

THE ANTI-BIOFOULING EFFECT OF PIPER BETLE EXTRACT ON MEMBRANE
BIOFOULING IN BIOREACTOR FOR BATIK WASTEWATER TREATMENT

MUHAMMAD FAISAL SIDDIQUI

Thesis submitted in fulfillment of the requirements
for the award of degree of
Doctor of Philosophy (Bioprocess Engineering)

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

NOVEMBER 2012

ABSTRACT

Navigating novel biological route to mitigate biofouling is of great worth in order to allow sustainable performance of MBRs in wastewater treatment technology. Recently, it was confirmed that a number of natural compounds in plants have an anti-biofouling effect, reducing the formation of biofilm. The main objectives of this study were to investigate the anti-biofouling effects of *Piper betle* extract (PBE) on membrane biofouling and how PBE mitigates biofouling based on quorum sensing (QS). Membrane biofouling propensity was investigated for a bacterial consortium and bacterial strains of batik wastewater. During MBR operation with bacterial consortium, a significant relationship ($R^2= 0.9916$) between extracellular polymeric substances (EPS) and transmembrane pressure (TMP) was revealed. MBR showed increased removal performance for dye and chemical oxygen demand (COD) removal with operation time. Fourier transform infrared spectroscopy (FTIR) showed the presence of EPS in membrane foulants. Furthermore, scanning electron microscopy (SEM) confirmed the occurrence of biofouling. The microtiter plat assay suggested that strain FS5 to be the major biofilm contributor. Batch tests of the production of EPS indicated that the *Bacillus* strain (FS5) produced a large amount of EPS compared to the bacterial consortium. This study addressed the feasibility of *Piper betle* extract (PBE) as anti-biofouling agent against the model organism *Pseudomonas aeruginosa* PAO1 and bacterial consortium. The anti-biofouling effects of PBE were evaluated via a microtiter plate assay; changes in the growth rate (μ) and EPS production. SEM was employed to qualitatively illustrate the biofilm formation. The anti-biofouling effects of PBE revealed ≥ 80 % reduction in biofilm formation, growth rate (87%) and reduced the EPS production. Furthermore, it decreased the soluble EPS concentration, reduced the cake resistance, and a two-fold increase in time required to reach 33 kPa of TMP. The PBE indicated a negligible effect on endogenous decay rate and biomass yield. SEM of sludge particles in PBE bioreactor showed the presence of a mixture of bacteria on its surface with a clear spherical shaped boundary. Besides that PBE indicated negligible effects on biological treatment performance. Response surface methodology (RSM) has been employed to mitigate EPS, TMP rise-up control, and dye removal in ultrafiltration MBR. The optimum conditions found to be biofouling reducer (BFR) of 0.23 mg/mg MLSS, HRT of 30.16 h and air flow rate of 0.60 l/min, with predicted values as 28.28 mg/l of EPS, 24.16 kPa of TMP and 95.65% dye removal, respectively. Validatory tests were closely agreed with the predicted values. The autoinducers production in bioreactor was confirmed using an indicator strain *Agrobacterium tumefaciens*. Moreover, three different AHLs were found in biocake using thin layer chromatographic analysis. An increase in EPS and TMP was observed with AHL activity of the biocake during continuous MBR operation, which shows that membrane biofouling was in close relationship with QS activity. PBE was verified to mitigate membrane biofouling via inhibiting AHLs production. These results exhibited that PBE could be a novel agent to target AHLs for mitigation of membrane biofouling based QS.

ABSTRAK

Penerokaan novel penggunaan biologi untuk mengurangkan kotoran adalah amat signifikan bagi prestasi MBR yang mapan dalam teknologi rawatan air sisa. Kini, telah dibuktikan terdapat beberapa sebatian semulajadi dalam tumbuhan boleh bertindak sebagai kesan anti sumbat bio yang boleh mengurangkan pembentukan biofilem. Objektif utama kajian ini adalah untuk mengenalpasti kesan anti sumbat bio daripada ekstrak daun sireh (PBE) terhadap membran yang tersumbat dan bagaimana PBE menyingkirkan kotoran berdasarkan kuorum penderiaan (QS). Kecenderungan membran tersumbat telah dikenalpasti bagi konsortium bakteria dan strain bakteria dalam air sisa batik. Semasa operasi MBR dengan konsortium bakteria, hubungan yang signifikan ($R^2 = 0.9916$) antara bahan polimer luar sel (EPS) dan tekanan transmемbran (TMP) yang telah ditunjukkan. MBR menunjukkan penyingkiran prestasi yang meningkat untuk pewarna dan penyingkiran permintaan oksigen secara kimia (COD) dengan masa operasi. Spektroskopi jelmamaan Fourier inframerah (FTIR) menunjukkan kehadiran EPS dalam kotoran membran. Tambahan pula, mikroskopi imbasan elektron (SEM) mengesahkan berlakunya kesumbatan. Plat mikrotiter asai mencadangkan bahawa strain FS5 menjadi penyumbang lapisan yang utama. Ujian kelompok pengeluaran EPS menunjukkan bahawa EPS yang dihasilkan oleh strain *Bacillus* (FS5) adalah dalam jumlah yang besar berbanding dengan konsortium bakteria. Kajian ini memberi tumpuan kepada kemungkinan ekstrak daun sireh (PBE) sebagai agen anti-sumbat bio terhadap organisma model *Pseudomonas aeruginosa* PAO1 dan konsortium bakteria. Kesan-kesan anti-sumbat bio terhadap PBE dinilai melalui plat mikrotiter asai; perubahan dalam kadar pertumbuhan (μ) dan pengeluaran EPS. SEM telah digunakan untuk menggambarkan pembentukan biofilem secara kualitatif. Kesan anti-sumbat bio terhadap PBE menunjukkan pengurangan $\geq 80\%$ dalam pembentukan biofilem, kadar pertumbuhan (87%) dan mengurangkan pengeluaran EPS. Tambahan pula, ia menurun kepekatan EPS larut, mengurangkan rintangan kek, dan meningkat dua kali ganda dalam masa yang diperlukan untuk mencapai 33 kPa bagi TMP. PBE menunjukkan kesan yang tidak signifikan pada kadar pereputan dalaman dan hasil biojisim. SEM zarah enapcemar di dalam bioreaktor PBE menunjukkan kehadiran campuran bakteria pada permukaannya dengan sempadan berbentuk sfera yang jelas. Selain itu, PBE menunjukkan kesan yang tidak signifikan mengenai prestasi rawatan biologi. Kaedah gerak balas permukaan (RSM) telah digunakan untuk mengurangkan EPS, maningkatkan kawalan TMP, dan penyingkiran pewarna dalam MBR ultraturasan. Keadaan optimum didapati pada pengurang kotoran (BFR) sebanyak 0.23 mg/mg MLSS, HRT pada 30.16 jam dan kadar aliran udara pada 0.60 l/min, dengan nilai-nilai yang diramalkan sebagai 28.28 mg/l EPS, 24.16 kPa bagi TMP dan 95.65% penyingkiran pewarna. Ujian pengesahan menunjukkan nilai yang sangat hampir dengan nilai-nilai yang diramalkan. Pengeluaran auto-pencetus di dalam bioreaktor telah disahkan dengan menggunakan penunjuk strain *Agrobacterium tumefaciens*. Selain itu, tiga AHLs yang berbeza telah didapati di kek menggunakan analisis kromatografi lapisan nipis. Peningkatan dalam EPS dan TMP diperhatikan dengan aktiviti AHL daripada kek semasa operasi MBR secara berterusan, menunjukkan bahawa membran yang tersumbat mempunyai hubungan yang rapat dengan aktiviti QS. PBE telah disahkan boleh mengurangkan membran yang tersumbat dengan menghalang pengeluaran AHLs. Keputusan ini menunjukkan bahawa pihak PBE boleh menjadi ejen novel untuk mensasarkan AHLs bagi mengurangkan membran yang tersumbat berdasarkan QS.

TABLES OF CONTENTS

		Page
SUPERVISOR’S DECLARATION		ii
STUDENT’S DECLARATION		iii
DEDICATION		iv
ACKNOWLEDGEMENTS		v
ABSTRACT		vi
ABSTRAK		vii
TABLE OF CONTENTS		viii
LIST OF TABLES		xiv
LIST OF FIGURES		xvi
LIST OF ABBREVIATIONS/SYMBOLS		xx
CHAPTER 1	INTRODUCTION	
1.1	Introduction	1
1.2	Background	1
1.3	Problem Statement	5
1.4	Research Objectives	6
1.5	Scope of the Study	7
1.6	Overview of the Thesis	8
CHAPTER 2	LITERATURE REVIEW	
2.1	Introduction	10
2.2	Textile Wastewater and its Effects	10
2.3	Batik wastewater	11
	2.3.1 Batik	11
	2.3.2 Process of Making Batik	11
2.4	Nature of Textile Effluent and its hazards	13
2.5	Dye Treatment technologies	14

2.6	Membrane bioreactors in Treatment of dyes	14
2.7	Introduction to Membrane and Membrane Bioreactor	16
	2.7.1 Fundamentals of Membrane	16
	2.7.2 Membrane bioreactor	18
2.8	Membrane Fouling	19
	2.8.1 Background	19
	2.8.1 Membrane biofouling	21
	2.8.3 Techniques to Characterize the Biofouled Membrane	22
	2.8.4 Major Causes of Biofouling	24
2.9	Biological Mitigation of Membrane Biofouling	27
	2.9.1 Enzymatic Control	29
	2.9.2 Control by Nitric Oxide	31
	2.9.3 Control by Bacteriophage	32
	2.9.4 Quorum Sensing Mitigation	32

CHAPTER 3 MATERIALS AND METHODS

3.1	Introduction	46
3.2	The biofouling propensity of Batik wastewater by indigenous bacteria	47
	3.2.1 Materials	47
	3.2.2 Sludge sample collection and preparation of activated sludge	47
	3.2.3 Submerged membrane bioreactor system and operation	47
	3.2.4 Analysis of main contributor to membrane fouling	50
	3.2.5 Isolation of bacterial Strains from fouled membrane	50
	3.2.6 Microtiter plate assay for biofouling	51
	3.2.7 Molecular characterization	51
	3.2.8 Batch tests for EPS production and harvesting	52
3.3	The anti-biofouling effect of <i>Piper betle</i> extract against <i>P. aeruginosa</i> and bacterial consortium	53
	3.3.1 Materials	53
	3.3.2 Model bacterial strain	53
	3.3.3 Sludge sample collection and preparation of activated sludge	53
	3.3.4 Preparation of plant extract	53
	3.3.5 Chemical components and microbial activity analysis of PBE	54
	3.3.6 Determination of Minimum inhibitory concentration of PBE	55
	3.3.7 Biofilm control assay	56
	3.3.8 Effect of PBE on growth profile	57

3.3.9	Effect of PBE on EPS production	57
3.3.10	Biofilm study on membrane	58
3.4	Influence of PBE on extracellular polymeric substances, sludge and filterability in MBR	59
3.4.1	Sludge sample collection and preparation of activated sludge	59
3.4.2	Preparation of Plant extract	59
3.4.3	Submerged membrane bioreactor system	60
3.4.4	The optimum dosage of BFR	60
3.4.5	Resistance in series model	61
3.4.6	Determining the effect of PBE on biokinetic parameters of activated sludge	61
3.4.7	Effect of PBE on biological treatment performance and characteristics of activated sludge	61
3.5	Design of process parameters for mitigation of EPS, TMP rise-up and decolorization of dye in MBR	62
3.5.1	Dye, activated sludge and BFR	62
3.5.2	Optimization using one factor at a time	63
3.6	Application of Response Surface Methodology for biofouling mitigation using PBE in MBR	64
3.6.1	Dye solution, activated sludge and BFR	64
3.6.2	Submerged membrane bioreactor system and operation	64
3.6.2	Experimental Design and optimization	64
3.7	Membrane biofouling control based on Quorum sensing using PBE	67
3.7.1	Biomonitor microorganism	67
3.7.2	Quorum sensing signal compounds	67
3.7.3	Luria-Bertani medium (LB)	67
3.7.4	Preparation of activated sludge, BFR, and reactor operation	67
3.7.5	Verification for autoinducer production	68
3.7.6	Crude extraction of AHL and TLC for AHL identification	68
3.7.7	Membrane biofouling mitigation based on QS	69
3.8	Analytical Methods	69
3.9	Statistical Analysis	73

CHAPTER 4 RESULTS AND DISCUSSION

4.1	Introduction	74
4.2	The biofouling propensity of batik wastewater by indigenous bacteria	74
	4.2.1 Main Contributor to Membrane Fouling	74
	4.2.2 Change of Soluble EPS and its Relationship with TMP	75
	4.2.3 Biological Treatment Performance	77
	4.2.4 FTIR Spectroscopy	78
	4.2.5 SEM Profiles	79
	4.2.6 Biofouling Potential of Bacterial Isolates and Bacterial Consortium	80
	4.2.7 Molecular Characterization	81
	4.2.8 EPS Production and Biochemical Characteristics	82
4.3	The anti-biofouling effect of <i>Piper betle</i> extract against <i>P. aeruginosa</i> and bacterial consortium	85
	4.3.1 Chemical components and microbial activity of PBE	85
	4.3.2 Minimum Inhibitory concentration (MIC) of PBE	87
	4.3.3 Biofilm reduction assay	88
	4.3.4 Growth profile and growth rate	89
	4.3.5 Effect of PBE on EPS production	90
	4.3.6 SEM analysis	93
4.4	Influence of biofouling reducer on EPS, sludge properties and filterability in a bioreactor	95
	4.4.1 The determination of BFR optimum dosage	95
	4.4.2 The effect of BFR concentration on soluble and bound EPS	96
	4.4.3 The effect of BFR on the characteristics of activated sludge	97
	4.4.4 The effect of BFR on biological treatment performance	100
	4.4.5 The effect of BFR on biokinetic parameters	101
	4.4.6 Effect of BFR on Membrane resistances	102
4.5	Design of Process parameters for mitigation of EPS, TMP rise-up control and decolorization of dye in MBR	103
	4.5.1 Effect of PBE dosage	103
	4.5.2 Effect of HRT	103
	4.5.3 Effect of Air flow rate	106
4.6	Application of Response Surface Methodology for biofouling mitigation using PBE in MBR	108
	4.6.1 Overall Performance	108
	4.6.2 Fitting model and analysis of variance (ANOVA)	109
	4.6.3 Adequacy check of the model	112

4.6.4	Optimization conditions and response surface analysis	113
4.6.5	Model validation and experimental confirmation	119
4.7	Membrane biofouling control based on Quorum Sensing using PBE	119
4.7.1	Quorum sensing identification in MBR	119
4.7.2	Relationship between biofouling and QS activity	120
4.7.3	Membrane biofouling mitigation based on QS	122

CHAPTER 5 CONCLUSION AND RECOMMENDATIONS

5.1	Conclusions	125
5.2	Recommendations	127

REFERENCES		128
-------------------	--	-----

APPENDICES		146
-------------------	--	-----

A1	Changes of EPS and TMP with operation time	146
A2	Biofouling activity of different bacterial isolates	147
A3	Extracted EPS (slime, capsular and broth (total)) concentrations for single bacterium	148
A4	Extracted EPS (slime, capsular and broth (total)) concentrations for Bacterial Consortium	149
A5	The log (CFU/ml) results for the cells measured for control and PBE treated	150
A6	The optical density (OD) results for the cells measured for control and PBE treated	151
A7	Extracted EPS (slime, capsular and broth (total)) concentrations for single bacterium	152
A8	Extracted EPS (slime, capsular and broth (total)) concentrations for Bacterial consortium	153
A9	Variations in soluble and bound EPS concentration as a function of BFR dosage	154
A10	Variations of MLSS in BFR (0.3 mg/mg MLSS) and control bioreactors	155

A11	Variations of SVI in BFR(0.3 mg/mg MLSS) and control bioreactors	156
A12	Variations in soluble and bound EPS concentration as a function of HRTs	157
A13	Effect of different HRTs on dye removal	158
A14	Variations in soluble and bound EPS concentration as a function of different air flow rates	159
A15	List of Publications	160

LIST OF TABLES

Table No.	Title	Page
2.1	General features of a textile dyeing wastewater	13
2.2	Different dye removal techniques	15
2.3	Advantages and disadvantages of side stream and submerged MBR	18
2.4	Bacterial biofilm and EPS/SMP characterized by different techniques	23
2.5	Relationship between major fouling factors and membrane biofouling	24
2.6	Capabilities, advantages and limitations of current biological methods to control membrane biofouling	28
2.7	Abbreviations used for quorum sensing signal molecules	35
2.8	Autoinducers detection, identification and characterization in membrane biofouling by different techniques	38
2.9	Relationship between quorum sensing and biofouling	40
2.10	Recent strategies to mitigate membrane biofouling based on quorum sensing	44
3.1	Membrane and membrane module specifications	49
3.2	Composition of synthetic dye wastewater	49
3.3	Operating conditions used for reactor	49
3.4	Operating conditions used for control and BFR bioreactor	60
3.5	Coefficient of biokinetic parameters	62
3.6	The chemical structure and properties of Reactive black 5	63
3.7	Variation of process factors for EPS, TMP and dye removal	63

3.8	Experimental range and levels of independent variables	65
3.9	Central composite design (CCD) with experimental and predicted results	66
4.1	GCMS data of the spots (antibacterial activity) for chemical components in PBE	87
4.2	Absorbance of biofilm and the percentage reduction of biofilm	89
4.3	EPS produced by single bacterium and bacterial consortium after 6 days and the percentage of reduction from control	93
4.4	The results of PBE optimum dosage tests	95
4.5	Biokinetic coefficients of aerobic sludge of control and PBE bioreactor	101
4.6	Resistances in the control and PBE bioreactor after 8 days operation	102
4.7	Central composite design (CCD) with experimental and predicted results	109
4.8	ANOVA of the quadratic models for EPS, TMP and Dye removal	111

LIST OF FIGURES

Figure No.	Title	Page
2.1	Flow chart shows the process of making batik	12
2.2	Annual publications on membrane biofouling	20
2.3	Membrane fouling: a) Pore blocking and cell attachment b) biofilm formation	21
2.4	Different biological methods to control membrane biofouling	29
2.5	Concept of membrane biofouling control based on QS	33
2.6	Bacterial QS systems. A: AHL mediated QS system in Gram negative bacteria. B: Autoinducer Peptide (AIP) QS in gram positive bacteria	34
2.7	Schematic overview of QS applications in biofouling	41
3.1	The reactor setup: 1- Feed tank, 2- Hollow fibre membrane, 3- Air splitter, 4- Pressure gauge, 5- Peristaltic pump, 6- Air compressor, 7- Effluent storage tank, 8- Air flow meter, 9- Drain valve, 10- Feed pump	48
3.2	Microtiter plat assay for biofouling activity of different bacterial isolates (FS-FS9) and bacterial consortium (C)	52
3.3	<i>Piper betle</i> Leaves	54
3.4	Microtitre plat assay for biofilm activity of PBE against <i>P. aeruginosa</i>	56
3.5	Schematic diagram of MBR setup	59
4.1	Changes of EPS and TMP with operation time	76
4.2	Relationship of EPS with TMP	77
4.3	Profile of removal performance of SMBR system for dye and COD	78

4.4	FTIR spectra of membrane foulants	79
4.5	SEM micrographs of biofilm developed on the membranes during ultrafiltration as a function of operating time; a: virgin membrane, b: 1 day, c: 4 days, d: 8 days	80
4.6	Biofouling activity of different bacterial isolates (at 570 nm) and bacterial consortium (at 600 nm)	81
4.7	Phylogenetic tree of <i>Bacillus</i> sp. FS5 with other <i>Bacillus</i> spp. based on 16S rDNA via neighbor joining method	82
4.8	Extracted EPS (Slime, capsular and broth (total)) concentrations for single Bacterium	84
4.9	Extracted EPS (Slime, capsular and broth (total)) concentrations for bacterial Consortium	84
4.10	TLC plates; Reference plate (A), microbial activity plate (B) and plate C was to identify spots with phenolic compound	85
4.11	GCMS chromatogram of <i>Piper betle</i> extract of leaves	86
4.12	The log (CFU/mL) results for the cells measured for control and PBE treated	88
4.13	Absorbance of biofilm formed at different time intervals at MIC of PBE	89
4.14	The growth profile of <i>P. aeruginosa</i> at control and PBE treated	90
4.15	Extracted EPS (slime, capsular, broth) concentrations of control and PBE treated for single bacterium	92
4.16	Extracted EPS (slime, capsular, broth) concentrations of control and PBE treated for bacterial consortium	92
4.17	SEM micrographs, (a) Virgin membrane (b) control [single bacterium]; showing bacterial population and EPS, (c) treated with PBE [single bacterium]; Bacterial cells attached to membrane, (d) control [consortium]; showing bacterial population and EPS, (e) treated with PBE [consortium]	94
4.18	Variations in soluble and bound EPS concentration as a function of PBE dosage	97

4.19	Variations of MLSS in BFR (0.3 mg/mg MLSS) and control bioreactors	98
4.20	Variations of SVI in BFR (0.3 mg/mg MLSS) and control bioreactors	99
4.21	SEM micrographs of sludge particles, (a) Control: showing outer surface of sludge particle, (b) PBE treated: showing spherical shaped outlined boundary, (c) Control: Bacterial cells embedded in EPS matrix, (d) PBE treated: dense mixture of bacterial cells packed in EPS	100
4.22	Effect of different HRTs on TMP	104
4.23	Variations in soluble and bound EPS concentration as a function of different HRTs	105
4.24	Effect of different HRTs on dye removal	105
4.25	Effect of different air flow rates on TMP	106
4.26	Variations in soluble and bound EPS concentration as a function of different air flow rates	107
4.27	Effect of different air flow rates on dye removal	108
4.28	Actual and predicted values of (a) EPS, (b) TMP and (c) dye removal	116
4.29	The internally studentized residuals and normal % probability plot for EPS (a), TMP (b), and dye removal (c)	117
4.30	Response surface for EPS (a)-(b), TMP (c)-(e), and for dye removal (f)-(g)	118
4.31	Identification of AHL signal activity in biocake of membrane: (a) bioassay for detection of AHL activity (b) TLC profile for AHL identification	120
4.32	Presence of AHL signals in biocake (a-d) (a- 48 h, b- 96, c-144, d-192) EPS in biocake (e) and variations of TMP and AHL level (f) in biocake in MBR during continuous membrane operation	121
4.33	TMP profiles: verification for biofouling mitigation by quorum sensing	123
4.34	Evidence for membrane biofouling mitigation by QS: (a), (b), (c)	124

A. tumefaciens bioassay results. Bioassay was carried out after 4 days operation, (d) quantitative analysis of EPSs in biocake in the MBR under various operating conditions

LIST OF ABBREVIATIONS/SYMBOLS

AFM	Atomic Force Microscopy
AHL	Acyl homoserine lactones
AI	Autoinducer
ATP	Adenosine triphosphate
BOD	Biological oxygen demand
CCD	Central composite design
CLSM	Confocal laser scanning microscopy
COD	Chemical oxygen demand
DGGE	Denaturing gradient gel electrophoresis
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DOE	Department of Environment
DOTM	Direct observation through the membrane
EPS	Extracellular polymeric substances
F/M	Food to microorganism ratio
FISH	Fluorescence <i>in situ</i> hybridization
FTIR	Fourier transform infrared spectroscopy
GCMS	Gas chromatography-mass spectrometry
HHL	Hexanoyl homoserine lactone
HPLC	High pressure liquid chromatography
HRT	Hydraulic retention time
MEC	Magnetic enzyme carrier
MLSS	Mixed liquor suspended solids

OD	Optical Density
OFAT	One-factor-at-a-time
OHL	Octanoyl homoserine lactone
OLR	Organic loading rate
PBE	<i>Piper betle extract</i>
PCR	Polymerase Chain reaction
QS	Quorum Sensing
R_c	Cake resistance
RSM	Response surface methodology
SEM	Scanning electron microscopy
SMBRs	Submerged membrane bioreactors
SMP	Soluble microbial product
SNP	S-nitroso-N-acetylpenicillamine
SRT	Sludge retention Time
TLC	Thin layer chromatography
TMP	Transmembrane pressure
UF	Ultrafiltration membrane
$^{\circ}\text{C}$	Degree Celsius
min	Minute
cm^2	Square centimeter
g	Gram
H	Height
h	Hour

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

This chapter describes the background of batik dye wastewater, treatment methods, membrane biofouling and biological mitigation of membrane biofouling. The first part of chapter explains about batik wastewater; nature and hazards of dye wastewater. Meanwhile, the second part explains a brief background of the treatment methods for dye wastewater. The third part explains about membrane biofouling. The fourth part discusses mitigation of membrane biofouling. Finally, for the last part of this chapter, problem statement, objectives and the scopes of study were described.

1.2 BACKGROUND

1.2.1 BATIK WASTEWATER, NATURE AND HAZARDS OF DYES

In Malaysia, the textile sector is the third largest foreign exchange earner after the palm oil and electronic industries (Malaysian Textile Manufacturers Association (MTMA), 2008). Approximately 1500 textile industries operate in Malaysia, most of which are backyard industries making the local 'batik'. These homemade textile industries are well known in Malaysia. These industries are traditionally inherited from generation to generation. The batik making process can generally be categorized into four processes, cloth preparation, application of wax, dyeing of cloth and removal of wax in boiling water (Ahmad et al., 2002).

These textile facilities discharge a large amount of wastewater, which contains many types of dyes, solvents, salts and detergents (Marcucci et al., 2003, and Ali et al., 2009). Wastewater dye affects water transparency, gas solubility, and aesthetics of aquatic systems, and can be toxic to aquatic organisms (Vandevivere et al., 1998, and Siddiqui et al., 2010). Moreover, most synthetic azo dyes are noxious, mutagenic and carcinogenic, causing danger to organisms (Nilsson et al., 1993, and Siddiqui et al., 2009). The limits for the discharge of color effluents are 100 and 200 Platinum–Cobalt units according to standards A and B, respectively (Department of Environment (DOE), 2010).

1.2.2 TREATMENT TECHNIQUES

Textile wastewater is often treated with physio-chemical methods, but these methods are generally very expensive (Robinson et al., 2001). Moreover, the complex molecular structure of dyes makes them more resistant to degradation via biological methods. Thus, there is an urgent need to develop a suitable technology for treatment of dyes in textile effluent.

In recent years, submerged membrane bioreactors (SMBRs) have received significant interest, because they eliminate the need of a secondary settling tank. SMBRs also having small reactor space and it usually produce very less amount of sludge (Yun et al., 2006). However, the membrane fouling prevents its large scale application, as it is a major hitch (Meng et al., 2009). Fouling is of various types, e.g. organic, inorganic, and physical and biofouling (Kramer and Tracey, 1995). Of these, “biofouling” resulting from extracellular polymeric substances and microbial cells is a difficult task (Yu et al., 2010). It reduces the membrane life span; decreases the permeate flux, and ultimately add an extra capital cost for the replacement of a membrane (Yu et al., 2010).

1.2.3 MEMBRANE BIOFOULING

Membrane biofouling is a pervasive membrane system problem. Biofouling process involves adhesion and growth of microorganisms on the membrane surface (Flemming et al., 1997, and Wang et al., 2005). Biofouling is hard to control, even by reducing the number of microorganisms in the feed water, because they can multiply even if their number is strongly diminished, and they will do so if nutrients are available (Ridgway and Flemming, 1996). Since microorganisms are abundant in wastewater effluents and due to prevention methods such as disinfection or MF/UF pretreatment in technical systems neither leads to sterility nor is maintained over a long period of time. Moreover, the microorganisms will always invade and colonize the system. Thus if they removed to 99.99% there are still enough cells left which can grow at the expense of biodegradable substances in the feed stream. In membrane systems biofouling represents the Achilles heel of the process because all other fouling components such as organic and inorganic dissolved substances can be removed by pretreatment (Ridgway and Flemming, 1996). Biofouling leads to considerable technical problems and economic loss. During the past two decades, the membrane bioreactor (MBR) has emerged as one of the innovative technologies in wastewater treatment. Biofouling is still an unsolved problem (Yeon et al. 2009).

Extracellular polymeric substances (EPSs) are found to be key substances to cause membrane biofouling (Massé et al., 2002, and Rosenberger et al., 2002). These compounds are high molecular weights that are produced by bacterial cells. These compounds comprised of polysaccharides, lipoproteins, proteins, and deoxyribonucleic acid (DNA). Recently, studies on EPS in either soluble microbial product (SMP) or bound form have attained growing interest (Yeon et al., 2009). EPS shows an essential component of biofilm development and structure, particularly mechanical strength, attachment, and protection against environmental deleterious effects (Tansel et al., 2008). It is necessary to remove EPS (soluble) from the activated sludge, because they can pose internal fouling and hence decrease the membrane flux (Lee et al., 2003).

It has been known that biofilm formation in liquid–solid interfaces is controlled via quorum sensing (QS). QS refers to the density-dependent regulation of gene expression in microorganisms (Freeman and Bassler, 1999). During the bacterial quorum sensing, *N*-acyl homoserine lactones (AHL) are produced from the microorganisms. AHL has been known as quorum sensing molecules (i.e., autoinducer (AI)) transferring bacterial signals from one to another and, thus controlling the rate and the extent of biofilm formation (Hu et al., 2003).

1.2.4 MEMBRANE BIOFOULING CONTROL

So far, extensive research has been pursued to investigate the possible methods to control membrane biofouling. Many physico-chemical methods have been used, for regular physical and chemical cleaning, etc. (Ramesh et al., 2006), and they may not be effective and energy efficient. Although several biofouling control techniques have been developed through engineering (Yeon et al., 2005), material (Yu et al., 2007), and chemistry approaches (Lee et al., 2001), all these attempts have the limitation that they are essentially not able to prevent the biofilm formation because it is intrinsically a natural biological process. Sometimes it is hard to reach all the areas that are contaminated with biofilm. Acidic and alkaline solutions are occasionally used to remove biofilm from surfaces by washing, but there is an issue of adverse environmental impact. Thus, it appears that biological control of microbial attachment would be a novel promising alternative for mitigating membrane biofouling and would be a new niche that deserves further study (Xiong and Liu, 2010). It would be better to prevent biofilm formation rather than killing the cells after it forms. However, killing the cells using antibiotics, as practiced in industry, for example, does not always work, because it is not usually possible to kill all the cells completely for an extended time, and some cells still can attach onto the solid surface to form a biofilm (Costerton, 1999).

Recently, a number of QS inhibitors have been discovered and it has revealed a novel way to target QS to mitigate biofilm. Not surprisingly, the QS inhibitors of plants such as *Vanilla planifolia* (Choo et al., 2006), *Bucida buceras* (Adnonizio et al., 2006), and

Terminalia catappa (Taganna et al., 2011) inhibit bacterial QS. Results from these studies enabled us to hypothesize that, in principle; membrane biofouling originating from biofilm formation could also be alleviated through QS control, e.g., the blocking of intercellular communication to mitigate membrane biofouling.

Thus, it is clear that mitigation of biofilm attachment on membrane would be a new alternative to control membrane biofouling and it would be a new route which needs further research (Xiong and Liu, 2010). Several plant products are known for their antibacterial activities (Kubo et al., 2006), and in this study, it was hypothesized that these may help reduce biofilm formation (Sendamangalam et al., 2009). The extract of *Piper betle* plant (PBE) leaves has been reported to possess many biological activities that contributed its role as an antibacterial agent (Nair and Chanda, 2008). *Piper betle* extract control the growth of many gram-positive and gram- negative microbes (Nair and Chanda, 2008).

1.3 PROBLEM STATEMENT

Biofouling control is a difficult and challenging task because some microorganisms survive and grow rapidly. Biofouling control techniques have been developed, but they are not able to prevent the biofilm formation because it is intrinsically a natural biological process. Therefore, elucidating mechanisms involved in biofilm formation as well as developing the methods for controlling biofilm formation is important for the efficient application of membrane technologies. It would be better to prevent biofilm (biofouling) formation rather than killing the cells after it form biofilm because once biofilm form it's 10-1000 times more resistant. Several plant products are known for their antibacterial activities (Kubo et al., 2006, and Sendamangalam et al., 2011), and in this study, it was hypothesized that these may help reduce biofilm formation. No information, however, is available on the *Piper betle* (L.) extract to mitigate membrane biofouling. In this study, the concept of bacteriostatic effect and quorum sensing (QS) of *Piper betle* extract on biofouling control was evaluated. For this research, we hypothesized that in principal, preventing biofilm formation, targeting QS system and extracellular polymeric substances (EPS) production, membrane biofouling could also be alleviated through the use of *Piper*

betle extract (PBE), to control its growth, formation of biofilm, disruption of QS and production of EPS.

1.4 RESEARCH OBJECTIVES

a) Main objective

This study seeks to evaluate the biological control agent (*Piper betle* extract) to control membrane biofouling in a submerged membrane bioreactor.

b) Specific Objectives

- i. To determine the biofouling potential of batik wastewater by indigenous bacteria.
- ii. To evaluate the anti-biofouling effect of *Piper betle* extract (PBE) against model bacterium *Pseudomonas aeruginosa* and bacterial consortium.
- iii. To determine the influence of PBE on extracellular polymeric substances (EPS), biokinetic parameters, sludge properties, membrane filterability and reactor treatment performance.
- iv. To determine the optimized conditions for EPS removal, transmembrane pressure rise-up control and dye removal in membrane reactor using response surface methodology (RSM).
- v. To determine how PBE target the Quorum Sensing (QS) (autoinducer signals) to mitigate membrane biofouling.

1.5 SCOPE OF STUDY

To accomplish the above objectives, the following tasks were undertaken:

1. The resistance-in-series model was applied to find out the main contributor to membrane fouling. The bacterial consortium in membrane bioreactor (MBR) exhibited a significant relationship ($R^2= 0.9916$) between EPS and transmembrane pressure (TMP).
2. Biofilm formation was qualitatively illustrated via scanning electron microscopy (SEM), to confirm the occurrence of biofouling. FTIR spectra of membrane foulants qualitatively confirmed the presence of polysaccharides and proteins as major components.
3. Microtiter plat assay was carried out to determine the biofouling activity of indigenous bacteria from batik wastewater. Batch tests of the production of extracellular polymeric substances (EPS) by *Bacillus* strain (FS5) was compared to the bacterial consortium.
4. The anti-biofouling effects of PBE against model bacterium *Pseudomonas aeruginosa* and bacterial consortium were evaluated via a microtiter plate assay; changes in the growth rate (μ) and EPS production. SEM was employed to qualitatively illustrate the biofilm formation.
5. The influence of different concentrations of *Piper betle* extract (PBE) as a biofouling reducer, on soluble and bound EPS was carried out in submerged membrane bioreactor.
6. The effect of addition of PBE on cake resistance and time to reach 33 kPa of TMP was also determined.
7. The effect of PBE on biokinetic properties of sludge, sludge volume index, biomass in reactor, chemical oxygen demand and color removal was also carried out.
8. The effects of PBE dosage (mg/mg MLSS), HRT (h), and air flow rate (l/min) on EPS mitigation, TMP rise-up control and dye removal were