BIOLOGICAL TREATMENT OF POME (HIGH ACIDIC CONTENT): FACTORIAL ANALYSIS

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ABSTRACT

Palm Oil Mill Effluent (POME) is an effluent discharged from unrecovered palm oil extraction process and typically acidic (pH 4 to 5.5). Disposing POME without treatment would give adverse effect to environment as well as human being. Therefore, biological treatment using various microbes is suitable to employ to increase the pH of POME since it is low cost and safe. The aim of this study was to study the factors which are influencing the biological pH treatment of acidic POME. The factors involved in this study are agitation speed, temperature, reaction time, inoculum to POME ratio and light intensity. To begin, sample of acidic POME was collected from a nearby palm oil mill in Gambang, Pahang while soil mixed culture was obtained from soil near to plants root system. Preliminary study on reaction time and potential microbes was done in 10 days and at the end, soil mixed culture performed better utilization process than *Enterobacter* sp. with pH 5.58. Then, an acclimatization of soil mixed culture was conducted and 32 experimental runs were done according to two level factorial. An analysis using Design Expert Software shows that three factors are contribute in increasing the value of pH which is agitation speed, temperature and reaction time with 19.08%, 7.58% and 4.68% respectively. Interaction between factor temperature and agitation speed gives highest percentage of contribution with 17.30% in existence of interaction, followed by interaction of factor temperature and inoculum to POME ratio and interaction of factor reaction time and agitation speed with 8.41% and 7.03% respectively. It is predicted the best conditions regarding this study for agitation speed, temperature, reaction time, inoculum to POME ratio and light intensity lies on the value of 138.89 rpm, 28.98 ºC, 8.81 days, ratio 1:3 and with light respectively. All the best conditions provided a stable pH value which is 6.9999. Overall, factors of agitation speed, temperature and reaction time are suitable to be the factors for optimization experiment.

RAWATAN BIOLOGI SISA KUMBAHAN KELAPA SAWIT (KANDUNGAN ASID TINGGI): ANALISA FAKTORIAL

ABSTRAK

Sisa kumbahan kilang minyak kelapa sawit ialah sisa kumbahan yang dilepaskan daripada proses pengekstrakan minyak yang belum diperoleh dan biasanya berasid (pH 4 ke 5.5). Melupuskan sisa kumbahan tanpa rawatan boleh memberi kesan besar kepada persekitaran serta manusia. Oleh itu, rawatan biologi menggunakan pelbagai mikroorganisma adalah sesuai digunakan bagi menaikkan nilai pH sisa kumbahan selain ianya berkos rendah dan selamat. Tujuan kajian ini ialah untuk mengkaji faktor-faktor yang mempengaruhi rawatan pH bagi sisa kumbahan berasid secara biologi. Faktor-faktor yang terlibat ialah kelajuan agitasi, suhu, reaksi masa, nisbah inokulum kepada sisa kumbahan dan keamatan cahaya. Sampel sisa kumbahan berasid didapati daripada kilang kelapa sawit berhampiran Gambang, Pahang manakala kultur campuran tanah didapati daripada tanah berhampiran sistem akar pokok. Ujian awal terhadap reaksi masa dan potensi mikrob dilakukan selama 10 hari dengan kultur campuran tanah melaksanakan proses penggunaan yang lebih baik berbanding spesis *Enterobacter* dengan pH 5.58. Kemudian, penyesuaian kultur campuran tanah dilakukan dan 32 eksperimen dijalankan berdasarkan analisis faktorial dua peringkat. Analisis menggunakan perisian Design Expert menunjukkan tiga faktor menyumbang kepada kenaikan pH iaitu kelajuan agitasi, suhu dan reaksi masa dengan peratus sebanyak 19.08, 7.58 dan 4.68. Interaksi antara faktor suhu dan kelajuan agitasi menyumbang kepada peratusan tinggi sebanyak 17.3 peratus dengan kehadiran interaksi, diikuti dengan interaksi antara faktor suhu dan nisbah inokulum kepada sisa kumbahan serta interaksi antara faktor reaksi masa dan kelajuan agitasi dengan peratusan 8.41 dan 7.03. Diramalkan keadaan yang terbaik untuk kajian ini ialah 138.89 rpm, 28.98 ºC, 8.81 hari, nisbah 1:3 dan dengan kehadiran cahaya. Kesemua keadaan yang terbaik menyediakan nilai pH yang stabil iaitu 6.9999. Keseluruhan, faktor kelajuan agitasi, suhu dan reaksi masa adalah sesuai untuk dijadikan faktor bagi eksperimen pengoptimuman.

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LIST OF SYMBOLS

ºC	Degree Celcius
%	Percentage
mg/L	Milligrams per Liter
rpm	Rotation per Minute

LIST OF ABBREVIATIONS

F/M	Food to Microorganism
DOE	Design of Experiment
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
WHO	World Health Organization
RSM	Response Surface Methodology
UASB	Upflow Anaerobic Sludge Blanket
POME	Palm Oil Mill Effluent
sp.	Species
vs.	Versus
d	Day
R^2	Coefficient of Determination
F-	Flouride Ion

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Palm oil is produced from oil palm, primarily *Elaeis guineenis*, which originated from West Africa but has adapted well to other tropical lowland regions such as Malaysia. The African oil palm belongs to the family *Palmae* is classified as *Elaeis guineensis* and it is believed to be indigenous to West Africa because the specific name, *guineensis* shows that the first specimen described was collected in Guinea, West Africa. The oil palm (*Elaeis guineesis*- an unbranched monoecious plant) is not a native plant of Malaysia; it was introduced in 1875 as an ornamental plant (Ahmed, 2009). There are over 500 million oil palm trees across various plantations in Malaysia (Malaysia Palm Oil Board-MPOB, 2004).

Generally, production of oil palm requires it to go through an extraction process where it started from fresh fruit bunch until it is completely producing the desired product. The process to extract the oil requires significantly large quantities of water in order to sterilize the palm fruit bunches and clarifying the extracted oil. In 2006, Abu Bakar investigated that for one tonne of crude palm oil produced, 5-7.5 tonnes of water are required, and more than 50% of the water will end up as palm oil mill effluent (POME).

Despite its long clinical success in being a major foreign exchange earner for Malaysia, production of oil palm has a number of problems in use. POME has been identified to be one of the major sources of water pollution due to its high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) concentrations (Chan et al., 2010). It is also identified as the single largest source of water pollution (Abdullah et al., 2004). In advantage, solid waste products from a process can put to economically useful purposes such as fuel material and mulch in agriculture (Okwute et al., 2010).

Instead of thinking much about the profit gain, consideration on the pollution is necessary in view of the fact that it can gives adverse effect to human being and environment. Nwoko and Ogunyemi (2010) claimed that POME can cause land and aquatic pollutant when it is discharged directly into the environment without having a treatment. Therefore, a wide range of approaches for the treatment of POME have been developed to alleviate the pollution problems such as chemical, physical and biological treatment with variety of microorganisms used.

1.2 Problem Statement

Higher amount of POME which is directly discharged without treatment would give effect to land and aquatic life. The raw or partially treated POME has an extremely high content of degradable organic matter, which is due in part to the presence of unrecovered palm oil (Abu Bakar, 2006). This highly polluting wastewater can, therefore, cause pollution of waterways due to oxygen depletion and other related effects. According to Abu Bakar (2006), in order to regulate the discharge of effluent from the crude palm oil industry as well as to implement the environmental controls, the Environmental Quality Order, 1977 (Prescribed Premises) (Crude Palm Oil) were promulgated under the Environmental Quality Act, 1974. This law was enforced in order to ensure that palm oil industry will follow the standards quality of discharge POME characteristics. The POME characteristics and standard discharge limit is illustrated in Table 1.1

Table 1.1 Characteristics of POME and its respective standard discharge limit by the Malaysian Department of the Environment

Parameter	Concentration, mg/L	Standard limit, mg/L
pH	4.7	5-9
Oil and grease	4,000	50
BOD	25,000	100
COD	50,000	-
Total solids	40,500	-
Suspended solids	18,000	400
Total nitrogen	750	150

(Source: Abu Bakar, 2006)

Therefore, this research aims to increase the pH value of an acidic POME according to a standard discharged limit by biological treatment and using soil mixed culture. The usage of this bacterium seems to have a potential in increasing the pH value of acidic wastewater.

1.3 Research Objective

The main objective of this research is:

To study the factors which is influencing biological pH treatment of acidic POME

1.4 Scope of Research

The scopes of this research are:

i. To do preliminary study in order to determine suitable reaction time and potential microbes
ii. To use soil mixed culture for pH treatment of acidic POME
iii. To study the factors in pH treatment of acidic POME which are agitation speed, reaction time, temperature, light intensity and inoculum to POME ratio
iv. To use two-level factorial in experimental design
v. To screen the factors using Design Expert Software

1.5 Significance of Research

By doing this research, when considering the parameters such as agitation speed, reaction time, temperature, light intensity and inoculum to POME ratio, factors affecting an overall treatment of acidic POME can be achieved by using Design Expert Software. Apart from that, this treatment process also can increase pH value of an acidic POME to be higher than the initial pH of raw POME. Besides that, the cost of pH treatment of acidic POME can be reduced since it does not consumed any chemicals to increase the pH such as lime and soda ash which is practically used in industry, but it is biologically treated and provide safe environment to human being. Not only that, the effects on soil chemical properties which is affecting supply of macro and micro nutrient for plant growth also can be reduce

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Mill Effluent (POME)

In general, palm oil mill effluent is resulting from oil palm extraction process. In some West African countries, POME, a by-product of palm oil production is produced in large quantity (Nwoko and Ogunyemi, 2010). ZInatizadeh et al., (2006) analyzed that the characteristics of fresh POME are thick brownish slurry, hot (80-90ºC), acidic (pH 3.8-4.5) and contains very high concentration of organic matter (COD = 40,000-50,000 mg/l, BOD = 20,000–25,000 mg/l). The effluent is non-toxic as no chemical is added in the oil extraction process (Rupani et al., 2007). Figure 2.1 shows an overview of raw palm oil mill effluent which is discharge from palm oil mill industry.

In auxiliary analysis on characteristics of acidic POME, Lorestani (2006) have come out with typical characteristics and composition of POME sample as shown in Table 2.1

Table 2.1 Typical Characteristics and Composition of POME

Parameter	Average	Metal	Average
pH	4.7	Phosphorus	180
Oil and grease	4000	Potassium	2270
Biochemical Oxygen Demand (BOD_5)	25,000	Magnesium	615
Chemical Oxygen Demand (COD)	50,000	Calcium	439
Total solids	40,500	Boron	7.6
Suspended solids	18,000	Iron	46.5
Total volatile solids	34,000	Manganese	2.0
Ammonical nitrogen	35	Copper	0.89
Total nitrogen	750	Zinc	2.3

(Source: Lorestani, 2006)

Consecutively, to regulate the discharge of effluent from the crude palm oil industry as well as to exercise the environmental controls, the Environmental Quality (Prescribed Premises) (Crude Palm Oil) Order, 1977, was promulgated under the

Environmental Quality Act, 1974. The POME characteristics and standard discharge limit is illustrated in Table 1.1 (Ahmad et al., 2003)

Besides following the standard discharge limit of POME, the most common propose technologies in relation to wastewater treatment require extremely high cost. Due to these factors, the palm oil mill industry faces the challenge of balancing the environmental protection, its economic viability and sustainable development. Therefore, there is an urgent need to find a way to preserve the environment while keeping the economic growth.

Even though POME which has been discussed to give an effect to environment, it also could become a very good carbon feedstock for hydrogen production in fermentation process if it is well prepared and being utilised based on its fermentable constituents composed in POME (Kamal et.al, 2012).

Figure 2.1 Palm Oil Mill Effluent (Source: http://ecoideal.com.my/wp)

2.2 Effect of Low pH Wastewater to Environment

Fresh water is essential to the survival of humans and most other land-based life forms. Nowadays, it is practically harsh to have fresh water since Malaysia have become a developing country and there are many developments which cause a large production of wastewater either classified as domestic wastewater or industrial wastewater. In definition, domestic wastewater may come from municipal wastewater which is including the disposed material from housing area, restaurants, and business centre while industrial wastewater is classified as a wastewater which is discharged from industrial processes such as palm oil mill which is generally producing palm oil mill effluent (Peavy et al., 1985).

Along with that, adverse effects to environment were distinguished. As analyzed by Hamdi and Srasra (2006), in acidic waste solution, it contained fluoride ion (F-) which is according to World Health Organization (WHO) guidelines, it is troublesome for health of the humans and the animals. If its concentration in water is too high which is higher than 1.5 mg/L, it can damage the bones. Moreover, industrial wastewaters from mining and mineral processing which are often characterized by low pH and high metal and sulphate concentrations is toxic to many organisms as well as to environment and it result in changes in the food web (Kaksonen et al., 2003).

Another case to be pointed out is the monosodium glutamate industrial wastewater (MSGW) which is generally contains high concentration of organic matter, COD, ammonium, sulphate and low in pH (Yang et al., 2005). From the characteristics highlighted, Liu et al., (2006) carried out a number of experiments with the purpose of studying the effect of monosodium glutamate industrial wastewater on wheat, Chinese cabbage and tomato. The experiments were experimentally done by using the wastewater discharged from different processing phases of monosodium glutamate production and as a results, it gives effect on seed germination and root elongation of the three crops.

In broad perspective discussed by Rusan et al., (2007), they claimed that low pH wastewater is recognized to have direct effect on soil chemical properties. It affects supply of mineral macro and micro nutrients for plant growth, soil pH and soil buffer capacity. However, Mohammad and Mazahreh (2003) found that when implementing irrigation with source from low pH wastewater, it increased the level of soil salinity due to the wastewater salt content.

2.3 Biological Treatment of Low pH Wastewater

According to Howard (1985), a wastewater-treatment system is composed of a combination of unit operations and unit processed designed to reduce certain constituents of wastewater to an acceptable level. It comprises of primary, secondary and tertiary treatment. Biological treatment process is categorized as secondary treatment and usually consists of biological conversion of dissolved and colloidal organics into biomass that can subsequently be removed by sedimentation.

In biological treatment, microorganisms are employed to utilize the organic matter, represented as BOD and COD contained in the effluent as their food supply. Wide variety of species is used to perform their work in treating wastewater. Meesap et al., (2011) claimed that anaerobic treatment is one of the successful and powerful biological methods for POME treatment. In fact, biological treatment has been found as one of the simplest treatment for wastewater and it is low cost and safe.

Initial treatment of the POME was with the marine *Yarrowia* strain. This strain has the ability to grow both in seawater and in fresh water over a broad range of pH (3.0–8.5). These properties made it a suitable candidate in waste management, as had the ability to sustain such shock loads. The acidic pH of POME became alkaline after the first treatment with *Yarrowia* probably due to the utilization of fatty acids present in the raw POME by this yeast (Oswal et al,. 2002).

Apart from that, in Wellington, South Africa, they separate alcohol from fermented liquid where the resulting effluent stream from the process is highly polluted with Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) and low pH ranging from 3 to 4 respectively. Thus, with the intention to pre-treat this effluent, the winery installed an up flow anaerobic sludge blanket (UASB) process in 1994 to reduce the COD concentration to acceptable levels for discharge to the municipal sewer. Based on Rajeshwari et al., (2000), anaerobic biological treatment of high-strength distillery effluent is a proven technology that has been widely applied.

Another biological treatment proposed by Gersberg et al., (n.d), they were introduced the concept of wetland (both constructed and natural) as an alternative biological treatment system for effluent purification has developed rapidly over the last

25 years. Pollutants are removed through a complex variety of biological, physical and chemical processes, in particular, the combination of saturated soil, plants, and microorganisms which provide both aerobic and anaerobic conditions for the reduction of pollutants from the overlying wastewater. The G-bag approach, which used a bag of adsorbent to capture the pollutants and degrade the pollutants with the immobilized microorganisms on the adsorbent, seems to be a good alternative only if the system can be designed simple and free from fouling (Chen et al., 2000).

It was decided that the best method to adopt for this research was anaerobic process due to the organic characteristic of POME (Perez et al., 2001). Therefore, ponding system is the most conventional method for treating POME (Khalid et al., (1992); Ma et al., (1985). It has been applied in Malaysia for POME treatment since 1982 and is classified as waste stabilization pond (Onyia et al., 2001). More than 85% of palm oil mills exclusively use ponding systems due to their low costs and easy to operate the systems but the disadvantages are large amount of land required, relatively long reaction time of 45-60 d for the effective performance, bad odour and difficulty in maintaining the liquor distribution which gives harmful effect on the environment.

2.4 Soil Mixed Culture as a Potential Microbe to Increase pH of POME

Amongst the different microorganisms inhabiting in the soil, bacteria are the most abundant and predominant organisms. These are primitive, prokaryotic, microscopic and unicellular microorganisms without chlorophyll. Morphologically, soil bacteria are divided into three groups namely *Cocci* (round/spherical), (rod-shaped) and *Spirilla I Spirllum* (cells with long wavy chains). *Bacilli* are most numerous followed by *Cocci and Spirilla in* soil (My Agriculture Information Bank).

According to research done by Charathirakup et al., (2004), it was found that this mixed culture was a gram negative bacterium with 7 different isolates. Mixed indigenous cultures were two to three times more efficient than single cultures (Dutta et al., 2003). An advantage of using mixed culture over pure culture are lower cost and can reduce the cost for sterilization while septic organic wastes can be used as substrate, and process using mixed culture gave stable yield of hydrogen production from non-sterile organic wastes (Noike and Mizuno, 2000).

Anaerobic microorganisms from palm oil mill wastewater treatment plant have been utilized as inocula for pH treatment of POME in batch cultivation (Morimoto et al., 2004; Atif et al., 2005). Vijayarahavan and Ahmad (2006) supported that mixed culture can increase the utilization process of acidic wastewater rather than pure cultures. pH treatment of acidic POME has been studied for a large group of pure fermentative bacteria, such as *Clostridia* and *Enterobacteria*, however, mixed cultures have attracted the researchers to further studied on it for increasing the pH (Fang and Liu, 2001).