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

MOHD FAIZAL BIN HAMZAH

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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 oryoides*, *Pistia stratiotes* and *Ludwigia peploides*. The phytoremediation performance was evaluated by monitoring the changes and removal efficiency (%) of BOD_5, COD, N-NH_3, 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 BOD_5, COD, N-NH_3 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 aeroginosa* and *Bacillus cereus* that usually involved in denitrification process was identified in *Pistia stratiotes*, *Ludwigia pepoides* 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 MENGGUNAKAN 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 Tanjong 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 (%) BOD_5, COD, N-NH_3, 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 BOD_5, COD, N-NH_3 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 aeroginosa* 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.

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LIST OF ABBREVIATIONS

BCF – Bioconcentration Factor

BLAST – Basic Local Alignment Search Tool

BOD_3 – 3-day Biochemical Oxygen Demand

BOD_5 - 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

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.

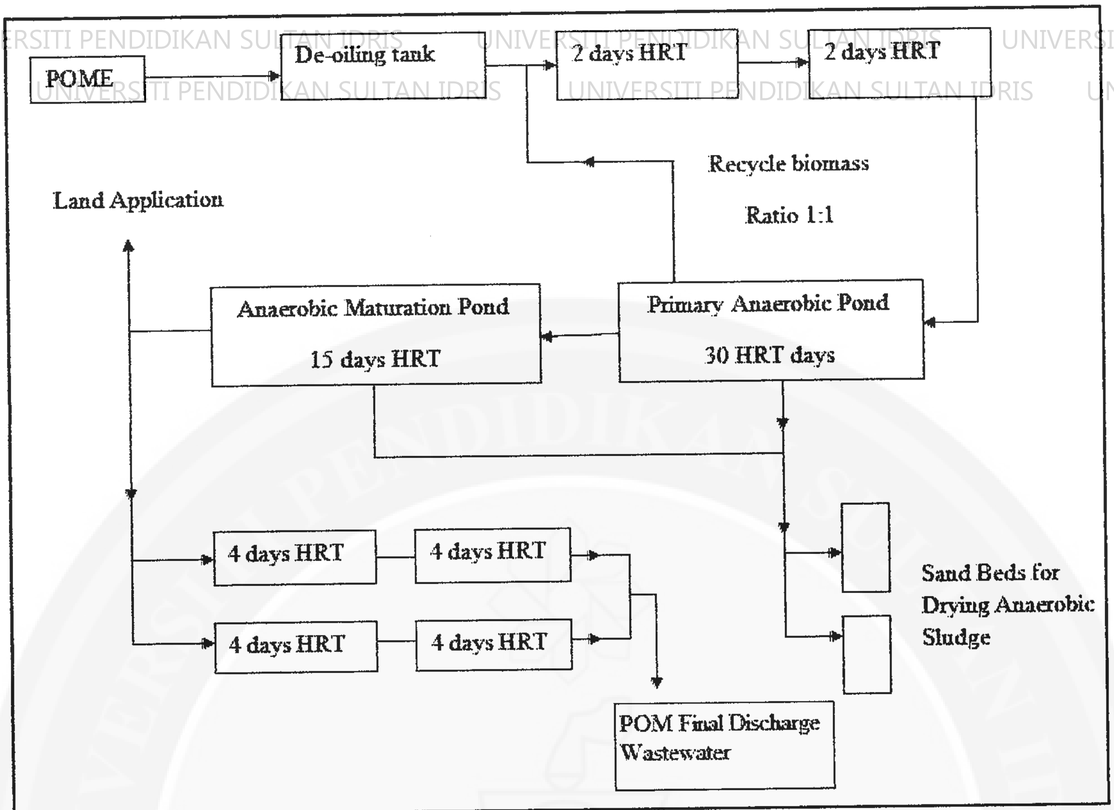

Figure 1.1. Schematic flow diagram of ponding system for POME treatment (Ma, 2009)

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 mesoscosm 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] BOD_5 express for POM final discharge wastewater and BOD_3 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 BOD_5 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 $kg/m^3/day$ of BOD (Ahmad et al., 2005). In addition, the large size and configuration of the ponds made the biological process difficult to be controled and monitored. Usually, the mixing process at the facultative and aerobic zones could be only done by the biogass 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.