internal).

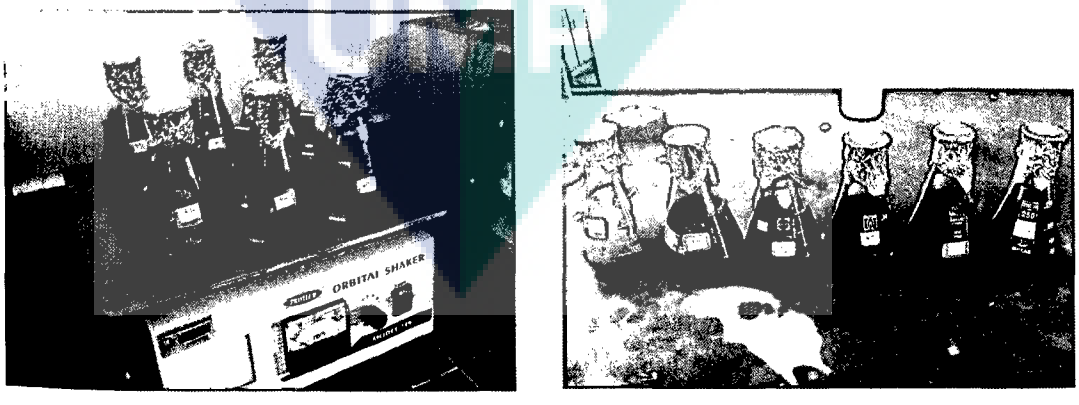
3.3 BFR Batch Test

In this study, three types of BFR were used – powder activated carbon (PAC), powdered eggshell (ES) and zeolite (ZEO) – due to their ability in adsorption as reviewed in Chapter 2 (Fouling Mitigation) and the BFR specification can be found in Table 3.3. All BFRs were chosen based on the adsorption capability of contaminants. PAC and ZEO have known as good adsorption and also in formed biofloc while ES able to remove contaminant (heavy metal and phenol) but it is new in biofloc formation. BFRs have unique surface characters and are able to adsorb organic and inorganic compounds by physisorption (pore size or surface area by van der Waals forces), chemisorption (polar or nonpolar surface charge or chemical bonding) or both characteristics of absorbent (El-Naas *et al.*, 2010; Wen *et al.*, 2006). Eggshell were collected from a local hatchery waste and washed with distilled water overnight before being dried at 105°C for 24 hours, was grounded and sieved without further chemical or physical process (Hassan and Hassan, 2013).

BFR was chosen based on the behaviour and characterisation as mention above. The adsorption test was carried out in batch test by using continuous shaker in various ranges of design, 2 to 12 g L^{-1}. Each beaker contained 0.25 L of SSC wastewater, dried BFR and shaken for eight hours as detention time (Damayanti *et al.*, 2011) at a speed of 200 rpm and 60 minutes sedimentation. Protein and carbohydrate are factors of biofouling, hence the adsorption test for protein and carbohydrate was done in batches using albumin (for protein) and glucose (for carbohydrate) at a constant concentration 2 g L^{-1}. These tests were proven and represented of the ability of BFR on adsorption, adhesion and flocculation that may influence membrane biofouling in reactor. The adsorption was observed for COD, protein and carbohydrate removal which create the similar behaviour in reactor. The adsorption isotherms on all BFR concentrations were studied (Figure 3.5).

Table 3.3: BFRs specification

No	Type of BFR	Size	Type	Source
1	PAC commercial (PAC)	4mm mesh	Powder	PAC Master
2	Eggshell (ES)	4mm mesh	Powder	Local hatchery waste
3	Zeolite commercial (ZEO)	4mm mesh	Powder	Zeo Master



(a) (b)

Figure 3.5 BFRs adsorption batch test; (a) adsorption process; (b) settlement process.

3.3.1 BET Analysis

The surface areas of BFRs were identified by nitrogen gas (physical adsorption) using Micromeritics ASAP 2010 (Figure 3.6). BFRs were dried at 105°C for 24 hours. Then 0.02±1g of dried BFRs was inserted into the sample holder. The samples were degassed at 130°C for 1 hour using vacuum pump before being analysed using nitrogen gas that includes the area of micropores and mesopores (Bansal and Goyal, 2005; Eisazadeh *et al.*, 2013).

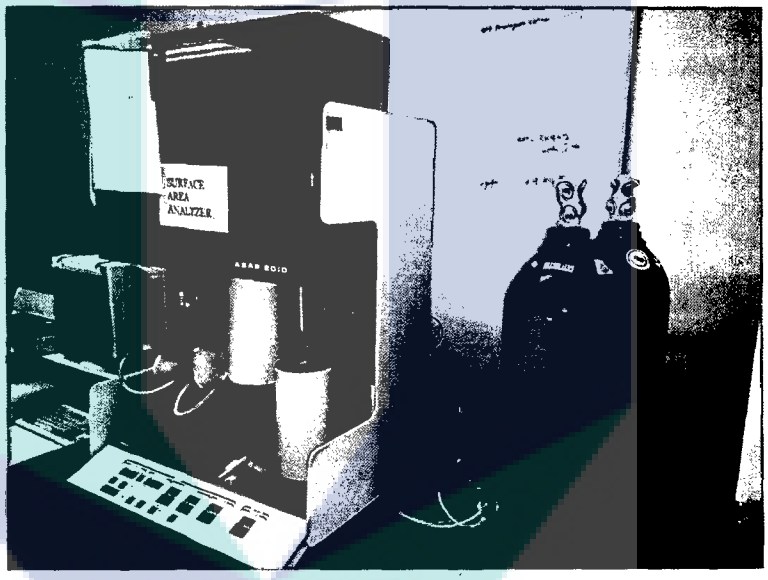


Figure 3.6 Micromeritics ASAP 2010 as surface area analyzer

3.4 Application of BFRs in ASMBR Operation

During acclimatisation process, 6L of SSC was fed until the COD removal achieved 80% and desired MLSS was obtained. After two weeks acclimation process in semi-batch column, 4L sludge biomass was transferred into ASMBR and it acclimatized again in ASMBR according to operating condition in Table 3.4. This stage of acclimation in ASMBR was carried out until range of COD removals

achieved (80%) and desired MLSS was obtained (5 g L^{-1}). This process took two weeks to reach steady state condition and it run 24 hours continuously.

BFR was prepared before it can be applied in ASMBR. BFRs were washed with deionized water and dried in the oven for 24 hours at 105°C to remove contaminants and moisture. Consequently, optimum concentrations of BFRs were applied into ASMBR with new membrane under the same condition.

Table 3.4: Operational condition of lab-scale ASMBR

Operational Parameter	Value
MLSS (mg L^{-1})	5000±23
MLVSS (mg L^{-1})	4700±19
Flux (LMH)	4
pH	7.2-7.8
DO (mg L^{-1})	2-7
HRT (hours)	16

3.5 Analytical Methods

Analysis was carried out at Pollution Control Laboratory, Faculty of Chemical Engineering (FChE), UTM by using Standard Method (APHA, 2005). The regular parameters analysed include biological oxygen demand (BOD), chemical oxygen demand (COD), total sulphide (S^{2-}), total sulphate (SO_4^{2-}), MLSS, MLVSS, dissolved oxygen (DO) concentration, pH, soluble microbial product (SMP) and extrapolymeric substance (EPS). Average total phosphate (TP), total nitrogen (TN) and phenol were also analysed. COD, S^{2-}, SO_4^{2-}, TP, TN and phenol were analysed using UV-visible spectrophotometer (HACH/DR5000) (Figure 3.8) according to Standard Methods (APHA, 2005). The DO concentration, temperature, and trans-

membrane pressure (TMP) were monitored and logged via online automatic control system.

SMP and EPS were released into the bulk solution and were analysed using heating extraction method (Figure 3.7). 30 mL sludge biomass solution was initially centrifuged for 10 minutes to extract the solution as supernatant and the sludge biomass precipitate. Later the supernatant was filtered using 0.22μm syringe filter to keep SMP solution free of suspended precipitate while sludge precipitate was mixed with deionized water. After that the mixer was heated in water bath at a temperature of 80°C for 10 minutes to release intracellular products. The mixture was then extracted and filtered again to get the EPS free of sludge precipitate (Antonelli *et al.*, 2011).

Protein contained in SMP and EPS extract was determined using Bradford's method. 0.1mL sample and 0.1 mL deionized water (blank sample) in 1.5mL cuvettes were added with 1mL Bradford solution each. Then, vials were vigorously shaken before being analysed by UV-visible spectrophotometer. The carbohydrate were analysed by phenol-sulphuric acid method. The 2mL sample and 2mL deionized water (blank sample) were placed in vials and added with 0.05mL of phenol (98% concentration) and 5mL H_2SO_4 (97% concentration) in each vials. Then, vials were shaken before analysis by UV-visible spectrophotometer (Nielsen, 2010; Walker, 2002). Figure 3.9 shows the protein and carbohydrate analysis. Several methods have been proposed to analyse protein that are Lowry, Bradford and Biuret. In this study, Bradford's assay was chosen and the only interference is by detergent whereas Lowry's assay and Biuret are interfered by phenol in wastewater (Upstone, 2000).

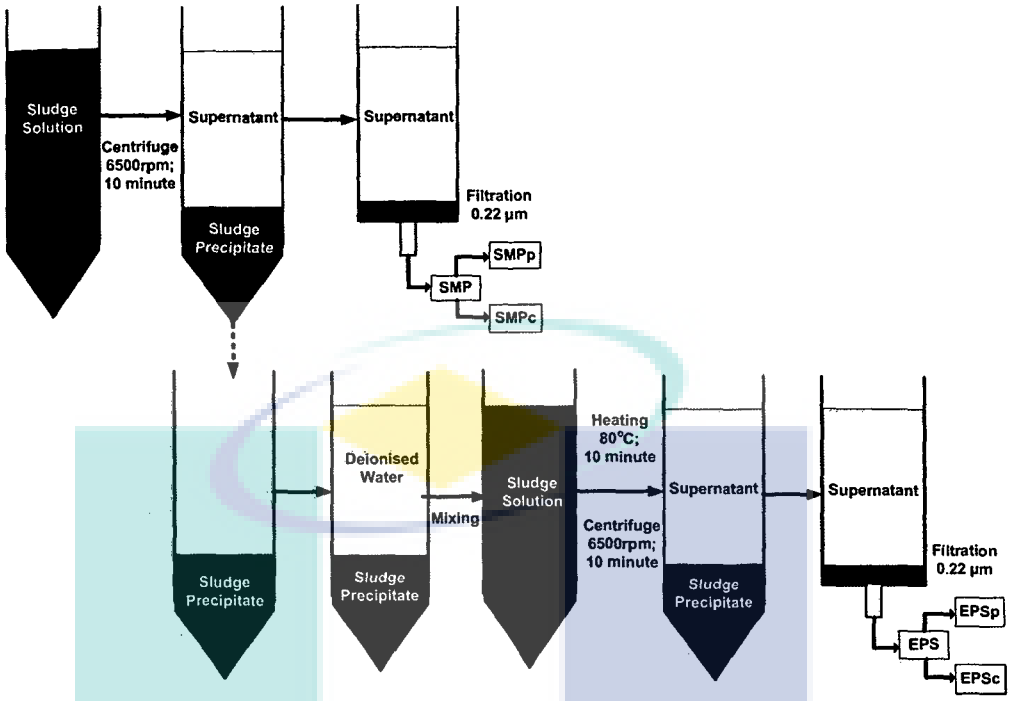


Figure 3.7 Heating extraction method for SMP and EPS measurement

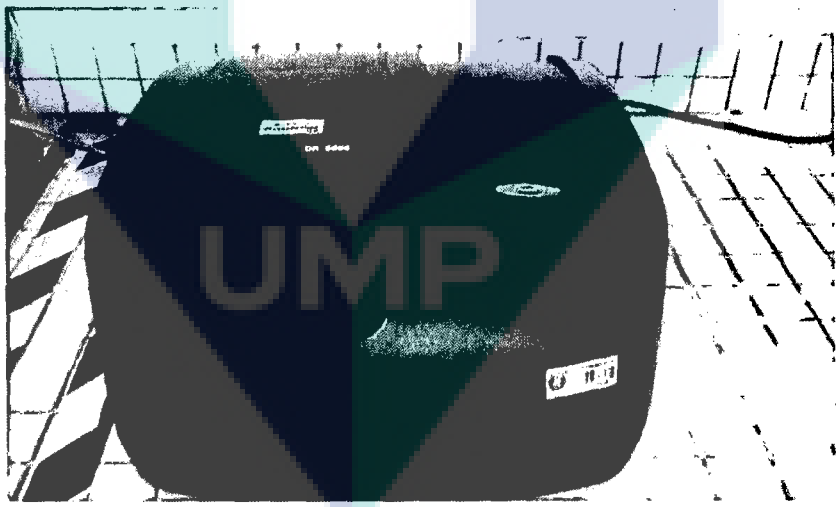


Figure 3.8 HACH/DR5000 UV-visible spectrophotometer

(a) (b)

Figure 3.9 (a) Protein analysis by Bradford's assay; and (b) Carbohydrate analysis by phenol-sulphuric acid method

3.6 Microscopy Analysis

Meiji EMT2-PBH –1x/3x Dual-Power Stereo Microscope attached with Pax Cam Leica and PAXIT Image analyser (USA) was used to capture the sludge biomass progress. This equipment was used to analyse the biomass morphology in ASMBR either with or without BFRs. Samples were taken fresh from the reactor and placed on the microscope glass slide and covered with another glass. Then the slides containing samples were placed on the microscope's stage and clipped by slide's clamp. Samples were analysed by using PAXIT analyser. Figure 3.10 shows the biomass that had been analysed using PAXIT.

JEOL JSM-6701F field emission scanning electron microscope (FESEM) was used to image and analyse the chemical element before and after fouling. For FESEM analysis, membranes and BFRs samples were dried for 24 hours at room temperature. Random hollow fibre membrane samples were selected and cut using liquid nitrogen to avoid membrane structure destruction. The membrane samples were placed vertically to observe cross-sectional membrane on a FESEM holder.

Then membrane and BFRs samples were sputtered with AU/Pt and analysed with FESEM.

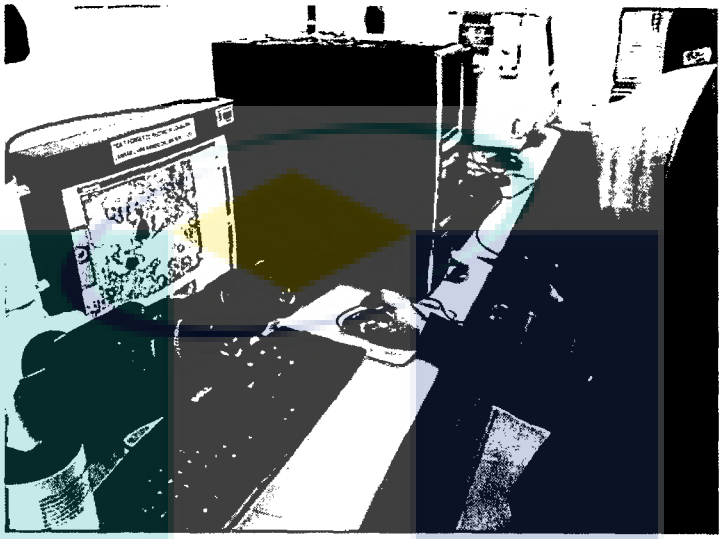


Figure 3.10 Microscopy analysis by Meiji EMT 2-PBH – 1x/3x Dual-Power Stereo Microscope attached with Pax Cam Leica and PAXIT

3.7 Bacterial Identification of ASMBR Sludge Biomass

Two approaches of bacterial identification in ASMBR were employed. Biochemical method is a traditional method that is limited to biochemical characterization and commonly used as a touchstone for bacterial identification. On the other hand, molecular method was applied for clear genetic characterization. The combination of both methods gives accurateness of the analysis (Janda and Abbott, 2002).

3.7.1 Pour Plate Method

Sludge in ASMBR was analysed for aerobic bacteria due to its ability to oxidize elements in neutral and alkaline condition (Bitton, 2005). Standard plate count was prepared according Standard Method (APHA, 2005). A volume of 1 mL of sludge sample from ASMBR or dilutions was taken and transferred onto a sterile, empty petri dish. 15mL of agar medium was poured into petri dish containing sample and mixed thoroughly by rotating the plate several times. The media was left until it solidified before the plate was inverted and incubated at 33°C for 48 hours. These pour plate method purposely to reduce the numerous colonies in mixed culture until clear colonies were obtained.

3.7.2 Isolation of Bacteria

From the dilution plate, five different colonies were transferred to the nutrient broth and incubated at 33°C for 24 hours for cell revival. Then 1 mL of grown (turbid) nutrient broth was transferred to a new nutrient broth and incubated again at 33°C for 24 hours to produce pure culture of a particular strain of bacteria. After that, the bacteria were streaked to new agar to obtain a single colony and became ready for the identification stage.

3.7.3 Biochemical Identification Bacteria

At the identification stage, GEN III MicroPlate test was used. The isolated pure culture was transferred to Biolog's nutrient agar and incubated again at 33°C for

4 to 24 hours to freshly grown the strains. Then MicroPlate inoculum was prepared at a cell density in the range of 90 to 98% turbidity. With a cotton-flipped swab, 3mm diameter area of cell growth was taken from the surface of agar plate, grasped the swab at its tip and held the swab vertically before touching the cell growth. The cell suspension was poured into multichannel pipet reservoir and soft gel formed. The Micro Plates was covered before the plates weas placed into OmniLog incubator for 3 to 36 hours at 33°C and the result was read using Biolog's Microbial Identification System software (Abel *et al.*, 2012).

3.7.4 DNA Extraction Method

With the same samples from isolation, the DNA extraction was continued for molecular identification method. The samples were scraped and extracted using GF-1 bacterial DNA extraction kits (Vivantis Technologies, Malaysia). Waterbath was set to 37°C to 65°C to ensure the longer DNA was breakdown. 1mL samples culture was centrifuged at 7000 rpm for 2 minutes at room temperature. Then the supernatant was decanted and the pellet was resuspended with 10µL Buffer R1 (Vivantis). The mixture was then resuspended by pippeting up and down. Next, lysozyme treatment took place for Gram-negative bacteria strains, 10µL lysozyme was added into the cell suspension while 20µL lysozyme was added to cell suspension for Gram-positive bacteria strains. Both mixtures were incubated at 37°C for 20 minutes.

The pellet was centrifuged again at 9,000 rpm for 3 minutes. After that, it was mixed with 180µL of Buffer R2 (Vivantis) and 20µL of Proteinase K (Vivantis) for protein denaturation. The mixture was then incubated at 65°C for 20 minutes in a shaking water bath. At the homogenization stage, 400µL of Buffer BG (Vivantis) was added into two tubes (with and without RNase) and the mixtures were inverted several times until they were homogenized before being incubated at 65°C for 10

minutes. Then 200µL of absolute ethanol was added and mixed immediately. The samples were transferred into columns for centrifugation at 10,000x*g* for 1 minute. The columns were washed with 750µL mixture of ethanol and Wash Buffer (Vivantis) and centrifuged at 10,000x*g* for 1 minute for ethanol removal. For DNA elution, samples in the column were transferred into microcentrifuge tube and 50µL of preheated Elution Buffer (Vivantis) was added before the samples were centrifuged at 10,000x*g* for 1 minute. Then the concentration and yield quantity of DNA extracts were checked by using UV-visible spectrophotometer (Eppendorf BioPhotometer Plus) (Zin *et al.*, 2011).

3.7.5 Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) amplification was carried out using PCR instrument (Mastercycler Pro Eppendorf). The reaction mixture each contained a primer, 1X PCR buffer, 400µM deoxynucleoside triphosphate, 5 mM $MgCl_2$ and 2.5 µM of Taq DNA polymerase. 20 to 50 cycles of PCR reaction was performed until clear 16S rRNA was obtained and the PCR cycles consisted of (i) the DNA was heating to 95°C for 1 minute by denaturing process; (ii) the primers were annealed to complementary target DNA sequences at 60°C for 1 minute; (iii) the new copies of the DNA were extended at 72°C for 1 minute; (iv) the new copies of the DNA were repeated. In the final cycle, the extension was run at 72°C for 10 minutes for full extension (Baharuddin *et al.*, 2010; Suria *et al.*, 2013).

3.7.6 Gel Electrophoresis

Gel electrophoresis process is a qualitative method to isolate mixed populations of DNA and RNA fragments. DNA extraction and PCR products were then analysed in a 1% (w/v) agarose (Vivantis) that were appropriately melted and 0.5 μg mL^{-1} of Ethidium bromide (EtBr) was added. The mixer was then poured in gel apparatus before solidification took place. The apparatus was then immersed into Tris-Acetate EDTA (TAE) buffer (Vivantis). After that, 2μL of dye and sample were added and mixed together. The mixed samples were electrophoresed at a voltage gadient (80V) for 90 minutes. DNA Ladder from Gene Ruller™ MBI Fermentas was used as a marker and mixed with 1μL of dye. The fragments were observed and imaged under automated UV transluminator (Gel Logic 212 PRO) with 1.4 megapixel CCD camera (Baharuddin *et al.*, 2010; Suria *et al.*, 2013).

3.7.7 PCR Purification

Products from PCR were purified with GF-1 PCR Clean-up kit (VivantisTechnogy, Malaysia). Samples were adjusted with sterile distilled water to 100μL and then mixed with 5 volumes of Buffer PCR (Vivantis) and inverted several times until they turned to yellow. They were then transferred into the columns and centrifuged at 9000 rpm for 1 minute. The columns were washed with 750μL of Wash Buffer and centrifuged at 9000 rpm for 1 minute. The columns were then transferred again to microcentrifuge tubes and 30μL of Elution Buffer was added and centrifuged again at 9000 rpm for 1 minute for DNA elution.

3.7.8 DNA Sequencing

The samples were analysed for DNA sequence at 1st base Asia (http://www.base-asia.com).

3.8 Activated Sludge Model No. 1 (ASM1)

Lab-scale ASMBR was modeled using ASM1. Stoichiometric and kinetic coefficients and COD fraction were the main data that had to be obtained to characterize the overall ASMBR behaviour as proposed by Henze *et al.* (2000). Combination physical-chemical and biological characterization method were used. At the beginning, COD was obtained to fit into ASIM 4004. Readily biodegradable and slowly biodegradable can be obtained respirometric method. While, inert soluble COD, (S_i) obtained from soluble COD effluent and inert particulate COD (X_i) is obtained from the total COD balance from Equation 2.15.

3.8.1 Respirometric Test

Respirometric tests were performed by using respirometric vessel that was connected to the aerobic submerged membrane bioreactor (ASMBR) (Figures 3.11 and Figure 3.12). A dissolved oxygen (DO) probe was immersed into the fabricated respirometer vessel of 80 ml with stirrer. The biomass from ASMBR was pumped into the OUR vessel with 6 minutes recirculation time and 4 minutes off and DO data was logged for every 30 seconds by using JENCO DO meter. The decrease of DO during off recirculation period was measured. The influent was kept fed for 24 hours

and feeding was stopped and the OUR reading dropped to approximately zero (Canudas, 2005; Metcalf and Eddy, 2004; Salmiati *et al.*, 2010)

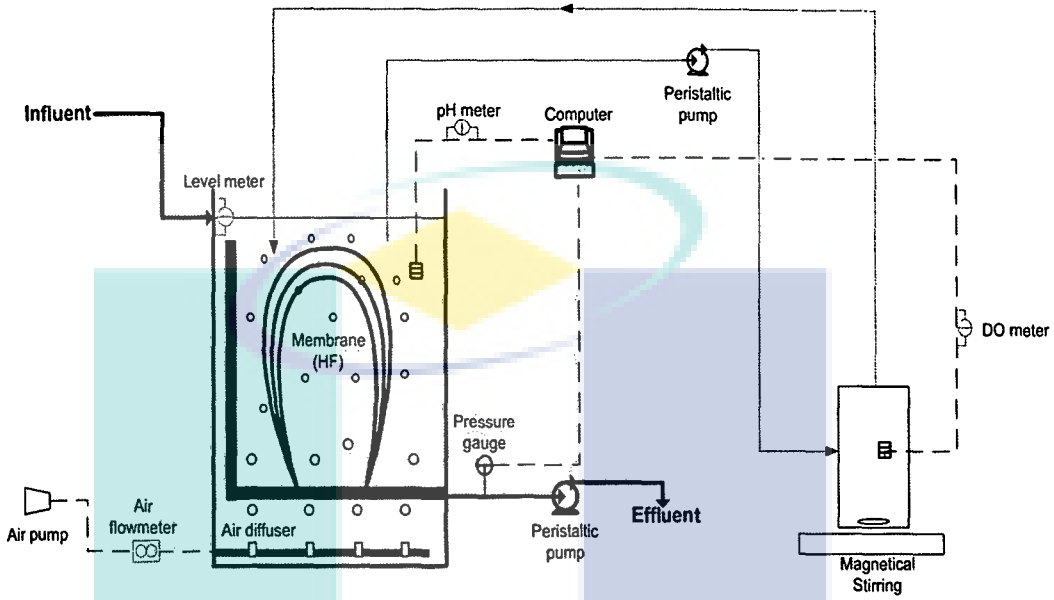


Figure 3.11 Schematic flow of respirometric vessel connected with ASMBR

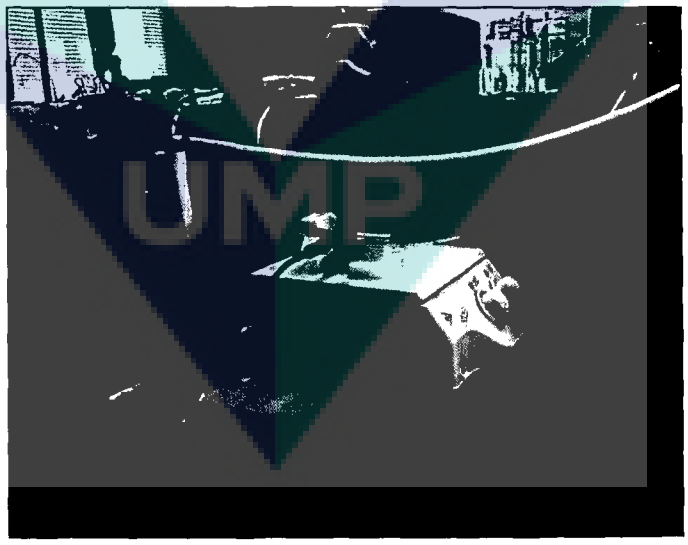


Figure 3.12 Respirometric vessel connected with ASMBR

3.8.2 Influent COD Fraction and Stoichiometric and Kinetic Coefficients

The scope for ASM1 was COD fractions that contained readily biodegradable (S_s), slowly biodegradable (X_s) and inert suspended organic matter (X_i and S_i). In ASM1 effectively all state variables are directly influenced by a change in a parameter value while ASM3, the direct influence is considerably low (Henze *et al.*, 2008). According to Henze *et al.* (2000), readily and slowly biodegradable organic matters depend on heterotrophic yield coefficient (Y_H) and respirometric profile. Y_H was obtained by observing the mass of cell material formed during removal of soluble COD. 2 L of wastewater settled and filtered to remove the particulate matter. Wastewater that contanin soluble COD seeded with 2 mL biomass from ASMBR. 0.22 μm of syringe filter was used to filter SCOD from TCOD periodically until stable CODs were obtained. S_i is the COD fraction with same concentration that enters and leaves the system ($SCOD_{effluent}$) while inert particulate organic (X_i) was calculated using Equation 3.10 while S_s and X_s were obtained by including OUR differenciation from respirotoric graph and Y_H (Petersen *et al.*, 2003; Vanrolleghem *et al.*, 2003). DO data from experiment were fit into ASM1 and stoichiometric and kinetic coefficients were obtained. COD fractions were fit into ASIM 4004 software to identify the variable coefficients.

$$X_i = TCOD - S_s - S_i - X_s \tag{3.10}$$

3.9 Conclusion

Synthetic spent caustic (SSC) was treated by aerobic submerged membrane bioreactor ASMBR using U-shaped hollow fiber membrane. MLSS concentration and SRT are important parameters needed to optimize the ASMBR operation. However, membrane biofouling is a major problem during operating ASMBR.

Hence, biofouling reducer (BFR) was used to minimize the biofouling effect. Unknown bacteria were identified in mixed culture ASMBR and the behaviour of this system was characterized using ASM1 (ASIM 4004 software).

Nutrients analyses were carried out using UV-visible spectrophotometer under Standard Method for concentration analysis. SMP and EPS were extracted from sludge solution using heating extraction method. Then protein SMP and EPS were analysed using Bradford's method while carbohydrate SMP and EPS used phenol-sulphuric acid method for concentration analysis. Meanwhile PAXIT was used to analyse the morphology of biomass and morphology of membrane and BFRs were examined by FESEM.

In bacteria identification, two methods were used which are biochemical method and molecular method. Both methods were used for comparison and to ensure the accurateness of the identification. Biochemical method was used as touchstone for bacteria identification and molecular method for clear genetic characterization for ASMBR sludge. In the Activated Sludge Modeling (ASM1), respirometric tests were used to characterize the COD fraction of SSC and to obtain the stoichiometric and kinetic coefficients of ASMBR-BFRs systems. In this model, respirometric vessel connected with ASMBR and DO data were collected.

CHAPTER 4

FOULING BEHAVIOUR AND EFFICIENCY IN ASMBR

4.1 Introduction

This chapter discusses the important operation parameters during the running of the ASMBR. To date, there is no study detailing the parameters used in treating spent caustic wastewater using MBR which is known as high strength wastewater. These parameters were examined on the organic and nutrients removal performance (especially on sulphide and COD removal) and directly reduced the membrane biofouling.

Controlling parameters is part of reducing membrane fouling. Therefore, to enhance reduction in membrane fouling, biofouling reducers (BFRs) were added. Biofouling reducer (BFR) had been used in wastewater separation technology treatment and has the capability to adsorb contaminants. The morphology of biomass, membrane and BFRs will be seen at the end of this chapter.

4.2 Synthetic Spent Caustic Characteristics

A synthetic spent caustic (SSC) wastewater contains stable composition and in this study it was used to reduce fluctuation of disturbance by unknown compounds that are present when using real spent caustic. Fluctuation occurs due to the different composition of raw materials used and processes involved. It is important to control the compounds fluctuation to optimize the comparative result later. Hence, a preliminary study of SSC wastewater was done on the effect of aeration and membrane on COD removal without biomass presence. COD analysis was done on effluent in a reactor without sludge biomass and effluent after the membrane to observe the removal influence.

The results in Table 4.1 indicate 4.4±1% removal by nature, 8.03±1.2% of average COD removal by aeration in the reactor and 19±3% of average COD removal by membrane which indicated that COD of SSC is hard to remove by nature, aeration and membrane filtration. Stable components in SSC such as phenol (Sheu and Weng, 2000) are hard to be oxidized by aeration itself hence SSC wastewater is stable for several days leading to slight removal of COD. According to study of Yang *et al.* (2010), water quality from lakes treated by direct membrane filtration shows 10% of COD removal by ultrafiltration of PVDF hollow fibre membrane (Yang *et al.*, 2010). In another study, data from pre-treatment of poultry wastewater showed 18.4% of COD removal by polysulphone membrane microfiltration and membrane material played a significant role in filtration process (Afari and Kiepper, 2011). It is apparent that SSC is stable and difficult to treat by aeration or filtration independently and need another mechanism to improve the removal performance.

Table 4.1: COD trend analysis for wastewater characterization

Time (day)	Influence	Reactor	Effluent
1	2370±29	2150±31	1820±19
2	2230±15	2080±27	1630±23
3	2150±19	1990±27	1570±21
4	2070±16	1890±26	1550±16

In pH adjustment process, the sulphuric acid (H_2SO_4) was chosen because it is more economical and has less thermal impact and less corrosive when compared to hydrochloric acid (HCl) (Berne and Cordonnier, 1995). In this study, SSC with pH 10±1was adjusted to 7. Thus according to Table 4.2, removal by pH adjustment shows 7.2% of COD removal and 8.3% of phenol removal. While other component shows the removal was below 10% except for sulphide with 13% removal. Sulphate product from sulphide oxidation showed an increment up to 79%. In the industry, phenol is the indicator for pH effect due to its difficulty of oxidation compared to sulphide. pH adjustment also shows part of the COD have been removed and it might be from components that were easy to oxidize by acid, for instance, sulphide and heat can be recovered since it is exothermic reaction. Non-sulphide components like phenol might be part of remaining COD that are more stable and need further process for removal (Berne and Cordonnier, 1995; Sheu and Weng, 2000).

Table 4.2: Characteristic of synthetic spent caustic wastewater for OL1

Parameter	Before pH Adjustment	After pH Adjustment
COD (mg L^{-1})	2500±32	2320±44
BOD (mg L^{-1})	810±14	754±12
Sulphide (mg L^{-1})	102±7	88±6
Sulphate (mg L^{-1})	83±5	403±30
Phenol (mg L^{-1})	48±2	44±3
Total Nitrogen (mg L^{-1})	30±2	29±3
Total Phosphate (mg L^{-1})	15±1	14±1

4.3 MLSS Concentration and SRT

Identification of optimal MLSS and SRT for operation of ASMBR is crucial to improve the treatment of SSC in contaminant removal and helps to lengthen the form of severe biofouling (reduce the lifespan of the ASMBR operation). MLSS helped in sludge production, aeration demand and reduce membrane fouling clogging while SRT improved in controlling the sludge production, microbial products and reduced the rate of membrane fouling (Judd, 2006). The idea is to examine the organic and nutrients removal and the reduction in biofouling so as to prolong ASMBR operation. In this study, ASMBR was examined for organic and nutrient removal, RIS, TMP trend and SMP and EPS production. At the beginning, the critical flux of ASMBR was identified as guideline for flux operation. The activity of biomass in bulk solution directly affect to membrane fouling since membrane was submerged into bulk solution in reactor. Therefore, it is important to analyse the viability of biomass towards membrane fouling.

4.3.1 Critical flux of MLSS

At the MLSS of preliminary test, steady state was achieved with more than 80% COD removal (Appendix E) in MLSS started from 5 g L^{-1} with MLVSS/MLSS average ratio of 0.94. During pre-experiment, the MLSS concentration was increased gradually batch by batch until flux reading does not give significant result (less production or permeate). Subsequently, the appropriate permeate flux (J) for operation was identified which should be below the critical flux (J_c) as a flux operation limitation to avoid fouling severity. However the values are subjective but the concepts of critical flux for this study are that the rate of TMP change should be greater than 0.5 kPa min^{-1} (Bottino *et al.*, 2009; Damayanti *et al.*, 2011). When J is above J_c, physical or chemical cleaning is required in order to maintain membrane performance (Bottino *et al.*, 2009; Judd, 2006). Critical flux at 5 g L^{-1}, 7 g L^{-1} and 9

g L^{-1} of MLSS concentration were identified by flux-step method. In the flux-step method, one step constant was applied at 15 minutes intervals while the corresponding TMP was recorded at every 1 minute. The flux-step method result is shown in Appendix C. New membranes were used at respective MLSS concentrations.

Data from the flux step method (Appendix C) were used to identify average TMP (TMP_{ave}) and the rate of change of TMP (dTMP dt^{-1}) (Figure 4.1). In this study, the flux with dTMP $dt^{-1} \geq 0.5$ kPa min^{-1} was defined as the critical flux. The critical flux reading decreases at MLSS increase. According to Figure 4.1, the constant rate of increasing TMP up to 8.4 LMH while its rate became significant at higher fluxes due to fouling for MLSS 5 g L^{-1}. However the constant rate of increasing TMP decrease as MLSS increase to 7 g L^{-1} (6 LMH) and 9 g L^{-1} (3.5 LMH) due to high suspended solid that attached on to membrane surface and reduced the filterability capability. Damayanti *et al.* (2011) reported that at dTMP $dt^{-1} \geq 0.5$, by increasing the MLSS from 5 to 20 g L^{-1}, the critical flux becomes four times lower. Meanwhile, Bottino *et al.* (2009) recorded critical flux decreases 2.5 times from MLSS 3 to 14 g L^{-1}.

With the ability of MF membrane to withstand vacuum pressure up to 70 kPa, the severity of membrane fouling was observed by ascending and descending TMP_{ave} (Figure 4.2). At the ascending run, flux increased proportionally with TMP increase until flux levelled off, more quickly at higher MLSS and volume rate of permeate reduced due to high membrane fouling and more rapidly at MLSS 9 g L^{-1}. The membrane fouling severity were observed during the descending run, where at zero flux (TMP_{ave}), the reading was recorded. The ΔTMP_{ave} at zero flux shows increments as MLSS increase that indicate the degree of membrane fouling severity increased with ΔTMP_{ave} 0.85 kPa (MLSS 5), 18.5 kPa (MLSS 7) and 21.5 kPa (MLSS 9). Meanwhile, average dynamic plot of graph recorded increase as MLSS increased; 5 g L^{-1} (23.2 kPa), 7 g L^{-1} (24 kPa) and 9 g L^{-1} (28 kPa). It is because during filtration, a part of water filtered and retained the colloids and macromolecular matter on the surface of the membrane (irreversible fouling) (Meng

et al., 2006) and more suspended solid presence as MLSS increased and deposited onto membrane surface to form more thick biocake layer. Bottino *et al.* (2009) reported that as MLSS increase from 3 to 14 g L^{-1}, the ascending run increase and more quickly at higher MLSS due to fouling. The detrimental effect of fouling on the membrane flux observed during the descending run and the same trend recorded by Damayanti *et al.* (2011).

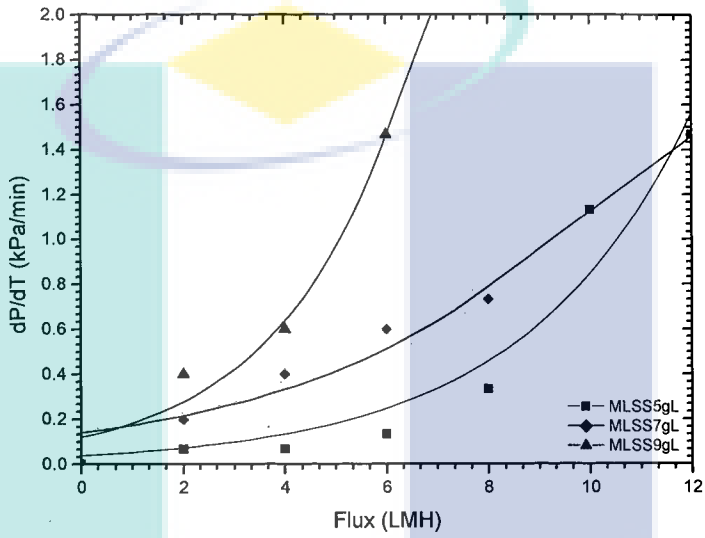


Figure 4.1 The rate of TMP change (dTMP dt^{-1}) for MLSS concentration

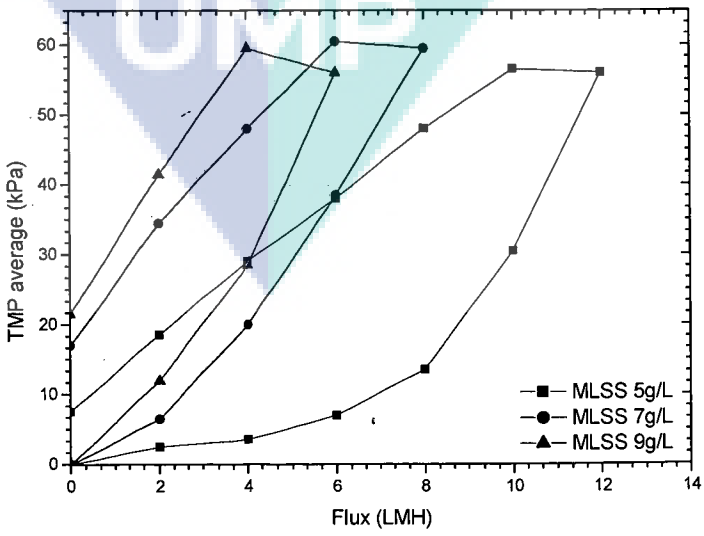


Figure 4.2 Hysteresis loop for TMP_{ave} and flux at each different MLSS concentration

4.3.2 MLSS and SRT Performance

The performance of MLSS and SRTs were analysed by nutrients removal, RIS, TMP trends and SMP and EPS concentrations. The hydraulic retention time (HRT) was fixed at 16 hours. MLSS concentrations as discussed in Chapter 3 (MLSS Concentration and SRT) were based on pre-experiment with 80% COD removal that can be seen at Appendix E. MLSS started from 5, 7 and 9 g L^{-1} and the concentration was increased gradually until flux reading does not give significant result (less production or permeate). The performance was mainly to observe the removal performances and the degree of membrane fouling as mention in Chapter 1 (Scope of Study). Operational flux must be below critical flux and hence operational flux (J) for the next ASMBR runs was 4 LMH which was below J_c at 6 L d^{-1} of SSC influent flow rate to prolong ASMBR lifespan of membrane.

4.3.2.1 Removal Performance in SSC wastewater

SSC is known to have high content of sulphide, organic and inorganic. The influents of SSC wastewater BOD_5/COD has a mean of 0.33, which is categorized as high strength wastewater and contain high levels of total sulphide and phenol contaminants. Hence the SSC was acclimatized in ASMBR that operated for 10 days until steady state was achieved (more than 80% of COD removal). SSC also contain a large number of compounds, thus the yield is based on measurable parameter reflecting the overall compound which is COD removal. Figure 4.3 shows the yield for MLSS and SRT that indicate the production of biosolid to the amount of COD consumed. The rate of cell growth increased as MLSS and SRT increased that may be related to the accumulation of biosolid growth in the reactor by consumed of organic matter.

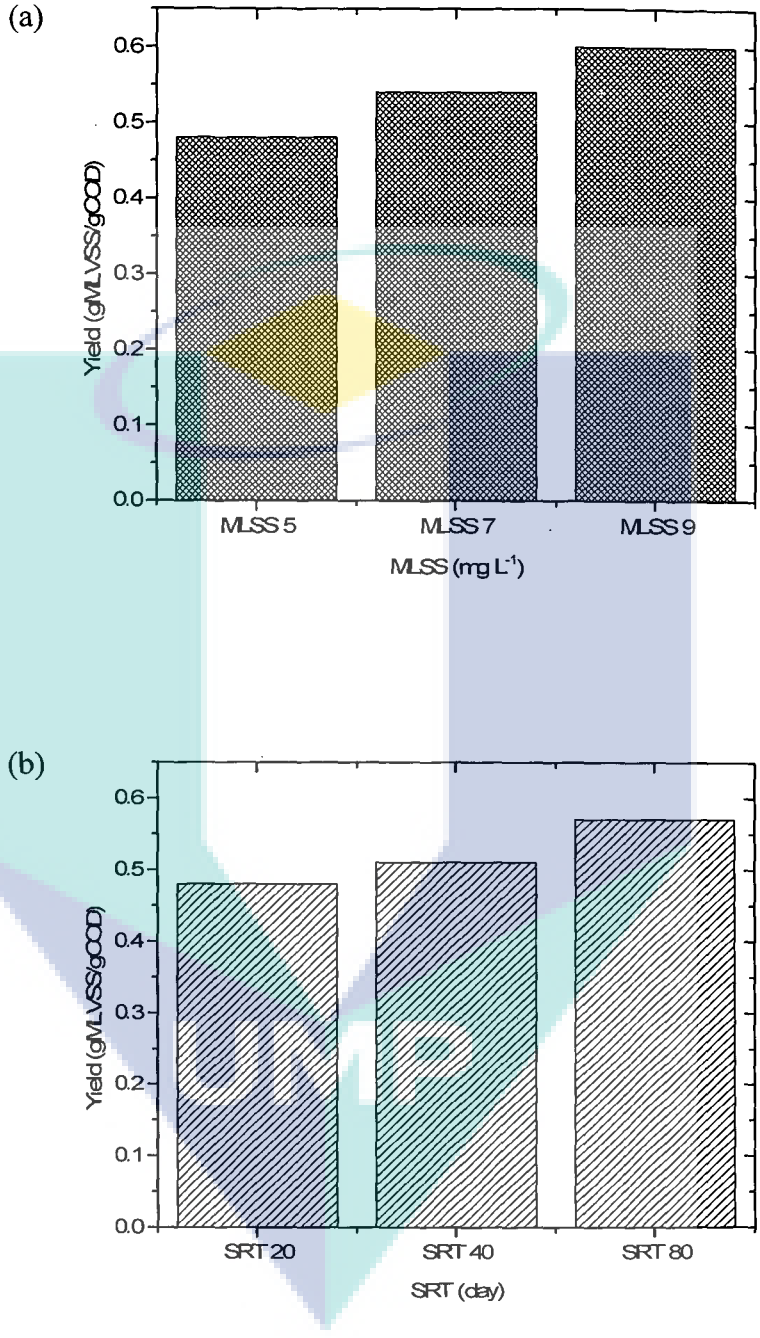
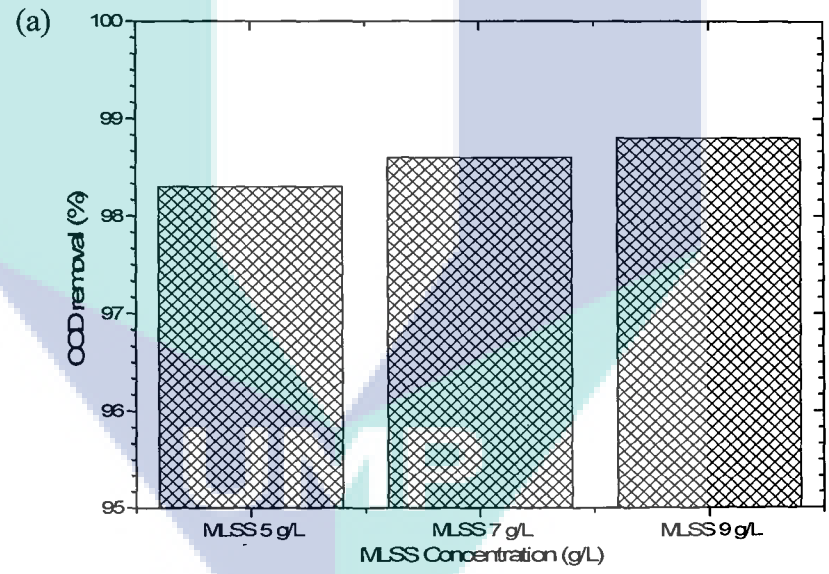


Figure 4.3 (a) Yield for MLSS; and (b) Yield for SRT

The F/M ratio also decreased as MLSS increased with result recorded as 0.78, 0.5 and 0.44 kg COD kg^{-1} MLVSS d^{-1}. It was significant with removal performance where at constant COD loading, the removal increased due to the increase accumulation of biomass in the reactor for degradation. Average COD percentage removal showed slightly increased as MLSS increased (Figure 4.4a) and average

effluent concentration recorded 43 mg L^{-1} (MLSS 5 g L^{-1}), 34 mg L^{-1} (MLSS 7 mg L^{-1}) and 28 mg L^{-1} (MLSS 9 g L^{-1}). Total sulphide recorded 99 to 100% (with average effluent 0.011 to 0.013 mg L^{-1}) of removal for all MLSS concentrations (Figure 4.4b). Meanwhile total sulphate showed 39 to 40% of production from complete sulphide oxidation (Figure 4.4c). These organic and nutrients result are also influenced by filtration of membrane and 'pseudo-membrane'. Overall, as MLSS increased, the performances of nutrients removal are slightly increased. It is due to high good and active biomass in high MLSS concentration that highly degraded the nutrients with less nutrient escaping.



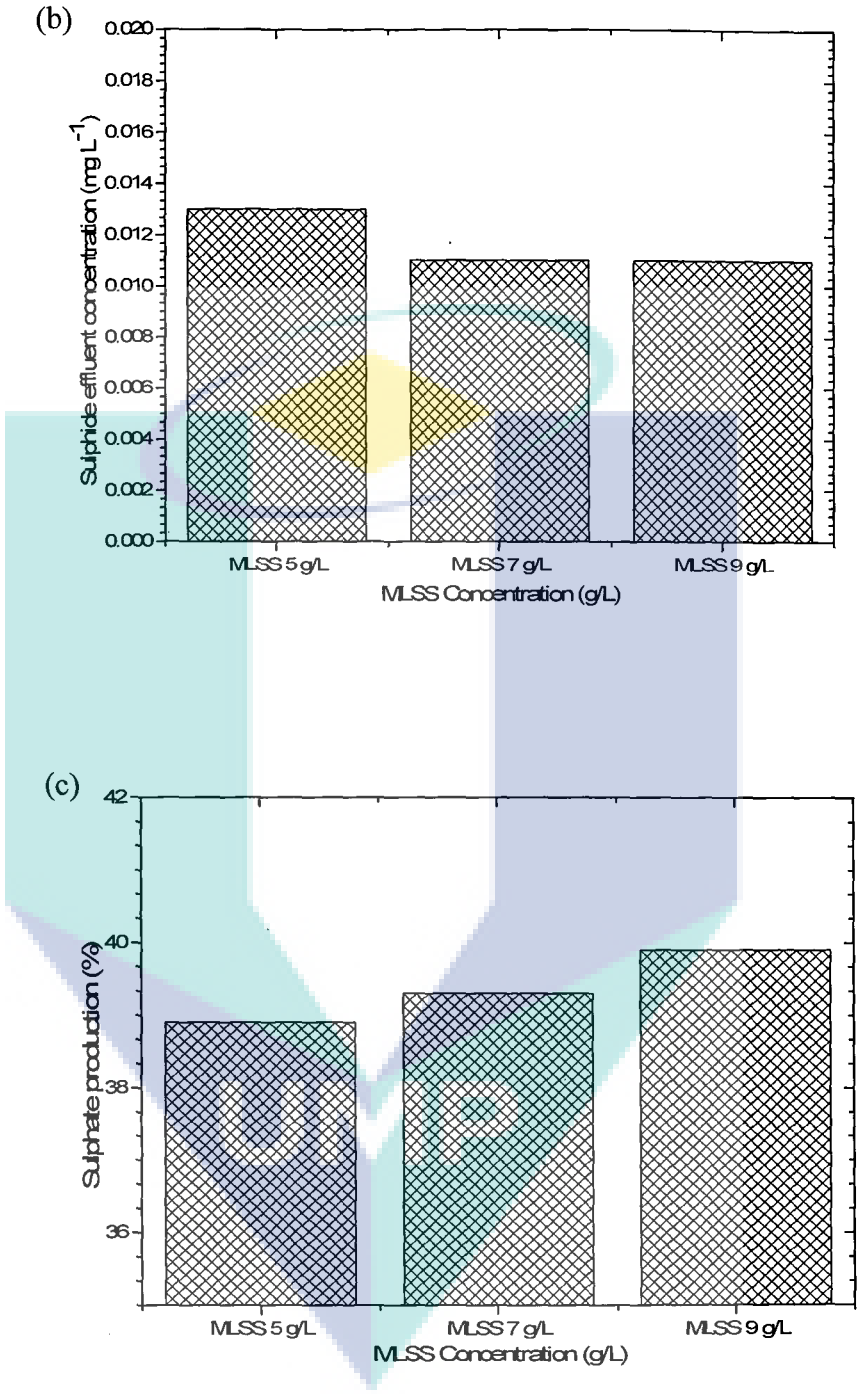


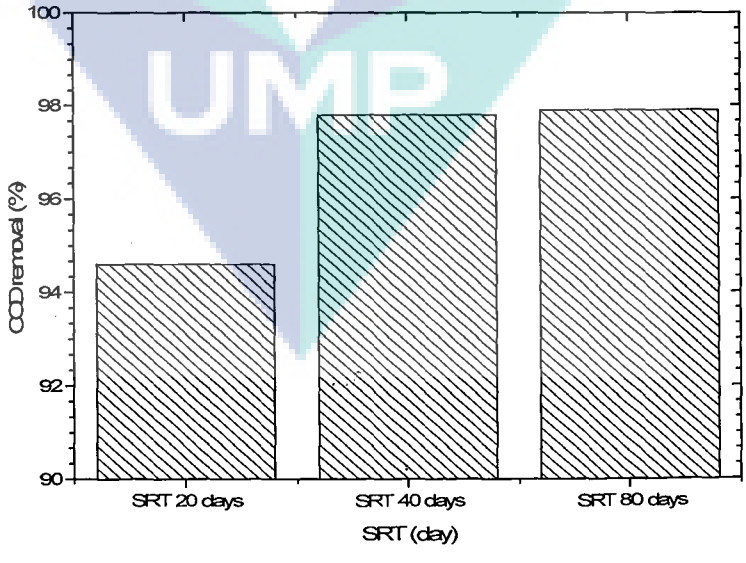
Figure 4.4 Performance of MLSS concentrations; (a) COD removal percentage; (b) sulphide effluent concentration; and (c) sulphate production percentage

The SRTs test showed more than 90% of COD removal for SRTs runs but from COD removal performance, SRT of 40 and 80 days gave slightly higher performance when compared with SRT of 20 days (Figure 4.5a). The F/M ratio average was recorded as 0.81, 0.72 and 0.64 kg COD kg^{-1} MLVSS d^{-1} for SRT of 20,

40 and 80 days respectively and high biomass accumulation as SRTs increased. Thus, the average effluent concentration recorded was 134 mg L^{-1} (SRT of 20 days), 53 mg L^{-1} (SRT of 40 days) and 28 mg L^{-1} (SRT of 80 days). Total sulphide recorded 99 – 100% (with average effluent 0.012 – 0.023 mg L^{-1}) (Figure 4.5b). While total sulphate showed 38 to 46% of production from the complete sulphide oxidation (Figure 4.5c). At low SRT (high sludge discharge) there was low removal due to high wash-out of good biomass from the system creating high F/M ratio at constant organic loading. This high F/M ratio created less biomass that degraded the substrate since food is larger than biomass and more food escaped from the system.

As a result, better removals occurred at high MLSS and high SRT. However, membrane fouling became a limitation of ASMBR performance and tended to be increased as MLSS increased. Therefore, membrane biofouling analysis was considered in MLSS concentration and SRT characterization. The characterization analysis continued with membrane fouling performance on RIS, TMP trends and SMP and EPS production.

(a)



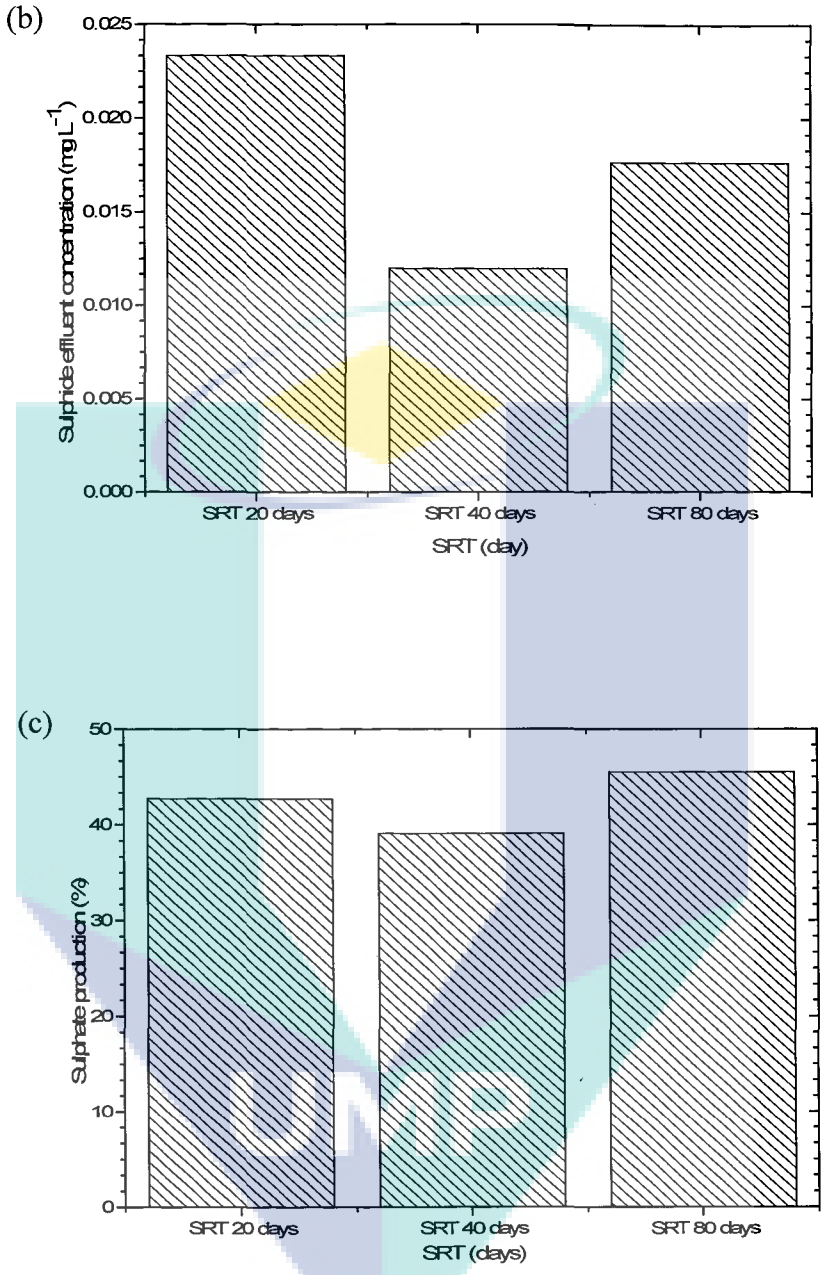


Figure 4.5 Performance SRTs; (a) COD removal percentage; (b) sulphide effluent concentration; and (c) sulphate production percentage.

4.3.2.2 Resistance-In-Series (RIS)

Total resistance (R_{tot}) for RIS was disaggregated into sludge resistance (R_s) and clean membrane resistance (R_m). R_s and R_m acted independently. R_s is the sum of external fouling (cake layer) and internal fouling. Table 4.3 showed that MLSS increased proportionately with increasing total resistance (R_{tot}) without sludge discharge. High total resistance led to detrimental flux operation. In this case, the sludge resistance dominated the total resistance and this was due to cake formation and fouling of the membrane. From clean water flux test, R_m recorded has the same value due to the same membranes that had been used. The value of R_m affected by the type of membrane, membrane material, configuration, surface area and pore size (Judd, 2006). In this study, R_m is inherent to the system since the membranes used have the same characteristics and the same observation as reported by Damayanti *et al.* (2011).

The concerns in this analysis were R_s value that kept on increasing as the MLSS (Table 4.3) and SRT increased (Table 4.4). In addition, no sludge discharge (high accumulation of MLSS) was recorded that enhanced the total high resistance. The fouling is referred to as the loss of filterability due to fouling resistance and it was represented by R_s. By increasing the SRT, less sludge was discharged and biomass tended to accumulate in the reactor and laterally increased the MLSS concentration and R_{tot}.

Consequently, high biocake layer was formed on the surface of the membrane and increased the percentage of R_s. The biocake layer formed on the membrane surface acted as 'pseudo-membrane' and helped to avoid nutrients in SSC wastewater passing through the membrane but it reduced the efficiency of filterability until cleaning process took place. These are due to adsorption of soluble matter and pore blockage within the membrane and similar observation was reported by Chang *et al.* (2008) where cake resistance, R_c increased when MLSS was increased because of higher suspended solids in the reactor (Chang *et al.*, 2008). In

addition, R_m may be impeded by the membrane characteristic e.g. membrane porosity or membrane material and pure water permeability (Bottino *et al.*, 2009) that contributed detrimental to flux operation. Damayanti *et al.* (2011) recorded that higher MLSS will lead to higher R_{tot}. Meanwhile, Lee *et al.* (2010) reported that as SRTs increased, the MLVSS increased and small MLVSS accumulation had been recorded at low SRT, hence the operation stabilised after 10 days operation (Lee *et al.*, 2010). Lee *et al.* (2003) study shows fouling resistance high at SRT 60 days (3.09×10^{11} m^{-1}) and at SRT 40 days recorded the lowest fouling resistance with 2.56×10^{11} m^{-1}. This is due to suspended solid, solutes and colloids mainly resulting from the lysis of biomass accumulated in MBR as dominant factors. The substrate characteristics, the physiological state of sludge and the membrane properties contributed in membrane fouling (Lee *et al.*, 2003).

Table 4.3: Resistance-in-series (RIS) in different MLSS concentration without sludge discharge.

MLSS ($mg\ L^{-1}$)	R_{tot} ($10^{13}\ m^{-1}$)	R_m ($10^{13}\ m^{-1}$)	R_s ($10^{13}\ m^{-1}$)	R_s/R_t (%)
5000	2.5	0.46	2.04	81.5
7000	2.52	0.46	2.06	81.6
9000	2.58	0.46	2.12	83

Table 4.4: Resistance-in-series (RIS) in different SRTs.

SRT (day)	R_{tot} ($10^{13}\ m^{-1}$)	R_m ($10^{13}\ m^{-1}$)	R_s ($10^{13}\ m^{-1}$)	R_s/R_t (%)
20	2.52	0.46	2.06	81.3
40	2.45	0.46	1.99	81.1
80	2.64	0.46	2.18	82.4

4.3.2.3 TMP Trends

Figure 4.6 showed the TMP performance of MLSSs and SRTs. The results illustrated the initial TMP for MLSS concentrations and SRTs were slightly different. Rapid increase could be seen for MLSSs rather than SRT due to no sludge discharge in MLSS concentration runs. The TMP started to increase drastically in day 4 for MLSS 9 g L^{-1} due to drastic increase in the accumulation of biomass and inert suspended in the reactor since there were no sludge discharges for MLSS runs. Same results have been reported in Bottino *et al.* (2009) and Melin *et al.* (2006) where increased MLSS hastens membrane fouling (Bottino *et al.*, 2009; Melin *et al.*, 2006). Meanwhile, at SRT of 80 days, the TMP rapidly increased when compared to SRT of 20 and 40 days. This was due to less sludge discharges that increased the inert compound and biomass accumulated in the reactor. Similar result had been recorded in Lee *et al.* (2003), Kimura *et al.* (2009) and Wu *et al.* (2011). Wu *et al.* (2011) recorded that higher membrane fouling occurred in the high MLSS (SRT infinity) due to small floc size. Meanwhile, Kimura *et al.* (2009) stated that the degree of membrane fouling in the MBR was not directly related to the concentration of SMP in the reactor. The concentration of SMP do not correspond to the fouling trends where at SRT 102 days, the TMP trend shows rapid fouling (severe membrane fouling) even though the SMP recorded lower.

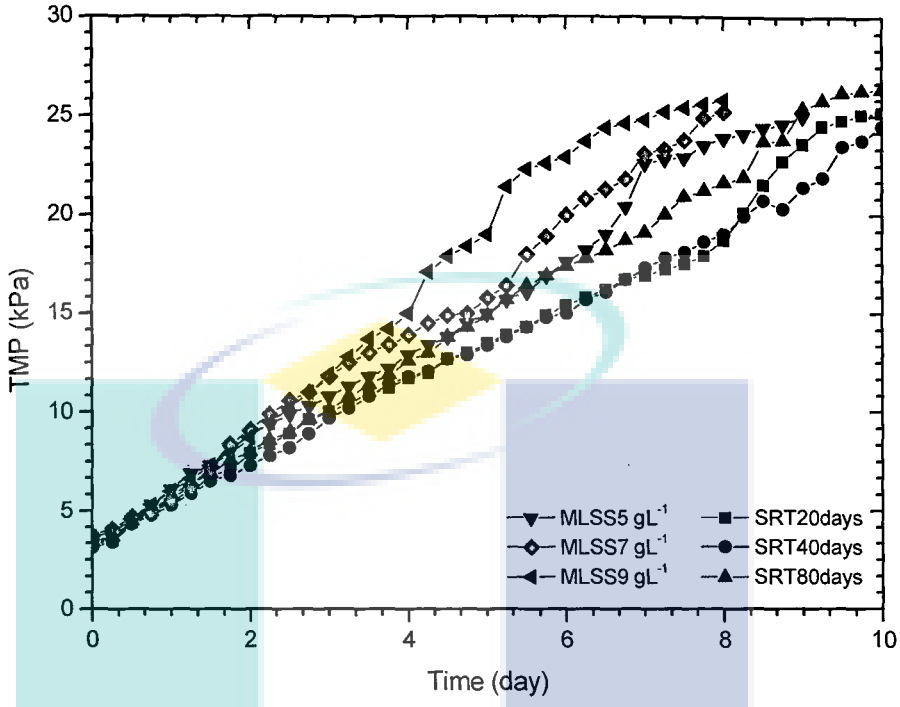


Figure 4.6 TMP performance for MLSS concentration and SRTs

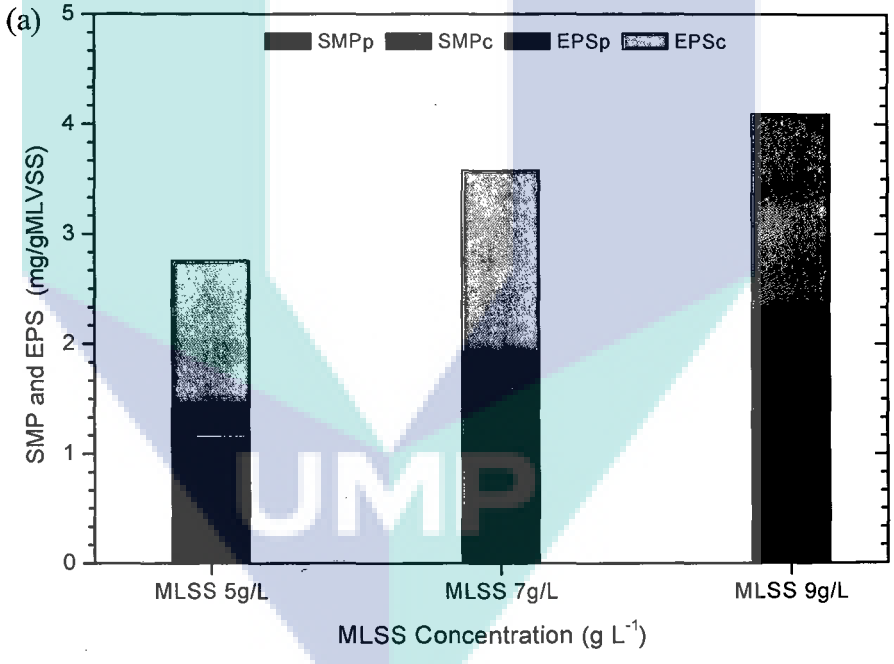
4.2.3.4 SMP and EPS Performance

The performances of MLSSs and SRTs on the production of microbial product (SMP and EPS) were examined. SMP is known as soluble cellular components and was released during cell lysis and diffused through the cell membrane. SMP also became part of effluents (Antonelli *et al.*, 2011). EPS are located at or outside the cell surface. Hence, EPS is a medium connecting cells in microbial aggregates. EPS contributed into many organic compounds such as polysaccharides, amino polysaccharide and protein (Ying and Ping, 2006). SMP and EPS in common microbial produced organic materials that contain electrons and carbon but they are not active cells (Laspidou and Rittmann, 2002).

Figure 4.7 (a) on MLSS showed the average SMP and EPS. In order to get the best performance of ASMBR in treating high strength spent caustic, MLVSS concentration must be high to increase degradation process and led to increased SMP and EPS. Y_{obs} (mg(SMP and EPS) g(COD utilised)$^{-1}$ d^{-1}) recorded increase as MLSS increased at constant utilised COD that correlated to high biomass growth (high microbial product produced); MLSS 5 (0.104), MLSS 7 (0.159) and MLSS 9 (0.291). Meanwhile, as MLSS increased, there was a decrease of F/M ratio. This meant that the increase MLSS was correlated to the increase accumulation of biomass product (SMP and EPS) since there was no sludge discharge (Mutamim *et al.*, 2013b). The reading showed that average SMP and EPS increased by increased MLSS and led to membrane fouling and the same result was reported in Wu *et al.* (2011). The study showed that the performance of EPS was high at high MLSS with SRT at infinity having serious biofouling due to low flocs size (Wu *et al.*, 2011). Besides SMP and EPS from biomass, MLSS concentration also consist of inert suspended solid, inert compounds, dead and old biomass that accumulated in the reactor since there were no sludge discharge in these runs that contributed to membrane fouling (Hasar *et al.*, 2002). As a result, accumulation of SMP and EPS and also inert particulate influenced the speed of membrane biofouling due to deposition of biocake layer and biolayer of membrane surface.

Y_{obs} (mg(SMP and EPS) g(COD utilised)$^{-1}$ d^{-1}) recorded increase as SRT increase that is correlated to high microbial product due to high biomass present in constant utilised COD; SRT 20 (0.12), SRT 40 (0.117) and SRT 80 (0.132). Even though Figure 4.7 (b) showed that accumulation of SMP and EPS are low at SRT of 80 days and 40 days, the TMP result showed rapid increase at SRT of 80 days due to less sludge discharge and high inert particulate accumulation in the reactor and it contrasted with SRT of 20 days. The same result has been reported in Ng and Hermanoicz (2005) where SRT parameter related to flocculation of biomass. As SRT decreased, the amount of non-flocculating biomass increased (biomass more dispersed) that evenly deposit on membrane surface and formed biocake layer (Ng and Hermanowicz, 2005). The effluent result also recorded the existing of SMP and EPS due to soluble SMP and EPS for both MLSS and SRTs runs. A part of soluble microbial products adsorbed and tend to deposit in membrane pore to formed

standard blocking that contributed to increase fouling rate. Although, the SMP and EPS from biomass synthesis released in bulk solution led to fouling, it needed to be taken into account. Figure 4.7 (a) and (b) showed SMPc and EPSc highly accumulated in bulk solution that most influenced the form of biolayer due to domination in the reactor and enhanced the fouling to occur and this result is consistent with a study by Pan *et al.* (2010). In this study also shows the amount of carbohydrates SMP and EPS in bulk solution is 5.7 (MLSS) and 4.9 (SRT) times more than proteins SMP and EPS. The lower release of proteins SMP and EPS indicates that the biomass was active and there was no situation of stress (Andrade *et al.*, 2013).



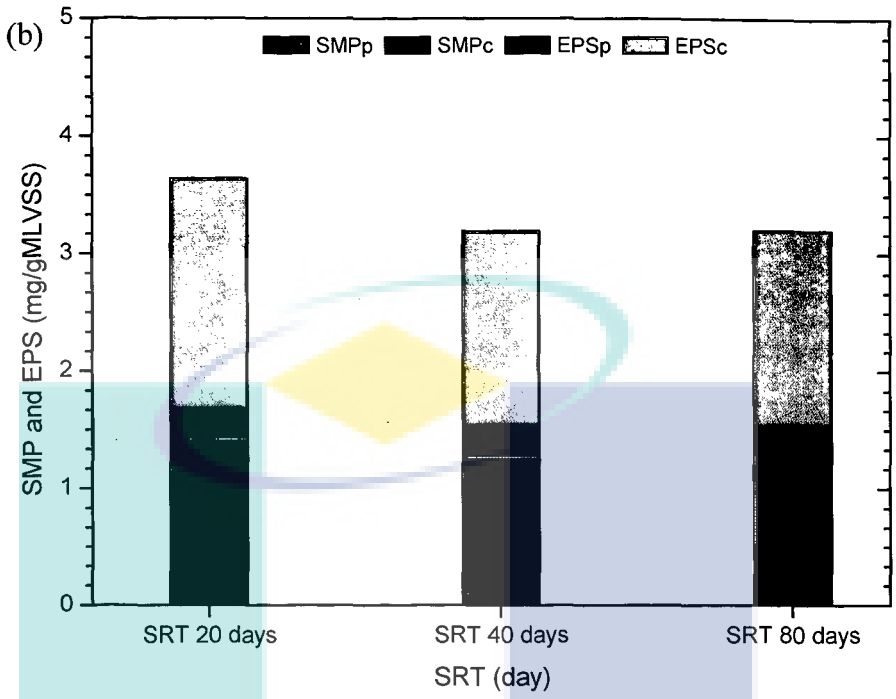


Figure 4.7 SMP and EPS concentration for (a) MLSS concentrations and (b) SRTs

The mean of COD effluent was 0.088 gCOD L^{-1} and 0.06 gS L^{-1} for sulphide effluent in all runs. COD removal showed good performance in all runs (above 90% COD removal). Total sulphide recorded 99 – 100% removal while total sulphate was observed to increase from 0.423 to 0.783 gSO_4 L^{-1}. Sulphate production showed high percentage of complete oxidisation of sulphide at high MLSS concentration and SRT. However, at high MLSS concentration and SRT there was drastic TMP increased. From the RIS and TMP trends, it could be seen that SMP and EPS were not directly affected the degree of membrane fouling for MLSS concentration and SRT runs. As MLSS concentration increased, the SMP and EPS accumulation increased and TMP trends showed drastic increase at high MLSS concentration. This could be due to no sludge discharge being recorded and the ability of biomass, solid inert particulate and SMP and EPS to accumulate in the reactor. It is opposite to the SRTs result where the SMP and EPS decreased as SRT increased but the TMP trend showed rapid increase at high SRT. Longer SRT cause stress to the biomass in the reactor that needed more energy for cell maintenance and therefore leave less energy production (less microbial production). Too high SRT increased suspended

solid accumulation due too low sludge discharge. While at too low SRT (high sludge discharge), a large portion of good biomass had the tendency to be washed out and it took a long time for biomass to stabilise for nutrients removal (Judd, 2006).

The significant of membrane fouling not only related with microbial product, but it is also related with accumulation suspended solid. It can be seen as SRT increase, the microbial products decrease but the trend of TMP increase. It shows that the factors of membrane fouling does not only depend on microbial products but also debris, biomass decay and non-biodegradable substrate that led to accumulation of inert particulate matter and it can be seen from RIS and TMP as fouling effect as mention by Hasar *et al.* (2002), Bottino *et al.* (2009) and Damayanti *et al.* (2011). Hasar *et al.* (2002) reported that non-biodegradable compounds accumulate in SMBR from microorganism production and MLVSS can be included in the dead biomass and inert compounds and it reduced viability of biomass at the 50% level at high MLVSS. Damayanti *et al.* (2011) recorded the increment trend of R_c from 1.54 to 3.71 10^{12} m^{-1} as MLSS increase from 5 to 20 g L^{-1} and the removal achieved up to 99%. Meanwhile Grelier *et al.* (2006) reported that the best operating performances at SRT 40 days with the lowest fouling rate and degradation achieved the most efficient result. Judd (2006) mentions that CASP operating at SRT of 8 days with MLSS 2.5 g L-1 while MBR operating at SRT 40 days with MLSS up to 12 g L^{-1}. High SRT (low F/M ratio) implies high MLSS and low sludge yield that increase SRT is advantageous with respect to waste generation. Judd (2006) also mentions too high MLSS are to some extent detrimental to process performance due to accumulation of inert compounds, reflected in a decrease in MLVSS/MLSS ratio and high solid levels increase the propensity for clogging or 'sludging' that led to reduce aeration efficiency.

As a result, the change in MLSS concentration clearly affected critical flux, RIS and TMP performance with 5 gMLSS L^{-1} giving a good performance when compared with others leading to less formation of SMP and EPS. High MLSS concentration was found to be detrimental to system performance by reducing aeration efficiency and increasing membrane biofouling. Though high SRT was

advantageous in generating less sludge waste but it increased the accumulation of suspended solids. SRT of 80 days had high R_s formation and TMP increased drastically when compared with SRT of 20 and 40 days due to high MLSS accumulation (less sludge discharge) even though the SMP and EPS concentration decreased. However, SRT of 40 days gave a good performance (low SMP and EPS formation, slow rate of TMP increase and low R_s) in fouling rate reduction more than SRT of 20 days. Thus, by controlling SRT, substrate degradation, excess sludge production and biomass concentration were controlled. In addition, carbohydrates are synthesized extracellularly for a specific function and proteins often result in the excretion of intracellular polymers or cell lysis. Thus, the lower proteins SMP and EPS released indicate that the biomass was active and there was no situation of stress.

The accumulation of solids in the system had the tendency to clog the membrane especially at the area of membrane epoxy (Appendix B) due to compact space and difficulty for aeration bubble to reach this area. Hence, high solids accumulation also affected the biomass activities as it reduced the efficiency of aeration. Therefore, the factors that affect MLSS concentration and SRT performances were a combination of (i) accumulation of biomass; (ii) accumulation of non-biodegradable and (iii) accumulation of biomass products (SMP and EPS) in ASMBR.

4.4 BFR Dosage Identification

Nutrients removal of SSC wastewater showed a good performance in ASMBR, but fouling became a major problem since this system was connected to the membrane. Controlling the operational parameters is part of reducing the fouling problem. Besides, changing the sludge characteristic by adding BFR is the one of promising approach to prolong ASMBR operation. Natural organics like humic acid

and lipid that basically from bacteria product (Antonelli *et al.*, 2011) have large molecules and have polar groups that naturally contact with surface charge of adsorbents for adsorption. Meanwhile, inorganic matters that have polar charges like oxygen, nitrogen, halogen and sulphur have the tendency to contact with surface charge of adsorbents (Bansal and Goyal, 2005).

4.4.1 BFR Adsorption Performance

PAC mechanisms of adsorption are commonly a combination of physical and chemical adsorption by electrostatic interaction with the carbon surfaces and pollutants (Salman, 2009). Eggshell consists of membrane with protein and calcium carbonate composed in the crystalline shell (Stadelman and Cotterill, 1995; Tsai *et al.*, 2006) and is estimated to have 7000 to 17000 pores (Stadelman and Cotterill, 1995). It is efficient as an adsorbent for organic and inorganic compounds by molecular or ionic bond within its surface (Carvalho *et al.*, 2011; Koumanova *et al.*, 2002; Zulfikar *et al.*, 2013). Zeolite is a porous mineral which consists of hydrated alumino silicate mineral that is able to adsorb effectively for wide range of pollutants with ion exchange property of specific surface area and reversible cavities structure that can easily undergo ion exchange by other ion compounds (Wen *et al.*, 2006). Figure 4.8 showed the image of three different types of BFR; zeolite, eggshell and powdered activated carbon under FESEM analysis. All BFRs showed the craggy and porous surface area that influenced adsorption process.

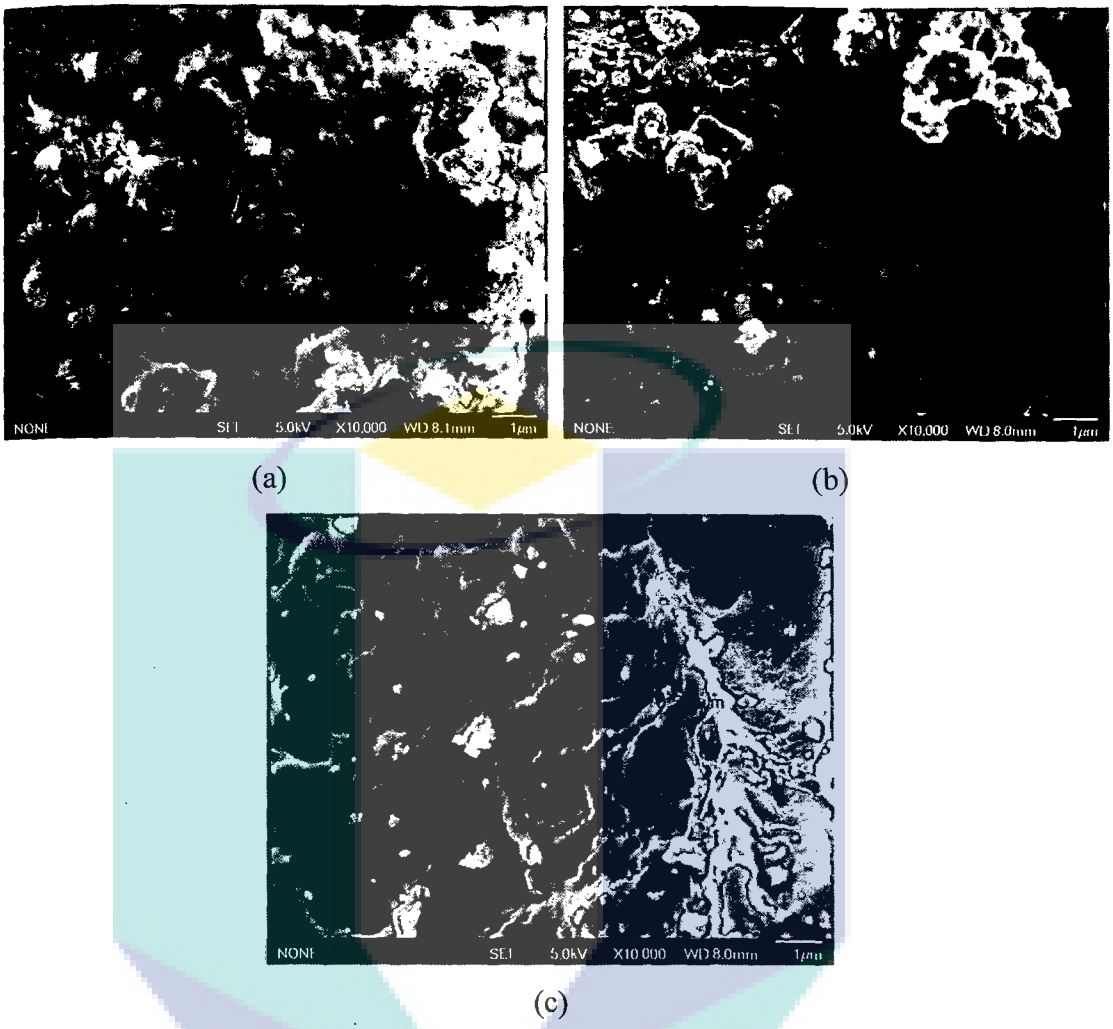


Figure 4.8 Three types of BFRs FESEM (magnification x10000); (a) zeolite (ZEO); (b) egg shell (ES); and (c) powdered activated carbon (PAC).

The BFRs were tested for COD, protein and carbohydrate removal. The optimum dosages were identified through shaker batch experiments and were tested from 2 to 12 g L^{-1}. COD removal result is shown in Figure 4.8. Since protein and carbohydrate are one of the factors that led to biofouling as mentioned in literature, the batch test for both were taken into consideration. Protein and carbohydrate removal have been used to represent microbial product in sludge solution. The adsorption results are shown in Figures 4.9 and 4.11. For the three types of BFRs shown, PAC has the highest percentage adsorption when compared with zeolite and eggshell. Concentrations of 8 to 12 gL^{-1} showed slightly significant differences for COD, protein and carbohydrate removal percentages by adsorption and adhesion processes. As a result, the optimum dosages corresponding with COD, protein and

carbohydrate was 8 gL^{-1} and this dosage was applied in future runs since as higher dosages do not show removal improvement. The same study had been reported in Yuniarto *et al.* (2008), where the dosage was chosen based on higher removal of organics (Yuniarto *et al.*, 2008). Damayanti *et al.* (2011) reported PAC gave a good result in COD removal and SMP as compared ZEO and Mo (*Moringa oleifera*) and optimum removal occurred at 8 g L^{-1}.

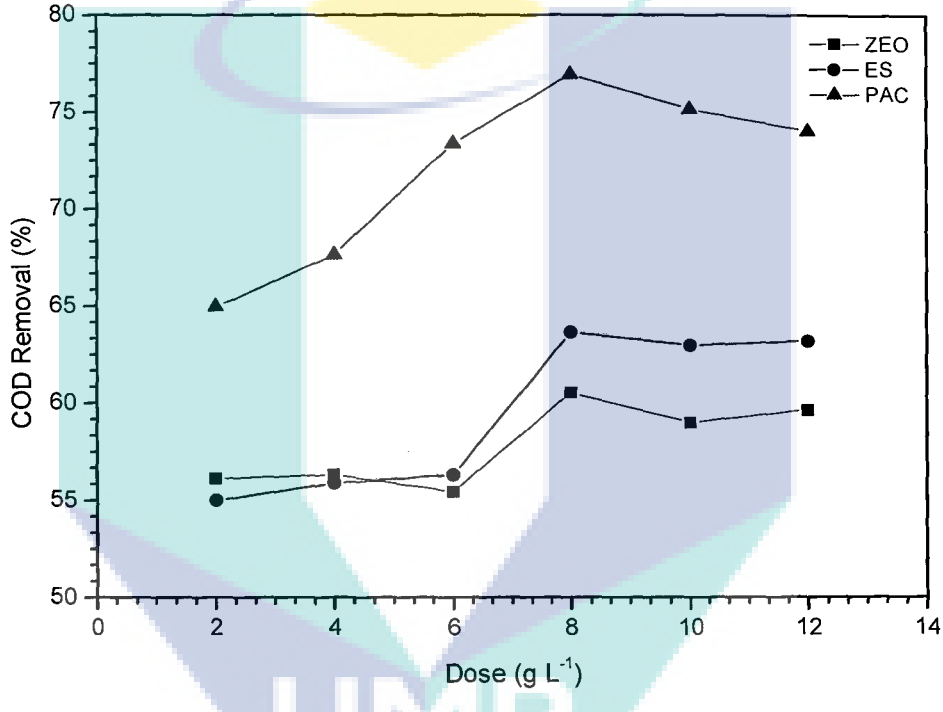


Figure 4.9 Percentage of COD removal for adsorption

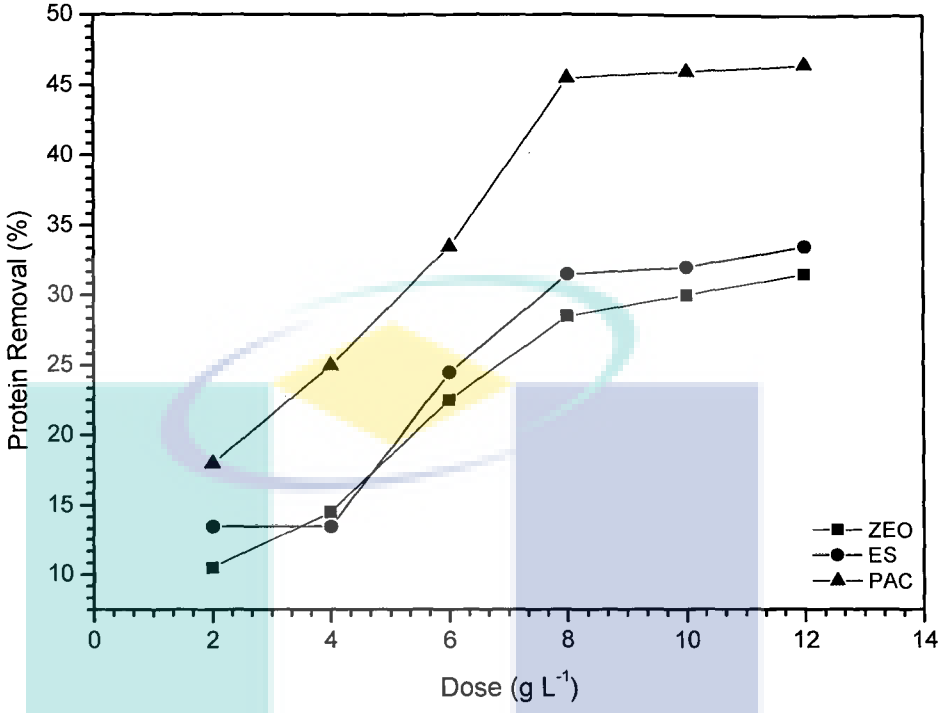


Figure 4.10 Percentage of protein removal for adsorption

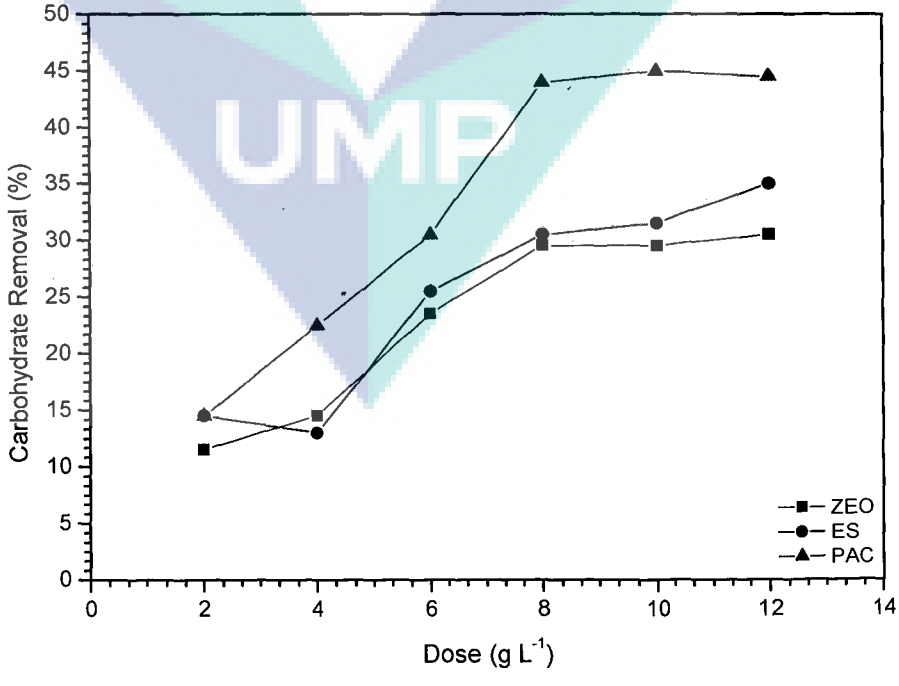


Figure 4.11 Percentage of carbohydrate removal for adsorption

The SSC wastewater connection with BFRs in batch runs were analysed by observing the behaviour of BFRs towards isotherms. Figure 4.10 (a) and (b) showed the adsorption performance for BFRs. Langmuir and Freundlich isotherms were carried out to analyse the adsorption characteristic. These isotherms are most commonly used to weigh the characteristics of the adsorbent surface. Equilibrium adsorption capacity has been illustrated in Equation 4.1. Equation 4.2 was derived and Equation 4.2 expressed Langmuir isotherm while Equation 4.3 expressed Freundlich isotherm.

$$q_e = \frac{V(C_0 - C_e)}{W} \tag{4.1}$$

Langmuir isotherm:

$$\frac{1}{q_e} = \frac{1}{bQ}\left(\frac{1}{C_e}\right) + \frac{1}{Q} \tag{4.2}$$

Freundlich isotherm:

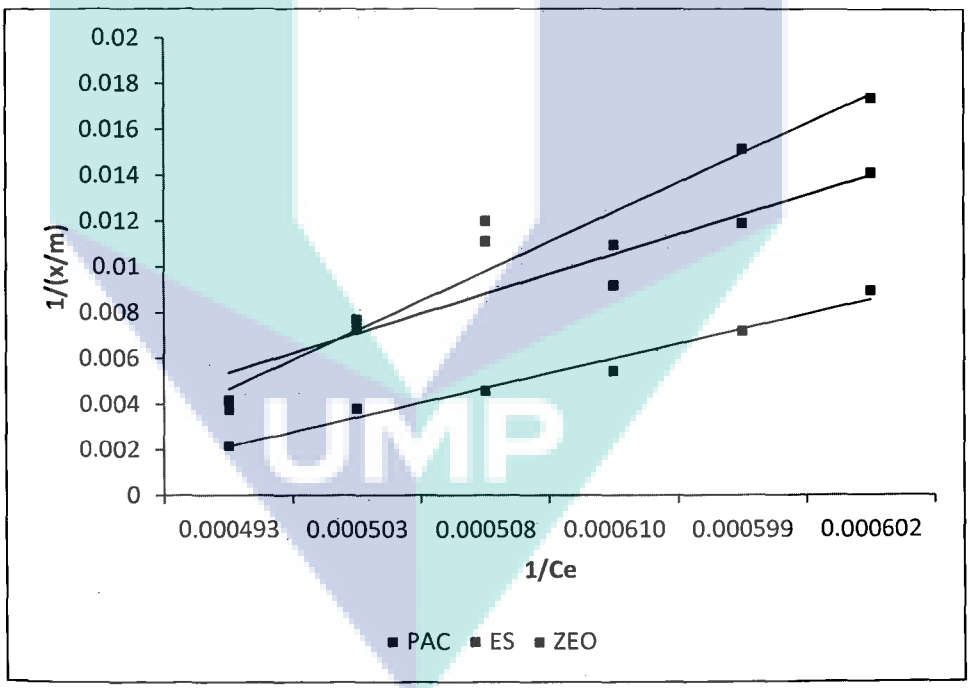
$$\log q_e = \frac{1}{n}\log C_e + K_f \tag{4.3}$$

where q_e or $\frac{x}{m}$ is adsorbent after equilibrium (mg adsorbate/g adsorbent). V is the volume solution where W is the weight of adsorbent. C_0 is the initial adsorbate concentration (mg L^{-1}). C_e is adsorbate equilibrium concentration after adsorption (mg L^{-1}). Q (mg g^{-1}) and b (L mg^{-1}) are Langmuir constants while K_f (mg g^{-1}) (L mg^{-1}) $^{1/n}$ and 1/n are the Freundlich constants (Lin *et al.*, 2014; Maarof *et al.*, 2004).

The data for Langmuir isotherm was plotted and the linear plot was shown in Figure 4.12 (a). The slope (1/bQ) and intercept (1/Q) are shown in Table 4.5. Data for Freundlich isotherm was plotted and the linear plot was shown in Figure 4.12(b). The slope 1/n and intercept K_f are shown Table 4.5. All the constants indicated the maximum adsorption capacity. R^2 value showed the characteristic of adsorbent that measured goodness-of-fit either in Langmuir or Freundlich isotherms, hence the high

value for R^2 recorded for Langmuir isotherm. The negative value for Freundlich isotherm pointed out the insufficiency of the isotherm to describe the adsorption characteristics. This could be an indication that the BFRs have monolayer adsorption surface for SSC in batch runs. These Langmuir adsorptions occurred at absorbent free surfaces (no deposition of adsorbate or the other adsorbate that already adsorbed) (Bansal and Goyal, 2005; Toth, 2001; Tsai *et al.*, 2006). Subsequently, the BFRs were applied in ASMBR treatment of SSC to assess their ability to reduce membrane fouling by their characteristics, hence the prolonged the treatment process. A study of tannery wastewater by Munz *et al.* (2007) showed the adsorption by PAC was fitted into Langmuir isotherm with optimum adsorption at 3 g L^{-1} of PAC concentration and was applied into MBR pilot plant (Munz *et al.*, 2007).

(a)



(b)

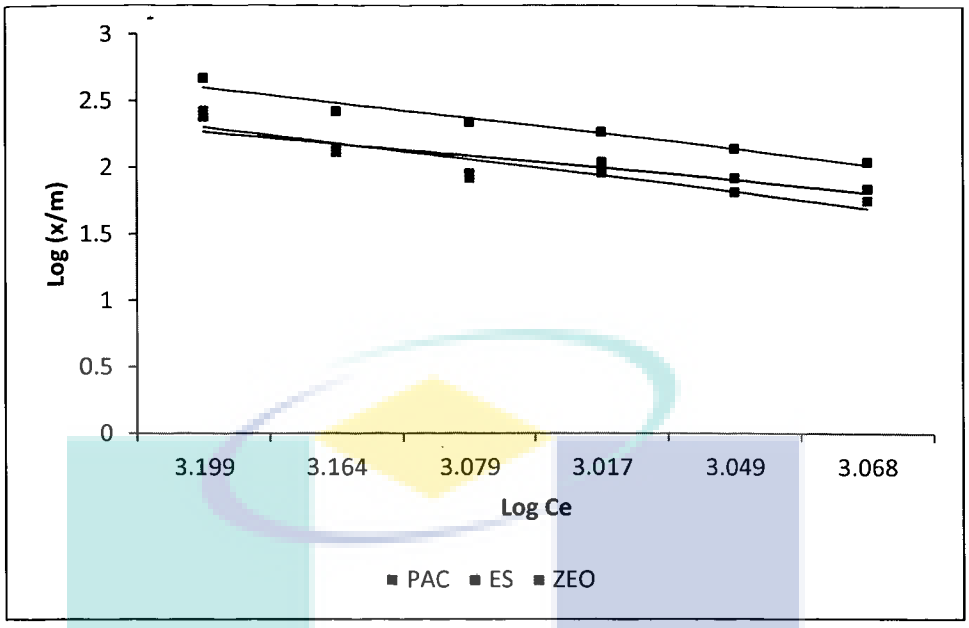


Figure 4.12 BFRs isotherm of (a) Langmuir and (b) Freundlich

Table 4.5: Langmuir and Freundlich expressions for BFRs

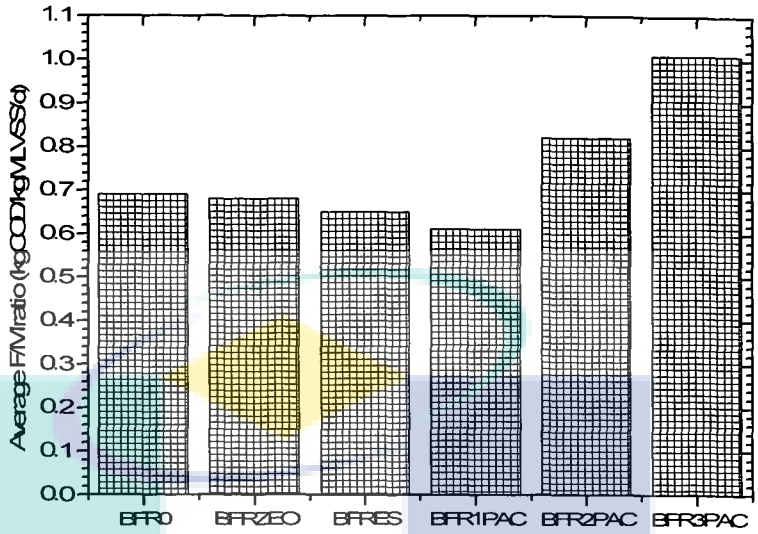
BFR Type	Langmuir		Freundlich	
	Regression Linear	R^2	Regression Linear	R^2
PAC	y = 0.0013x + 0.0009	0.979	y = -0.1143x + 2.7136	0.956
ES	y = 0.0017x + 0.0037	0.853	y = -0.0896x + 2.3569	0.79
ZEO	y = 0.0026x + 0.0021	0.938	y = -0.1203x + 2.4234	0.861

4.5 ASMBR-BFRs Effluent Performance

There are three possible mechanisms that influence pollutant removal in MBR - by adding adsorbent like biological, adsorption and filtration. In aerobic condition, sulphide tends to oxidize and produce sulphate and energy (Bosch, 2008; Bosch *et al.*, 2006) while phenol initially monohydroxylates to form catechol before ring cleavage at ortho position that forms pyruvate and succinate or at meta position that forms pyruvate and acetaldehyde by aerobic microorganism (Martinez *et al.*,

2006; Basha *et al.*, 2010; Sridevi *et al.*, 2012). Microbes utilize phenol for energy and carbon source (Sridevi *et al.*, 2012; Tuah *et al.*, 2009). Adsorbent are well known in purification processes and bacteria tend to bond on surfaces of absorbent in activated sludge to form biosorption trait (Bitton, 2005; Utrilla *et al.*, 2013). Direct filtration by membrane led to cake formation on membrane surfaces that are known as 'pseudo-membrane' which help in pollutants removal by filtration process (Guell *et al.*, 1999; Wu *et al.*, 2011)

ASMBR was operated in varying OL of SSC wastewater from 3.5 to 6.7 kg COD d^{-1} m^{-3} and biodegradability of 0.33 to 0.38 which were considered as high strength wastewater. The operation started with 5 g MLSS L^{-1} at constant operational flux 4 LMH and 40 days of SRT as discussed previously. The sample was taken once in every two days and the test was repeated three times. At constant COD loading, the cell biomass growth increased as when BFRs were added and it can be seen in Figure 4.13 where the value of MLVSS is larger than COD loading that decrease the value of F/M ratio, but F/M ratio increase as COD loading increased. As COD loading increase, the F/M ratios also increase due to high food that needs to degrade by biomass. The presence of BFRs, F/M ratio decreased at constant COD. There were three causes that affect the MLVSS reading which were (i) tendency of BFR to adsorb nutrients and attract bacteria to attach on BFR surface; (ii) the aggressive growth of bacteria on the surface of BFR; and (iii) tendency of BFR to form biosolid as compared to ASMBR without BFR. While Figure 4.14 shows the yield of ASMBR-BRFs runs shows increment as BFRs were added concurrently with COD removal increment.



.Figure 4.13 Average F/M ratio for all BFRs

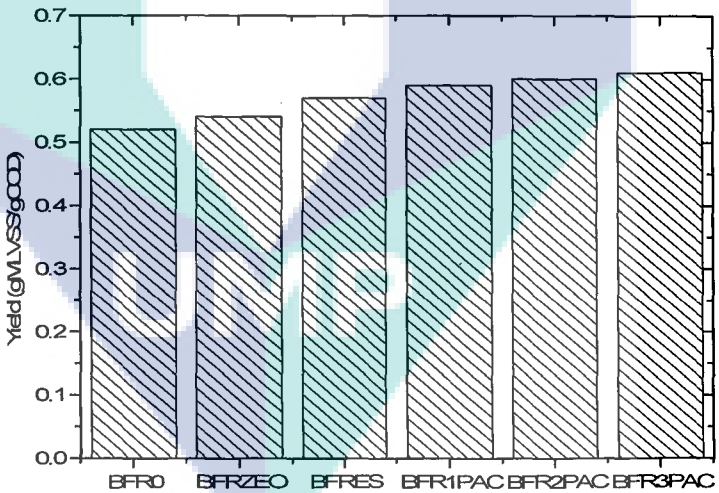


Figure 4.14 Average yield for all BFRs

It was shown in Figure 4.15 (a) where the average of COD removal for BFR0 was 95.1% and it was slightly and significantly different from ASMBR-BFRs runs which had more than 96% COD removal. ASMBR-BFR gave slightly significant different results in compounds removal when compared with ASMBR without BFR.

In ASMBR-BFR0, ASMBR-BFRZEO and ASMBR-BFRES runs, the operations were repeated after chemical cleaning and the MLSS concentration were reduced to original concentration. After the cleaning process, the result showed slight change of COD and sulphate effluent due to change of MLSS concentration and adaptation process after cleaning.

Furthermore, the effectiveness of operation in terms of COD removal for ASMBR-BFRs was compared with ASMBR-BFR0 is 63.2% (BFR1PAC), 36.3% (BFRES) and 24.2% (BFRZEO). This significant result may be affected by adsorption, flocculation processes and the same result have been shown by Damayanti *et al.* (2010) and Yuniarto *et al.* (2013). Damayanti *et al.* (2011) reported that BFRPAC achieved up to 99% while 96% by BFRZEO and 97% by BFRMo (*Moringa oleifera*) and BFRPAC has a favourable nitrification compared to other types of BFRs in POME treatment. Yuniarto *et al.* (2013) recorded that 95% of average organic removal but without BFR, needed longer time to achieved constant organic removal. BFR helped to improve the start-up runs in reducing the fouling rate and the same result could be seen in Akram and Stuckey (2008). Without PAC, the production of SMP increased due to catabolism and cell lysis and SMP was adsorbed by addition of PAC (Akram and Stuckey, 2008).

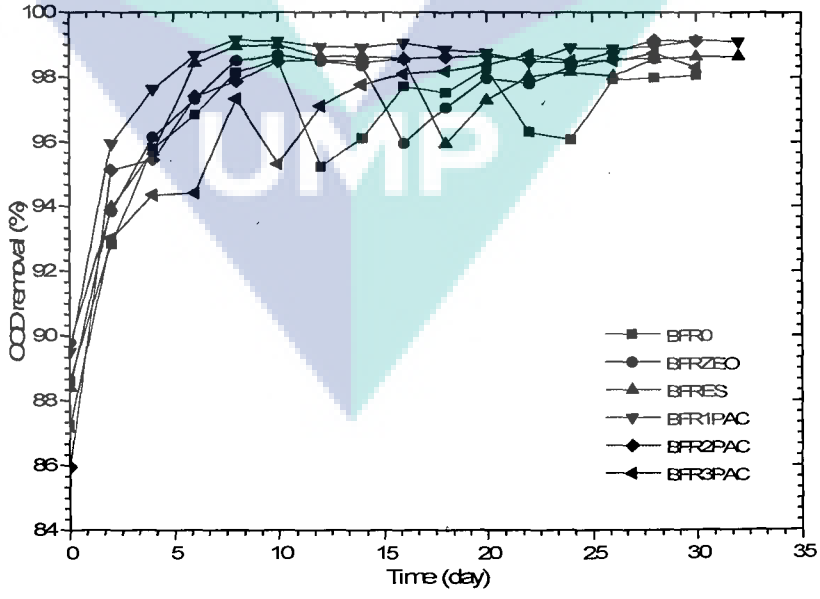
By increasing the OL (COD increase) in ASMBR-BFR2PAC and ASMBR-BFR3PAC as shown in Table 4.6, the COD effluent concentration showed an increment. In ASMBR-BFR1PAC, it took only 2 days to stabilise the effluent concentration below 50 mg L^{-1} while ASMBR-BFR2PAC took 6 days to keep the effluent COD stable below 50 mg L^{-1}. Meanwhile, ASMBR-BFR3PAC took 14 days to stabilise the effluent concentration above 50 mg L^{-1} and it could be seen in Figure 4.15 (b). ASMBR-BFRPAC showed that as OL increased, the inefficiency increased from 50.6% (ASMBR-BFR2PAC) to 70.1% (ASMBR-BFR3PAC) with respect to ASMBR-BFR1PAC with average COD effluent concentration of 89 mg L^{-1} (ASMBR-BFR2PAC) and 152 mg L^{-1} (ASMBR-BFR3PAC). It may be due to food biomass ratio being too high for the system to adapt that were over the limitation for biomass to degrade and/or over the limitation for saturation adsorption and

flocculation activities. The increase of the loading rate (more food supplied) may reduce the oxidation of complex compounds (uncompleted oxidation) (Radjenovic *et al.*, 2008). Yuniarto *et al.* (2013) reported at higher OL, the removal performance decrease with and without BFR were added and BFRPAC gave the stable removal started from day 4 as compared BFRZEO (day 10) and without BFR (day 10).

Table 4.6: The influents for ASMBR-BFR2PAC and ASMBR-BFR3PAC

Parameter	**OL2 (BFR2PAC)**	**OL3 (BFR3PAC)**
COD (mg L^{-1})	3360±83	4450±151
BOD (mg L^{-1})	1180±25	1710±17
Sulphide (mg L^{-1})	173±13	241±11
Sulphate (mg L^{-1})	530±22	623±20
Phenol (mg L^{-1})	61±9	170±5
Total Nitrogen (mg L^{-1})	27.2±0.5	27.8±1
Total Phosphate (mg L^{-1})	15±1	14±2

(a)



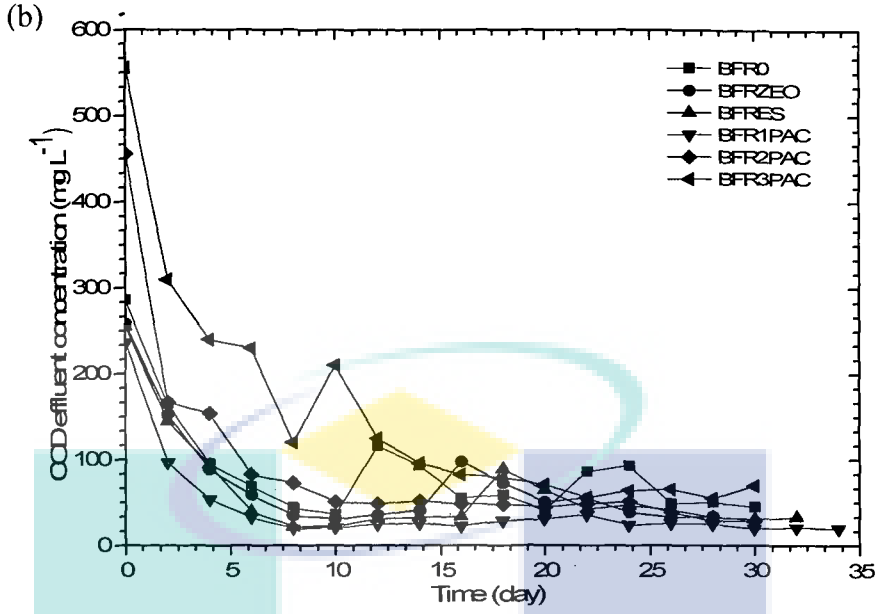


Figure 4.15 COD performance in ASMBR-BFRs; (a) percentage of COD removal; and (b) COD effluent concentration

The sulphide analysis showed 99% of sulphide removal for every run with loading rate from 0.14 to 0.36 kgS m^{-3} d^{-1}. The effluent results shown in Figure 4.16 recorded 9 and 2.6 times of sulphide effluent concentration ratio for BFR2PAC and BFR3PAC with regard to BFR1PAC respectively. ASMBR-BFR3PAC recorded high effluent concentration that may be due to very high loading of sulphide that was over the capability of biomass to degrade, adsorption and flocculation activities. Sulphide effluent concentration inefficiency increased as the loading rate was increased from 61.8% (ASMBR-BFR2PAC) to 88.8% (ASMBR-BFR3PAC) with sulphide effluent concentration 0.013 mgL^{-1} (ASMBR-BFR2PAC) and 0.045 mgL^{-1} (ASMBR-BFR3PAC) with respect to ASMBR-BFR1PAC.

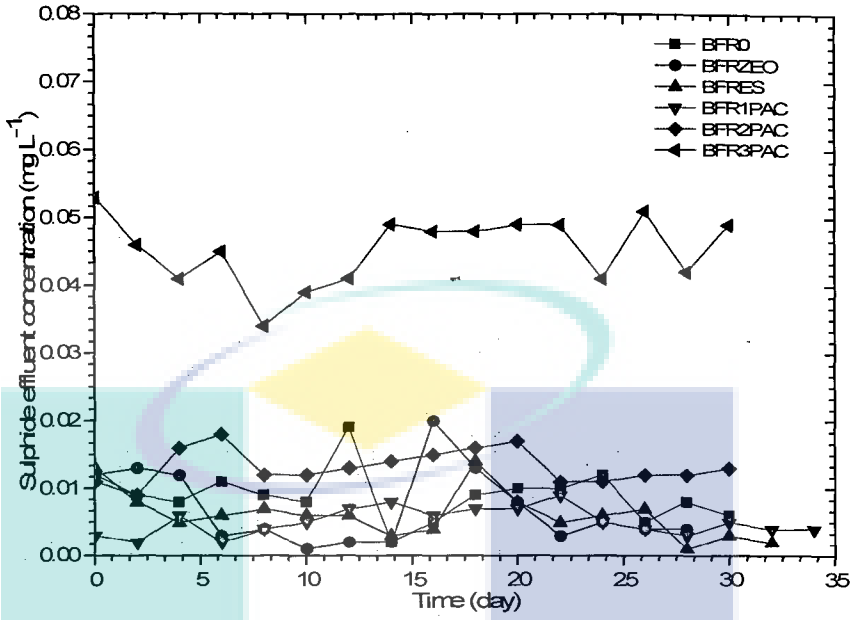


Figure 4.16 Effluent performance for sulphide in ASMBR-BFRs

Figure 4.17 (a) showed the average sulphate performance at 46% of sulphate formed in BFR0 run. The effluent concentration reading decreased showed statistical significance in ASMBR with BFR runs (Figure 4.17 (b)). Hence the effectiveness of operations in terms of sulphate reduction was 39.3% (ASMBR-BFR1PAC), 26.9% (ASMBR-BFR2PAC) and 19.9% (ASMBR-BFR3PAC) with respect to ASMBR-BFR0. This reduction may have occurred due to adsorption and flocculation activities in the reactors. ASMBR-BFR2PAC and ASMBR-BFR3PAC showed that the sulphate effluent concentration increased from 30% (707.1 mg L^{-1}) and 43% (868.1 mg L^{-1}) with respect to ASMBR-BFR1PAC. This may have occurred due to high sulphate production that was over the limitation of adsorption and flocculation activities in the reactors that led more sulphate escaping from the system. Figure 4.18 showed the average total nitrogen, total phosphate and phenol effluent result. Phenol loading rate was from 0.066 to 0.255 kg Phenol m^{-3} d^{-1} and it showed that more than 99% of phenol removal in all ASMBR runs with and without BFR. The reading of TN and TP were statistically significant with almost 98% and 97% average removal of TN and TP respectively.

The successful removal could be seen in ASMBR-BFR0, but by applying BFRs, it enhanced the removal. The difference of effluents reading for each BFR run may be due to adsorption and flocculation processes occurring in the reactor besides degradation by biomass and filtration by membrane and 'pseudo membrane'. The problem within ASMBR-BFR0 was that the membrane biofouling increased drastically which increased the chemical cleaning process (reduce membrane lifespan). In a study of membrane flocculation adsorption by Vigneswaran *et al.* (2004) it showed similar mechanisms that affected the removal of organic matter.

There is no study reported regarding the treatment of SSC by ASMBR-BFRs and also no study recorded on the use of powdered eggshell as BFR in MBR. Hence, this study is considered as a new finding in MBR area. The study of sulphide removal by Lohwacharin and Annachhatre (2012) using airlift bioreactor showed 93% of sulphide removal and 90% sulphate was formed when sulphide influent increased up to 2.2 kgS $m^{-3}d^{-1}$ with 290 mgL^{-1} of sulphide concentration and high sulphur formed (incomplete oxidation) as sulphide concentration increased due to sulphide oxidation to sulphates reaches the maximum electron transferring capacity. It led to more metabolic pathway was shifted to oxidation of sulphide to sulphur as sulphide loading was increased (Lohwacharin and Annachhatre, 2010). Barrios-Martinez *et al.* (2006) studied on treating phenol wastewater by MBR showed 100% efficiency for COD removal and 98.6% for phenol removal when biodegradability of influent was 0.43 (Martinez *et al.*, 2006). In a study by G. Munz *et al.* (2007), they determined that organic pollutants possibly were partly adsorbed by PAC (Munz *et al.*, 2007).

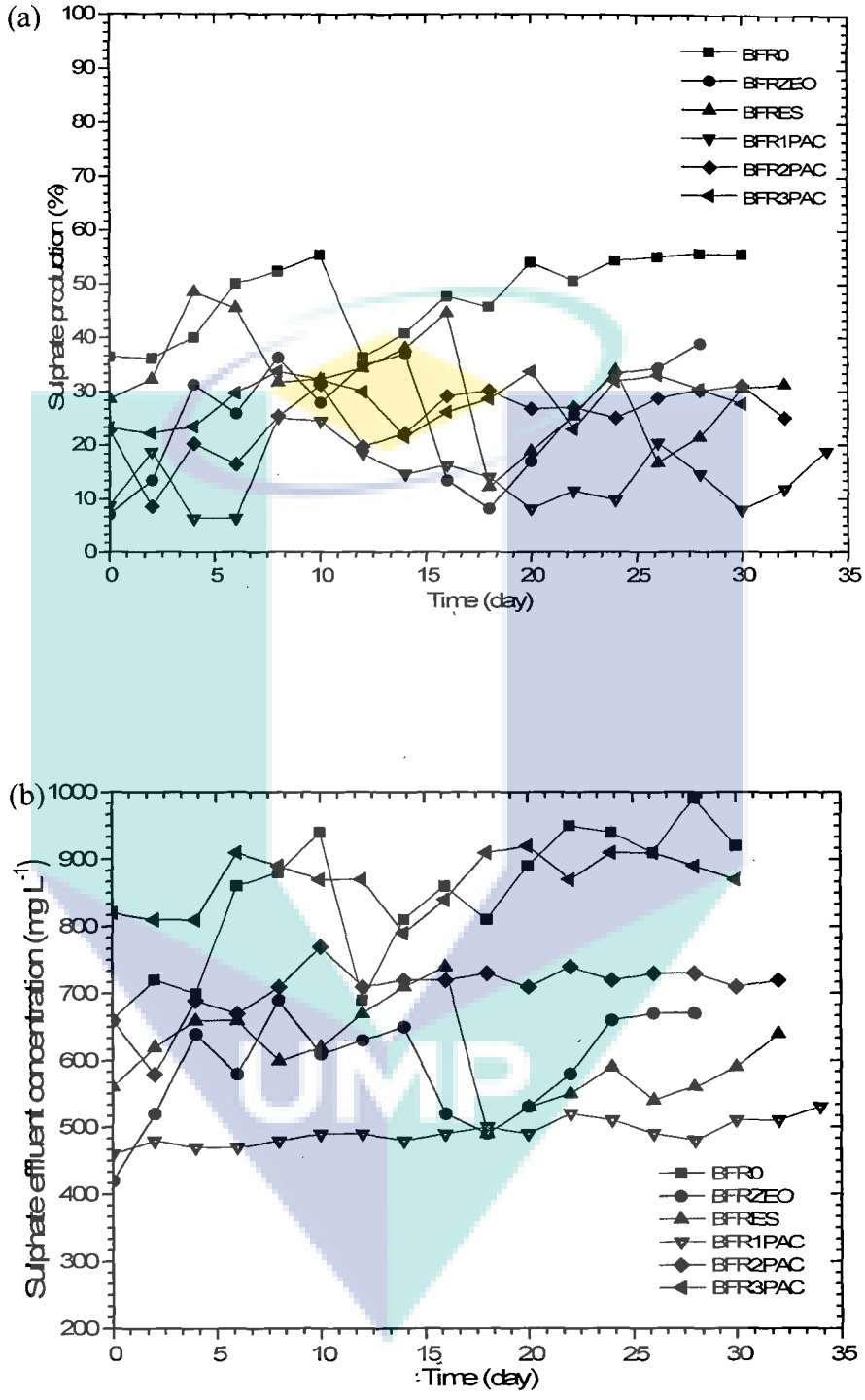


Figure 4.17 Sulphate performance in ASMBR-BFRs; (a) sulphate production percentage; and (b) sulphate effluent concentration

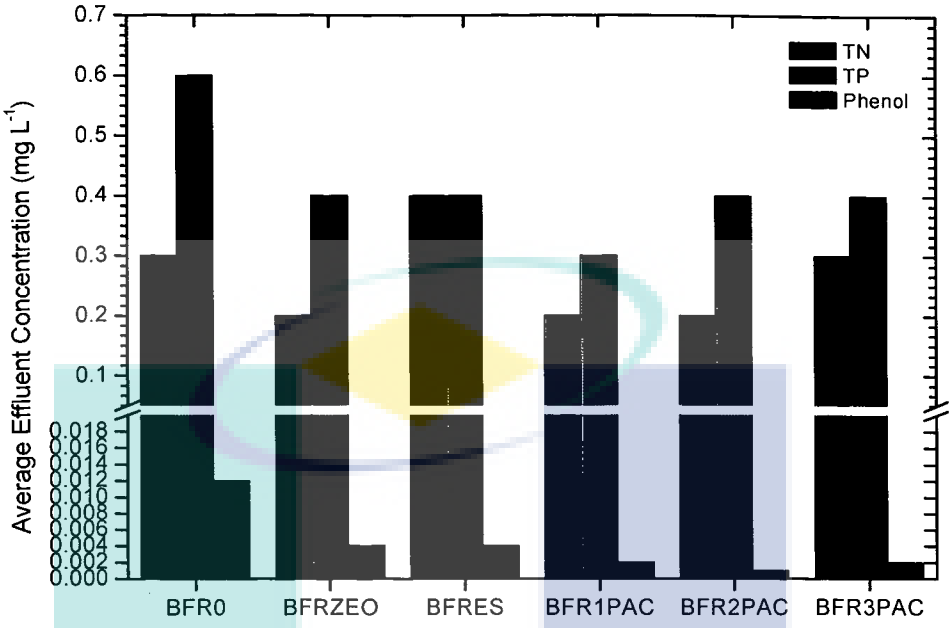


Figure 4.18 Effluent performance for total nitrogen, total phosphate and phenol in ASMBR-BFRs

4.6 ASMBR-BFRs SPM and EPS Performance

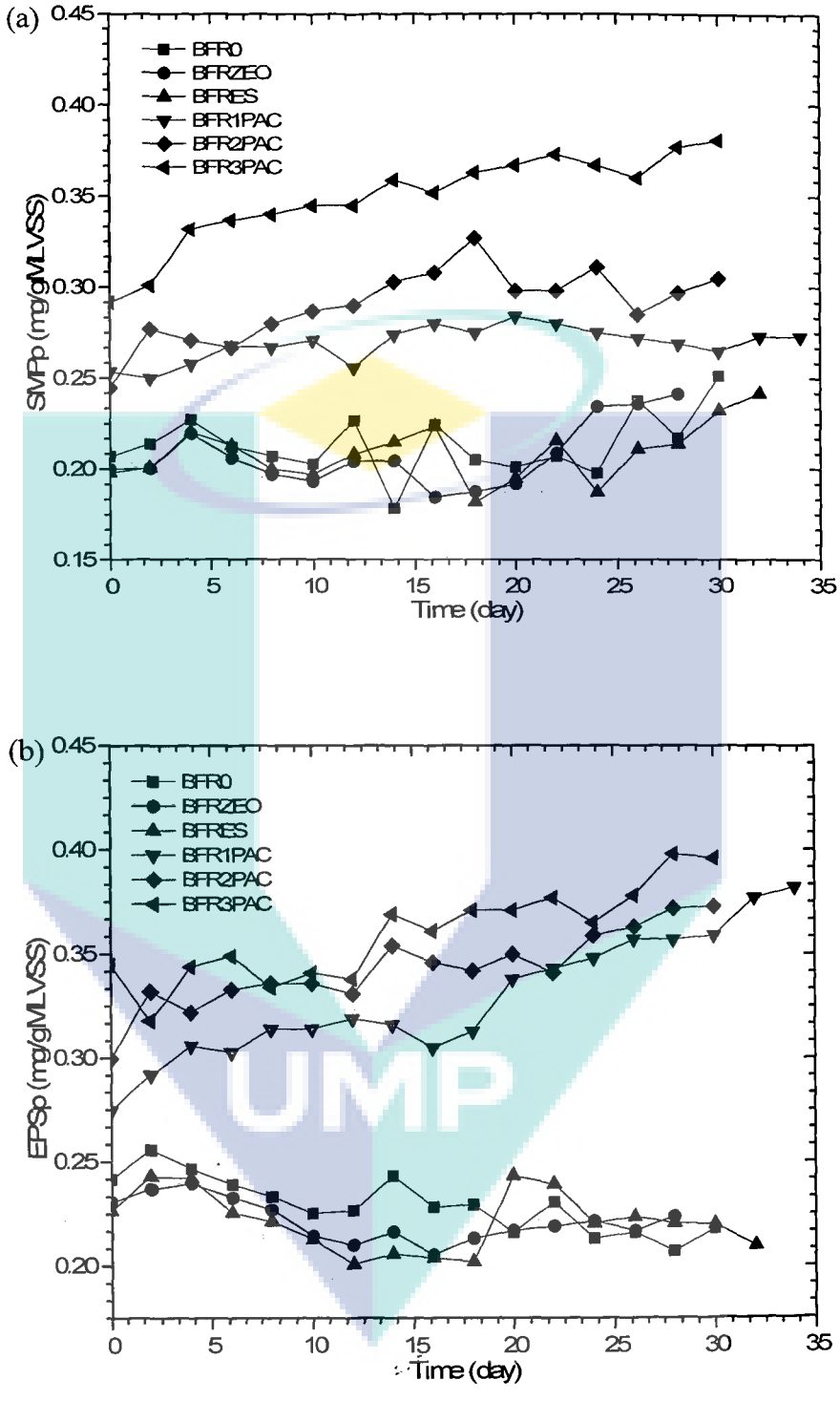
Metabolisms of microorganism produce products like SMP and EPS. SMP are released during substrate metabolism or microorganism decay while EPS are located outside the cell surface. SMP and EPS are composed of protein and carbohydrate concentration and EPS are categorised as bound EPS and soluble EPS while SMP as pool of organic compounds (Mutamim *et al.*, 2013b; Pan *et al.*, 2010). Proteins are more hydrophobic than carbohydrate but both have tendencies to deposit on the membrane surface due to vacuum driving force of the membrane as reported by Pan *et al.* (2010) and Liang *et al.* (2007) (Liang *et al.*, 2007; Pan *et al.*, 2010). SMP and EPS also have the tendencies to deposit on membrane surfaces to form dense biolayer.

Figure 4.19 (a), (b), (c) and (d) showed the results of SMP and EPS in the reactor. This study showed that carbohydrate dominates in bulk solution after normalization with MLVSS has been done. As a result there was an increment in the amounts of SMP and EPS when BFRs were added. The increment shows that there were biosorption and bioflocculation occurred in bulk solution. Constant COD concentration recorded high amounts of SMP and EPS with average total of 3.95 mg $gMLVSS^{-1}$ of BFR1PAC when compared with BFRZEO and BFRES with 2.89 and 3.129 mg $gMLVSS^{-1}$ respectively. BFRZEO, BFRES and BFR1PAC showed high accumulation of SMP and EPS when compared with BFR0 and that could be due to biosorption and bioflocculation process. Observed yield (mg(SMP and EPS) $gCOD^{-1}$ d^{-1}) was recorded increase at constant utilised COD as BFRs were added; BFR0 (0.127), BFRZEO (0.135), BFRES (0.147) and BFR1PAC (0.218). These were correlated to the tendency of BFR to adsorb microbial products and formed large biofloc (high microbial product flocculate in biofloc) in bulk solution and/or increase the growth of biomass (produce high microbial products) due to tendency of BFR to attach, grow and develop biomass on BFR surface (Remy *et al.,* 2010; Yuniarto *et al.,* 2013).

By increasing the COD influent, SMP and EPS accumulation in bulk solution increased with average total of 4.42 and 4.75 mg $gMLVSS^{-1}$ for BFR2PAC and BFR3PAC respectively. When the concentration of OL was increased, there was high degradation of the substrate and cell lysis and released of high formation of biomass products and free soluble biomass product that over the limit capacity of BFR and reduced the BFR capability efficiency. However, the increase of SMP and EPS in bulk solution had low effect on TMP performance of ASMBR-BFR2PAC and ASMBR-BFR3PAC since TMP rate slightly increased with the increase in OL when compared with ASMBR-BFR1PAC. This could be due to high free soluble biomass products and non-biodegradable at high OL. Meanwhile, Y_{obs} (mg(SMP and EPS) $gCOD^{-1}$ d^{-1}) was recorded decrease as OL increased that may be correlated to high utilised COD; BFR2PAC (0.183) and BFR3PAC (0.162). Carbohydrate SMP and EPS show higher concentration as compared protein SMP and EPS. It also indicated that carbohydrate were most likely the major foulant and the same result recorded by Yuniarto *et al.* (2013).

In the effluent analysis, BFR0 showed the highest containment of SMP and EPS, but lowest in bulk solution. Although ASMBR with BFR recorded high SMP and EPS in reactor bulk solution, but they were lower in the effluent (Figure 4.20). This may be due to BFRs flocculation and adsorption of the organic compounds, SMP and EPS were taking place in the bulk solution and small parts of free soluble SMP and EPS were discharged through as soluble organic compounds in the effluent. BFRPAC had the possibility to form large and stable biofloc structure that may bind the SMP and EPS throughout the adsorptive process since all BFRs used have the ability in adsorption and hence, enhanced the removal of compounds. BFRPAC has high tendency to adsorb SMP in bulk solution and release less soluble SMP in the effluent. Besides, EPS also had the propensity to enhance bioflocculation to form bigger bioflocs and this led to high EPS results for BFRPAC and less soluble EPS effluent.

The bigger bioflocs also tended to reduce membrane fouling due to more permeable flocs being formed and reducing hydraulic resistance. According to Yuniarto *et al.* (2013), SMP and EPS normalized to biomass in the reactor (as MLVSS) over the experiment period of each variation. The result confirmed that the concentration of SMP and EPS in bulk solution which was regarded as soluble SMP and EPS released during cell lysis, decreased due to adsorption process (Damayanti *et al.*, 2011), and/or lost during synthesis biomass, and/or already attached onto membrane surface as bio-layer (Remy *et al.*, 2010). In this study also shows carbohydrates SMP and EPS in bulk solution is 6.7 time in average more than proteins SMP and EPS. The lower release of proteins SMP and EPS indicates that the biomass was active and there was no situation of stress (Andrade *et al.*, 2013).


(a)
BFR0
BFR2EO
BFRES
BFR1PAC
BFR2PAC
BFR3PAC
SMPp (mg/gMLVSS)
Time (day)
(b)
BFR0
BFR2EO
BFRES
BFR1PAC
BFR2PAC
BFR3PAC
EPSp (mg/gMLVSS)
Time (day)

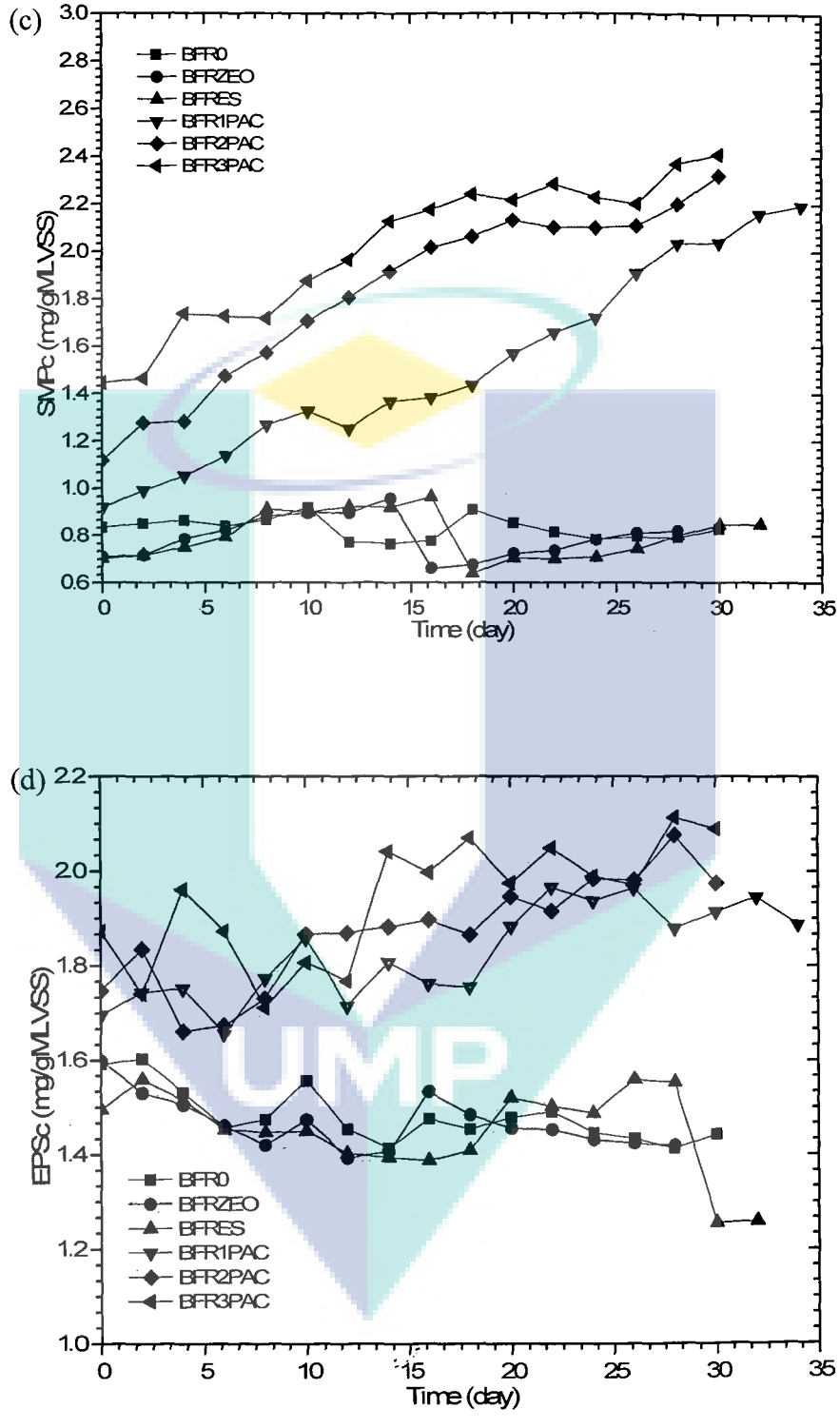


Figure 4.19 Protein and carbohydrate of SMP and EPS concentration in ASMBR-BFRs; (a) SMPp; (b) EPSp; (c) SMPc; and (d) EPSc.

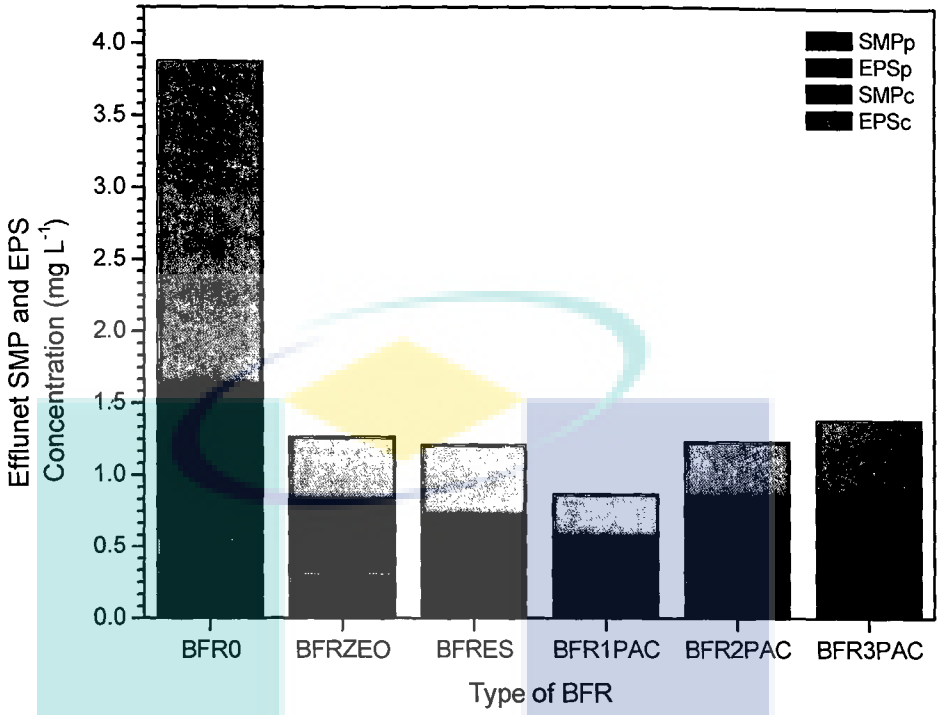


Figure 4.20 The effluent for SMP and EPS for all BFR runs

4.7 ASMBR-BFRs TMP Performance

The maximum adsorption dosage of BFR from batch experiment was added to ASMBR to examine its effect on fouling reducer performance. Flux is an important factor in membrane fouling process and the determination of critical flux is the limitation operation of ASMBR with or without BFR and to prolong operation time. Critical flux was the initial examination for the short-term fouling effect test. Data from flux step method (Appendix C) were used to evaluate the rate of TMP change (dTMP dt^{-1}) and the plot is shown in Figure 4.21. The flux region was divided into two regions; dTMP dt^{-1}<0.5 kPa min^{-1} which was considered as the sub-critical flux while dTMP dt^{-1}≥0.5 kPa min^{-1} was the super-critical flux. The value of dTMP dt^{-1}≥0.5 kPa min^{-1} was defined as the critical flux and BFRPAC shows the

highest value of critical flux which was followed by BFRES and BFRZEO with 26.8LMH, 13.4LMH and 12LMH respectively. All the BFRs critical flux were statistically significant and enhanced the ASMBR operation in short term run. The critical flux enhancement recorded were 30.6%, 38.29% and 69.1% for BFRZEO, BFRES and BFRPAC respectively corresponding to BFR0. Yuniarto *et al.* (2013) reported increase trend of critical flux as BFR added and BFRPAC gave higher critical flux reading with 42LMH (110%) more than BFR0.

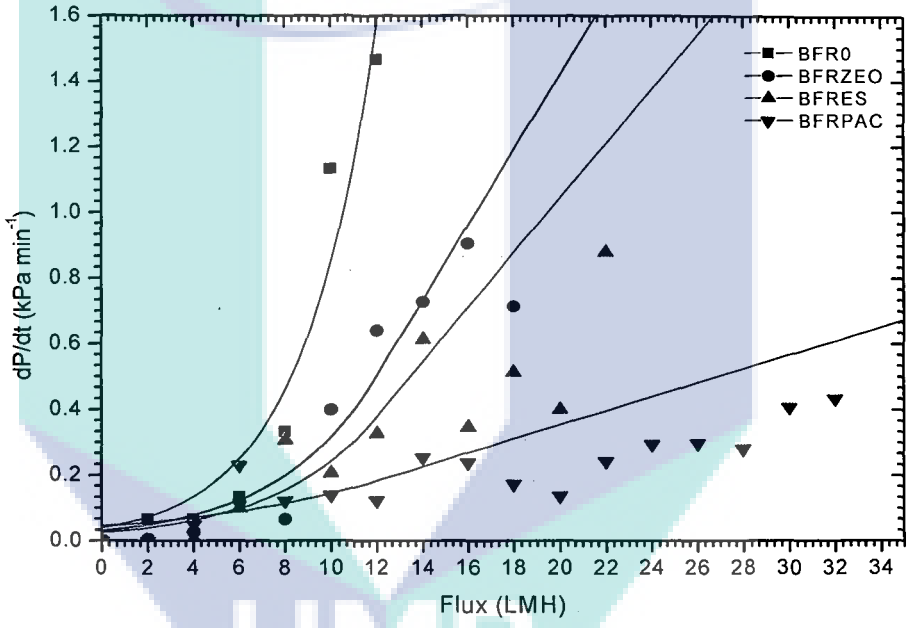


Figure 4.21 Rate of TMP for BFRs

The effect of BFR was continued by observing long-term run for TMP performance using fixed flux 4LMH that lied on the sub-critical flux region for all critical flux examination tests either ASMBR without or with BFRs. Figure 4.22 showed the TMP trends for ASMBR-BFRs runs. The operations were run with SRT of 40 days with 5 gMLSS L^{-1} for starting. Permeate/relax protocol was also applied for all runs. The increase in TMP was observed until it levelled off. From day 0 to 5, it showed a small TMP increment that indicated biofouling that might gradually have occurred. The ASMBR-BFRs showed 35, 51 and 92% of fouling reduction of BFRZEO, BFRES and BFRPAC when compared with BFR0 respectively. After a

long operation, progressive blocking of membrane pore occurred due to deposition of biofoulant that reduced filtration effectiveness. In some extension of membrane fouling, part of membrane regions reached critical local filtration and formed a thick biocake layer that led to a sharp turning point of TMP curve. Yuniarto *et al.* (2013) stated the gradual TMP rise and values continued to be lower as BFR added and BFRPAC produced lower TMP (below 43 mbar) for 68 days as compared to without BFR (below 73 mbar; 43 days).

ASMBR-BFR1PAC recorded less TMP increment in the beginning and the curve started to increase gradually on the 11th day. It could be clearly seen that BFR1PAC significantly improved prolonging the ASMBR operation without cleaning process. BFR1PAC was also applied to different COD loading and the result showed slight differences between BFR1PAC, BFR2PAC and BFR3PAC. At different COD loading, BFR2PAC had performed with rate of change of 0.14 kPa day^{-1} of TMP when compared with BFR1PAC and BFR3PAC with dP dt^{-1} 0.15 and 0.21 kPa day^{-1} respectively. There was fouling rate increase of 12% and 19% for ASMBR-BFR2PAC and ASMBR-BFR3PAC with regard to ASMBR-BFR1PAC that was due to increase of inert particulate matter with increase in the OL. The gradual change of TMP performance can be seen from day 11 towards the end and showed that BFR1PAC contributed less increment when compared to BFR2PAC and BFR3PAC at 0.98, 1.07 and 1.06 kPa day^{-1} respectively. According to Damayanti *et al.* (2011), by adding BFR produced flocs that had better structure and/or formation and possibility to form bigger and better flocs structure (Damayanti *et al.*, 2011; Remy *et al.*, 2010). Yuniarto *et al.* (2013) recorded sudden TMP increase as OL increase with or without BFR but BFRPAC had rapid recovery as OL maintained to original loading.

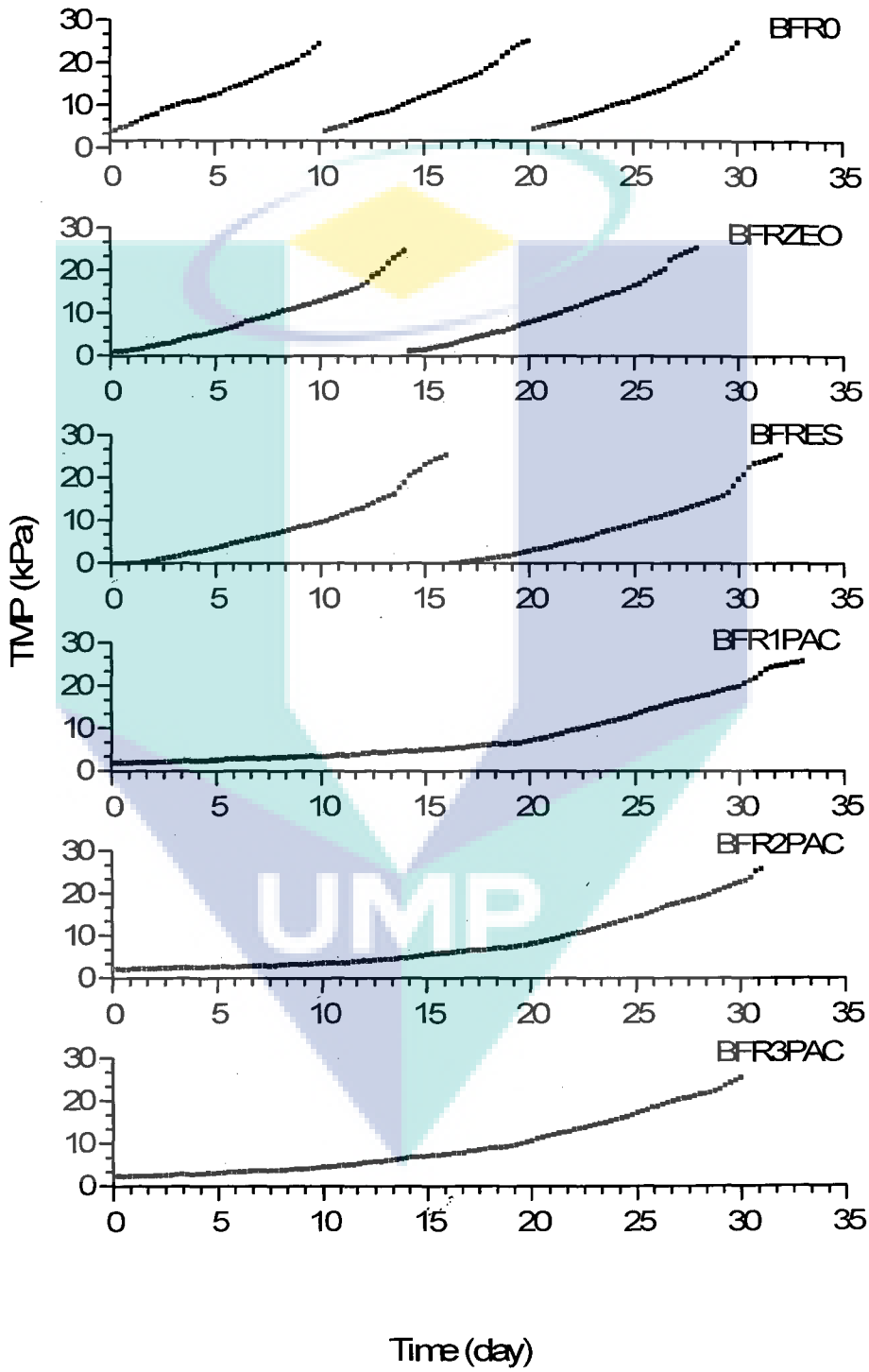


Figure 4.22 TMP trend of BFR0, BFRZEO, BFRES, BFR1PAC, BFR2PAC and BFR3PAC.

membrane especially at the area of membrane epoxy. These were due to compact spaces and difficulty for aeration bubbles to reach this area for scouring effect.

In conventional activated sludge plant, PAC is used to increase the biofilm activated carbon adsorption in and increase the settleability of activated sludge by increased the biofloc size (Munz *et al.*, 2007). A study by Satyawali and Balakrishnan (2009) reported that MBR with PAC additive was 8 days longer than MBR without PAC in distillery wastewater that indicated through small rise of TMP. In this study BFR corresponded to (i) adsorb and flocculate foulant and form 'filter' in bulk solution and reduce the foulant from settling on membrane surface; (ii) increase the porosity of biocake layer (and/or bio-layer) by forming a loose biocake layer structure (Li *et al.*, 2005; Satyawali and Balakrishnana, 2009b). The deposition of biocake layer on the membrane surface are mainly caused by permeate flux velocity (Drews, 2010). Hence increasing the porosity of biocake layer increased the filterability and reduced the membrane resistance.

The operation of ASMBR without BFR, BFRES and BFRZEO showed the repetition of run after cleaning process had been taken. Running operation without BFR showed the repetition occurred at every 10 days of operation while BFRZEO was for every 14 days and BFRES was for every 16 days caused by biofouling effect. Physical and chemical cleaning processes had taken place in this process due to reversible and irreversible fouling. Reversible fouling was due to external biocake deposition and it is removed by physical cleaning process including back flushing and permeate/relax cycle while irreversible fouling was removed by chemical cleaning process. When irrecoverable fouling occurred, the deposition cannot be removed by any physical cleaning process and it commonly happens for long period operation (Drews, 2010). On the systems with BFR, biolayer was even thinner as compared without BFR and it should be noted that biolayer was not happed uniformly on the entire membrane surface.

4.8 Morphology of Biomass, Membrane and BFRs

By using PAXIT Image analyser, wet sample biomass activity have been captured with x40 magnification and shown in Figure 4.23 (a) – (d). Figure 4.23 (a) showed that the biomass was more dispersed when compared with Figure 4.23 (b) – (d) where with the presence of BFR's tendency to attach on the surface of BFRs before the bigger and stable biofloc were formed, grew and developed. The bulk solution without BFR turned to slimy with the tendency to stick on the surface of membrane to form gel layer (biolayer) and led to severe fouling and weak flocs were formed which were easily broken to free suspended matter under turbulent aeration. The accumulation of SMP and EPS formed flocs, gel layers and freely suspended matter with the same result reported by Drews (2010).

The same result can also be seen in Li *et al.* (2005) where the destruction of flocs released colloidal, soluble components and soluble microbial products. Consequently, the released particles filled the void space between biomass in biocake layer and formed a dense biocake layer that led to increased filtration resistance. In ASMBR-BFR runs, part of SMP and EPS and other compounds attached and accumulated with BFRs to form slimy and sticky surfaces. They reduced the amount of foulants deposited in membrane pores and reduced a dense mass of biocake layer formed on the membrane surface. In Li *et al.* (2005), the study showed that addition of PAC in the systems was surrounded by microbial which formed a strong flocs structure and a loose and rigid biocake layer with higher porosity of 'psuedo-membrane'. Hence it filtered out microbial cells and microbial products from deposits on membrane surface.

Figure 4.24 showed the result of BFRs before and after runs and the deposit could be seen at BFRs after runs. All BFRs had porous surfaces that have the ability to adsorb by disperse force. The result showed the hole with craggy, crevasses and ridges surface of BFRs that have the tendency to become surfaces for biomass attach. Attachment, growth and development of bioflocs occurred due to adhesion and

adsorption of BFR surface to the substrate, nutrient and oxygen that formed suitable environment (for biomass biodegradation) to attract more biomass to attach. Figure 4.25 (a) – (b) showed the deposition of biocake layer on the surface of the membrane. In FESEM analysis for BFRs and membranes, dry samples had been used. Figure 4.25 (b) - (d) showed cross section of membrane after BFR was added. The biocake layer deposited on the membrane surface and was found that the biocake formed were uneven and were found on a few spots of membrane surface due to uneven air scouring and water turbulence from aeration. More porous biocake layers were formed in ASMBR-BFRs and when compared with ASMBR-BFR0 and ASMBR-BFRPAC showed the highest porosity by observation using the FESEM analysis.

The mechanisms that affected bacteria attachment to the BFR surface are biospecific (protein-carbohydrate or protein-protein) and non-biospecific (hydrophobic or electrostatic) (Daeschel and McGuire, 1998; Ostuni *et al.*, 2001). BFR was also characterized as hydrophobic and had interaction with bacteria to form strong flocs and reduced deflocculation (Chapman *et al.*, 2001; Daeschel and McGuire, 1998; Liu *et al.*, 2013; Ostuni *et al.*, 2001; Remy *et al.*, 2010; Toth, 2001). Based on BET analysis, BFR surface area was 1178.44m^2 g^{-1} (BFRPAC), 3.25m^2 g^{-1} (BFRES) and 32.59m^2 g^{-1} (BFRZEO). BFRPAC have shown the highest removal performance due to larger surface area for the highest adsorption capacity and highest adhesion surface area and had successfully reduced the biofouling that may be due to its ability to improve and develop strong biofloc.

BFRPAC also showed higher SMP and EPS in bulk solution and less soluble microbial product at effluent that indicated higher adsorption, adhesion and flocculation. Bacteria that adhered on BFR surface also have the tendency to adhere to each other due to carbohydrate-protein or protein-protein attraction to form bigger bioflocs besides interaction between biomass and surface of BFRs that increase the strength structure of bioflocs. The deposition of mixed biomass and BFRs on membrane surface still occurred due to vacuum driving force. However, BFRPAC changed the bioflocs structure that formed high porosity of biofloc and deposited to

form porous biocake layer on the membrane surface. The porous biocake layer was more rigid and loose (pseudo-membrane) and helped to increase the filterability and reduced the membrane biofouling. The same result was reported in Li *et al.* (2005) (Li *et al.*, 2005).

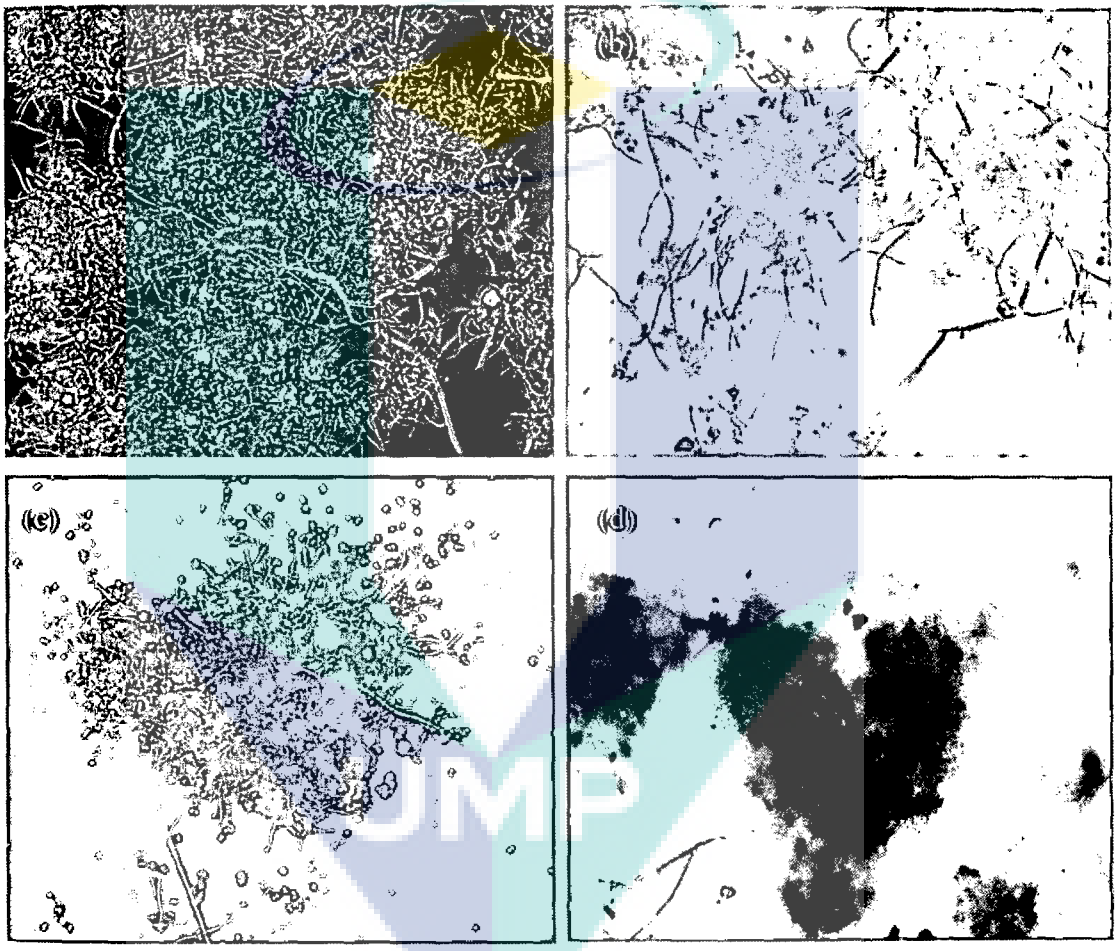


Figure 4.23 PAXIT (magnification x40) image for different BFR, (a) BFR0; (b) BFRZEO; (c) BFRES and (d) BFRPAC

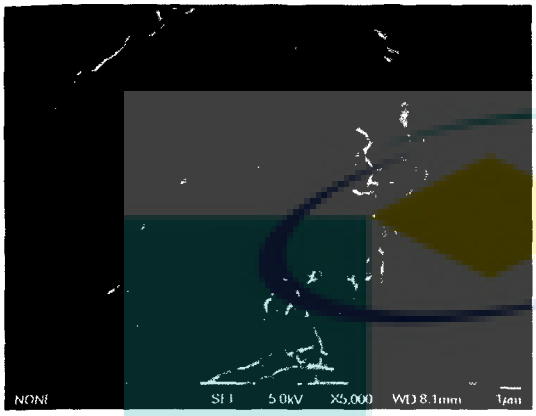
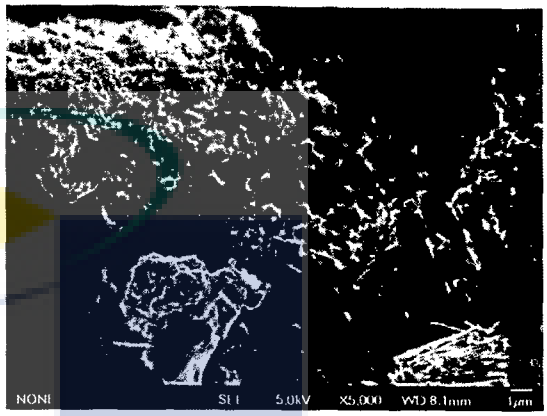
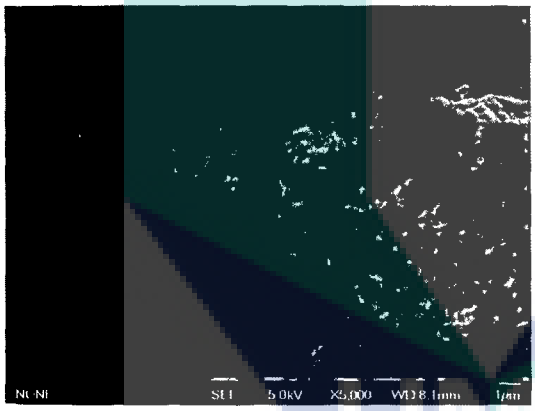
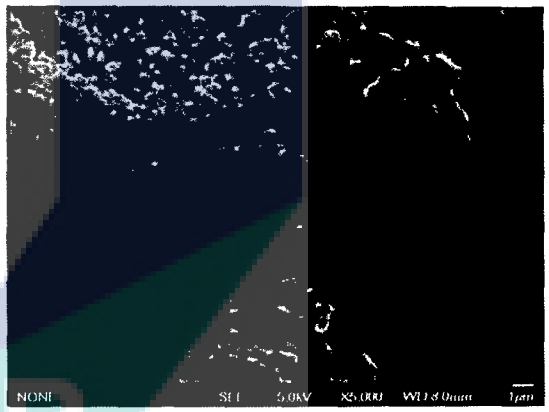
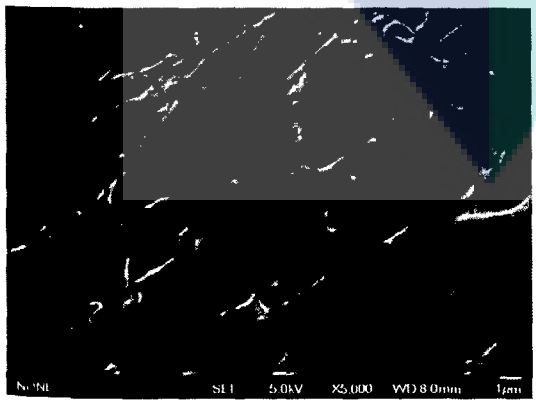
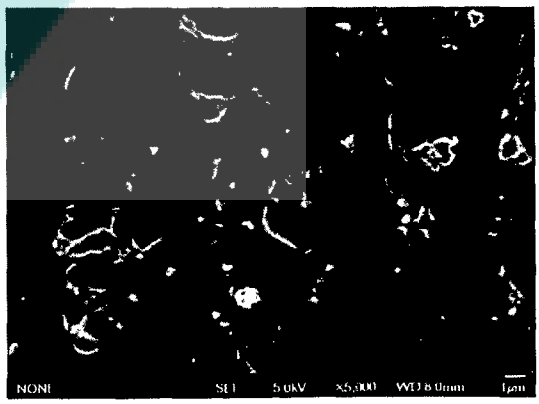
	Before	After
BFRZEO		
BFRES		
BFRPAC		

Figure 4.24 BFRs before and after treatments (magnification x5000)

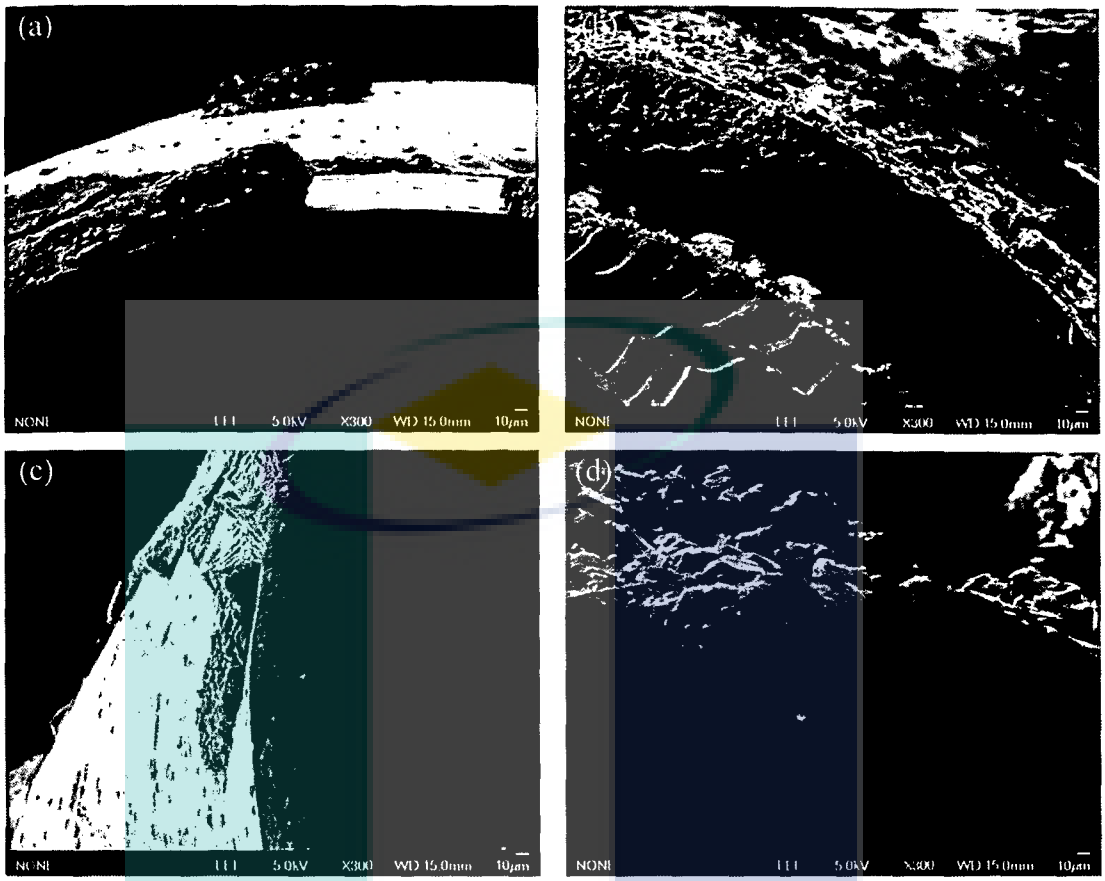


Figure 4.25 Different types of BFRs biocake layer on membrane surface (magnification x300); (a) BFR0; (b) BFRZEO; (c) BFRES and (d) BFRPAC

4.9 Conclusion

In the study of ASMBR in treating SSC wastewater, the biofouling and the performance removal were examined. MLSS and SRT have affected the ASMBR performance in terms of resistance in series (RIS), TMP and SMP and EPS. Removal performance for all MLSS concentration and SRT tests showed more than 90% of COD removal and 99% of sulphide removal were recorded. RIS and TMP trends showed the SMP and EPS directly affected the degree of membrane fouling and both SMP and EPS have the tendencies to deposit on membrane surfaces by

vacuum driving force. However, to get the optimum performance, BFRs were added and the removal performance and biofouling were examined.

Adsorptive characteristic of BFRs improved the filtration of MF membrane in ASMBR due to attachment, growth and development of bigger bioflocs that resisted bioflocs passing through the membrane pores. The protein-carbohydrate and protein-protein interaction also have the tendencies to form strong bioflocs and reduce deflocculation. ASMBR-BFR1PAC showed the optimum performance in reducing biofouling and substrate removal resulting from high surface area of BFRPAC hence, improved the operation (TMP performance) and SMP and ESP trends. The ability of BFRPAC in adsorption of organic substrate increased the attraction of biomass to attach on the surface of BFRPAC and formed suitable environment for biomass biodegradation. Moreover, high surface area of BFRPAC had the tendency to offer high capacity of foulants (organic and inorganic substrate and biomass product) to adsorb and adhere hence, formed bigger and strong bioflocs. BFRPAC in the reactor had the tendency to change the structure of bioflocs in ASMBR-BFRPAC to form more porous bioflocs that deposited on the membrane surface, thus increasing the filterability and reducing the biofouling.

CHAPTER 5

BACTERIA STRAIN IDENTIFICATION AND ACTIVATED SLUDGE MODEL NO. 1

5.1 Introduction

Different activated sludge treatment has different population of bacteria. It depends on the type of wastewater. Consideration of bacteria identification is important to recognize the bacteria species that perform in ASMBR with addition of BFR in spent caustic treatment. Meanwhile, ASM1 was applied for each ASMBR with or without BFR for getting insight into treatment performance. It was purposely to reveal the unforeseen behaviour of design and problems. The system was maintained in aerobic and heterotrophic conditions by controlling the dissolved oxygen (DO) above 2 mg L^{-1} and food to microorganism (F/M) ratio or organic loading (OL) in stages of equilibrium. Heterotroph uses organic matter as energy source and oxygen as electron acceptor and where the bacteria population reached the carrying capacity in treating SSC wastewater biologically (Gerardi, 2006; Judd, 2006; Metcalf and Eddy, 2004).

5.2 Biomass Morphology in ASMBR

Nutrient removal and membrane fouling of ASMBR-BFRs occurred in the presence of biomass in the system. The biomass in ASMBRs was assumed to be the same since the same SSC wastewater was used in the research. The addition of BFRs were considered not to change the bacteria strain since BFRs had been characterized as adsorbent, flocculent and places for biomass to attach and grow (Carvalho *et al.*, 2011; Koumanova *et al.*, 2002; Utrilla *et al.*, 2013; Zulfikar *et al.*, 2013). Figure 5.1 shows the chronology of biomass in ASMBR-BFR1PAC. It could be seen that the change of biomass was physically due to the domination of the strain in the reactors. The beginning of growth of 'long' filament could be seen at Figure 5.1 (b) and grew until the end of the experiments. At the end of this run, samples were taken at steady state with constant COD removal for bacteria strain identification that was dominant in the system.

5.3 Bacteria Identification of ASMBR-BFRs Sludge Biomass

The objective of the bacteria identification was to screen the main bacteria strain dominant in ASMBR that was implicated in treating spent caustic wastewater. Biochemical method is a rapid method that was used as preliminary and as touchstone step for identification of the isolation. This method can be proved and was assisted by the molecular method. Sample was collected from ASMBR-BFR1PAC run and this sample was considered to have the same bacteria colonies with all ASMBR-BFRs runs.

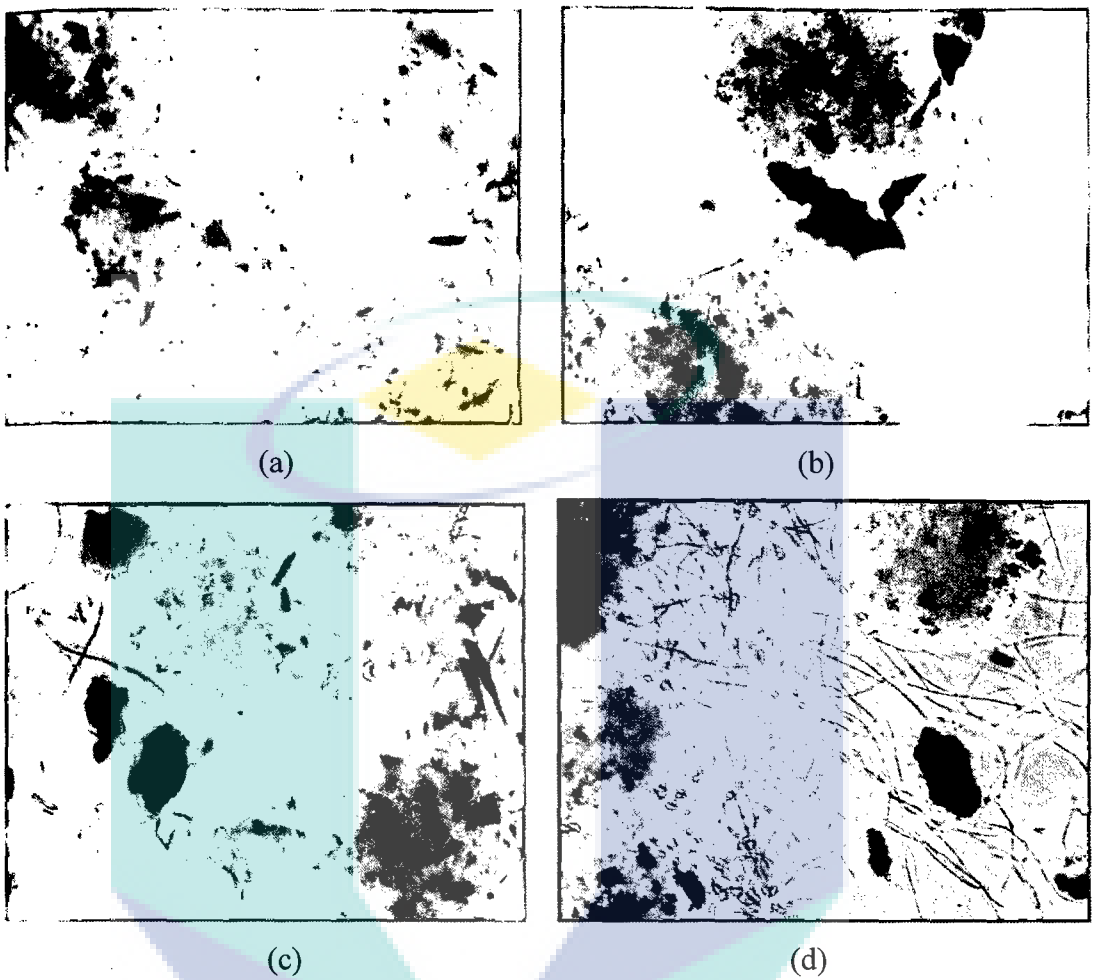


Figure 5.1 PAXIT (magnification x40) for biomass morphology in ASMBR-BFR1PAC at (a) 29th April 2013; (b) 7th May 2013; (c) 15th May 2013; (d) 30th May 2013

5.3.1 Bacteria Isolation

Pour plate dilution method was to ensure that the pure isolate colonies were formed. Objectively, an aerobic bacterium was identified; therefore standard plate count was used in this study. The isolation was done until there were no growths on the plate observed and in this study it occurred above 10^{-6}. From the isolation, five dominant pure culture colonies (produced excessive colonies) were swabbed for the

next isolation in biochemical and molecular identification. Figure 5.2 shows the plate count for bacteria isolation.

5.3.2 Biochemical Identification

In biochemical tests, non-identification colony may be due to low biochemical reactivity that lead to strains to match with database or the strains are not matched with database (Tasic *et al.*, 2012). Table 5.1 showed the biochemical result for five colonies. Gram-positive and negative bacteria indicated the different chemical composition of external cell wall. Colony A showed the spore forming gram positive with rod shape under *Bacillus sp.* with 50.9% probability of *Bacillus cereus* or *Bacillus thuringiensis*. Colony C shows nonspore forming gram-positive bacteria with rod shape characterized as *Rhodococcusequi* with 52.2% probability. Colony E identifies the gram negative fastidious of *Bordetellaholmesii* with 64.6% probability. However biochemical identification shows unclear results with probability less than 80% and need further process of identification and hence, molecular identification method was implemented.

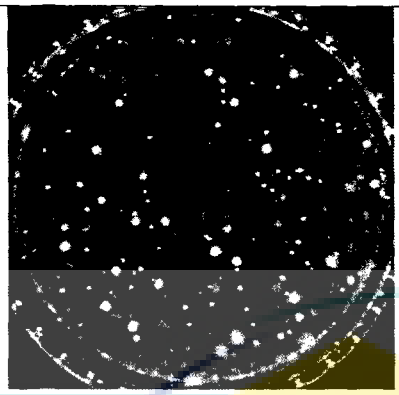
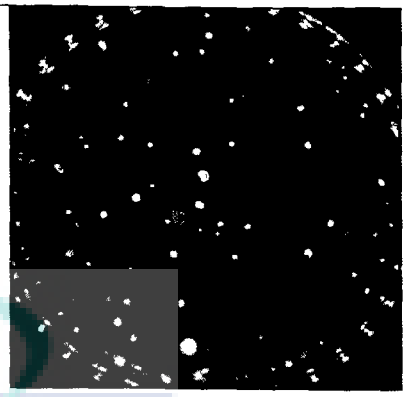
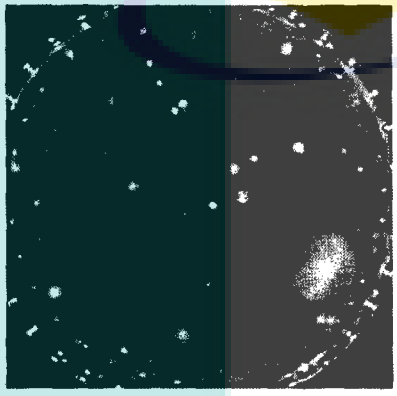
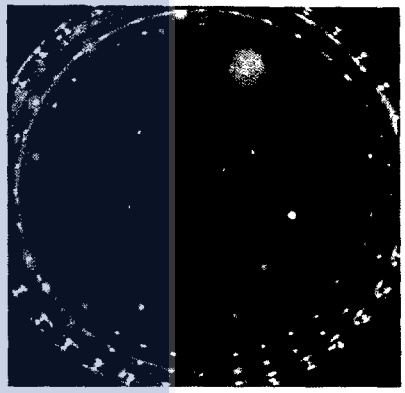
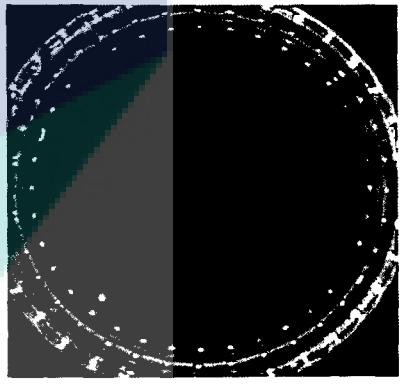
Sample Marking	Plate	Sample Marking	Plate
10^{-1}		10^{-2}	
10^{-3}		10^{-4}	
10^{-5}		10^{-6}	

Figure 5.2 Pour plate count

Table 5.1: Biochemical identification result

Sample Marking	Result		
	Organism Type	**Bacteria Identification**	**Probability (%)**
Colony A	Gram positive-Rod-spore forming bacillus	*Bacillus cereus/thuringiensis*	50.9
Colony B	-	-	-
Colony C	Gram positive-Rod	*Rhodococcusequi*	52.2
Colony D	-	-	-
Colony E	Gram negative- Fastidious	*Bordetellaholmesii*	64.6

5.3.3 Molecular Identification

In molecular identification, the samples used for DNA extraction were the same colonies samples that had been used in biochemical identification. DNA extraction is to take DNA from whole cell and purify them from protein bound. Success of DNA extracts and purity can be measured quantitatively by adsorption with A260 wavelength between 0.1±0.1 to 1.0±0.1 and range of A260/A280 ratio is between 1.7±0.1 to 2.0±0.1. Readings below the range indicate the presence of contaminants (Nguyen *et al.*, 2009; Ranjan *et al.*, 2010). Table 5.2 showed the adsorption reading for five sample colonies (A to E) and the reading lied in the range mentioned above, hence high yield purity were recorded.

The quantity of DNA extraction supported by agarose gel electrophoresis result is shown in Figure 5.3. This qualitative test was to image the DNA isolation and confirm DNA purity with 1kb (250bp to 10,000bp) DNA ladder marker. The analysis had been done and the imaging showed very clear and visible DNA bands and it indicated the excellent purity of DNA extraction. Hence, it is ready for PCR amplification test.

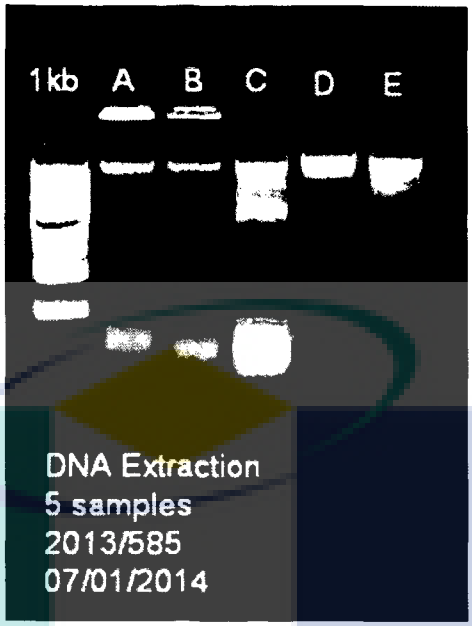


Figure 5.3 1% of agarose gel electrophoresis of qualitative genomic DNA extraction.

Table 5.2: Quantitative adsorption of DNA extraction

Colony	Yield (µg/mL)	A260	A280	A260/A280
A	16.2	0.336	0.172	2.03
B	29.9	0.671	0.376	1.97
C	35.2	0.720	0.366	2.01
D	8.9	0.179	0.092	1.97
E	9.7	0.196	0.102	1.92

The DNA fragments were successfully amplified using the control primers. The success of PCR was qualified by the presence of clear 16S rRNA (Figure 5.4). Afterward, the successful primer were sequenced and matched to GenBank to identify the closest sequence data by BLAST. Table 5.3 showed the result from the sequencing test.

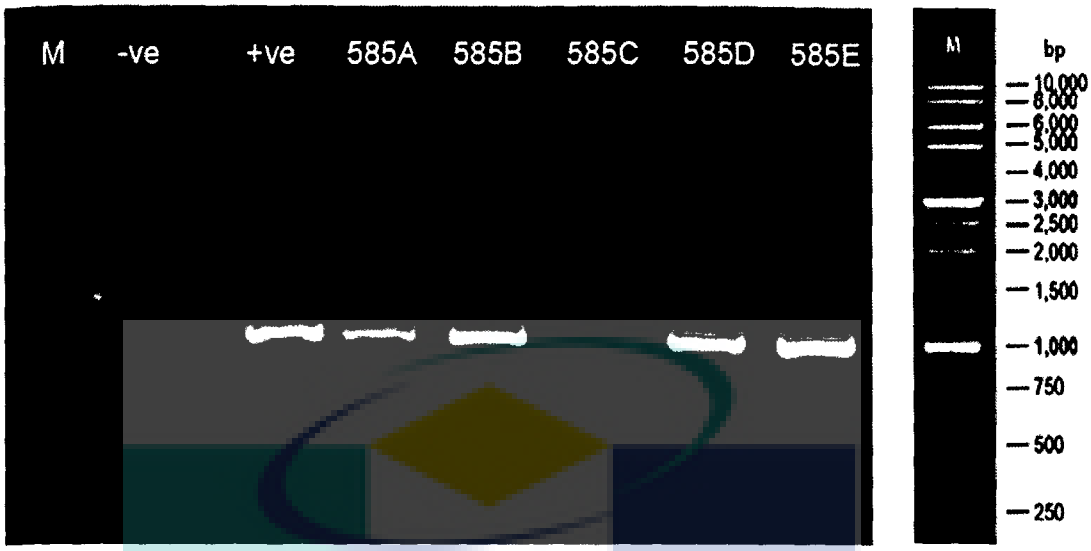


Figure 5.4 1% of agarose gel electrophoresis of PCR amplicons from DNA extraction. +ve and -ve indicate control reactions with or without purified DNA.

Table 5.3: The result from sequencing

Sample	Bacteria	Identify (%)
A	*Bacillus thuringiesis Bt407*	99
B	*Carnobacterium maltaromaticum LMA28*	99
C	*Bacillus sp.*	99
D	*Carnobacterium maltaromaticum LMA28*	99
E	*Carnobacterium maltaromaticum LMA28*	99

In this study, five samples have been identified with *Bacillus thuringiesis Bt407*, *Carnobacterium maltaromaticum LMA28* and *Bacillus sp.* and dominating in sludge of ASMBR. These strain of bacteria are dominant in this system may be because of their capability to degrade compounds in SSC wastewater. Genus *Bacillus* is categorized under heterotrophic sulphur-oxidizing bacteria that biologically oxidized sulphides to sulphates (Gerardi, 2006; Nakada and Ohta, 1999; Ryua *et al.*, 2009). This genus was also able to breakdown and utilizes phenol (Joseph, 1997). A study by Bratina *et al.* (1998) showed that the genus *Carnobacterium* sp. had been identified at Lake Vanda water column where sulphide compounds was present (Bratina *et al.*, 1998). Huang *et al.* (2014) shows genus

Carnobacterium sp. had been identify in treating high strength wastewater using microbial fuel cell (Huang *et al.*, 2014). Cheng *et al.* (2010) shows the genus *Carnobacterium sp.* identified in diversity of the bacteria community in bioreactor during ammonia removal (Cheng *et al.*, 2010). In the study of phosphorus removal by bed biofilm reactor, this genus was identified as 99% of *Carnobacterium* sequenced clone similarity (Helness, 2007). Table 5.4 showed dominant bacteria in bio treatment of spent caustic wastewater and majority of the treatment identified *Thiobacillus* as the common bacteria in treating spent caustic and was listed in a group of sulphur-oxidizing bacteria (Gerardi, 2006).

Table 5.4: Biotreatment of spent caustic

Process	Compound	Bacteria	Condition
Aerobic submerged membrane bioreactor (**This study**)	Sulphide, phenol, $NaHCO_3$	*Bacillus thuringiesis Bt407; Carnobacterium maltaromaticum LMA28*	T: 25°C; pH: 7; HRT: 16 hours; aeration: 5 L min^{-1} ; MLSS: 5 - 9 g L^{-1}
Bubble column reactor (Sipma *et al.*, 2004)	Sulphide, phenol, methanethiol dimethylsulphide	*Thiobacillusthioparus*	T : 30°C; pH : 7; HRT: 6 hour; sulfide loading: 31 mmolS^{2-} L^{-1} d^{-1}; Pure oxygen
Fluidized-bed bioreactor (Graaff *et al.*, 2011)	Sulphide, benzene, sodium	*Thiobacillusthioparus*	T: 35°C; pH:9.5; HRT: 3.0 - 3.5 days; sulfide loading: 16 – 27 mmolS^{2-} L^{-1} d^{-1}
Bench and pilot scale stirred tank reactors (Kolhatkar and Sublette, 1996)	Sulphide	*Thiobacillusdenitrificans*	T: 20 – 25°C; MLSS: initial 3.6 g L^{-1}
Fluidized-bed column bioreactor (Conner *et al.*,	Sulphide, mercaptans	*Thiobacillusthioparus*	T: 30°C; pH: 7; aeration: 300 mL min^{-1}

2000)

Biotreatment stirred-tank reactor (Rajganesh and Sublette, 1995)	Sulphide	*Thiobacillusdenitrificans*	T: 30°C; pH: 7

5.4 ASM1 of ASMBR-BFRs

The determination of kinetic model components is an expensive and time-consuming process. Therefore, the objective of ASM1 calibration is to adjust kinetic coefficients to match the result (from respirometric experiment) for accurate determination of kinetic coefficients using programmer (ASIM 4004). The decision to use ASM1 was to characterize the COD fraction of SSC wastewater besides determining heterotroph coefficient of ASMBR with or without BFR in treating SSC wastewater. The concept is quantitative for better definition system and to determine the unforeseen coefficient. The coefficient is indirectly measurable by experimental studies that may exist in the ASMBR operation and become system monitoring practices (Jeppsson, 1996b; Makinia, 2010). In this model, dynamic state had been applied according short-term operation and time-variation. It also consists of simple parameters and the system is assumed to be uniform for over the volume space and the system represented by a respiratory vessel in definition of continued stir tank reactor (CSTR) (Henze *et al.*, 2008; Jeppsson, 1996b; Makinia, 2010).

Heterotrophic bacteria become a second major constituent in aerobic activated sludge system after suspended and dissolved organic matter (Jeppsson, 1996b; Judd, 2006). Heterotrophic bacteria are generally able to convert complex organic compounds to simpler organic or inorganic compounds. Besides, aerobic heterotrophic sulphur oxidizing bacteria are able to convert organic sulphide to the sulphate and sulphur by the presence of enough oxygen supply as electron acceptor

and organic carbon as carbon source (Bitton, 2005; Gerardi, 2006; Hui *et al.*, 2010). Aerobic autotrophic bacteria are for nitrification and inorganic compounds that use carbon dioxide as carbon source and oxygen as electron acceptor but this condition might occur in the presence of carbon dioxide as carbon source.

In this study, the volume reactor used is small and homogenous. Aeration had been applied to maintain the system in aerobic condition. Besides, DO and F/M ratio was controlled (Bitton, 2005) with more than 2 mg $L^{-1}$and F/M ratio set in the range 0.5 to 1 with continuous aeration to reduce aerobic autotrophic to occur. Autotroph bacteria grew more slowly rather than heterotroph since autotrophs have less ability to gather their energy (Judd, 2006). ASM1 does not deal with terms like nitrogen and alkalinity limitation (Jeppsson, 1996a) and only COD fractions are considered in this study due to the main concern being on COD removal rather than nitrogen removal (Kantachote *et al.*, 2007).

5.4.1 COD Fractionation of ASMBR-BFRs

Table 5.7 shows the list of COD fractionation in ASMBR with and without BFR and in different COD loading. Readily biodegradable substrate is an important fraction and is conceived as the limiting substrate for heterotrophic growth (Orhon *et al.*, 1996). In this study, respirometric was measured in aerobic condition for 48 hours. Since the reactor was maintained in aerobic heterotrophic condition, only part of autotrophs were co-opted into biomass denitrification (Kose, 2006). Endogenous respiration is known as one unit of biomass COD loss that leads to one unit of oxygen utilization minus inert particulate COD products that are formed (Petersen *et al.*, 2003) or it can be defined as OUR in the absence of substrate (Vanrolleghem, 2002). Meanwhile, exogenous respiration is known as immediate OUR needed to degrade a substrate (Vanrolleghem *et al.*, 1998). Table 5.6 shows calculation procedure to identify COD fractions.

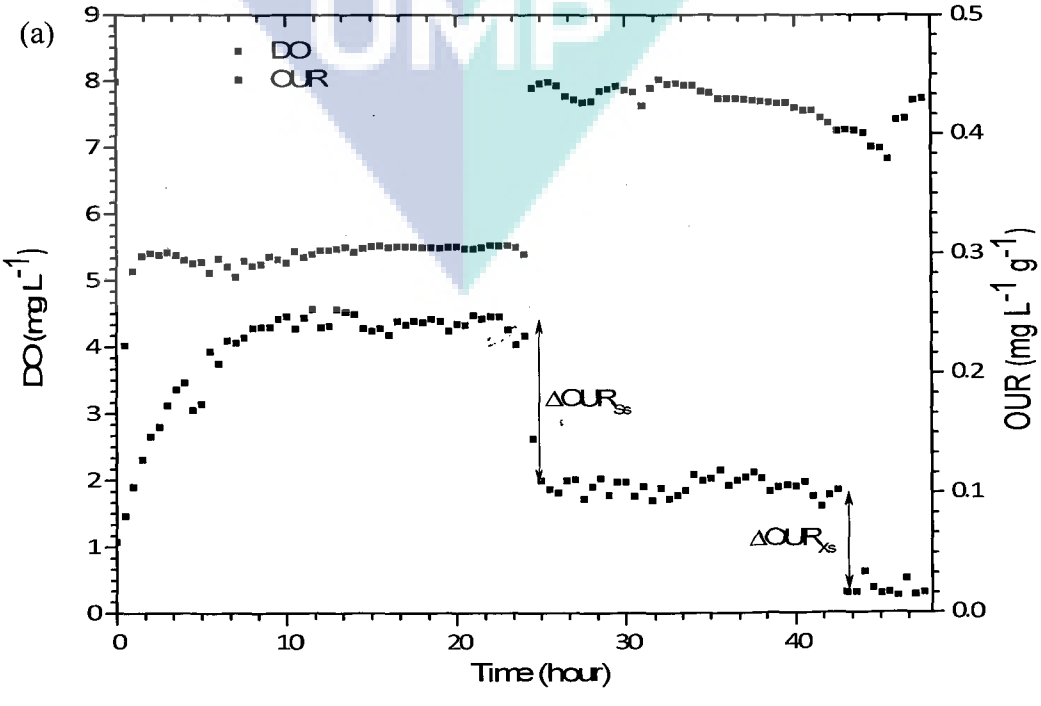
COD fraction was divided into non-biodegradable and biodegradable. Non-biodegradable matter can be categorized as biologically inert that passes through the reactor without any change. The inert soluble COD (S_i) is classified as having the same concentration during entering and leaving the reactor while inert particulate COD (X_i) is a matter that is trapped in the reactor and can only be removed by sludge discharge. Biodegradable matter includes soluble readily biodegradable COD (S_s) and slowly biodegradable COD (X_s). S_s is a simpler matter that might be adsorbed and utilized directly by aerobic heterotroph bacteria and also used for new biomass growth. X_s is a complex organic matter that requires enzymes for degradation to S_s before absorption and utilization occur.

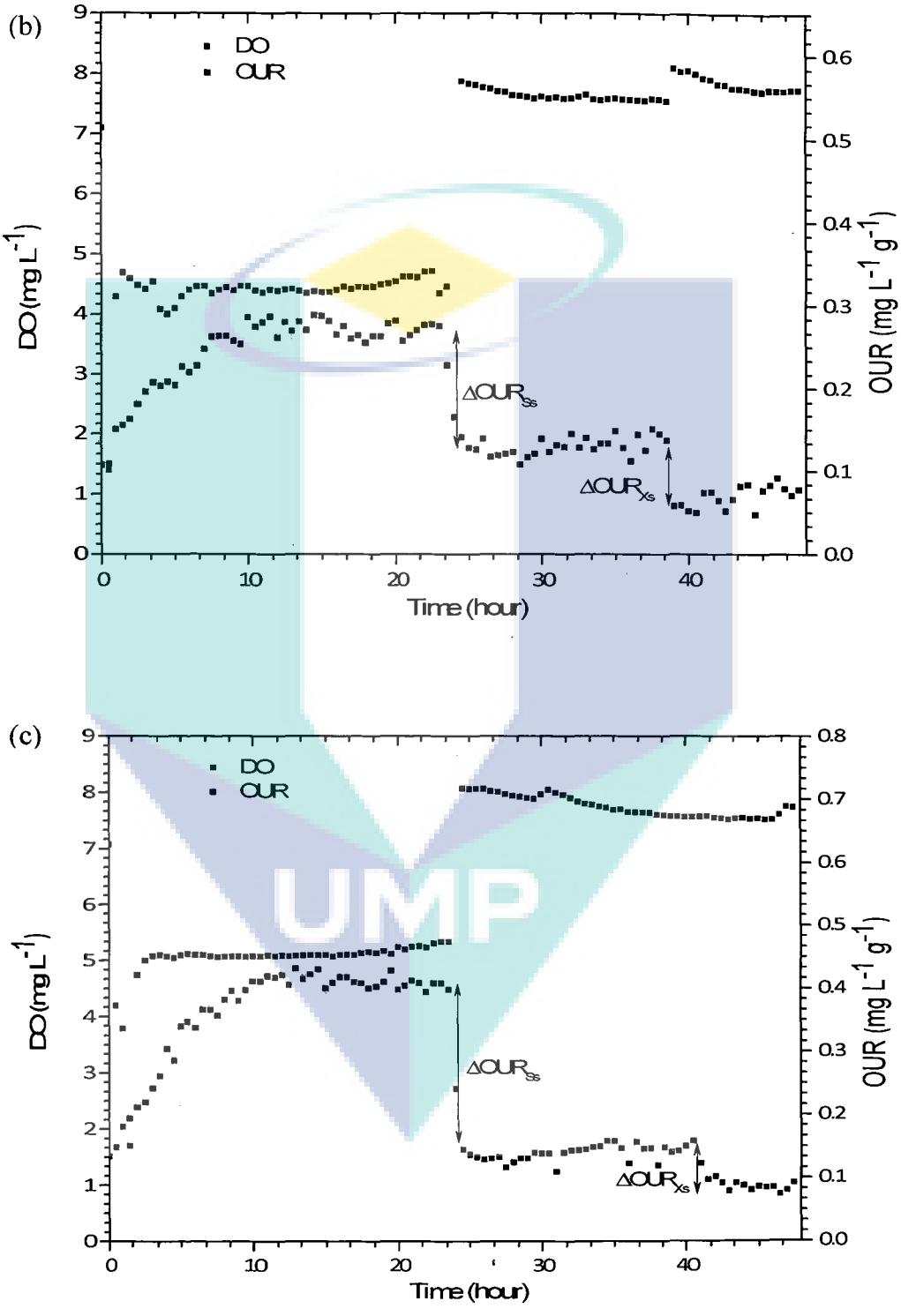
Figure 5.5 shows the OUR profile. Initial SSC wastewater was fed to the endogenous sludge and OUR increased drastically from 0 to 8 hours and the oxygen consumption (OUR) was between 0.05 to 0.9 $mgO_2\ L^{-1}\ min^{-1}$ and DO reading reported at a range of 4 to 6 $mgO_2\ L^{-1}$. In 9 to 24 hours, the equilibrium phase occurred until OUR was fully depleted and dropped to the lower level after feed was stopped at hour 24. This stage is known as exogenous respiration and the differential of OUR readily influenced the biodegradable substrate (S_s). It was represented by the readily biodegradable in SSC wastewater that was degraded. After that, OUR profile was brought into the next phase that was endogenous respiration. In this stage, the feeding was stopped and no substrate was supplied. Slowly biodegradable (X_s) phase was identified by differential of second drop of OUR. At the endogenous respiration, OUR was recorded in a range of 0.1 to 0.15 $mgO_2\ L^{-1}\ min^{-1}$ and DO 7.5 to 8 $mgO_2\ L^{-1}$. In this study, S_i was determined by the soluble effluent COD.

The fractions S_s, S_i and X_s are known and X_i can be identified via simple mass COD balance as shown in Table 5.5 and the COD fraction result can be seen in Table 5.6. Different BFRs does not have much influence towards COD fraction but overall, X_i is dominating in total COD. Different OL showed differences in COD fractions for ASMBR-BFR2PAC and ASMBR-BFR3PAC towards ASMBR-BFR1PAC especially S_s and X_i. Overall, the percentage of COD fraction for S_s (10 to 24%) was higher than X_s (3 to 10%) in ASMBR-BFRs runs and the S_s percentage

increased 4 times when the OL of SSC wastewater increased. Fall *et al.* (2012) reported 39% of COD fraction came from inert soluble COD, 23% from inert particulate COD and the rest from soluble hydrolysable (Fall *et al.*, 2012).

X_i is particulate COD that is 'non-biodegradable' at high loading of SSC wastewater but in low loading or in phases where there is no external substrate, X_i can be biodegraded. X_i was recorded in the range of 72 to 82% and the reading decreased from 82% to 47% and 50% when the OL loading increased in the same BFR. By increasing OL, the value of X_i was still in the range of 1700 to 2300 mg L^{-1} and only the increase of S_s reduced the percentage of X_i. However the X_i percentage still dominated the COD fraction. High accumulation of X_i is due to very slow biodegradation of inert organic matter such as precipitation that formed during neutralization processes and complex compounds of SSC wastewater during retention in ASMBR. According to Henze *et al.* (2008) and Peterson *et al.* (2006), X_i was also produced during decay in the system. X_i accumulated the cell wall from biomass that decay very slowly and can be considered as non-biodegradable. Accumulation of X_i also becomes a part of membrane fouling rate increased as mentioned in Chapter 4.




(b)
DO
OUR
DO (mg L-1)
OUR (mg L-1 g-1)
ΔOURSs
ΔOURXs
Time (hour)
(c)
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OUR (mg L-1 g-1)
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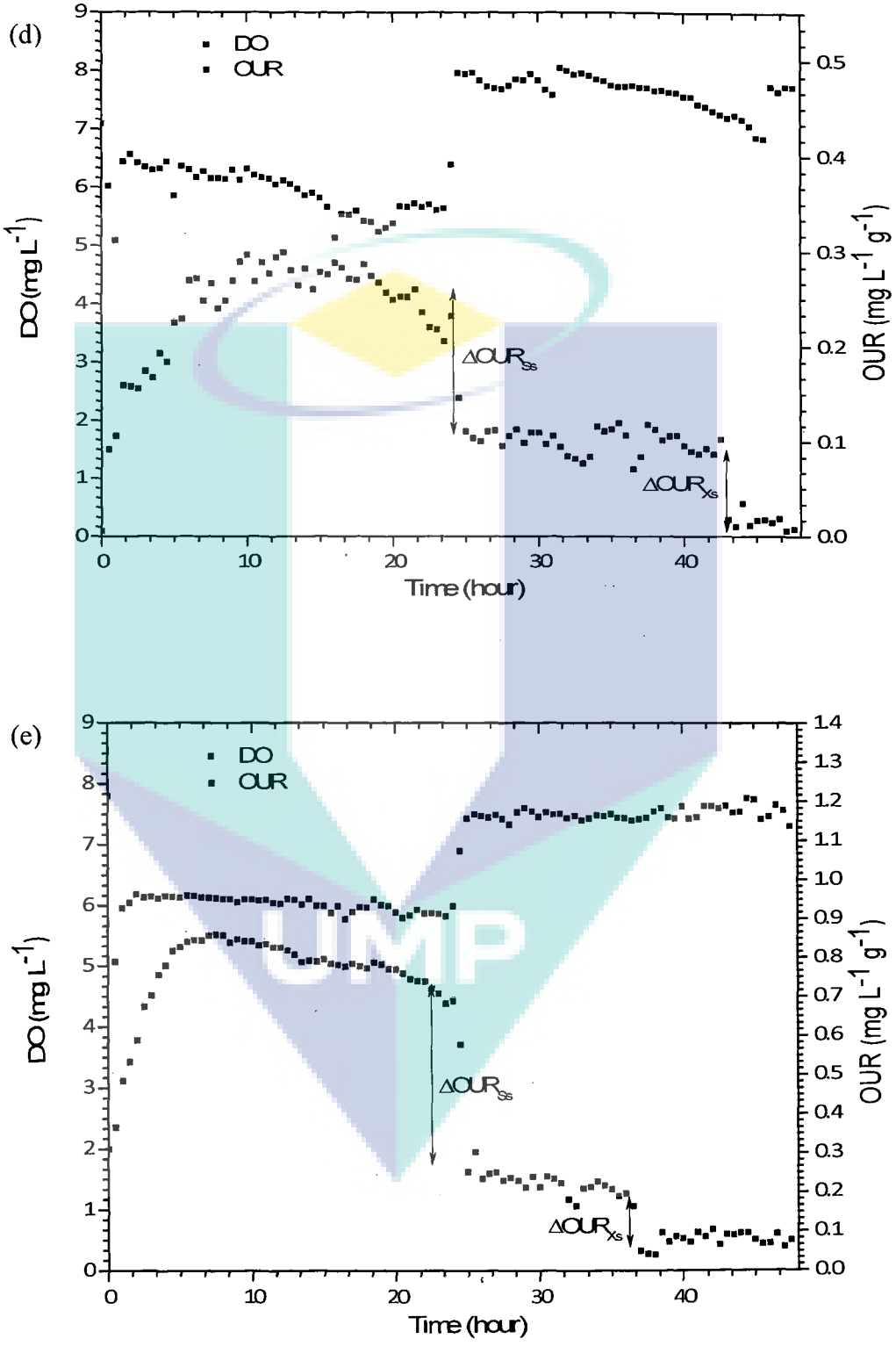
ΔOURXs
Time (hour)


(d)
DO
OUR
DO (mg L-1)
OUR (mg L-1 g-1)
ΔOURSs
ΔOURXs
Time (hour)
(e)
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OUR (mg L-1 g-1)
ΔOURSs
ΔOURXs
Time (hour)

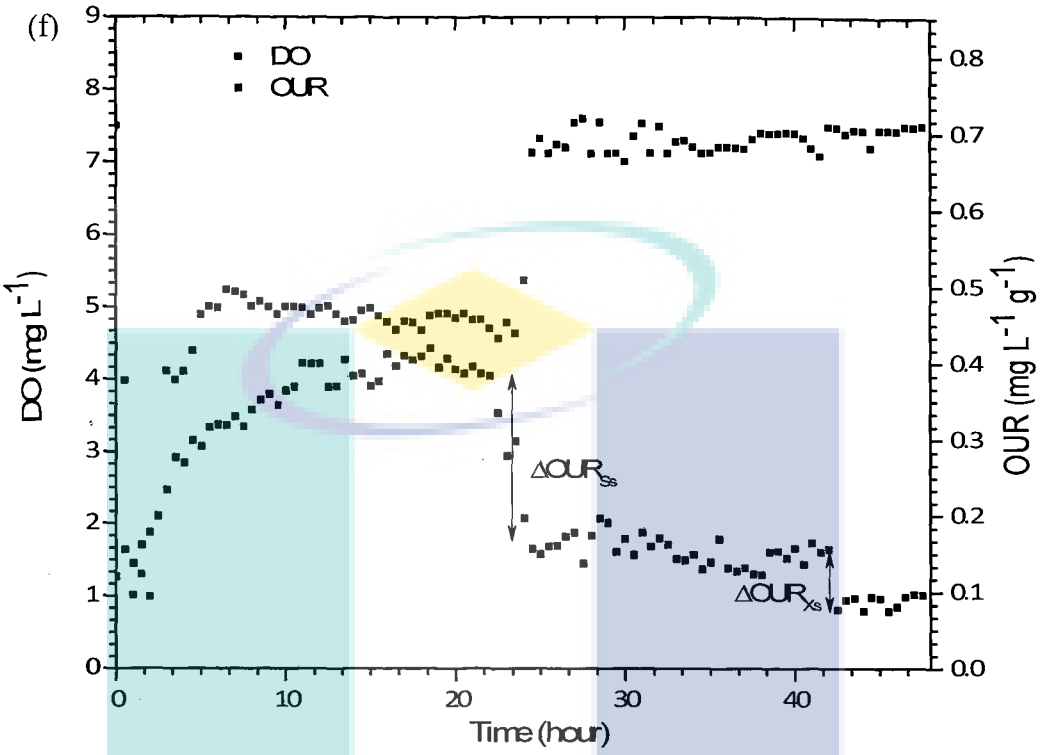


Figure 5.5 DO and OUR performance for ASMBR system (a) BFR0; (b) BFRZEO; (c) BFRES; (d) BFRPAC; (e) BFR2PAC; and (f) BFR3PAC

Table 5.5: COD fractionation of wastewaters formulation procedures.

Parameter (mg L^{-1})	Equation
Readily biodegradable COD (S_s)	$S_s = \frac{(\Delta OUR_{t0-t1})V}{Q\,(1 - Y_H)}$
Slowly biodegradable COD (X_s)	$X_s = \frac{(\Delta OUR_{t1-t2})V}{Q\,(1 - Y_H)}$
Inert soluble organic COD (S_i)	$S_{i,effluent} = S_{i,influent} = SCOD_{effluent}$
Inert particulate organic COD (X_i)	$X_i = TCOD - S_s - S_i - X_s$

Table 5.6: COD fractionation of SSC wastewater in different types of BFRs and organic loading

Parameter	BFR0	BFRZEO	BFRES	BFR1PAC	BFR2PAC	BFR3PAC
TCOD ($g\ m^{-3}$)	2310	2290	2320	2340	3890	4580
SCOD ($g\ m^{-3}$)	2080	2040	2150	2270	3510	4400
S_s ($g\ m^{-3}$)	324 (14%)	322 (14.1%)	327 (14.1%)	322 (14.6%)	1576 (40.5%)	1868 (40.8%)
X_s ($g\ m^{-3}$)	174 (7.5%)	176 (7.7%)	170 (7.3%)	175 (7.5%)	505 (13%)	378 (8.3%)
S_i ($g\ m^{-3}$)	45 (1.9%)	36 (1.6%)	31 (1.3%)	25 (1.1%)	55 (1.4%)	75 (1.6%)
X_i ($g\ m^{-3}$)	1767 (76.5%)	1756 (76.7%)	1762 (75.9%)	1818 (77.7%)	1754 (46.1)	2259 (49.3%)

5.4.2 Coefficients Dynamic Simulation

ASM1 data from the experiment were simulated and estimated to determine the optimum value of unforeseen kinetic coefficients. μ_{maxH}, μ_{maxA}, K_s, $K_{O,H}$, $K_{O,A}$ and b_H coefficients and to predict the effluent result. For the dynamic calibration, the calculated influent wastewater fractions were used as input to the model. Then, the coefficients had been changed by trial and error. Therefore the calibrated model was able to fit to OUR experiment curve by adjusting variable coefficients (Keskitalo and Leiviskä, 2010; Sperandio and Espinosa, 2008). Membrane separation was considered as perfect particles separator with a negligible volume. Two main basic processes involved in ASM1 are growth of biomass and decay of biomass. In dynamic ASM1, aerobic heterotrophic growth is factor of concern in this study since organic matter is present as carbon source. However, aerobic autotroph growth to some extent that takes part due to the presence of ammonia in SSC wastewater.

Hence, aerobic autotrophic growth occurs when ammonia nitrogen is oxidized to nitrate to produce autotrophic biomass. This occurred by presenting CO_2 as carbon source in alkaline condition and the effect of biomass growth is small due to the low autotrophic nitrifiers yield (Jeppsson, 1996a). Ammonia concentration is not the main component in SSC wastewater hence it does not affect the change of stoichiometry and kinetics directly. The temperature of operation was averagely maintained at 25°C.

The mass transfer coefficient (K_{La}) was identified according to 'two film theory'. The concept of this theory is the existence of two films on each side of the interface. The dissolved oxygen diffuses into the liquid and must pass through two films as defined by Henry's Law. The reactor is small and assumed to be well mixed and the oxygen is uniform at all points by aeration mixing. Equation (5.1) showed transfer rate of oxygen in water (Damayanti *et al.*, 2010; Hwang, 1983):

$$\frac{dC}{dt} = K_L a(C_s - C_t) \qquad (5.1)$$

where C_S is saturated dissolved oxygen at a temperature of 25°C

C_t is concentration at time t

Aerobic growth indicates that the biomass reproduces by binary fission. The aerobic heterotrophic biomass growth occurred during degradation of soluble S_s under oxygen consumption S_s and oxygen becomes a limitation factor for growth process (Jeppsson, 1996a; Vanrolleghem *et al.*, 2003). In heterotrophic growth, ammonia also incorporated and becomes a limitation factor (Petersen *et al.*, 2003) For this study, ammonia was only used for additional nutrient in SSC wastewater. Biomass decay in all processes may reduce the number of biomass or weight and specific biomass activities (Makinia, 2010). Growth and decay of biomass occurred simultaneously and when both increased, net growth rate may produce identical

behaviour and hence increase the oxygen consumption and accelerate the substrate cycling (Jeppsson, 1996a).

Heterotrophic yield (Y_H) is known as the ratio rate of cell growth in the absence of maintenance energy requirement and is the most stable and sensitive stoichiometric coefficient (Makinia, 2010). Y_H which is identified through physiochemical method was influenced by cell growth that occur concurrently with oxidation of organic and inorganic matter and it is present as ratio of the amount of biomass produced to the amount of substrate consumed. This yield indicates the COD fraction that was converted to cell mass. For this study, Y_H was identified as soluble COD long-term batch test (physiochemical method) by using 0.45 µm syringe filter with an aliquot from ASMBR as mentioned in Henze *et al.* (2000) and the data were calculated using Equations (2.13) and (2.14). It showed that Y_H did not differ significantly for all BFRs when applied with the same OL loading. However, when OL increased, the Y_H also increased. This may be due to the readily biodegradable SSC wastewater that becomes the limitation factor of Y_H where simultaneous biomass growth drastically and easily degraded high organic matter. In a study by Bizukoic and Leakowicz (2011), Y_H recorded 0.67 mg cellCOD·mg COD^{-1} (Bizukoic and Leakowicz, 2011). Salmiati (2008) reported that with biodegradability less than 0.5 (low S_s fraction), Y_H coefficient is 0.42 mg cell COD mg COD^{-1}.

Figure 5.6 (a), (b), (c), (d), (e) and (f) shows the performance results of OUR data and ASM1 simulation that fitted well. The t-Test analyses were used to ensure data from ASM1 fit with experimental data. A test value of $p>0.005$ (one and two tail) indicated null hypothesis (mean or average for experiments data and ASM1 data are equal) were accepted and the result can be seen in Appendix F. Oxygen was used for substrate degradation (exogenous respiration) and endogenous respiration. In excess oxygen, the substrate degraded and the concentration does not reach zero since there are some substrate production from decay that needed oxygen for the process (Petersen, 2000). Hence, OUR concept model was used to illustrate substrate degradation. At hour 0 (endogenous stage), SSC wastewater was fed and

OUR increased drastically due to the fast growth rate of biomass. The system was continuously fed with constant COD concentration for 24 hours and the net growth rate was recorded. Net growth rate showed the balance of growth and decay of biomass. Then the feed stopped after 24 hours. Endogenous respiration occurred at this stage where decay rate was larger than growth rate and X_s was degraded to S_s. The degradation still occurred at this stage since substrate was being produced from decay process. The decay occurred until certain level before the second drop occurred. At this stage, growth rate was very low but still with some degradation since OUR did not reach zero.

In ASM1, μ_{maxH}, μ_{maxA}, K_s, $K_{O,H}$, $K_{O,A}$ and b_H were adjusted and calibrated for fast dynamic model and the results could be seen in Table 5.8. As reported in Henze *et al.* (2000) that every system has different biomass activities and difficulties to control, factors of limitation include carbon source, electron donor, electron acceptor and nutrient. It is difficult to compare qualitatively hence the result is necessarily to be described quantitatively for better definition (Henze *et al.*, 2008). Monod equation was used to describe biomass growth rate with growth limitation concentration that involved biomass growth. μ_{maxH} is maximum specific growth rate that characterize heterotrophic growth in the system. μ_{maxA} is maximum specific growth rate that characterize autotrophic growth in the system. Since ammonia is present in SSC wastewater as a minor nutrient, small activities of nitrification was taking place. Under unlimited growth condition, μ_H and μ_A were considered to approach μ_{maxH} and μ_{maxA}.

The importance of dynamic model calibration in ASM1 is to obtain a more reliable estimation of the maximum specific growth rate (Petersen *et al.*, 2003). From the result, it showed μ_{maxH} value was slightly increased in different BFRs runs with the same OL when compared with ASMBR-BFR0 and it was difficult to distinguish the factor that affected μ_{maxH}. However in this case, substrate did not influence μ_{maxH} as reported in Henze *et al.* (2000) and Makinia (2010) (Henze *et al.*, 2000; Makinia, 2010). The increment may be due to the presence of BFRs that became media for biomass to attach, grow and develop biomass flocs. The

adsorption characteristics of BFR with craggy surface served and enhanced the adsorption of substrate, nutrients and oxygen which attracted biomass to attach and grow on the surface. Figure 5.7 also showed that the MLVSS production increased when BFRs was added and BFRPAC showed the highest MLVSS production. Likewise in ASM1, BFRPAC gave slightly higher μ_{maxH} as compared with other BFRs. In different OL, μ_{maxH} showed almost the same result of μ_{maxH} from BFR1PAC to BFR3PAC and it indicated substrate which did not affect the μ_{maxH}. Difference with $\mu_{H,}$ it was defined from mathematical method and Equation (5.4) as shown below. Change in μ_H was influenced by substrate in the presence of high dissolved oxygen. As shown in the Table, μ_H has reached the limitation growth (μ_{maxH}) and these systems were operated under unlimited growth.

$$\mu_H = \frac{\mu_{maxH} \cdot S_S}{S_S + K_S} \qquad (5.4)$$

The results of μ_{maxA}, show slight differences in all the runs. μ_{maxA} showed the maximum specific growth rate of biomass in the sludge that underwent nitrification. However, ammonia substrate had small effect towards μ_{maxA} due to small amounts of autotroph nitrifier biomass growth in the sludge and the ammonium present only acted as biomass nutrient. Heterotrophic decay (b_H) was the result in the loss of biomass that released X_s and recycled it to soluble matter. It was used for more new cell growth which directly consumed the oxygen (Makinia, 2010). At high oxygen in the system, the substrate was not a limitation factor. This study showed b_H result for ASMBR with BFRZEO, BFRES and BFRPAC was below ASMBR-BFR0 in the same OL. It was difficult to identify the factor of b_H since the scope of decay process was wide. As reported by Makinia (2010), b_H is influenced by the loss of substrate through maintenance for energy requirement, endogenous respiration, inhibition by higher (strong) biomass (Makinia, 2010). In addition SMP and EPS are products from substrate degradation and biomass decay (Yuniarto *et al.*, 2013) that have influenced in decay processes.

Jacek (2010) reported that substrate saturation coefficients (K_s) is to determine the rapidness at which μ_H approaches μ_{maxH}. From Table 5.7, K_s is marginally different between all BFRs runs. Even though OL was increased, K_s showed slight increase from BFR1PAC up to BFR3PAC. K_s coefficient may be related to diffusion limitation such as substrate or oxygen into microbial flocs (Makinia, 2010). In completely mixed reactor and constant feed, μ_{maxH} and K_s tend to be lower as reported in Henze *et al.* (2000). $K_{O,H}$ and $K_{O,A}$ are known as switching functions. $K_{O,H}$ is oxygen heterotrophic half-saturation coefficient which becomes a benchmark to shut off heterotopic growth to anoxic growth when DO concentration drops (Henze *et al.*, 2000). $K_{O,A}$ is oxygen autotrophic half-saturation coefficient that indicates that the nitrification has stopped and it occurs when DO level is too low (Henze *et al.*, 2000). In every ASMBR run, the $K_{O,H}$ and $K_{O,A}$ showed slightly different result that may be due to different biological activities occurring for different runs. It is difficult to distinguish between factors that influence the coefficient since the limitation of this model is aerobic heterotrophic process and does not consider anoxic and nitrification processes and depend on biomass presence as mentioned in Petersen (2000).

5.4.3 Effluents of ASMBR Simulation

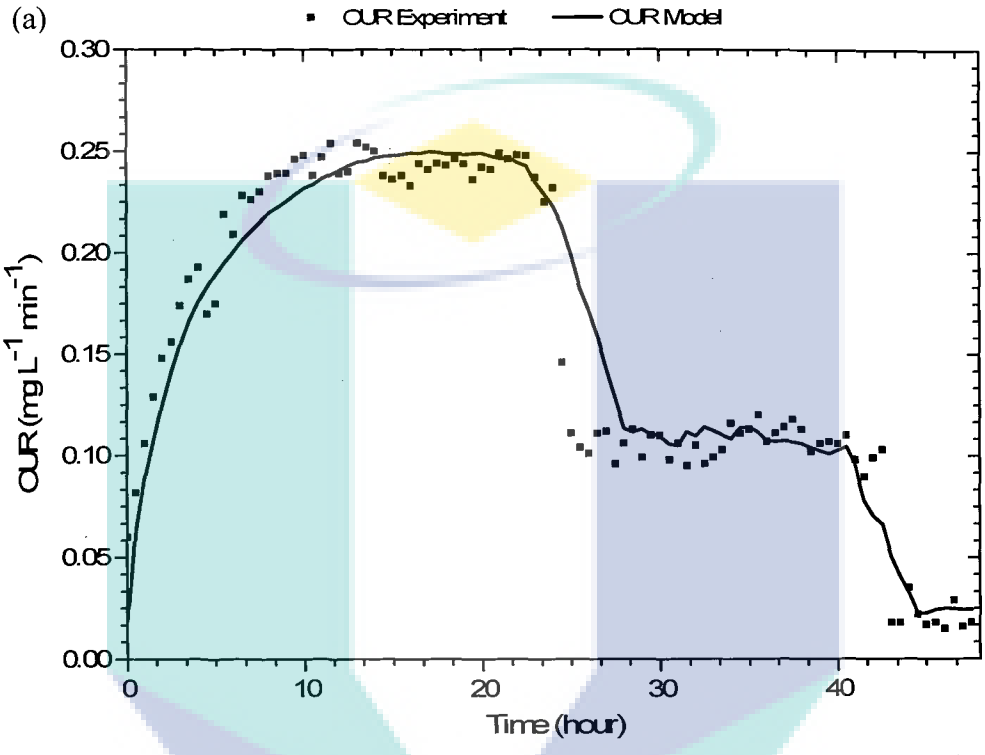
The dynamic simulation with ASM1 calibration was influenced by influent wastewater composition and validation (trial and error) parameters. These procedures were repeated until the best fit was obtained. The simulation predicted complete oxidation throughout the validation period. In this study, DO concentration was modelled at average 4.5±2 $gO_2\ m^{-3}$ to fit OUR experiments. Table 5.8 shows the large value effluent after models as compared to experiment result in all BFRs runs. Appendix G shows the trends of COD effluent (experiment and model). At first 24 hours, the trend shows increment as reactor was fed that correlated to accumulation in bulk solution in reactor. Then, COD reading maintained when fed was stopped

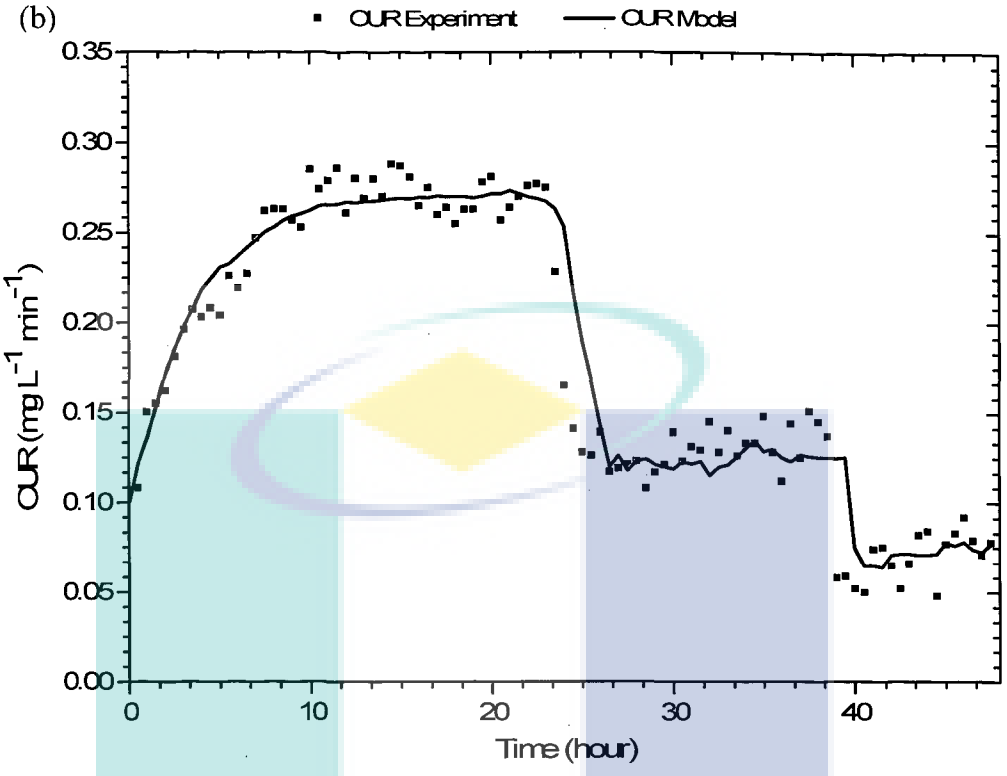
next 24 hours. In another point of view, the simulation result shows the change as BFRs were added and effluent simulation result increased when OLs were increased.

In this study, S_s and X_s were considered totally degraded and X_i were accumulated in the reactor. According to Peterson *et al.* (2003), X_i can be removed by sludge discharge. Meanwhile, simulated of this system is mainly sensitive to the inert soluble COD (S_i). In this experiment, the inert soluble COD is lower than simulated. It predicted that some organic inert compounds degraded in the reactor due to high MLSS concentration and/or trapped in large biofloc during flocculation and prevent the biofloc to pass through membrane pore (Remy *et al.*, 2010). In conventional activated sludge treatment, spent caustic was treated with MLSS 2 – 2.5 g L^{-1} (Berne and Cordonnier, 1995) while in this study, MLSS concentration was up to 5 – 9 g L^{-1}.

There were several factors that influence simulation result. They may be due to limit data collection which hydrolysis parameter simulation was neglected and particulate products (X_p) that were produced during growth and decay processes since they were considered to be very small and negligible during ASM1 simulation performance. As OLs were increased, the effluent simulation value increased and it could be caused by another possible factor that overestimates the high OL, possibly correlated to DO concentration in the bioreactor (Peterson *et al.*, 2003). At high OL, the DO requirement could be high to maintain high biomass growth rate and enhance the biodegradation. In ASM1 simulation, S_s and X_s were considered totally degraded. S_s was degraded under consumption of oxygen concurrent with growth and decay of heterotrophic biomass which release inert matter that include microbial product. S_s was produced while X_s was degraded during hydrolysis process. However, these factors seemed to have no significant changes on permeate soluble COD. As a result, most of S_s and X_s was biodegraded by biomass and only inert fraction of soluble COD passed through the membrane. Sperandio and Espinosa (2008) and Keskitalo and Leiviska (2010) mentioned that S_s and X_s were fully degraded and simulated sludge production sensitive to the inert COD (S_i and X_i) due

to some organic compounds that were considered inert degraded at high SRT (high MLSS).




(b)
OUR Experiment
OUR Model
OUR (mg L-1 min-1)
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0.30
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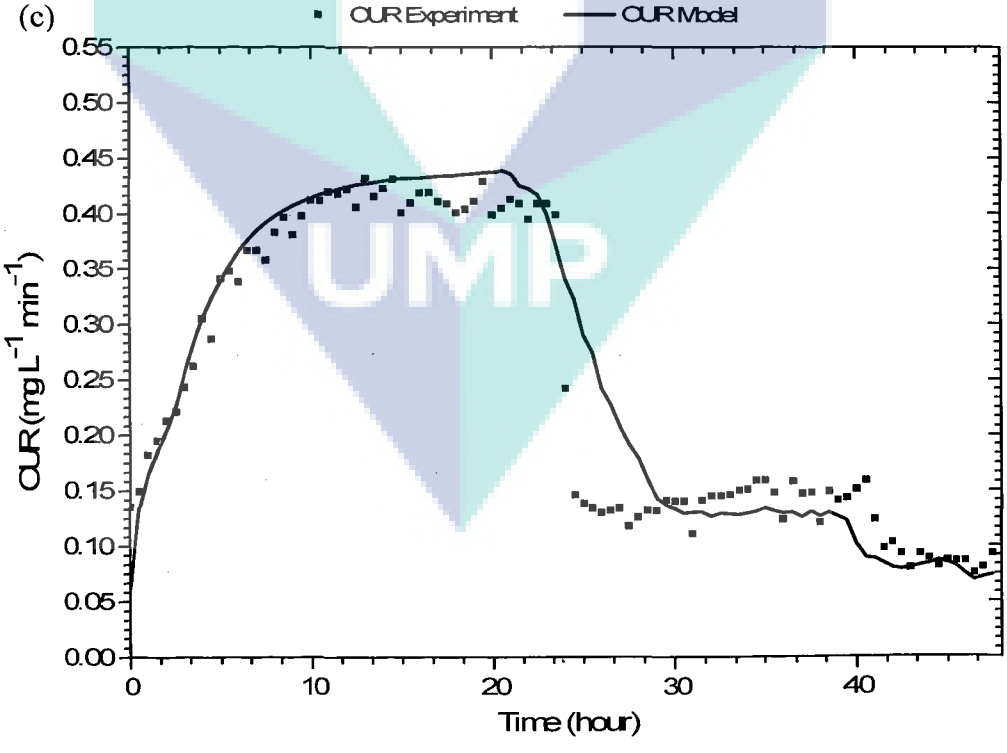
0.15
0.10
0.05
0.00
0
10
20
30
40
Time (hour)


(c)
OUR Experiment
OUR Model
OUR (mg L-1 min-1)
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0.35
0.30
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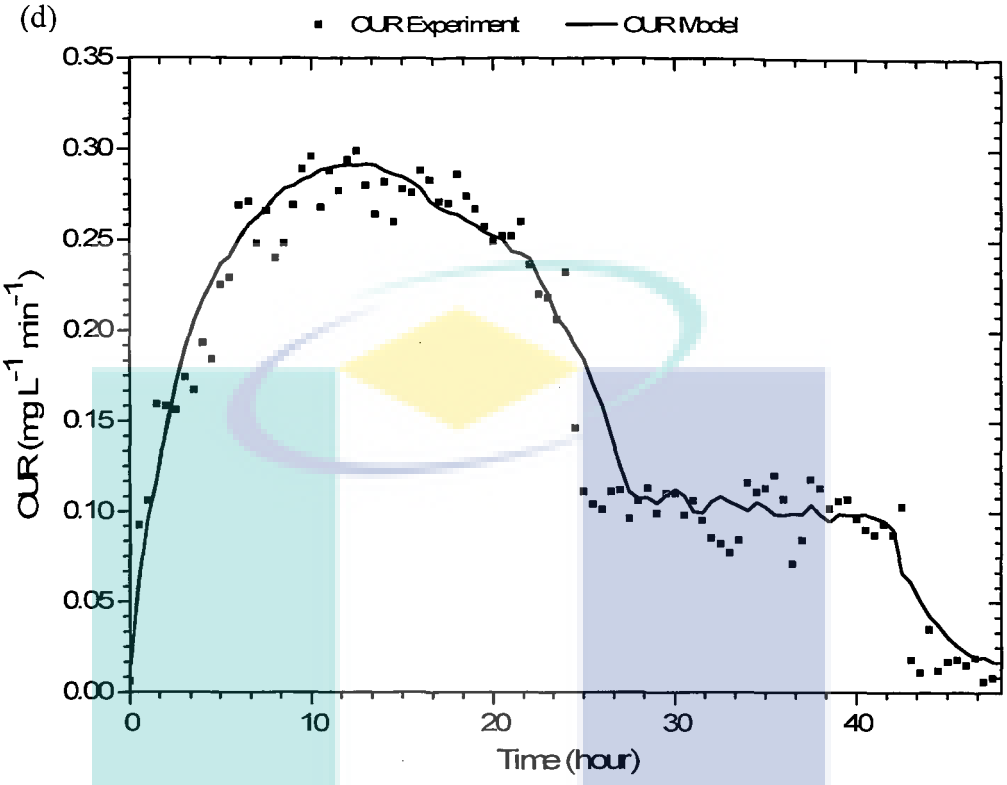
Time (hour)

(d)


OUR Experiment
OUR Model
OUR (mg L-1 min-1)
Time (hour)
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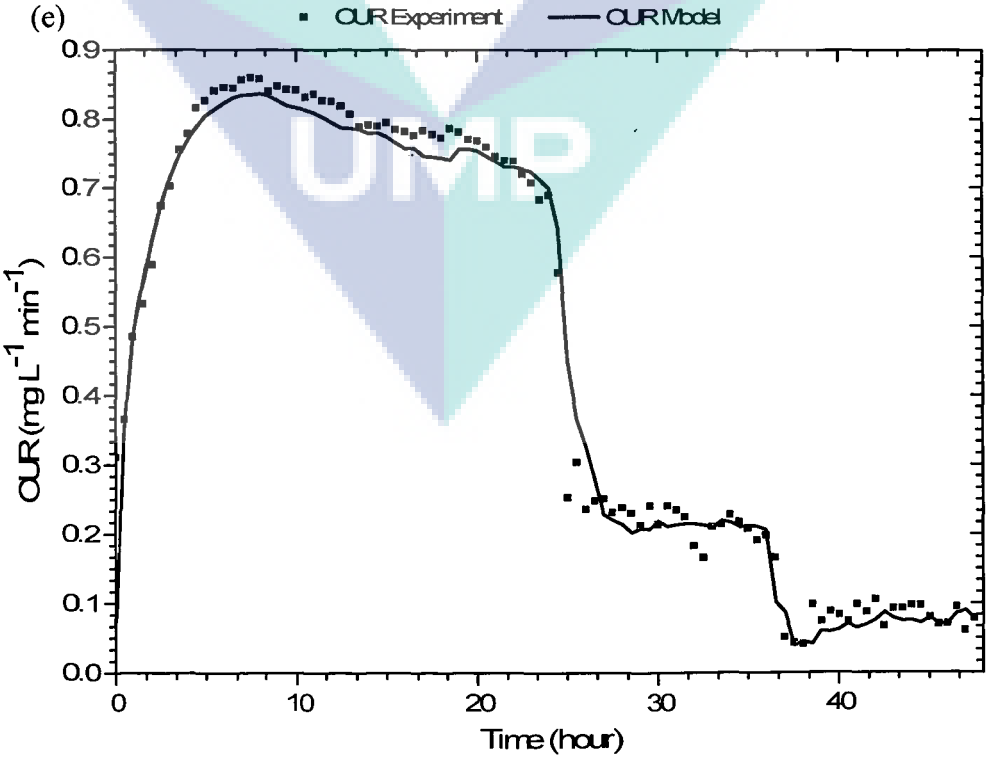
(e)


OUR Experiment
OUR Model
OUR (mg L-1 min-1)
Time (hour)
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0.7
0.8
0.9
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30
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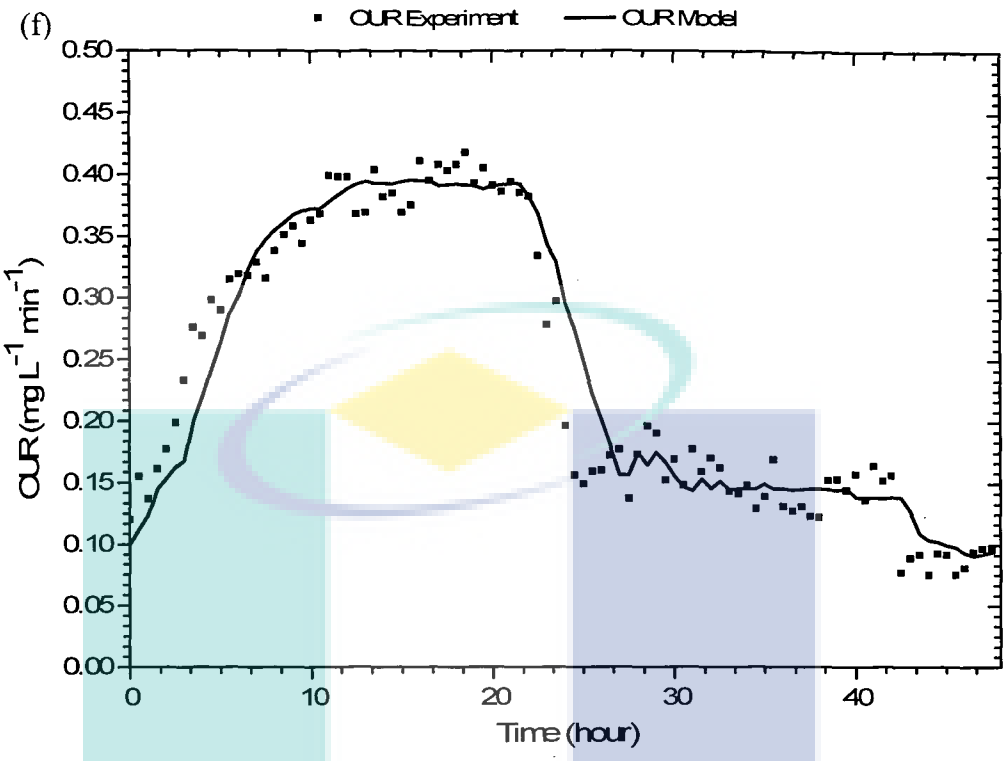

Figure 5.6 OUR data and the model simulation results for ASM1; (a) BFR0; (b) BFRZEO; (c) BFRES; (d) BFRPAC; (e) BFR2PAC and (f) BFR3PAC.

Table 5.7: Stoichiometric and kinetic coefficients for ASM1 in treating SSC wastewater for ASMBR in different types of BFRs and organic loading.

Parameter	BFR0	BFRZEO	BFRES	BFR1PAC	BFR2PAC	BFR3PAC
μ_{maxH} (d^{-1})	0.177	0.192	0.193	0.199	0.2	0.199
μ_H	0.176	0.191	0.192	0.198	0.2	0.199
μ_{maxA} (d^{-1})	0.276	0.286	0.276	0.235	0.276	0.288
b_H (d^{-1})	0.21	0.193	0.199	0.196	0.199	0.244
K_s (gCOD m^{-3})	0.92	0.93	0.95	0.93	0.95	0.97
$K_{O,H}$ (gCOD m^{-3})	0.08	0.15	0.55	0.55	0.45	0.5
$K_{O,A}$ (gCOD m^{-3})	1.3	1.2	0.93	0.45	0.41	0.89
Y_H (g cell COD g^{-1} COD)	0.53	0.52	0.52	0.51	0.7	0.88

Table 5.8: Experimental and model effluent concentration values for COD.

	BFR0	BFRZEO	BFRES	BFRPAC	BFR2PAC	BFR3PAC
Experiment	45	36	31	25	55	75
Model	81	73	72	59	89	109

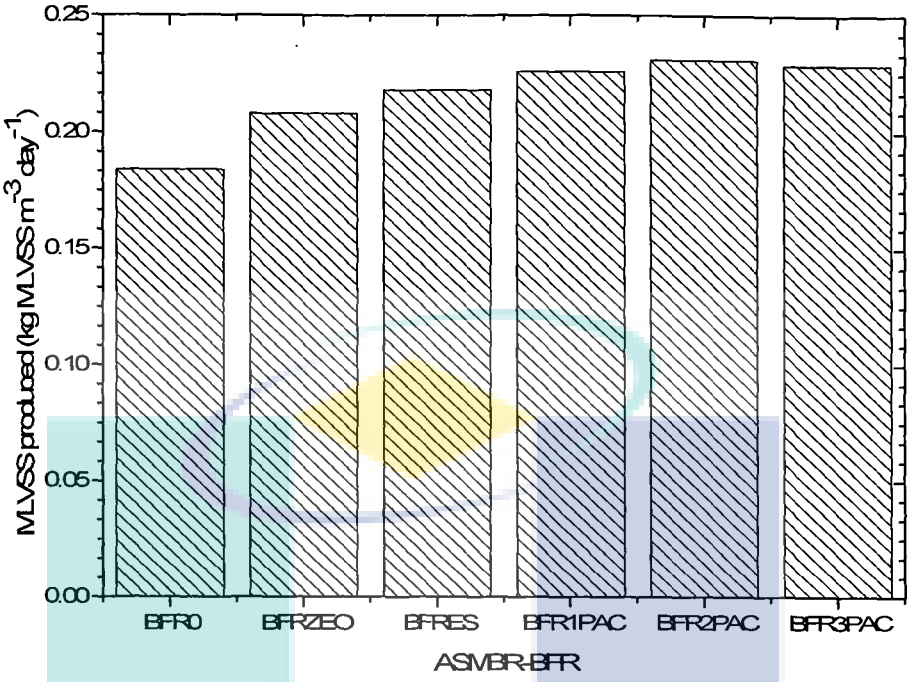

Figure 5.7 The MLVSS produced for first 10 days of experiments

5.4 Conclusion

Identification bacteria in ASMBR showed the *Bacillus thuringiesis Bt407* and *Carnobacterium maltaromaticum LMA28* are dominant and grow in the system to treat SSC wastewater. Aerobic heterotrophic bacteria are assumed to be grown in this system and for that reason, COD fraction and stoichiometric and kinetic parameters characteristics for this system have been identified and estimated. X_i showed that the higher percentage of the COD fraction that was identified from physiochemical method was due to the high complex components that were too slow to biodegrade. OUR from experiment data and ASM1 data fitted well for biological method to identify kinetic coefficients (μ_{maxH}, μ_{maxA}, K_s, $K_{O,H}$, $K_{O,A}$ and b_H) and the result showed BFRs affected μ_{maxH} in the system and BFRPAC proved to be the better agent for μ_{maxH}.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The conclusion that could be drawn from this study is as follows:

1. This study has successfully shown the ASMBR able to treat high strength spent caustic wastewater. At increased MLSS concentration and SRT, more than 94% - 98% of COD were removed. MLSS concentration at 5 gL^{-1} gave better performance resulting in less fouling effect according to critical flux and RIS result. At longer SRT, slightly low accumulation of SMP and EPS was observed at SRT of 40 and 80 days. However, the TMP increase rapidly at SRT of 80 days when compared with SRT of 40 days due to less sludge discharge and high suspended solid accumulation. The combination factors that effect the membrane fouling are (i) accumulation of biomass; (ii) accumulation of non-biodegradable; and (iii) accumulation of biomass products. The parameters with the optimum performance of organic and nutrients removal that able controlled membrane biofouling are at MLSS concentration of 5 g L^{-1} and 40 days of SRT in the ASMBR system based on the organic removal, microbial products, RIS and TMP performances;

2. This study also developed a novelty method by applying BFR from eggshell in ASMBR besides BFRPAC and BFRZEO. With optimum BFR dosage, the BFRs were added in short-term operation (critical flux) of ASMBR, and it showed the biofouling reducer effectiveness increased in ASMBR 30.6% (BFRZEO), 38.29% (BFRES) and 69.1% (BFRPAC) with respect to BFR0. BFRPAC is proved capable to become anti biofouling with 92% efficiency of fouling rate, 95% and 99% COD and sulphide removal with less soluble microbial products effluent recorded. This was due to its larger surface area that led to higher adsorption capacity and craggy surface for better adhesion. The morphology of biomass, membrane and BFRs showed positive physical change before (disperse) and after (adhesion, form bigger biofloc and porous of biocake layer) the experiment. As OL increase from 2 – 4 gCOD L^{-1} in BFRPAC run, the organic effluent achieved stable reading (50 mg L^{-1}) at day 6 for BFR2PAC and BFR3PAC stable above 50 mg L^{-1} at day 14. The fouling rate efficiency increased from 12% to 19% due to increase non-biodegradable that accumulate in ASMBR. As a result BFRPAC was more effective in organic and nutrient removal, caused less biofouling and was good in adsorption, adhesion, growth and developed large bioflocs and fouling rate efficiency decrease as OL increase;

3. In bacteria identification, molecular method was used to ascertain the accuracy of the result. The result 16S rRNA gene sequence analysis showed that *Bacillus thuringiesis Bt407* and *Carnobacterium maltaromaticum LMA28* dominated the growth process in the ASMBR. These strains were able to degrade compounds in SSC wastewater that had been categorized as high strength wastewater;

4. The characteristic of COD fractions shows inert particulate matter (X_i) dominated due to accumulation of very slowly biodegradable compounds that were considered as 'non-biodegradable'. This result proved that the accumulation of non-biodegradable increase membrane fouling rate. With COD fraction data and operational data, the design parameters coefficients

were obtained using ASIM 4004 for better understanding of the process. Overall, inert particulate matter (X_i) dominated the fraction of COD and OUR ASM1 data were fitted to experimental data. Five kinetic coefficients were obtained from the ASM1 simulation which were μ_{maxH}, μ_{maxA}, b_H, K_s, $K_{O,H}$ and $K_{O,A}$. The result from simulation showed the changes came when BFRs were added and compared with ASMBR-BFR0. The slight increase of μ_{maxH} showed that the maximum specific growth rate was affected by adding BFRs and BFRPAC gave a high value of μ_{maxH}. Thus, BFRPAC proved able to enhance the growth of biomass.

6.2 Recommendations

The ASMBR presented the better performance in treating spent caustic wastewater by adding BFRs as agent for biofouling reduction. With the ability of BFR, the continuity of the process and its commercial development will improve the effluent performance and MBR filterability. However, the challenge to bring this technology into commercial use is how to operate it at low cost. Future studies should be made to explore the process for more robustness based on the following recommendations:

1. BFRPAC showed the better performance in biofouling reduction, but it is costly to be applied industrially. The increase of BFRPAC concentration will increase the cost. The observation of low dosage implementation of BFRPAC in ASMBR needs the development of a strategy to reduce the cost and at the same time can optimise the permeability and the performance of the system. The membrane biofouling mechanism needs to explore more.

2. The design parameters of BFRPAC in ASMBR can be further study on ASM2 and ASM3. In this models were include nutrients and storage that effect the coefficients limitation.

3. Use the culture dominant bacteria like *Bacillus* sp. and *Carnobacterium* sp. that have the ability to treat spent caustic wastewater to minimise the acclimatization time. The use of mixed sludge bacteria takes a long time for bacteria to become dominant, stable and adapt to the wastewater. Long domination stage can increase the cost of operation.

4. Model SMP and EPS using ASM2d of BFRPAC to identify the factors that influence coefficients limitation. From the understanding of the influence of coefficients, the factor becomes the limitation one and can be controlled to maximize the operation and control the cost of operation.

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APPENDIX A

List of publications

JOURNALS

1) Mutamim, N.S.A., Noor, Z.Z., Abu Hassan, M.A., Olsson, G., (2012). Application of Membrane Bioreactor Technology in Treating High Strength Industrial Wastewater: A Performance Review, Desalination. (305) 1-11

2) Mutamim, N.S.A., Noor, Z.Z., Abu Hassan, M.A., Yuniarto, A., Olsson, G., (2013). Membrane Bioreactor: Application and Limitation in Treating High Strength Industrial Wastewater, Chemical Engineering Journal. (225) 109-119

CONFERENCES

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PATENT

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