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

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I hereby declare that this thesis entitled "Biological Treatment of POME (High Acidic Content)-Factorial Analysis" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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

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.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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).

Facultative anaerobe is one types of microbe in soil mixed culture which produces ATP by aerobic respiration if oxygen is present and is capable of switching to anaerobic fermentation. Therefore, it has an advantage compared to anaerobic bacteria which is sensitive to the presence of oxygen. Facultative bacteria can consume oxygen by aerobic respiration, leaving anaerobic condition that favours to hydrogen production. *Enterobacter* sp. is the most common gram negative and facultative anaerobe with the ability to produce hydrogen (Chong et al., 2009).

In terms of thermophilic and mesophilic bacteria, thermophilic bacteria are considered as more capable microorganisms than mesophilic bacteria for pH treatment of wastewater. Thermophilic bacteria are able to utilize a wide range of organic wastes such as an acidic POME and thermophilic mixed culture has been examined for their potential as pH increase microbes (Ahn et al., 2005; O-Thong et al., 2008). These properties formulate application of thermophiles for utilization process of acidic POME economically and technical sufficient.

2.5 Factors Affecting Soil Mixed Culture to Increase pH of Acidic POME

Treatment process of acidic wastewater may begin with various kinds of processes. It may be physical, chemical and biological treatment process. In this research, using soil mixed culture is considered as biological treatment process since naturally it use microbe other than chemical substances. Factors affecting biological hydrogen production from photosynthetic bacteria has been investigated previously

(Eroglu et al., 1999). Zabut et al., (2006) reported that continuous stirring, consistency in pH and moderate bacterial density enhanced the utilization of acidic POME. In addition to these factors, temperature effect is also crucial.

Eroglu et al., (1999) found that cell concentration and carbon to nitrogen ratio affect the utilization of acidic POME extensively and soil mixed culture should be under nitrogen limitation in order to increase the pH value. Atif et al., (2005) has studied the possibility of soil mixed culture to increase pH value from raw acidic POME. It was found out that the utilization of acidic POME is increase when the carbon to nitrogen ration is increase to a certain limit.

Another important factor is continuous stirring which influences the homogenous light and substrate distribution. pH treatment of acidic POME with a stirring at 100 rpm provided more effective utilization of acidic POME than in static fermentation (Reunsang et al., 2007). Higher value of agitation speed will increase the fermentation rate and for some microbes such as *Clostridia* and *Enterobacteria*, it enhances the increasing value of pH from acidic wastewater as stated by Fang and Liu (2001).

In terms of light intensity which can be considered as crucial factors for effective pH treatment process, Uyar et al., (2007) investigated the effect of light intensity and wavelength on hydrogen production. It was shown that light intensity plays a vital role for the photo-fermentation and near infrared light is essential to enhance the utilization of acidic POME. Conversely, the utilization process decreases with decreasing light intensity (Reunsang et al., 2007).

Sasikala et al., (1993) come out and investigated the behaviour of microorganisms and concluded that they have an optimum temperature to prolong their activities. The optimum temperature ranges for soil mixed cultures was reported as 25-35°C. In addition, optimum temperature can be achieved when they use various waste materials and wastewater from industrial processes as substrates and it is reported that the process can be operated at ambient temperature (30–40ºC) and normal pressure (Mohan et. al., 2007).

2.6 Factorial Analysis by using Design Expert Software

Experimental design can be regarded as a process by which certain factors are selected and deliberately varied in a controlled manner to obtain their effects on a response of interest, often followed by the analysis of the experimental results (Wang and Wan, 2009). There are actually many types of factorial experiments such as full factorial design or fractional factorial design, but Cochran et al., (1957) restricted to involve two level factorial experiments. The most commonly used full factorial design is two-level design, which can be denoted by 2^n when there are n factors (Kennedy and Krouse, 1999).

In a factorial analysis, it gives the ‘’main effects’’ and ‘’interactive effects’’ of changing the levels of the experimental variables from the lower level to upper level. The main effect of an experimental variable is defined as the average of the effects of changing its value from the lower level to the upper level among all experiments. It is derived by assuming that the experimental variable is an independent variable and all variations in its effects are due to experimental errors (The Optimization of Levels of Process Variables, 2012).

The interactive effects between two or more experimental variables are calculated on the assumption that the experimental variables are not independent but are in fact interacting between them. In mathematical method, Yate's method is used to analyse the main effects and interactive effects (The Optimization of Levels of Process Variables, 2012). Yet, advance analysis using Design of Experiment (DOE) software such as Design Expert Software was developed in order to simplify the analysis instead of using Yate's method.

DOE software was developed with the purpose of providing many powerful statistical tools such as two-level factorial screening designs, general factorial studies, response surface methods (RSM), mixture design techniques and combinations of process factors, mixture components, and categorical factors (Design-Expert® V8 Software for Design of Experiments (DOE), n.d).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Flowchart of Research Methodology

The brief description on process flow of biological treatment of POME is shown as in Figure 3.1.

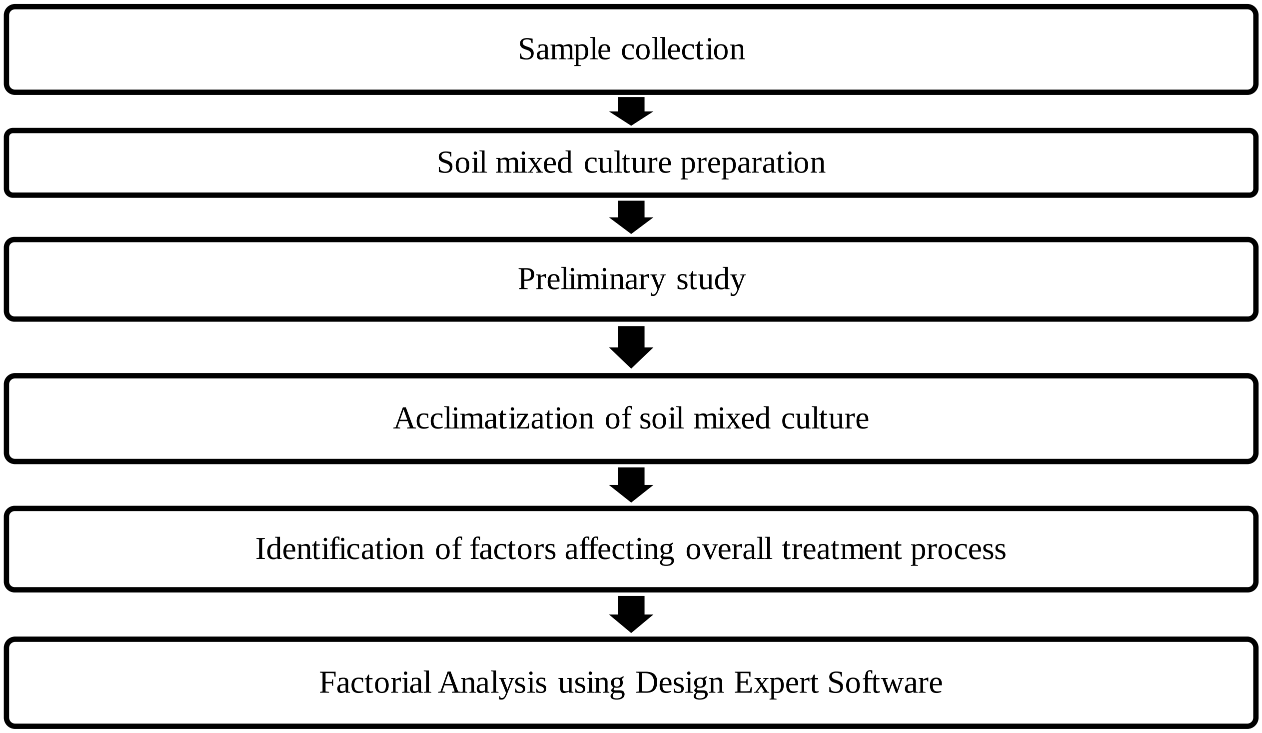

Figure 3.1 Process Flow Chart on pH Treatment of POME

3.1.1 Sample Collection

Sample of acidic POME in the present study was obtained from nearby palm oil mill around Gambang, Pahang Darul Makmur for about 17 L and was stored in freezer of 4^{o}C. Before used, the sample was cooled to room temperature for experimental study. Raw POME was a brown coloured suspension, which is acidic in range of 4 to 5.5 and consists mainly 95% of water. Freshly discharged POME is viscous and oily with obnoxious odor (Nwaogu et al., 2012)

3.1.2 Soil Mixed Culture Preparation

Soils mixed culture were collected from soils near to plant roots system in Universiti Malaysia Pahang. To collect the soils of 10 cm below the surface, a scoop was used. The collected soils were then mixed with a tap water for about 500 ml and directly stored in bottle of mineral water at room temperature before used.

3.1.3 Preliminary Study

Preliminary study was aims with the purpose of obtaining the suitable reaction time and potential microbes for POME, so that the changes in pH can be observed. In this study, *Enterobacter* species and soil mixed culture were used as potential microbes. Experiment was conducted using two conical flasks of 250 ml containing *Enterobacter* species and soil mixed culture with acidic POME as a substrate. The ratio of POME to potential microbes is 1:1. Afterward, it was shake in the incubator shaker with 150 rpm and 30ºC. pH changes were observed whether the potential microbes was treating the sample well or not while observing the reaction time in order to increase the pH. As a result, soil mixed culture provide an impressive value of pH rather than using Enterobacter sp. with value of 5.58 compared to 4.90 at day 3. The results were illustrated as in Figure below. Therefore, pH value is possible to increase in day ranging from 6 to 9 respectively.

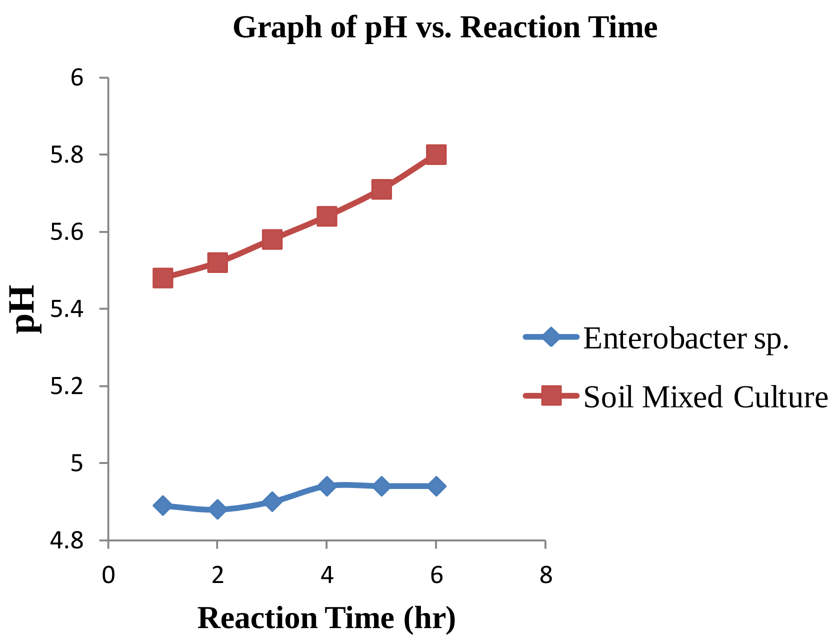

Figure 3.2 Graph of pH vs. reaction time

3.1 4 Acclimatization of Soil Mixed Culture

Since soil mixed culture have high potential to be used in this research, the acclimatization of soil mixed culture was taken about 10 days and for each day, similar amount of POME were added to potential microbe. The mean of acclimatizing the potential microbe is to make sure the suitability of it in changing an environment gradually besides maintaining performance across a range of environmental conditions. The experiment was conducted using Schott bottle of 2 L which consists of raw POME as pH control and raw POME with soil mixed culture bacteria. Subsequently, it was shake in incubator shaker with 150 rpm and 30ºC. After 24 hours time, the pH value were recorded.

3.1.5 Identification of Factors Affecting Overall Treatment Process

The factors identified for biological pH treatment of acidic POME was tabulated in Table 31.

Table 3.1 Factors and range involved in biological pH treatment of acidic POME

Factor	Range
Agitation Speed (rpm)	0-150
Temperature (ºC)	25-30
Reaction Time (days)	6-9
Light Intensity	Light/ No light
Inoculum to POME Ratio	1:3-1:5

3.1.6 Factorial Analysis using Design Expert Software

The experimental table design was constructed by using Design Expert Software as shown in Appendix A. Based on two-level factorial, it contains 32 experiments since involving five factors identified. Each run have dissimilar factors combination. In this experiment, 250 ml of shake flask were used. After finishing all the 32 experimental runs, the data were then analyzed using Design Expert Software. The data obtained was clearing up the suitable parameters for overall treatment of acidic POME.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Results for Factorial Analysis

The result obtained from the experiment was in form of percent contribution for factors affecting pH treatment of acidic POME. The percent contribution was ranging as low as 0.025% to 19.08%. In this experiment, 32 experimental runs were done and furtheranalyzedbyDesignExpertSoftware.

The model for pH treatment was stated in Equation 4.1.

$$pH = 5.55 + 0.22A + 0.17B + 0.029C + 0.35D - 0.11E + 0.098AB - 0.11AC + 0.33AD - 0.23AE - 0.043BC + 0.21BD + 0.033CD + 0.013CE - 0.038DE - 0.17ABC - 0.10ACD - 0.14ACE - 0.19ADE \qquad (4.1)$$

where A, B, C, D and E were referring to agitation speed, temperature, reaction time, inoculums to POME ratio and light intensity. From the equation obtained, the *R square* was 0.8448

4.2 Factors Affecting pH Treatment

From the results, the most important factor which gives highest percentage contribution for pH treatment of acidic POME was factor of agitation speed with 19.08%. Other factors which contribute to the effect on treatment process were factor of temperature, reaction time, inoculum to POME ratio and light intensity by having a value of 7.58%, 4.68%, 1.79% and 0.13% respectively.

The correlation between factors highlighted was tested and as outcomes, three interactions provide momentous effect towards treatment process where the most striking result to emerge from the data is the interaction between factor of temperature and agitation speed with 17.3% contribution. Meanwhile, interaction between factor of temperature with inoculum to POME ratio and interaction between factor reaction time with agitation speed gives 8.41% and 7.03% contribution respectively.

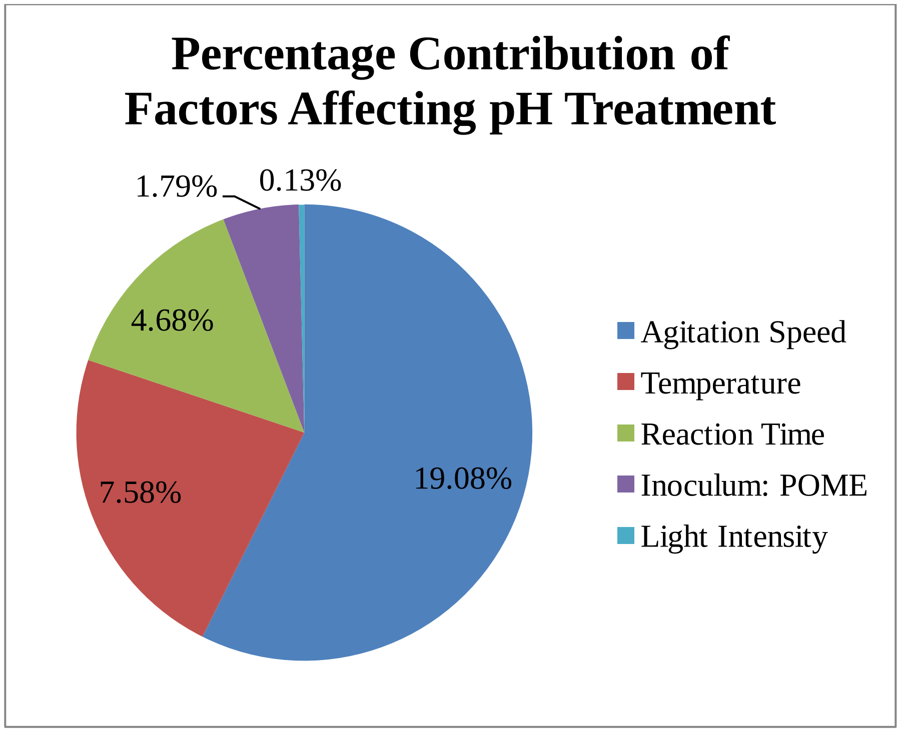

Figure 4.1 Percentage Contribution of the Five Factors Affecting pH Treatment

4.2.1 Agitation speed

As shown in Figure 4.1, the factor that contributes most for pH treatment of acidic POME is the agitation speed which is 19.08%. It can be understood that presence of agitation help in better utilization of acidic POME thus, increases the pH value.

Strong evidence related to agitation was found by Choi et al., (2008) when they declare that agitation itself is the key bioreactor operating parameter that is required for adequate mixing as it has high contribution to mass and heat transfer. Higher mass transfer means higher rate of acidic POME being utilized by the microorganism (Najafpour et. al., 2004).

In a similar way, Mussatto et al. (2006b) explained some agitation increases the yields, but excessive mixing can deactivate the utilization of acidic POME and reduce the pH value (Ingesson et al. 2001; Wright 1988). However, Jaapar et al., (n.d) point out with different opinion and claimed that in a static culture, acidic POME can be utilized five times higher than in the shake culture. Referring to their opinion, if excessive agitation was applied, it may detrimental to the cells, causing morphological changes and variation in their growth because of shearing stress and as a final point it lead to a less performance for utilization of an acidic POME (Kongkiattikajorn et al., 2007).

4.2.2 Temperature

The second factor which contributes to pH treatment of acidic POME is temperature with the value of 7.58%. Depending on the types of microbes used, different researchers claimed different values of temperature in order to utilize the acidic POME. There are several possible explanations from researchers for this result. Wang and Wan

(2008) were identified the utilization of acidic POME was increased with increasing temperatures from 20 ºC to 35 ºC, but it trended to decrease with further increasing temperature from 35 ºC to 55 ºC. It is supported by Amend and Shock (2001); Conrad and Wetter (1990) where the utilization processes were negatively affected with temperature increase.

For that reason, Lee et al., (2006) demonstrated the optimum temperature for utilization of acidic POME was 40 °C, while the optimal temperature reported by Valdez-Vazquez et al., (2005) was 55 °C. In other opinion, the utilization of acidic POME was low at mesophilic range (30–40 ◦C) but was rather efficient and high at thermophilic range (50–55 ◦C) (Lin et al., 2007). In extend, a change in system temperature might alter the efficiency of utilization process or microbial community. Thus, different temperatures may result from differences among their studies in terms of the substrates, their concentrations and ranges of the temperature studied.

4.2.3 Reaction Time

Another factor which is contributing 4.68% to the biological treatment of POME is the reaction time. The reaction time is corresponding on how long will it takes in order to have a reaction process. According to Wu et al., (2008), Alam et al., (2008) found that, the utilization of acidic POME could be obtained with longer time of fermentation (up to 7 days of fermentation) and addition of co-substrates (glucose & wheat flour) into POME.

Han and Shin, (2004) convinced that the utilization of acidic POME requires 1-2 days of reaction time, while claiming that too short reaction times could lead to bad hydrolysis of organic wastes.

4.2.4 Inoculum to POME Ratio

Inoculum to POME ratio had given 1.79% contribution to the treatment process. The amount of inoculum is responsible on how much the amount of acidic POME can be utilized. Fermentative bacteria such as *Clostridium sp.* (obligate anaerobe) and *Enterobacter sp*. (facultative anaerobe) are the main genera with the ability to utilize the acidic POME, where *Clostridium sp.* has been recognized as an effective microbe to utilize an acidic POME (Chong et. al, 2009).

Therefore, Hawkes et al., (2002) states it is advantageous to select for clostridia species in the inoculum instead of choosing facultative aerobic *Enterobacter sp.* Unfortunately, *Clostridium sp.* is very sensitive to minute amounts of dissolved oxygen. Due to its sensitivity to minute amounts of dissolved oxygen, it is difficult to cultivate a single culture to utilize an acidic POME and mixed culture is selected as seed inoculum rather than single culture. Therefore, some researchers use a mixed culture as an inoculum (Dong et al., 2009).

Contrary, Seengenyoung et al., (2011) studied the utilization of acidic POME was higher under no addition of the inoculum, compared to those obtained under the addition of *T. thermosaccharolyticum* PSU-2. Hence, they conclude that no inoculum need to be added since the POME itself has many types of microbes that can utilize the acidic POME under the optimum condition.

In all studies conducted by Cooney et al. (2007), the overall utilization of acidic POME is low with only 10-20% meanwhile acidic POME can be further utilized with increasing value of inoculum to POME ratio which is between 28-40%.

4.2.5 Light Intensity

The least percentage contribution to biological treatment of POME is light intensity which is only 0.13%. It can basically wrap up that light intensity was not contributed much in pH treatment of acidic POME. Barbosa et al., (2001) points out at a certain biomass concentration and light intensity, depending on the strain, light can become a limiting factor due to self shading and light absorption by the cells close to the illuminated surface.

However, light source from fluorescent give better results compared to tungsten. It gives about 8 times higher utilization of acidic POME compared to tungsten. Tungsten lamps are a convenient light source but they contain infrared light to a great extent. Infrared light over 1000 nm is not only useless for utilization of acidic POME but it also heats the culture suspension (Nakada et al., 1999).

4.3 Interaction between Factors

In a screening process, an interaction between factors also contributes to the biological treatment of POME. For overall process, there are 10 pairs of interaction between factors. However, only one interaction seems to be extensively contributes to the process. It can be shown in the following Figure 4.2.

Analysis on interaction factor showed that the highest percentage contribution is belonging to interaction between factor of temperature and agitation speed that is 17.30%. Another interaction factor which contributes to the treatment is interaction between factor of temperature and inoculum to POME ratio, factor of reaction time and agitation speed, factor of temperature and reaction time, factor of temperature and light intensity, factor of reaction time and light intensity, factor of reaction time and inoculum to POME ratio, factor of light intensity and agitation speed, factor of light intensity and inoculum to POME ratio and factor of agitation speed and inoculum to POME ratio percentage contribution of 8.41%, 7.03%, 1.50%, 1.71%, 0.28%, 0.59%, 0.17%, 0.025% and 0.23% respectively.

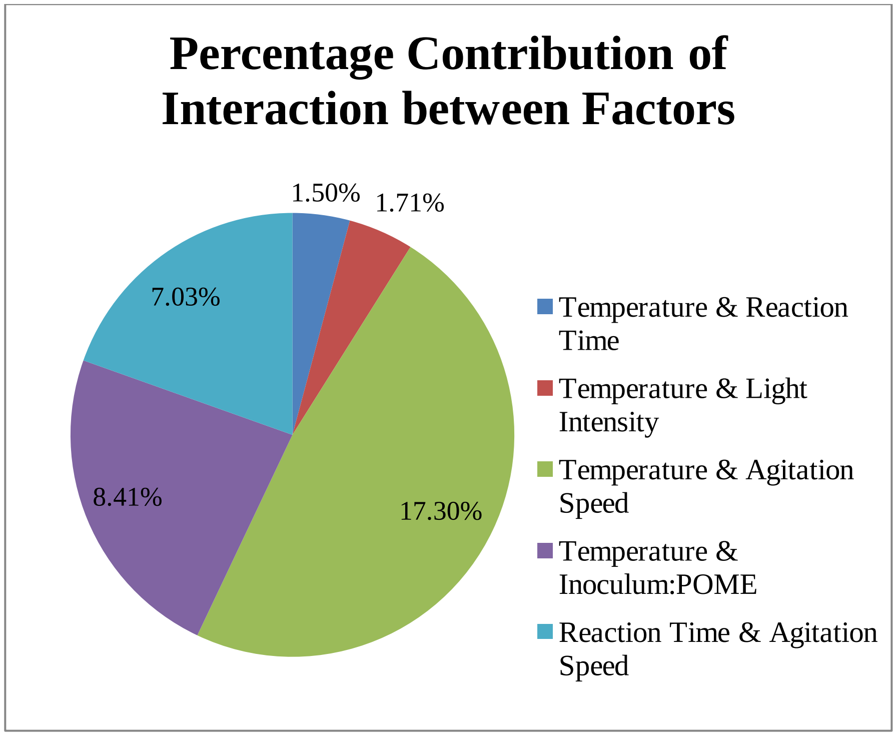

Figure 4.2 Percentage Contribution of the Interaction between Factors Affecting pH Treatment

4.3.1 Interaction between factor temperature with agitation speed

The interaction between factor temperature and agitation speed gives highest contribution of 17.30% to the treatment process which is by all accounts it becomes the most important interaction factor than another factors. It can be shown in the following Figure 4.3 where the trend of graph response to pH value of POME.

Referring to the graph, agitation gives effect to pH treatment of acidic POME. At temperature 25ºC, pH value was decrease with increasing value of agitation speed. Meanwhile, at temperature 30ºC, pH value was increase when agitation speed increased.

A comparison of results based on two different operating temperatures reveals that temperature and agitation speed is inter related in increasing the pH value.

This finding supports previous research into this brain area which links agitation speed and temperature. The result may be explained by the fact that agitation is the key bioreactor operating parameter that is required for adequate mixing since it has high contribution to mass and heat transfer (Choi et al., 2008). Microbes especially must collide with enough force to obtain surface to surface contact; only then bonding can occur between particles. With higher temperature, molecular collision frequency will increases, thus effective performance on utilization of acidic POME will be obtained.

In general, the rate of biochemical reactions and the utilization of acidic POME increases with increases in temperature and agitation (Durai et al., 2010). Thus, stirring and heating the culture had improved the treatment process, producing a better performance of utilization of acidic POME (Rubal et al., 2012).

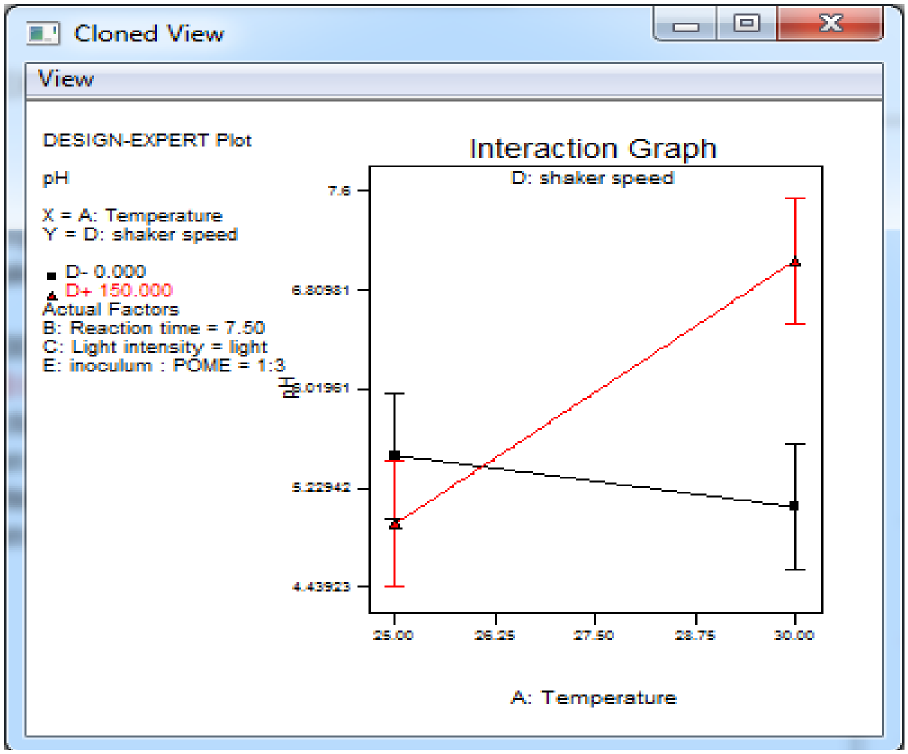

Figure 4.3 Interaction graph for factor of temperature with agitation speed

4.3.2 Interaction between factor temperature with inoculum to POME ratio

The interaction between factors of temperature with inoculum to POME ratio contributes 8.41% to the pH treatment of POME as shown in Figure 4.4. It shows the graph of the interaction factor with respect to response 1, where response 1 is the final value of pH after having a treatment process.

For general overview, the graph shows linear findings where pH value is directly proportional to the temperature for both inoculum to POME ratio. As temperature increase, pH value also increases. At beginning of the treatment process, the interaction between two factors is almost occurred. This is meaning that, temperature 25 ºC does affect the pH value for both inoculum to POME ratio with 1:3 and 1:5. In terms of effect of inoculum to POME ratio to overall process, the interaction between factors does not happened. This is due to the substrate ratio which is at the end of the process, it become toxic and inhibits the process.

Nwaogu et al., (2012) come out with the results that palm oil mill effluent contains some toxic substances which at toxic levels, it inhibit the utilization process of acidic POME. In general, plants should operate at low food to microorganisms (F/M) ratios during low temperature. A high F/M ratio correlates to a shorter reaction time while a low F/M correlates to a longer reaction time (Solids Inventory Control for Wastewater Treatment Plant Optimization, 2004). If high F/M ratio, filamentous bacteria will also grow resulting in low utilization process (Activated Sludge Process - Activated Sludge Wastewater Treatment Process, n.d).

In recent studies conducted by Jegatheesan et al., (n.d), they claimed that when operating with low organic matter (food) to microorganism's ratio (F/M) and long sludge ages, the culture may experience operational problems due to filamentous organisms which causing bulking sludge. In opposition, the high F/M ratio within the selector tanks promotes the growth of floc forming microorganisms while suppressing filamentous growth.

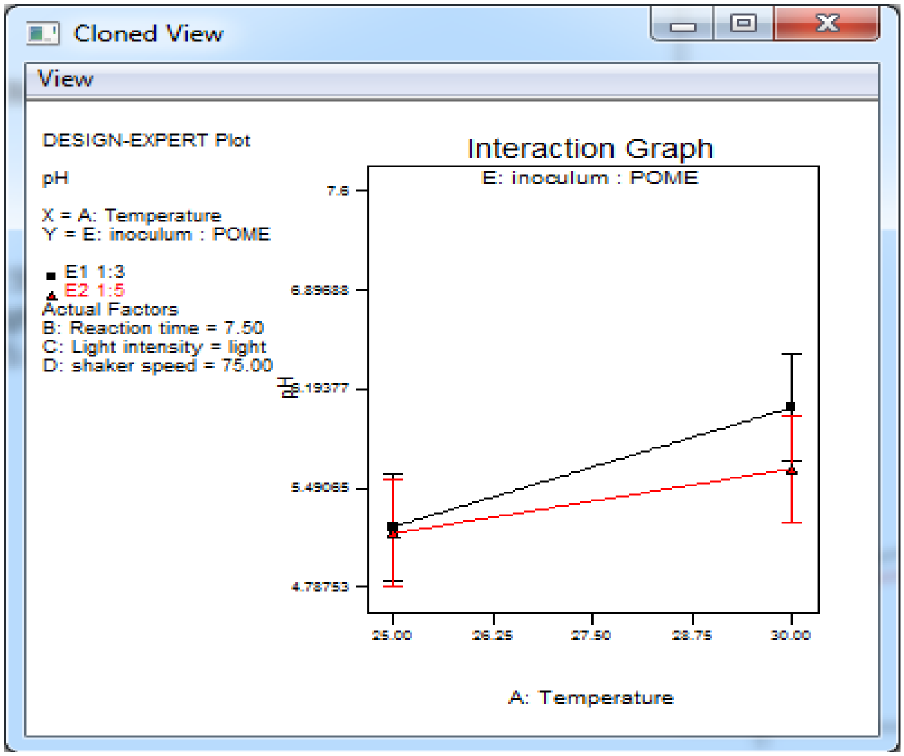

Figure 4.4 Interaction graph for factor of temperature with inoculum to POME ratio

4.3.3 Interaction between factors of reaction time with agitation speed

From Figure 4.5, there are no interaction occur between reaction time and agitation speed as the graph shows linear findings for this interaction. In days 6 as well as in days 9, the value of pH was increase when there was an agitation at 150 rpm. Meanwhile, without agitation; the value of pH was constant along respected reaction times. In other way round, it can be describes that the effective reaction to increase pH value of acidic POME may happened in less than six days.

Interaction does not occur because utilization of acidic POME is much better in performance when there is an agitation speed with lower reaction time and same goes when the treatment process is done in static culture with larger reaction time (Jaapar et al., 2009).

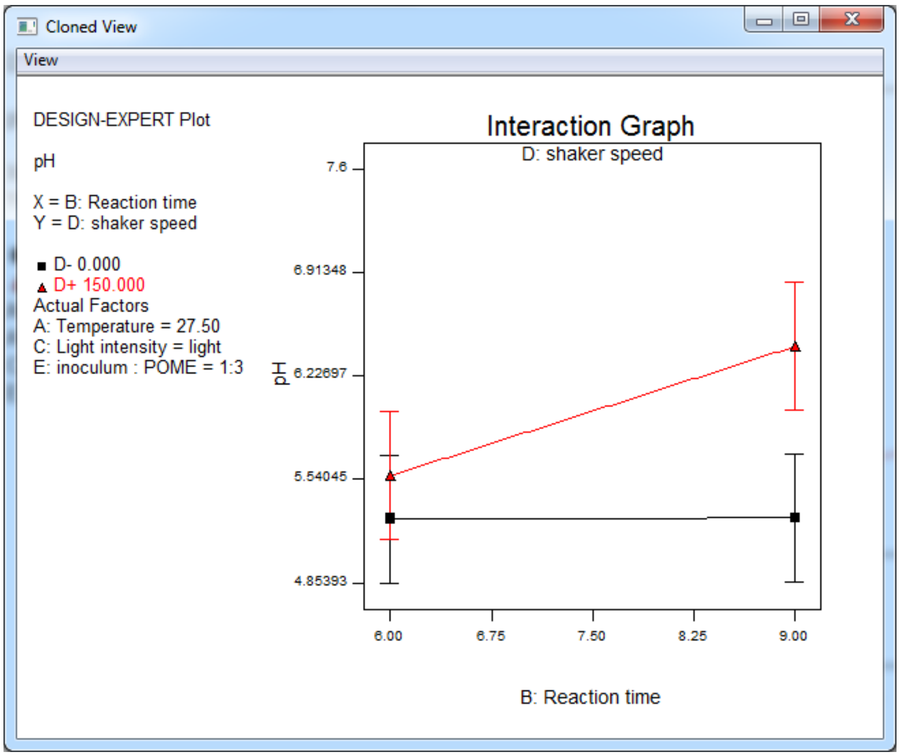

Figure 4.5 Interaction graph for factor of reaction time with agitation speed

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The present study was designed to determine the factors affecting an increasing pH of an acidic POME. As a conclusion, the objective of this experiment was successfully achieved. In this study, soil mixed culture was used to increase pH value of acidic POME; meanwhile the analysis on factors based on two level factorial analyses which is contributing to the treatment process was done by using Design Expert Software.

One of the most significant findings to emerge from this study is that in five factors tested, out of four factors were determined to give constructive result in treatment process. The factors are agitation speed, temperature and reaction time with percentage contribution of 19.08%, 7.58% and 4.68% respectively. Among the factors highlighted, agitation speed was the highest one because in the presence of agitation, it gives high involvement to mass and heat transfer to the utilization process.

In conditions of interaction between factors, the most significant interaction factor is between factor of agitation speed and temperature with 17.30% percentage contribution. Utilization of acidic POME needs soil mixed culture to have collision in order to get surface to surface contact. Therefore, agitation speed and temperature helps in obtaining better performance for treatment process by increasing the collision frequency.

It is predicted that best conditions regarding this study for temperature, reaction time, light intensity, agitation speed and inoculum to POME ratio lies on the value of 28.98 ºC, 8.81 days, with light, 138.89 rpm and ratio 1:3 respectively. All the best conditions provided a stable pH value which is 6.9999.

The model pH treatment is as in Equation 4.1 with *R square* of 0.8448

5.2 Recommendations

Generally, it seems that this study can be improved for further research by several amendments. So as to improve this research, optimization can be done since the parameters involved were already identified to give high contribution to pH treatment of acidic POME with the value of R^2 which is 0.8448 correspondingly.

In optimizing the overall process, it is highly recommended to involve only three factors. The factors are tabulated in Table 5.1 below. This optimization is to ensure that the utilization process is much efficient in increasing the pH value of acidic POME.

Table 5.1 Factors for optimization experiment

Factor	Range
Agitation Speed (rpm)	0-150
Temperature (ºC)	25-30
Reaction Time (days)	6-9

Then, microbe isolation also can be done as a part of amendment for this research. Previous researchers believe that pure culture can perform better instead of using soil mixed culture in pH treatment of acidic POME. Moreover, kinetic study also can be done in order to improve this research.

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APPENDIX A-MATERIALS AND METHODOLOGY

A.1 Sample of Acidic POME

Figure A.1 Sample of Acidic POME

A.2 Soil Mixed Culture

Figure A.2 Soil Mixed Culture

A.3 Inoculum Preparation

1. Prepare nutrient broth for 100 ml solution.
2. Autoclave the universal bottle and prepared nutrient broth for about one and half hour.
3. Pour 2 ml of nutrient broth into universal bottle.
4. To revive, take a loop of pure culture (*Enterobacter sp.*) and transfer it into the universal bottle. Make sure the process is in sterile conditions.

5. Seal universal bottle with parafilm and kept in anaerobic jar before incubated for about 24 hours.

6. In another 25 ml of nutrient broth, transfer the prepared inoculum to the universal bottle.

7. Seal the universal bottle and incubate it in incubator shaker with 150 rpm and 30ºC.

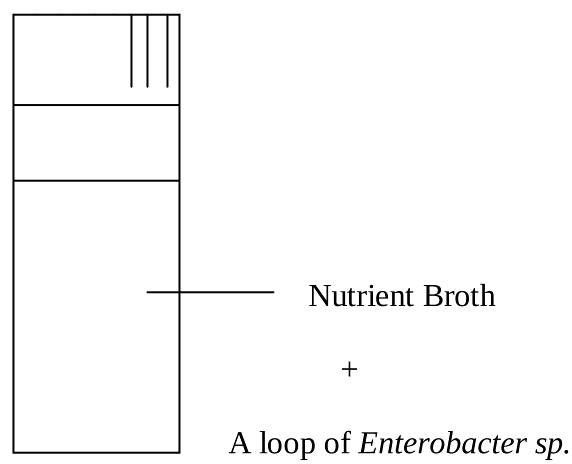


Figure A.3 Universal Bottle filled with Nutrient Broth and *Enterobacter sp.*

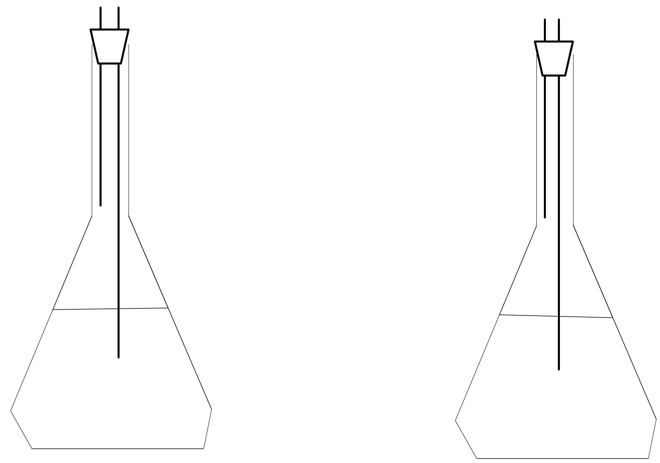
A.4 Preliminary Study on Reaction Time and Potential Microbes

1. In ratio 1:1 for inoculum to POME, *Enterobacter* sp. and soil mixed culture are added to 100 ml of acidic POME in two different conical flasks.

2. Purge the samples for 10 minutes.

2. Incubate the samples in incubator shaker with 150 rpm and 30ºC.

3. Observe and record the value of pH for every 12 hours in two days.



(a) (b)

Figure A.4 (a) Enterobacter sp. with acidic POME (b) Soil Mixed Culture with Acidic POME

Table A.4 Results for Preliminary Studies

Reaction Time (hours)	pH	
	Enterobacter sp.	Soil Mixed Culture
12	4.89	5.43
24	4.89	5.48
36	4.87	5.50
48	4.88	5.52
60	4.90	5.56
72	4.90	5.58

A.5 Acclimatization of Soil Mixed Culture

1. 100 ml of soil mixed culture is added to 100 ml of acidic POME in Schott bottle for each day.
2. Repeat step 1 until 10 days of acclimatization process.

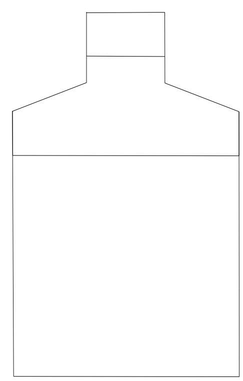


Figure A.5 2L of Soil Mixed Culture Bacteria in Schott Bottle

A.6 Experiment on pH treatment of POME

Table A.6 Experimental Table Design by Design Expert Software

Std	Run	Block	Factor 1 : Temperature (^0C)	Factor 2: Reaction Time (day)	Factor 3: Light Intensity	Factor 4: Shaker Speed (rpm)	Factor 5: Inoculum to POME ratio	Response 1 pH
30	1	Block 1	30.00	6.00	No light	150.00	1:5	
18	2	Block 1	30.00	6.00	Light	0.00	1:5	
7	3	Block 1	25.00	9.00	No light	0.00	1:3	

Table A.6 Experimental Table Design by Design Expert Software [Continued]

15	4	Block 1	25.00	9.00	No light	150.00	1:3	
32	5	Block 1	30.00	9.00	No light	150.00	1:5	
12	6	Block 1	30.00	6.00	Light	150.00	1:3	
13	7	Block 1	25.00	6.00	No light	150.00	1:3	
9	8	Block 1	25.00	6.00	Light	150.00	1:3	
26	9	Block 1	30.00	6.00	Light	150.00	1:5	
2	10	Block 1	30.00	6.00	Light	0.00	1:3	
22	11	Block 1	30.00	6.00	No light	0.00	1:5	
6	12	Block 1	30.00	6.00	No light	0.00	1:3	
14	13	Block 1	30.00	6.00	No light	150.00	1:3	
10	14	Block 1	30.00	6.00	Light	150.00	1:3	
28	15	Block 1	30.00	9.00	Light	150.00	1:5	
19	16	Block 1	25.00	9.00	Light	0.00	1:5	
4	17	Block 1	30.00	9.00	Light	0.00	1:3	
21	18	Block 1	25.00	6.00	No light	0.00	1:5	
8	19	Block 1	30.00	9.00	No light	0.00	1:3	
1	20	Block 1	25.00	6.00	Light	0.00	1:3	
5	21	Block 1	25.00	6.00	No light	0.00	1:3	
23	22	Block 1	25.00	9.00	No light	0.00	1:5	
27	23	Block 1	25.00	9.00	Light	150.00	1:5	
25	24	Block 1	25.00	6.00	Light	150.00	1:5	
16	25	Block 1	30.00	9.00	No light	150.00	1:3	
29	26	Block 1	25.00	6.00	No light	150.00	1:5	
11	27	Block 1	25.00	9.00	Light	150.00	1:3	
3	28	Block 1	25.00	9.00	Light	0.00	1:3	
24	29	Block 1	30.00	9.00	No light	0.00	1:5	
31	30	Block 1	25.00	9.00	No light	150.00	1:5	
17	31	Block 1	25.00	6.00	Light	0.00	1:5	
20	32	Block 1	30.00	9.00	Light	0.00	1:5	