

**PHYTOREMEDIATION OF PALM OIL MILL FINAL DISCHARGE
WASTEWATER USING SELECTED AQUATIC MACROPHYTES**

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ABSTRACT

This study assesses the treatment of palm oil mill (POM) final discharge wastewater using a cost effective technology approach of phytoremediation. POM final discharge wastewater obtained from final distribution pond at KL Kepong Berhad Palm Oil Mill, Tanjong Malim was treated with three species of local macrophytes, *Leersia oryzoides*, *Pistia stratiotes* and *Ludwigia peploides*. The phytoremediation performance was evaluated by monitoring the changes and removal efficiency (%) of BOD₅, COD, N-NH₃, TSS and pH level. The characteristics of nutrient uptake were determined by measuring the percentage of C, H and N elements, bioconcentration factor (BCF) and translocation factor (TF) of Ca, Mg, K, Na, Fe and Zn in leaf, stem and root organs of the macrophytes. The microbial population in the rhizosphere was identified using partial 16S rRNA molecular technique. It was found that BOD₅, COD, N-NH₃ removal efficiencies of 93%, 30%, and 82% were achieved for *Pistia stratiotes*, 90%, 27% and 80% for *Leersia oryzoides* and 93%, 20% and 80% for *Ludwigia peploides*, respectively after 15 days of treatment. The N percentages were increased in all studied macrophytes leaves. The BCF values for Fe and Zn were found highest in *Ludwigia peploides* than other elements, 1.47×10^4 L/kg and 1.18×10^4 L/kg, respectively. Low TF values (<1.0) obtained for Fe and Zn indicated that most of them were retained in root for phytostabilization. In this study, *Bacillus megaterium*, *Pseudomonas aeruginosa* and *Bacillus cereus* that usually involved in denitrification process was identified in *Pistia stratiotes*, *Ludwigia peploides* and *Leersia oryzoides* roots, respectively which confirmed the macrophytes-microorganisms interaction in the phytoremediation. These results suggest that phytoremediation of POM final discharge wastewater was feasible with *Ludwigia peploides* as the most tolerance macrophytes. The finding is of prominence important for improvement of the existing palm oil mill effluent (POME) treatment system.

FITOREMEDIASI AIR BUANGAN AKHIR KILANG KELAPA SAWIT MENGUNAKAN MAKROFITA AKUATIK TERPILIH

ABSTRAK

Kajian ini menilai kaedah rawatan air buangan akhir kilang kelapa sawit (POM) dengan menggunakan teknologi kos efektif iaitu fitoremediasi. Air buangan akhir kilang kelapa sawit yang diperolehi dari kolam pengagihan akhir kilang kelapa sawit KL Kepong Berhad Tanjung Malim dirawat dengan tiga spesis makrofita tempatan iaitu *Leersia oryzoides*, *Pistia stratiotes* dan *Ludwigia peploides*. Prestasi fitoremediasi dinilai melalui pemerhatian terhadap perubahan dan kecekapan penyingkiran (%) BOD₅, COD, N-NH₃, TSS dan pH. Ciri-ciri pengambilan nutrien ditentukan dengan mengukur peratusan elemen C, H dan N, faktor biokonsentrasi (BCF) dan faktor translokasi (TF) bagi Ca, Mg, K, Na, Fe dan Zn di dalam organ daun, batang dan akar makrofita. Populasi mikroorganisma di rizosfera dikenalpasti menggunakan teknik molekular 16S rRNA. Dapat diperhatikan bahawa kecekapan penyingkiran BOD₅, COD, N-NH₃ ialah masing-masing 93%, 30%, 82% bagi *Pistia stratiotes*, 90%, 27%, 80% bagi *Leersia oryzoides* dan 93%, 20%, 80% bagi *Ludwigia peploides* selepas 15 hari rawatan. Peratusan N meningkat di dalam semua daun makrofita yang dikaji. Nilai BCF bagi Fe dan Zn didapati paling tinggi di dalam *Ludwigia peploides* berbanding dengan elemen-elemen lain iaitu masing-masing 1.47×10^4 L/kg dan 1.18×10^4 L/kg. Nilai TF yang rendah (<1.0) yang diperolehi bagi Fe dan Zn menunjukkan bahawa kebanyakan elemen-elemen ini ditahan di akar bagi proses fitostabilisasi. Kajian mendapati *Bacillus megaterium*, *Pseudomonas aeruginosa* dan *Bacillus cereus* yang biasanya terlibat dalam proses denitrifikasi telah dikenalpasti pada akar *Pistia stratiotes*, *Ludwigia peploides* dan *Leersia oryzoides* yang mana mengesahkan interaksi makrofita-mikroorganisma di dalam fitoremediasi. Dapatan ini mencadangkan fitoremediasi air buangan akhir POM boleh dilakukan dengan *Ludwigia peploides* sebagai makrofita paling toleran. Dapatan kajian ini adalah sangat penting untuk sistem rawatan efluen kilang kelapa sawit (POME) sedia ada.

TABLE OF CONTENTS

| | Page |
|---|-------------|
| ACKNOWLEDGEMENT | iii |
| ABSTRACT | iv |
| ABSTRAK | v |
| TABLE OF CONTENTS | vi |
| LIST OF TABLE | xiii |
| LIST OF FIGURES | xv |
| LIST OF ABBREVIATIONS | xviii |
| | |
| CHAPTER 1 INTRODUCTION | 1 |
| 1.1 Background of Study | 1 |
| 1.2 Significance of Study | 6 |
| 1.2.1 Phytoremediation as an Environmental and Cost Effective Technology for Tertiary Treatment of POM Final Discharge Wastewater | 6 |
| 1.2.2 Compliance to Malaysian Environmental Regulation | 7 |
| 1.3 Objectives | 9 |
| | |
| CHAPTER 2 LITERATURE REVIEW | 10 |
| 2.1 Macrophytes for Phytoremediation | 10 |
| 2.1.1 Classification of Macrophytes | 10 |
| 2.1.2 Adaptation of Macrophytes | 12 |
| 2.1.3 Use of Macrophytes to Treat Water and Wastewater | 14 |

| | |
|--|----|
| 2.1.3.1 <i>Leersia oryzoides</i> | 15 |
| 2.1.3.2 <i>Ludwigia peploides</i> | 16 |
| 2.1.3.3 <i>Pistia stratiotes</i> | 17 |
| 2.1.4 Role of Macrophyte Roots in Phytoremediation | 18 |
| 2.2 Mechanisms of Phytoremediation | 19 |
| 2.2.1 Phytoextraction | 19 |
| 2.2.2 Phytoaccumulation | 20 |
| 2.2.3 Phytostabilization | 20 |
| 2.2.4 Phytodegradation | 21 |
| 2.2.5 Rhizodegradation | 22 |
| 2.2.6 Rhizofiltration | 23 |
| 2.2.7 Phytovolatilization | 24 |
| 2.3 Phytoremediation Performance of Wastewater | 25 |
| 2.3.1 Biodegradable organic removal | 26 |
| 2.3.2 Chemical Oxygen Demand (COD) Removal | 27 |
| 2.3.3 Ammoniacal Nitrogen Removal | 28 |
| 2.3.4 Solids Removal | 30 |
| 2.3.5 pH Changes | 30 |
| 2.4 Nutrients Uptake and their Functions in Phytoremediation | 32 |
| 2.4.1 Mechanism of Nutrient Uptake and Allocation | 32 |
| 2.4.2 Factors Influence Nutrient Uptake | 33 |
| 2.4.2.1 Growth Rate | 33 |
| 2.4.2.2 Bioavailability of Nutrient | 35 |
| 2.4.2.3 Bioconcentration Factor (BCF) and Translocation | 35 |

Factor (TF)

| | |
|---|-----------|
| 2.4.2.4 Age of Macrophytes and Decay Rate | 36 |
| 2.4.2.5 Types of Macrophyte Roots | 37 |
| 2.4.2.6 Effect of Phytotoxicity | 38 |
| 2.4.3 Allocation of Nutrients in Macrophytes Organs | 39 |
| 2.4.4 Transformation of Nutrients in Macrophytes | 40 |
| 2.4.4.1 Carbon (C) | 41 |
| 2.4.4.2 Nitrogen (N) | 42 |
| 2.4.4.3 Calcium (Ca) | 44 |
| 2.4.4.4 Magnesium (Mg) | 45 |
| 2.4.4.5 Potassium (K) | 46 |
| 2.4.4.6 Sodium (Na) | 47 |
| 2.4.4.7 Iron (Fe) | 47 |
| 2.4.4.8 Zinc (Zn) | 49 |
| 2.5 Microorganisms Involvement in Phytoremediation | 49 |
| 2.5.1 Role of Microorganism in Phytoremediation | 49 |
| 2.5.2 Microbial Population and Identification in Phytoremediation | 51 |
| CHAPTER 3 PHYTOREMEDIATION PERFORMANCE OF POM FINAL | 54 |
| DISCHARGE WASTEWATER USING <i>Leersia oryzoides</i>, | |
| <i>Ludwigia peploides</i> AND <i>Pistia stratiotes</i> | |
| 3.1 Introduction | 54 |
| 3.2 Methodology | 55 |
| 3.2.1 Selection of Tolerant Macrophytes to POM Final Discharge | 55 |

Wastewater

| | | |
|---------|--------------------------------------|----|
| 3.2.2 | Macrophyte Species | 56 |
| 3.2.2.1 | <i>Leersia oryzoides</i> | 56 |
| 3.2.2.2 | <i>Pistia stratiotes</i> | 57 |
| 3.2.2.3 | <i>Ludwigia peploides</i> | 58 |
| 3.2.3 | POM Final Discharge Wastewater | 59 |
| 3.2.4 | Experimental Set Up | 60 |
| 3.2.5 | Phytoremediation Process | 61 |
| 3.2.5.1 | Chemical Analyses | 62 |
| 3.2.5.2 | Plant Weight | 63 |
| 3.2.6 | Data Analysis | 63 |
| 3.2.7 | Statistical Analysis | 64 |
| 3.3 | Results and Discussions | 64 |
| 3.3.1 | Selection of Macrophytes | 64 |
| 3.3.2 | Phytoremediation Performance | 65 |
| 3.3.2.1 | BOD ₅ Removal | 66 |
| 3.3.3.2 | COD Removal | 69 |
| 3.3.3.3 | NH ₃ -N Removal | 71 |
| 3.3.3.4 | Changes in pH level | 73 |
| 3.3.3.5 | Changes in TSS | 74 |
| 3.3.4 | Plant Weight | 75 |
| 3.3.5 | Morphological Changes of Macrophytes | 77 |
| 3.4 | Conclusions | 79 |

CHAPTER 4 CHARACTERIZATION OF NUTRIENT UPTAKE IN MACROPHYTES DURING PHYTOREMEDIATION

| | |
|--|-----|
| 4.1 Introduction | 81 |
| 4.2 Methodology | 82 |
| 4.2.1 Preparation of Plant Sample for CHNO-S and Atomic Absorption Spectrometry (AAS) Analysis | 82 |
| 4.2.2 Atomic Absorption Spectrometry (AAS) | 83 |
| 4.2.3 CHNS-O Elemental Analysis | 83 |
| 4.2.4 Bioconcentration Factor (BCF) | 84 |
| 4.2.5 Translocation Factor (TF) | 84 |
| 4.3 Results and Discussions | 85 |
| 4.3.1 Nitrogen (N) Percentage in Macrophytes Organs | 85 |
| 4.3.2 Carbon (C) Percentage in Macrophytes Organs | 86 |
| 4.3.3 Hydrogen (H) Percentage in Macrophytes Organs | 88 |
| 4.3.4 Magnesium (Mg) | 90 |
| 4.3.5 Calcium (Ca) | 93 |
| 4.3.6 Potassium (K) | 96 |
| 4.3.7 Sodium (Na) | 98 |
| 4.3.8 Iron (Fe) | 100 |
| 4.3.9 Zinc (Zn) | 103 |
| 4.4 Conclusions | 105 |

CHAPTER 5 MICROBIAL POPULATION IDENTIFICATION AND CONTRIBUTION IN PHYTOREMEDIATION OF POM FINAL DISCHARGE WASTEWATER 106

| | |
|--|-----|
| 5.1 Introduction | 106 |
| 5.2 Methodology | 107 |
| 5.2.1 Scanning Electron Microscopy (SEM) | 107 |
| 5.2.2 Microbial Enumeration | 107 |
| 5.2.3 Isolation of Pure Colony | 108 |
| 5.2.4 Molecular Identification of Isolates | 108 |
| 5.2.5 DNA Extraction | 109 |
| 5.2.6 Polymerase Chain Reaction (PCR) Amplification | 110 |
| 5.2.7 PCR Purification and DNA Sequencing | 111 |
| 5.3 Results and Discussions | 111 |
| 5.3.1 Bacteria Morphology | 111 |
| 5.3.2 Microbial Populations | 114 |
| 5.3.3 DNA Extraction and Polymerase Chain Reaction (PCR) | 115 |
| 5.3.4 Molecular Identification of Bacteria Isolated from Macrophytes Root | 118 |
| 5.3.4.1 Selected Bacteria Sequence from <i>Pistia stratiotes</i> roots | 118 |
| 5.3.4.2 Selected Bacteria Sequence from <i>Ludwigia peploides</i> roots | 120 |
| 5.3.4.2 Selected Bacteria Sequence from <i>Leersia oryzoides</i> roots | 121 |

| | | | |
|--|------------------------------------|------------------------------------|--------------|
| UNIVERSITI PENDIDIKAN SULTAN IDRIS | UNIVERSITI PENDIDIKAN SULTAN IDRIS | UNIVERSITI PENDIDIKAN SULTAN IDRIS | 122 |
| 5.4 Conclusions | UNIVERSITI PENDIDIKAN SULTAN IDRIS | UNIVERSITI PENDIDIKAN SULTAN IDRIS | UNIVERSITI F |
| CHAPTER 6 GENERAL DISCUSSION | | | 123 |
| 6.1 Phytoremediation Performance | | | 123 |
| 6.2 Characterization of Nutrient Uptake | | | 125 |
| 6.3 Microbial Identification in Phytoremediation | | | 127 |
| CHAPTER 7 CONCLUSIONS AND RECOMMENDATIONS | | | 129 |
| 7.1 Conclusions | | | 129 |
| 7.2 Recommendations | | | 131 |
| REFERENCES | | | 133 |
| APPENDICES | | | 151 |



LIST OF TABLES

| TABLE | TITLE | PAGE |
|--------------|---|-------------|
| 1.1 | Characteristics of POME and POM final discharge wastewater | 5 |
| 1.2 | Comparison of three tertiary treatment of wastewater, dissolved air floatation (DAF), reverse osmosis (RO) and phytoremediation | 9 |
| 2.1 | Rooted macrophytes classification | 11 |
| 2.2 | Non-rooted macrophytes classification | 11 |
| 2.3 | Phytoremediation of various kind of wastewater using macrophytes | 25 |
| 2.4 | Sixteen essential plant nutrients | 40 |
| 3.1 | Characteristics of POM final discharge wastewater used in this study | 61 |
| 3.2 | Selection of macrophytes that tolerant to POM final discharge wastewater | 65 |
| 3.3 | Characteristics of POM final discharge wastewater after phytoremediation treatment | 66 |
| 4.1 | Nitrogen (N) level before and after phytoremediation study and its allocation percentage in macrophytes organs | 86 |
| 4.2 | Carbon (C) level in macrophytes organs before and after | 87 |

phytoremediation study

| | | |
|-----|---|-----|
| 4.3 | Hydrogen (H) level in macrophyte organ before and after phytoremediation study and its allocation percentage | 89 |
| 4.4 | BCF and TF of Mg in different macrophytes organs | 92 |
| 4.5 | BCF and TF of Ca in different macrophytes organs | 95 |
| 4.6 | BCF and TF of K in different macrophytes organs | 97 |
| 4.7 | BCF and TF of Na in different macrophytes organs | 99 |
| 4.8 | BCF and TF of Fe in different macrophytes organs | 102 |
| 4.9 | BCF and TF of Zn in different macrophytes organs | 104 |
| 5.1 | Heterotrophic bacteria count for the studied macrophytes roots and POM final discharge wastewater before and after the phytoremediation study | 116 |
| 5.2 | Bacterial similarity identification from BLAST database for selected bacterial DNA samples | 118 |
| 5.3 | Bacteria similarity identification from RDP II database for selected bacterial DNA samples | 119 |

LIST OF FIGURES

| FIGURE | TITLE | PAGE |
|--------|--|------|
| 1.1 | Schematic flow diagram of ponding system for POME treatment | 3 |
| 2.1 | Three main types of macrophytes | 12 |
| 2.2 | <i>Leersia oryzoides</i> | 15 |
| 2.3 | <i>Ludwigia peploides</i> | 16 |
| 2.4 | <i>Pistia stratiotes</i> | 17 |
| 2.5 | Molecular mechanisms proposed to be involved in transition metal accumulation by plants. | 34 |
| 2.6 | Relationship between nutrient supply and nutrient accumulation | 38 |
| 3.1 | <i>Leersia oryzoides</i> | 57 |
| 3.2 | <i>Pistia stratiotes</i> | 58 |
| 3.3 | <i>Ludwigia peploides</i> | 59 |
| 3.4 | Treatment process of POME at Tanjung Malim KL Kepong Palm Oil Mill at Changkat Asa, Tanjung Malim, Perak | 60 |
| 3.5 | Changes in BOD ₅ (a) and BOD ₅ removal efficiency (b) of POM final discharge wastewater treated with three macrophytes species | 68 |

| | | |
|------|--|----|
| 3.6 | Changes in COD (a) and COD removal efficiency (b) of POM final discharge wastewater treated with three macrophytes species | 70 |
| 3.7 | Changes in NH ₃ -N (a) and NH ₃ -N removal efficiency (b) of POM final discharge wastewater treated with three macrophytes species | 72 |
| 3.8 | Changes in pH level of POM final discharge treated with three macrophyte species | 74 |
| 3.9 | Changes in total suspended solid (TSS) of POM final discharge wastewater treated with three macrophytes species | 75 |
| 3.10 | Changes in plant weight during phytoremediation of POM final discharge wastewater | 76 |
| 3.11 | Wilting of macrophyte's leaves throughout phytoremediation study | 78 |
| 3.12 | Morphological changes of macrophytes throughout experiment | 79 |
| 4.1 | Nitrogen (N) percentage in macrophytes organs before and after phytoremediation experiment | 86 |
| 4.2 | Carbon (C) percentages in macrophyte organ before and after phytoremediation treatment | 88 |
| 4.3 | Hydrogen (H) percentages in macrophyte organ before and after phytoremediation treatment | 90 |

| | | |
|-----|---|-----|
| 4.4 | Mg concentration in macrophyte organ before and after phytoremediation treatment | 92 |
| 4.5 | Ca concentration in macrophyte organ before and after phytoremediation treatment | 95 |
| 4.6 | K concentration in macrophytes organs before and after phytoremediation treatment | 97 |
| 4.7 | Na concentration in macrophytes organs before and after phytoremediation treatment | 100 |
| 4.8 | Fe concentration in macrophytes organs before and after phytoremediation treatment | 102 |
| 4.9 | Zn concentration in macrophytes organs before and after phytoremediation treatment | 104 |
| 5.1 | Bacterial population observed in <i>Leersia oryzoides</i> root | 112 |
| 5.2 | Bacteria population observed in <i>Ludwigia peploides</i> root | 113 |
| 5.3 | Bacteria population observed in <i>Pistia stratiotes</i> root | 113 |
| 5.4 | Gel gradient electrophoresis of DNA extracts from six microorganisms isolated from macrophytes root | 117 |
| 5.5 | Gel gradient electrophoresis of PCR product after PCR amplification | 117 |

LIST OF ABBREVIATIONS

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS

UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS UNIVERSITI PENDIDIKAN SULTAN IDRIS

BLAST – Basic Local Alignment Search Tool

BOD₃ – 3-day Biochemical Oxygen Demand

BOD₅ - 5-day Biochemical Oxygen Demand

COD - Chemical Oxygen Demand

DO - Dissolved Oxygen

HPC – Heterotrophic Plate Count

HRT – Hydraulic Retention Time

POM – Palm Oil Mill

POME – Palm Oil Mill Effluent

POM FD – Palm Oil Mill Final Discharge

RDP II – Ribosomal Database Project II

TF – Translocation Factor

TS – Total Solid

TSS – Total Suspended Solid

VFA- Volatile Fatty Acids

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Phytoremediation is a method that use plants or plant organs to remove, degrade, or render harmless hazardous materials present in soil and water. The emerging technology may offer a cost effective, non-intrusive, and safe alternative to conventional soil clean up techniques by using the ability of certain tree, shrub, and grass species to remove, degrade or immobilize harmful chemical from soil or water (Vishnoi & Srivasti, 2008). For many years macrophytes have been known to play an important role in removing pollutants as tertiary treatment (Curia, Koppe, Costa, Feris & Gerber, 2011). The application of phytoremediation for various kind of wastewater such as landfill leachate, palm oil mill effluent (POME) and sewage might be an intriguing approach. In Malaysia, palm oil industry is among the largest agricultural

sector that contributes to the production of POME (Tang, Huang, Scholz & Li, 2009).

POME is a liquid waste produced during palm oil processing in palm oil mill. About 0.67 tonne of POME is generated for every fresh fruit bunch (FFB) processed (Ma, 2009).

In year 1970s, the subject of POME and its impact on the environment had become an issue of much concern to the government and the public. The government has taken action in enacting the Environment Quality Act in 1974 and specific regulations for POME in 1977. The regulations of governing discharges into water courses and lands came into force on 1st July 1978. It is mandatory for all palm oil mills to treat their wastewaters on site to an acceptable level before it is allowed to be discharged into watercourses (Table 1.1) (Environmental Quality Act 1974).

In response to these issues, most of the palm oil mills initiate POME treatment through physical and biological methods using open ponding system. Various designs and configurations of ponding systems are used in Malaysia by 421 palm oil mills in Malaysia (Ma, 2009; MPOB, 2010). Figure 1.1 shows a typical system of POME treatment with several ponds, each for different function as indicated. Usually the open ponding system is used as its involved low cost of maintenance and capital operating system. The treated POME also known as palm oil mill (POM) final discharge wastewater is produced after the biological and physical treatment and usually still contain several types of pollutants particularly BOD, COD N-NH₃ and TSS that requires tertiary treatment before being discharge into the watercourses.

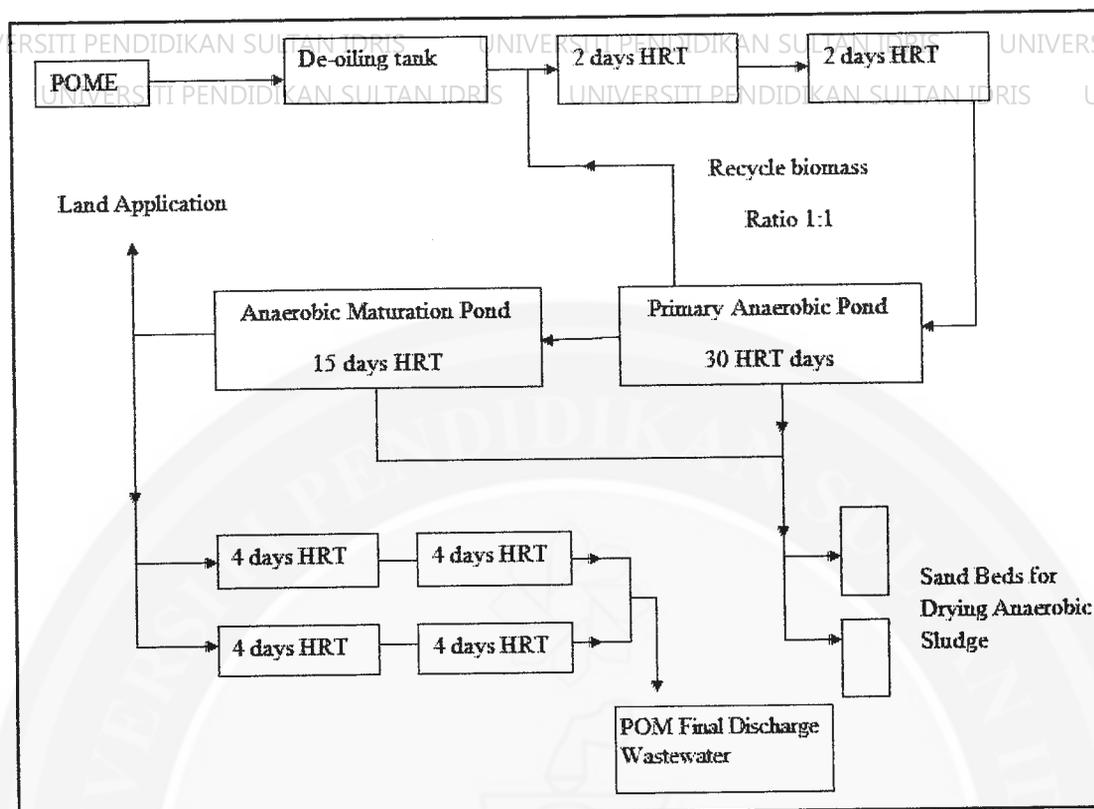


Figure 1.1. Schematic flow diagram of ponding system for POME treatment (Ma, 2009)

Phytoremediation is among the cost effective technology potentially used for bioremediation. This process depends on the plants to draw contaminated soil water to the rhizosphere (root zone) and in which plant uptake of contaminants or microbial activities provide desired removal of contaminants (Crites, Middlebrooks & Reed, 2006). Microbial community thrive up during phytoremediation because macrophytes provide large surface area for microbial biofilms and supply oxygen to microbial community through their roots (Greenway, 2007; Vymazal & Kropfelova, 2008). Furthermore, microbial communities also play important role in decomposition of organic matter (Cronk & Fennessy, 2001).

There is several factors influence phytoremediation efficiency using aquatic macrophytes. Among them are the rate of nutrient uptake, bioavailability of nutrient, age of macrophytes, types of macrophytes roots and phytotoxicity effect (Cronk & Fennessy, 2001; O'Sullivan, Moran, & Otte, 2004; Wu et al., 2011; Cheng et al., 2009; Pierce, Moore, Larsen & Pezeshki, 2010). The characteristics of wastewater such as recalcitrant properties also affect the performance of phytoremediation due to the limited biodegradation of organic matter (Calheiros et al., 2009). High strength of wastewater with large amount of chemical oxygen demand (COD) could inhibit the growth of macrophytes (Sooknah & Wilkie, 2004). For this reason, macrophytes are used to remediate palm oil mill (POM) final discharge wastewater which has lower strength of pollutants than the raw POME (Table 1.1). This research discussed phytoremediation of POM final discharge wastewater efficiency using selected aquatic macrophytes *Leersia oryzoides*, *Pistia stratoites* and *Ludwigia peploides*.

Previous studies have shown that aquatic macrophytes have great ability in removing pollutants during phytoremediation of various wastewaters. The ability of *Leersia oryzoides* in phytoremediation had been studied to remediate permethrin in mesocosm experiment (Moore, Kroger, Cooper & Smith, 2009) and different macronutrient allocations in its organs were observed (Pierce et al., 2010). *Pistia stratoites* was known could improve water quality by removing nitrogen and phosphorous from eutrophic stormwaters (Lu, He, Graetz, Stoffela & Yang, 2010).

Table 1.1

Characteristics of POME and POM final discharge wastewater

| Parameter (mg/L) ^a | Raw POME ^c | POM final discharge wastewater ^d | | Standard limits (mg/L) ^e |
|--|-----------------------|--|---------|--|
| | Range | Range | Average | |
| BOD ₃ ^b | 25,000 | 490-612 | 549 | 100 |
| Chemical Oxygen Demand (COD) | 30,000-50,400 | 1025-1073 | 1049 | - |
| Ammoniacal nitrogen (NH ₃ -N) | - | 38-42 | 40.5 | 100 |
| Total Suspended Solid (TSS) | 18,000 | 660-750 | 710 | 400 |
| Total Solid (TS) | 40,500 | 5150-5300 | 5233 | - |
| pH | 4.7 | 8.44-8.5 | 8.48 | 5.0-9.0 |
| Total Alkalinity (as CaCO ₃) | - | 13401- 13511 | 13457 | - |

Note. ^a mg/L except for pH,

^b BOD₅ express for POM final discharge wastewater and BOD₃ for raw POME at three days at 30°C

^c Adapted from Bhatia, Othman & Ahmad (2006)

^d This study

^e Environmental Quality Act (1974)

1.2 Significance of Study

1.2.1 Phytoremediation as an Environmental and Cost Effective Technology for Tertiary Treatment of POM Final Discharge Wastewater

As one of the leading agricultural industries in Malaysia, the palm oil industry has a yearly production of more than 13 million tons of crude palm oil and the plantations area covers 11 % of Malaysian land. However, the production of such amounts of crude palm oil had resulted in even larger amounts of POME produced, which estimated at nearly three times the quantity of crude palm oil (Wu, Mohammad, Jahim & Anuar, 2009).

POME can cause land and water pollution when discharged untreated due to the presence of high organic load and their phytotoxic properties (Nwoko, Ogunyemi, Nhwocha & Nnorom, 2010). Therefore it is imperative to find an economical and environmental friendly solution for this problem. Phytoremediation, being more cost-effective and fewer side effects than physical and chemical approaches, has gained increasing popularity in industry and research studies (Lone, He, Stoffela & Yang, 2008). Furthermore, Malaysia has a wide range of plant diversity, which provides high opportunity in finding the potential macrophytes to treat the environmental problem caused by POME discharges.

1.2.2 Compliance to Malaysian Environmental Regulation

Most of Malaysian palm oil mills are located near to the rivers for easy access to water supply. As the location of mill is close to the river, mills conveniently discharge POME into the river. To some extent, this benefits the ecosystem as some nutrients can fertilize the river and enhance the growth of microplanktons which are food to aquatic life. However, POME in excess, as it degrades will deplete oxygen in water and suffocates the aquatic life (Ma, 2000). Uncontrolled discharge of POME can easily pollute water resources (Lim et. al, 2009).

Watercourses are most vulnerable to pollution by unregulated discharge of POME (Lim et al., 2009). Because of that, Malaysia had enacted Environmental Quality Act (1978) to ensure palm oil mills comply with parameter limits for POME discharges, which is 100 mg/L of BOD₅ for watercourse and 5000 mg/L for land application (Ma, 2009). Nevertheless, treated POME discharged from ponding systems sometimes could not comply with these parameter limits (Mohammad, Alam, Kabbashi & Ahsan, 2012).

In Malaysia, pond system is widely used for a secondary treatment of POME (Ma, 2009). This secondary treatment is based on biological approach namely anaerobic and aerobic lagoons. However, it is not efficient enough to treat POME (Ahmad, Ismail & Bhatia, 2005). This is due to the low organic loading rate applied, which is about 0.2 to 0.35 kg/m³/day of BOD (Ahmad et al., 2005). In addition, the large size and configuration of the ponds made the biological process difficult to be controlled and monitored. Usually, the mixing process at the facultative and aerobic

zones could be only done by the biogas bubbling hence difficult to reduce BOD to the standard limit imposed by the government (Ma, 2009; Poh & Chong, 2009).

Therefore, an appropriate tertiary treatment is necessary to reduce strength of POM final discharge wastewater. In comparison with other tertiary treatments such as dissolved air floatation (DAF) and reverse osmosis (RO), phytoremediation approach is more cost effective, less chemical usage and energy saving. Table 1.2 shows several aspects of comparison between phytoremediation with other tertiary treatment such as DAF and reverse osmosis.

In response to these issues, POM final discharge wastewater using macrophytes is suggested. The feasibility study of phytoremediation using three macrophytes species was performed. Throughout the study, the performance of the phytoremediation was monitored and evaluated. The characteristics of nutrient uptake during phytoremediation were determined to obtain a better understanding of the mechanism of phytoremediation process. The microbial population isolated from macrophytes root was enumerated and identified using 16S rRNA molecular technique. This information is useful in understanding the contribution of microorganisms in the phytoremediation process. To date, very limited data on the phytoremediation of POM final discharge wastewater can be obtained for Malaysia, hence, it might be valuable to improve the existing POME treatment system.