PH TREATMENT OF POME VIA BIOLOGICAL PROCESS – OPTIMIZATION

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PH TREATMENT OF POME VIA BIOLOGICAL PROCESS – OPTIMIZATION

ANIS SAKINAH BINTI LOKMAN HAKIM

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
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SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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Dedication

I dedicate my dissertation work to my beloved

father and mother, Mr. Lokman Hakim bin Mohd Saman and Mrs. Nor Azhariah bt. Taman.

My friends, who always gave me inspiration, encouragements and support towards the success of this study.

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ABSTRACT

Palm oil mill effluent (POME) has been known as organic waste product from palm oil production which is featured by low pH of 3.5-4.5, high value of biological demand (BOD), chemical oxygen demand (COD) and suspended solids. It is found that palm oil industry is one of the contributors of environmental pollution if their waste not being treated well. So, 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 the water courses. Biological treatment appears less cost than chemical and physical methods, and also much faster than natural oxidation, with a lower environmental impact. Thus, this study is aims to optimize the factors which are influencing biological pH treatment of acidic POME by using research surface methodology (RSM) with CCD response of Design Expert software via biological treatment of using soil mixed culture. In the beginning, the sample of acidic POME was collected from a nearby palm oil mill whereas soil mixed culture was obtained from soil near to plants root system. Preliminary study to obtain the best reaction time was done in 5th days and at the end, the utilization was performed better in between 3rd-4th days. Then, an acclimatization of soil mixed culture together with POME was conducted and 13 experimental runs were done according to RSM. Lastly, all the experimental results data will be optimized using RSM with factorial central composite design (CCD) using the software package Design Expert Version 6.0. The model showed that pH will increase upon increasing of agitation and temperature between to optimum conditions, but declined with further increases of these factors. From these optimum condition, it can be concluded that agitation and temperature had an individual significant influence on pH. Yet, the agitation and temperature was interdependent or having significant interaction on pH treatment while pH and temperature were interdependent according to the study and slightly interdependent and their interactive effects were insignificant. Based on the results, the predicted optimum condition suggested by software is temperature of 32.50℃ and agitation speed of 125 rpm. The expected pH value of 8.03 may obtained. Thus, the experimental response variables were very close to those predicted by RSM, indicating that RSM was a useful tool to optimize the pH treatment. Besides, the error generated was 2.293% indicated that the data was valid since the result does not excess 10%. After all, it can be concluded that the agitation speed and temperature were optimized to the optimum conditions and can be applied in industrial applications.

ABSTRAK

Sisa minyak kumbahan kilang kelapa sawit telah dikenali sebagai bahan buangan organik daripada pengeluaran minyak sawit yang dicirikan seperti pH rendah diantara 3.5-4.5, mempunyai nilai tinggi keperluan oksigen biokimia (BOD), keperluan oksigen kimia (COD) dan pepejal terampai. Industri minyak sawit adalah salah satu daripada penyumbang pencemaran alam sekitar jika sisa mereka tidak dirawat dengan baik. Jadi, ia adalah wajib untuk kilang-kilang minyak sawit bagi merawat air buangan mereka ke tahap yang boleh diterima sebelum ia dibenarkan untuk dilepaskan ke dalam saluran air. Kebanyakan menggunakan rawatan biologi kerana kurangnya kos yang diperlukan daripada kaedah kimia dan fizikal, dan juga lebih cepat daripada pengoksidaan semula jadi, dengan kesan alam sekitar yang lebih rendah. Oleh itu, kajian ini adalah bertujuan untuk mengoptimumkan faktor yang mempengaruhi rawatan pH biologi POME berasid dengan menggunakan kaedah permukaan sambutan (RSM) dengan rekaan komposit pusat (CCD) menggunakan pakej perisian 'Design Expert' versi 6.0 melalui rawatan biologi menggunakan tanah pelbagai kultur. Pada mulanya, sampel POME berasid dikumpulkan dari kilang minyak sawit yang berhampiran manakala tanah pelbagai kultur telah diperolehi daripada tanah berhampiran sistem akar. Kajian awal untuk mendapatkan tindak balas masa yang terbaik telah dilakukan selama 5 hari dan pada akhirnya, keputusan eksperimen lebih baik adalah di antara hari ke-3-4. Kemudian, satu penyesuaian tanah pelbagai kulttur bersama-sama dengan POME telah dijalankan dan 13 eksperimen telah dilakukan mengikut RSM. Akhir sekali, semua keputusan data eksperimen akan dioptimumkan menggunakan RSM dengan reka bentuk CCD menggunakan pakej perisian Design Pakar Versi 6.0. Model telah menunjukkan bahawa pH akan meningkat apabila meningkatnya kelajuan putaran dan suhu hingga antara keadaan yang optimum, tetapi menurun dengan kenaikan lebih daripada faktor-faktor ini . Dari nilai optimum ini, ia boleh disimpulkan bahawa kelajuan putaran dan suhu mempunyai pengaruh besar ke atas pH. Namun, kelajuan putaran dan suhu adalah saling bergantung atau mempunyai interaksi yang ketara ke atas rawatan pH manakala pH dan suhu telah saling bergantung menurut kajian dan sedikit saling bergantung dan kesan interaktif mereka juga tidak ketara. Berdasarkan kepada keputusan , keadaan optimum yang diramalkan dicadangkan oleh perisian adalah suhu 32.50 ℃ dan kelajuan pergolakan 125 rpm. Jangkaan nilai pH 8.03 boleh diperolehi. Oleh itu, nilai pemboleh ubah tindak balas eksperimen sangat hampir dengan yang diramalkan oleh RSM, menunjukkan RSM adalah cara yang berguna untuk mengoptimumkan rawatan pH. Selain itu,

ralat yang dihasilkan adalah 2.293% menyatakan data itu adalah sah kerana ianya tidak melebihi 10%. Kesimpulannya, ia dapat disimpulkan bahawa kelajuan putaran dan suhu telah dioptimumkan untuk keadaan optimum dan boleh digunakan dalam aplikasi industri.

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LIST OF ABBREVIATION

AN	Ammonia Nitrogen
BBD	Box-Behnken Design
BOD	Biochemical Oxygen Demand
Ca	Calcium
CCD	Central Composite Design
COD	Chemical Oxygen Demand
CPO	Crude Palm Oil
CV	Coefficient Variations
DOE	Department of Environment
DoE	Design of Experiment
EQA	Environmental Quality Standard
FD	Factorial Design
FFB	Fresh Fruit Bunch
GDP	Gross Domestic Product
K	Potassium
Mg	Magnesium
MPOB	Malaysian Palm Oil Board
N	Nitrogen
P	Phosphorus

POME	Palm Oil Mill Effluent
RSM	Research Surface Methodology
SS	Suspended Solids
TN	Total Nitrogen
TS	Total Solids
TVS	Total Volatile Solids
VFA	Volatile Fatty Acids

TABLE OF NOMENCLATURE

F	Flow rate	tonnes/hr
t	Reaction time	min, s
V	Volume	m^3, mL
P	Pressure	Pa
T	Temperature	°C
m	Mass	tonnes, kg
c	Concentration	mg/L
s	Agitation speed	rpm

1 INTRODUCTION

1.1 Research Background

The Malaysian palm oil industry has seen as a new growth in the last four decades to emerge as the leading agricultural industry in the country. Palm oil was first introduced to Malaysia in year 1875 as an ornamental plant (Lang, 2007). Furthermore, palm oil is one of the main agricultural products of Malaysia which constitutes 41.37% of total world production for the year 2007 (Malaysian Palm Oil Board (MPOB), 2009). Currently, about half of the agricultural land in Malaysia is conquered by palm oil and the area is expanding. In 2003, more than 3.79 million hectares of land were under oil palm cultivation, occupying more than one-third of the total cultivated area in Malaysia and 11% of the total land area (Wu *et al*., 2009). Nowadays, Malaysia is the world's biggest producers and exporters of palm oil. The palm oil industry is primarily export-oriented. As cited by Wu *et al*. (2010), the amount of export earnings from palm oil, palm kernel oil and associating products in 1998 is almost US$5.6 billion, equivalent to 5.6% of the gross domestic product (GDP).

Moreover, palm oil has unique composition which makes it various in its application in food manufacturing and in the chemical such as manufacture of cleaning products, cosmetic and pharmaceutical industries. Its semi-solid physical properties are required in much food preparation, for example, being used as an ingredient in a wide range of foods including biscuits, bakery products, snacks and ice-cream. Animal feed may also contain palm oil and palm kernel meal. Besides, its non-cholesterol quality and digestibility make it popular as source of energy such as fuel, while its technical and economic superiority makes it preferable as base material in the manufacture of various non-edible products.

1.2 Motivation and problem statement

While it is recognized that the revenue from palm oil industry has contributed much towards the national development and improvement in the standard of the living of the people, the rapid expansion of the industry has also contributed to the environmental pollution. Oil palm cultivation and processing like other agricultural and industrial activities will raise environmental issues (Mustapa, 2008). Other than that, the palm oil mill industry in Malaysia is identified as the one contributing the largest pollution load in rivers throughout the country (Wu *et al*., 2010). An increasingly stringent environmental regulations in view of the government's commitment to the conservation of the environment and increased public awareness of pollution problems caused the palm oil industries facing tremendous challenges as palm oil mill effluent (POME) is a highly pollutant effluent. POME treatment requires an efficient system in facing the current challenges. There are many processing plants failed to achieve the standard discharge limits. 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 the water courses.

There are many current treatment methods, which are adopted by the palm oil industries. Currently, the ponding system is the most common treatment employed by most of the palm oil mills as their conventional treatment of POME (Wu *et al*., 2010) but other processes such as aerobic and anaerobic digestions, physicochemical treatments and membrane filtrations can provide the palm oil industries with a possible insight into the improvement of current POME treatment processes (Wu *et al*., 2009). On the other hand, the treatment that is based mainly on biological treatments of anaerobic and aerobic systems is quite inefficient to treat POME, which unfortunately leads to environmental pollution issues (Wu *et al*., 2009). This is because the high BOD loading and low pH of POME, together with the colloidal nature of the suspended solids, renders treatment by conventional methods difficult (Wu *et al*., 2009). A new and improved POME treatment would be required in order to meet the requirements of Department of Environment (DOE) discharge limits in order to increase the efficiency of biological methods used.

Although there has been successful industrial-scale of palm oil mill effluent treatment, generally the industry is still facing various challenges to ensure the standards for POME discharge into watercourses is achieved. Otherwise, this study was focusing on the pH where the pH of POME also plays an important role to make the effluent can be discharge. If pH of POME is not achieved the standards outlined by POME discharge standards under the Environmental Quality Act of Malaysia, 1974 it will not discharged or will cause the environmental pollution. In the Environmental Quality (Prescribed Premises) (Crude Palm Oil) (Amendment) Regulations 1982, the pH limits for POME discharge is between 5 and 9. Hence, this study will optimize the factors that can increase the pH from the previous study by using biological pH treatment. The pH changes can be influenced by many factors. These factors included agitation speed, temperature, and reaction time.

1.3 Objective

This work aims to optimize the factors which are influencing biological pH treatment of acidic POME.

1.4 Scope

To achieve the objectives of this research, the main research fields to be carried on which are;

- By using soil mixed culture.
- To acclimatize the POME and soil mixed culture to be inoculums.
- To analyze pH of POME sample after the treatment with soil mixed culture.
- To optimize the factors which are agitation speed, temperature and reaction time by using research surface methodology (RSM) with CCD response via Design Expert software.

2 LITERATURE REVIEW

2.1 Overview

This chapter provides a brief review that relating with this study. First of all, the review explains the palm oil mill processing for producing POME from CPO, the characteristics of POME, and the Enactment of Environmental Quality Act for discharging POME. Secondly, the review covers about the biological method involved in the treatment process; aerobic and anaerobic treatment processes of POME, inoculums used and factor that affecting biological pH treatment. Finally, the design of experiment using response surface methodology which was applied in this research to model and optimize the process is also elaborated.

2.2 Palm oil mill processing and process description

The wet palm oil milling process is the most standard and distinctive way of extracting palm oil, especially in Malaysia. In large factories, steam and water are used, thus giving rise to the wastewater known as palm oil mill effluent (POME). The palm oil milling process is more or less the same for all the mills throughout the country (Wu *et al*., 2010). The stages involved in the typical processing of crude palm oil (CPO) are depicted in Figure 2.1. The three main sources of POME are steriliser condensate, hydrocyclone waste and clarifier sludge. For a well-controlled conventional oil palm mill, about 0.9 m^3, 0.1 m^3 and 1.5 m^3 of steriliser condensate, clarifier sludge and hydrocyclone waste are generated for each tonne of crude palm oil produced (Lang, 2007).

CPO is extracted from the mesocarp of fresh fruit bunch (FFB). The capacity of a large scale mills range from 10 to 60 tonnes FFB/h. The first step consists in sterilizing the FFB in steam sterilizers for 50 min at about 140 ℃ and a pressure of 3 x 10^5 Pa in order to stop the rapid formation of free fatty acids during the pulping process. This process also allows the fruits that are still attached in bunch to be loosened. Secondly, the stripping is to separate the sterilized fruits from the bunch stalks by using a rotary drum thresher. The fruit are then mashed in the digester under steam heated condition with temperature around 90℃. Twin screw presses are generally used to press out the oil from the digested mashed fruits under high pressure. The oil is thus separated from the spent mesocarp and the nuts. But then, the crude oil extracted from digested palm fruit contains varying amounts of water as well as impurities consisting of vegetable matter. This matter is in the form of either insoluble solids or dissolved matter in water. By settling and centrifuging, the water present in the crude oil can be largely removed from the ‘bottom’ phase since most of it is free or non-dissolved. The ‘bottom’ phase of the clarification or settling tank is sent to a sludge separator or centrifuge where approximately 1.5 tonnes of sludge waste is obtained per tonnes of produced crude palm oil. During pressing of the digested fruit to extract the oil, a cake made up of nuts and fiber is produced. After separation of the fiber from the nuts, the latter are sent for further processing from which another product, the palm kernel is obtained. This processing section is constituted of a hydrocyclone that separates the kernels from the empty shells after cracking the nuts. Approximately 0.1 tonnes of liquid effluent per tonnes of produced crude palm oil is generated in this process. Any uncracked nuts must be removed and recycled, and the shell separated from the kernels. The kernel is dried to below 7% moisture in order to prevent the growth of mould for a longer storage time.

2.3 Crude palm oil (CPO)

The production of such large amounts of CPO results in even larger amounts of POME. For each tonne of CPO produced from the fresh fruits bunches, approximately 6 tonnes of waste palm fronds, 5 tonnes of empty fruit bunches, 1 tonne of palm trunks, 1 tonne of press fibre (from the mesocarp), 500 kg of palm kernel endocarp, 250 kg of palm kernel press cake, and 100 tonnes of POME can be obtained (Foo & Hameed, 2010). Table 2.1 show that the total productions of CPO for the month of January-February 2012 and 2013 respectively. Approximately 2.9 million tonnes of CPO was produced in the month Jan-Feb in 2013 which increased by 14.54% from 2.47 million tonnes in the month Jan-Feb in 2012 (MPOB, 2013). The production of POME has been increased lately due to the demand for palm oil in the world at an average rate of 7.36% per year during 2006-2010 (Yossan *et al.*, 2012).

Table 2.1: Malaysian production of CPO in Jan-Feb 2012 and 2013

States	Jan – Feb Total	
	2012	2013
Johor	372,928	417,141
Kedah	43,769	45,824
Kelantan	30,886	39,617
Melaka	16,185	13,447
Negeri Sembilan	85,082	102,397
Pahang	339,483	420,334
Perak	264,496	287,523
Pulau Pinang	14,206	15,404
Selangor	89,916	85,785
Terengganu	57,954	63,975
Sabah	779,710	979,533
Sarawak	382,035	427,088
Total	2,476,650	2,898,068

(Source: MPOB, 2013)

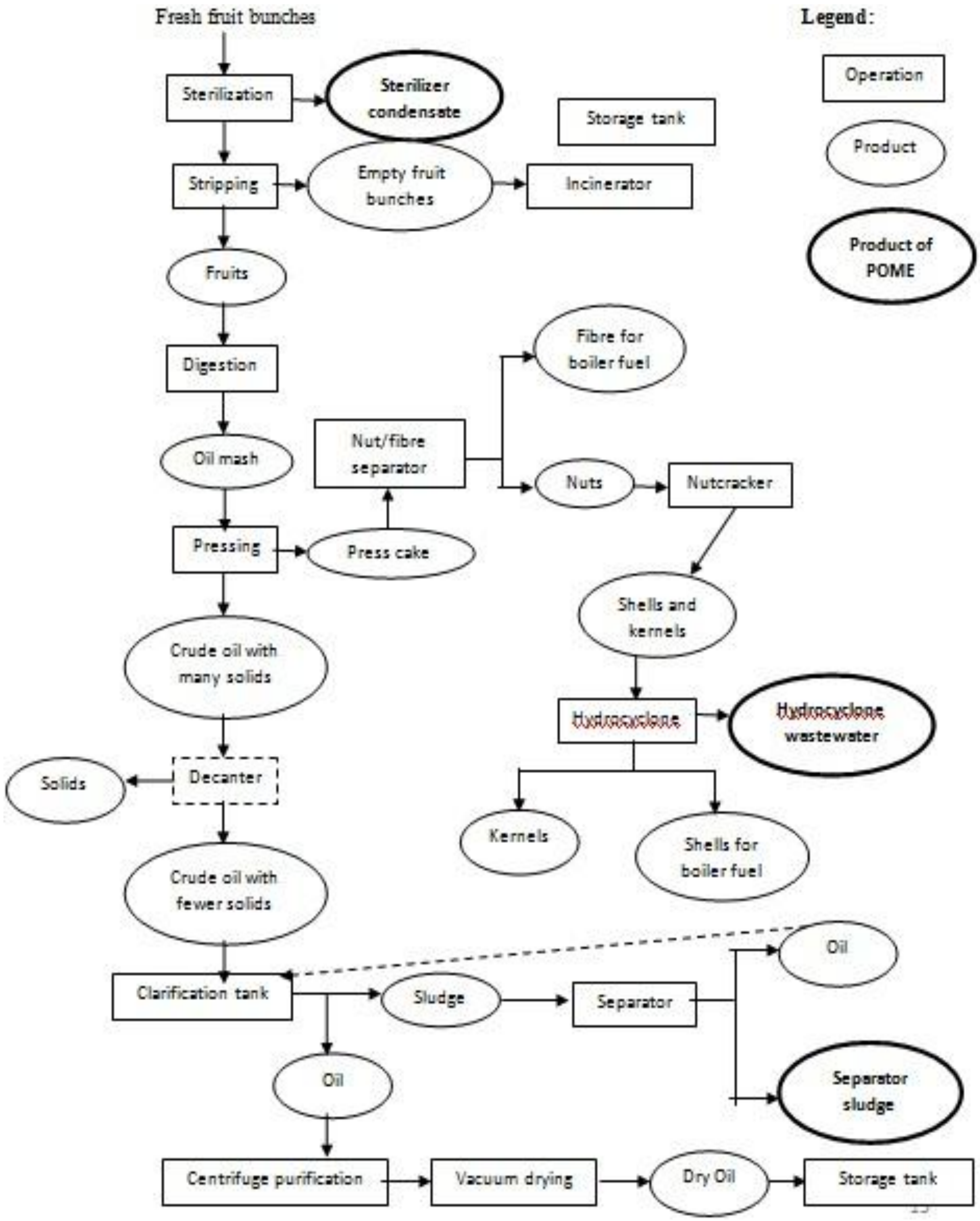

Figure 2.1: Process operations and products in a typical palm oil milling process

(Source: Wu *et al*., 2010)

2.4 Palm Oil Mill Effluent (POME)

POME is a type of organic waste which consists of a significant amount of solid wastes and wastewater (cellulosic material, fat, oil and grease etc) produced by palm oil industry during milling process. By nature, fresh POME is an acidic, thick, brownish, viscous and voluminous colloidal suspension with 95-96% of water, 0.6-0.7% of oil and 2-4% suspended solids. It also contains an essential inorganic nutrients (sodium, potassium, calcium, magnesium, manganese, and iron), cell walls, organelles, and short fibres, a range of nitrogeneous compounds from proteins to amino acids, free organic acids and a spectrum of carbohydrates ranging from hemicelluloses to simple sugars. Besides, raw POME is featured by low pH value of 3.5-4.5, high biological oxygen demand (BOD) of 10, 250-43,750 mg/L, chemical oxygen demand (COD) of 16,000-100,000 mg/L, suspended solids of 5000-54,000 mg/L, nitrogen content ranging from 200 to 500 mg/L as ammonia nitrogen and total nitrogen and discharge temperature of 80-90℃ (Foo & Hameed, 2010).

It can be seen in the Table 2.2, that the BOD: COD ratio of raw POME is approximately 1:2 which means that POME is considered to be suitably treated by biological processes. While the typical BOD: N: P ratio of 139: 4: 1 indicates the limitations of nutrient, which is required for bacterial growth and metabolic requirements of biomass to obtain optimum biological processes under aerobic conditions, which requires 100: 5:1. Nutrient deficiency can lead to increase the population of filamentous bacteria (Ujang & Lim, 2004).

Generally, the characteristics of POME may change substantially for different batches, days and factories; depend on the processing techniques, the age or type of fruit, the discharge limit of the factory, climate and condition of the palm oil processing as cited by Wu et al. (2010). Seasonal oil palm cropping, palm oil mill activities (such as occasional public holidays, closure of the mill, operation and quality control of individual mills) will also influence the quality and quantity of the discharge POME which in turn affect the biological treatment process of POME. Thus, the variation of treatment of POME in palm oil industries has been selected depending on the several of characteristics of POME, in terms of quality and quantity.

Table 2.2: Palm Oil Mill Effluent (POME) characteristics

GENERAL PARAMETERS		
PARAMETER*	MEAN	RANGE
pH	4.2	3.5-5.2
Oil & Grease	6,000	150-18,000
Biochemical Oxygen Demand (BOD)	25,000	10,000-44,000
Chemical Oxygen Demand (COD)	50,000	16,000-100,000
Total Solids (TS)	40,500	11,500-79,000
Suspended Solids (SS)	18,000	5,000-54,000
Total Volatile Solids (TVS)	34,000	9,000-72,000
Ammonia Nitrogen (AN)	35	4-80
Total Nitrogen (TN)	750	80-1,400
Phosphorus	180	
Magnesium	615	
Calcium	440	
Boron	7.6	
Iron	47	
Manganese	2.0	
Copper	0.9	
Zinc	2.3	

*All parameters in mg/L except pH

(Source: Industrial Processes & The Environment (Handbook No.3) - Crude Palm Oil Industry, 1999)

2.5 Environmental Quality Standard

With the rapid expansion of the palm oil industry and the public's increased awareness of environmental pollution, the industry is compelled both socially and aesthetically to treat its effluent before discharging it. After the enactment of the Environmental Quality Act (EQA), 1974 and the establishment of the Department of Environment in 1975, comprehensive environmental control of the crude palm oil industry was commenced. The Environmental Quality (Prescribed Premises) (Crude Palm Oil) Order, 1977 and the Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations, 1977 were proclaimed under the EQA, in order to regulate the discharge of effluent from the crude palm oil industry as well as to exercise other environmental controls (Pierzynski *et al.*, 2005). These were the first sets of industry specific subsidiary legislation to be proclaimed under the EQA for industrial pollution control. Table 2.3 presented the current effluent discharge standard ordinarily applicable to crude palm oil mills.

Table 2.3: Prevailing effluent discharge standard for CPO mills

Parameters	**Unit**	**Parameter Limits**
Biochemical Oxygen Demand (BOD) (BOD; 3 days, 30 ℃)	mg/L	100
Chemical Oxygen Demand (COD)	mg/L	*
Total Solids (TS)	mg/L	*
Suspended solids (SS)	mg/L	400
Oil & Grease (O&G)	mg/L	50
Ammonia Nitrogen (AN)	mg/L	150
Total Nitrogen (TN)	mg/L	200
pH		5-9
Temperature	℃	45

*no discharge standards after 1984

(Source: Pierzynski *et al.*, 2005)

2.6 Effects of acidic pH of POME

As mentioned earlier, Malaysia is identified as the country that produces the largest pollution load in the river. Even though POME is considered as non toxic, but it is identified as a major source of aquatic pollution by depleting dissolved oxygen when discharged untreated into water bodies. The acidity of POME has important consequences on the survival of aquatic organisms if it not being treated well. Acid pH levels can be harmful to fish, invertebrates, and other water organisms that are affected either directly or indirectly by acidic substances. The low pH can result in lakes, streams, and rivers that no longer support aquatic life.

Other than that, land application of POME is one of the disposal alternatives; however, it must be disposed by controlling of small quantities of POME at a time to avoid clogging and water logging of soil and kills the vegetation on contact (Wood *et al*., 1979). As discussed by Iwara *et al.* (2011), the low pH of raw POME will increase to near neutrality in soil as biodegradation takes place. This therefore, implies that POME increases soil pH which in effect increases the values of major nutrients (nitrogen (N), potassium (K) and phosphorus (P)) in the soil since POME also contains appreciable amounts of N, P, K, magnesium (Mg) and calcium (Ca), which are the vital nutrient elements for plant growth.

2.7 Biological treatment of POME

In biological treatment, microorganisms use the organics in wastewater as a food supply and convert them into biological cells, or biomass. Because wastewater contains a wide variety of organics, a wide variety of organisms, or a mixed culture, is required for complete treatment. Each type of organism in the mixed culture utilizes the food source most suitable to its metabolism.

Biological treatment of POME has been widely studied. Biological treatment appears less cost than chemical and physical methods, and also much faster than natural oxidation, with a lower environmental impact. The common biological processes that being used for treatment of waste products is aerobic treatment process and anaerobic treatment process.

2.7.1 *Aerobic treatment process*

Aerobic treatment process is a biological treatment that occurs in the presence of oxygen. Aerobic digestion actually refers to the use of aerobic bioreactors to stabilize particulate organic matter arising from primary clarification (predominantly biodegradable organic matter) and biological treatment (predominantly biomass) of wastewaters. Biodegradable particulate organic matter is hydrolyzed and converted into biodegradable soluble organic matter, releasing nutrients such as ammonia-N and phosphate. The biodegradable soluble organic matter is then converted into CO_2, water, active biomass through the action of heterotrophic bacteria.

2.7.2 *Anaerobic treatment process*

Anaerobic process is defined as biological treatment process that occurs in the absence of oxygen. Anaerobic treatment of wastewater is a complex biological process involving several groups of microorganisms (Badroldin, 2010). This process is time consuming as bacteria responsible for the degradation process requires time to adapt to the new environment before they start to consume on organic matters to grow. The principal function of anaerobic digestion, therefore, is to convert as much of the wastewater as possible to end products such as liquids and gases, while producing as little residual biomass as possible. Anaerobic digestion is the most suitable method for the treatment of effluents containing high concentration of organic carbon such as POME (Wu *et al*., 2010).

It has been established that the anaerobic process is in many ways ideal for wastewater treatment. It is being proved by Syed Jaapar *et al*. (2009) that the inoculums and substrate is more efficient when incubated in anaerobic rather than aerobic condition. Anaerobic digestion is a technology that has traditionally been viewed as symbol of 'energy from waste' that can provide a range of benefits in addition to the valuable renewable energy and reduce pollution, odours and disease. Furthermore, it could contribute to recycle the nutrients back to the soil (thereby reducing the requirements for artificial fertilizers); improve soil quality by recycling the organic matter as humus, thus preserving fragile topsoil, sanitization of the compost, reducing the spread of soil-borne pathogens and weeds (Liu, 2008).

There are several significant advantages over other available methods especially aerobic treatment and is almost certainly assured of increase usage in the future. The advantages of anaerobic process are as listed below (Singh, 2009):

i. Less energy is required as no aeration is needed
ii. Energy generation in the form of methane gas
iii. Minimizes the amount of final sludge disposal
iv. Less nutrients (nitrogen and phosphorus) requirement
v. Application of higher organic loading rate
vi. Space saving because application of higher loading rate requires smaller reactor volume thereby saving the land requirement
vii. Be able to transform several hazardous solvents to an easily degradable form.

However, this biological treatment system needs proper maintenance and monitoring as the processes rely solely on microorganisms to break down the pollutants. The microorganisms are very sensitive to changes in the environment and thus great care has to be taken to ensure that a conducive environment is maintained for the microorganisms in which to thrive (Ahmad *et al*., 2003). In addition, the treated wastewater cannot be reused in the plant, and it is being discharged into the environment.

2.8 Inoculums

2.8.1 Pure culture

Nowadays, studies on microbial activity have been conducted mostly by pure cultures and under mesophilic to thermophilic temperature ranges. Also, the pure cultures normally have special requirement on the medium for growth. The inoculum that has been widely used for POME treatment is *Clostridium* and *Enterobacter* (Wang & Wan, 2009). As discussed by Wu *et al.* (2010), it was more suitable to utilize single cultures rather than a mixed culture. Species of *Clostridium* species are gram-positive, rod-shaped, strict anaerobes and endospore formers; obligate anaerobes whereas species of *Enterobacter* are gram-negative, rod-shaped, and facultative anaerobes. However, *Clostridium* species acts as a strong microorganism that can enhance the microbial activity.

2.8.2 Mixed cultures

In fact, organic waste treatments by biological, sustainable, non-hazardous, safe and environmental friendly methods have been the main attention of several studies. Miles and Moy (1979) stated their product may be attributed to chemical hydrolysis in the medium made more alkaline by the microbial growth. Therefore, some of researchers used a mixed culture as inoculums. The mixed culture is more appropriate conducted with non-sterilized organic wastes under mesophilic temperatures at 20-40 ℃ or thermophilic conditions at 50-60 ℃ (Yossan *et al*., 2012). At present, the mixed culture of bacteria from anaerobic sludge, municipal sewage sludge, compost and soil have been widely used in the waste treatment. When it is conducted in anaerobic condition, mixed culture mainly consists of two types of anaerobic bacteria which are facultative (*Rhodopseudomonas* species) and obligate anaerobes (*Clostridium* species). A mixed culture study by Fang *et al.* (2002), found that it is consists of approximately 70% of the genus Clostridium and 14% were of Bacillus species. The facultative anaerobes usually can exist with or without oxygen whereas obligate anaerobes require an oxygen-free environment that sensitive to minute amount of dissolved oxygen.

These bacteria in a mixed culture can alter or break the waste constituents. According to Lin *et al.* (2008), mixed culture is helpful to degrade organic waste-based microbes feed stocks that might contain a variety of complex components. Processes using mixed cultures are more practical than those using pure cultures, because the mixed cultures are simpler to operate and easier to control, and may have a broader source of feedstock (Wang & Wan, 2008). It is believed that for future industrial applications, the use of mixed cultures for treatment of organic wastes might have more advantages because pure cultures can easily be contaminated. However, for a technologically practicable process, stable, mixed cultures easily to obtain from natural sources able to operate on non-sterile feedstock are required (Liu, 2008).

2.8.3 Soil mixed culture

The location of bacteria in soil commonly was found in the top few inches of soil where close to the organic matter on which they feed. Some bacteria species are very fragile and can be killed by slight changes in the soil environment. Other species are extremely tough, able to withstand severe heat, cold or drying. They mainly have one of two shapes which are spheres (called cocci) and rods (called bacilli). Other bacteria have more varied shapes including spirals and long thin hyphae.

On the basis of ecological characteristics, as cited by Conn (1948) was classified soil bacteria in particular into two broad categories such as Autochnotus (Indigenous species) and the Zymogenous (fermentative). The group of indigenous bacteria (eg. *Arthrobacter* and *Nocardia*) may be regarded as always numerous in soils and not fluctuating much in numbers, carrying on activities which require no nutrients or sources of energy other than those normally present in soil. Whereas the fermentative bacteria (eg. *Psesudomonas* and *Bacillus*) consists of actively fermenting forms which require for their activity ingredients that are consumed rapidly, hence under proper conditions will increase to large numbers. This type belongs to the nitrifiers, nitrogen fixing bacteria and cellulose decomposing organisms (Conn, 1948).

2.9 Factors affecting biological pH treatment

PH value can be affected because the microbial growth. More *et al.* (2010) mentioned that the pH level was changed during the population growth of microbes in treatment because the fungal growth may have led to excretion of acidic metabolites. It is due to the high capacity to explicit and release proteins, enzymes, organic acids and other metabolites. This might inhibit or reduce the intensity of the growth in alkaline pH of sludge. Meanwhile, the pH also plays a major role in microbial activity in fermentation since each bacterium is active only in a specific pH range and has maximum activity at its optimal pH. Wang and Wan (2008) described that the pH is affecting the activities of bacteria, and the fermentation itself. The microbial activity was dependent on the initial pH that might negatively affect its metabolic pathway (Yossan *et al*., 2012). Khanal *et al.* (2004) have stated that the higher the initial pH was, the lower the potential of microbial growth.

An initial pH of 4.5 might be delayed the microbial growth, but it lasted longer in comparison to a higher initial pH. It is due to the changes in environmental conditions caused by the rapid depletion of pH might have resulted in a metabolic alteration, and subsequent inhibition of microbial growth.

From these conditions, it needs further research and efforts to improve pH value of the fermentation system during mixed-culture process. To be considered, the microbial community in anaerobic fermentation are sensitive to pH changes. Optimum pH for most microbial growth is between 6.8 and 7.2 while pH lowers than 4 and higher than 9.5 are not tolerable (Poh & Chong, 2009). Hence, there are several factors that might affecting the biological pH treatment will be covered in this study.

2.9.1 Agitation speed

The performance of fermentation system could be improved by means of agitation. The two solutions are mixed well in given speeds; they called it as agitation. Agitation is another factor in biomass fermentation. The importance of agitation in achieving substrate conversion has been reported by several researchers (Kaparaju *et al.*, 2007). The microbial activities increased in the stirred culture (Lamed *et al.*, 1988). It is being supported by Clark *et al.* (2012) that mixing is a possible option to speed up the activity of microorganism from the liquid phase, and there is evidence of its effectiveness. The microbial activity is greater at faster stir rates in the range between 0 to 200 rpm. Mixing is expected to keep the organic material in suspension (Yuan *et al.*, 2011). Also, agitation provides good contact between microbes and substrates, reduces resistance to mass transfer, minimizes build up of inhibitory intermediates and stabilizes environmental conditions. When agitation is inefficient, overall rate of process will be affected the medium at different stages of digestion whereby every stage has a different pH and temperature (Poh & Chong, 2009). Otherwise, in other research provided that the microbial activity is higher in the static culture rather than in the shake culture (Syed Jaapar *et al.*, 2009). These conflicted results seem to be due to the types of culture being used that affected the metabolic pathway.

On the other hand, as discussed by Kaparaju *et al.* (2007), the effect of agitation speed (minimal, gentle or vigorous) has relationship with substrate to inoculums ratio. In batch assays at 55 ℃ showed that when the process was overloaded by high substrate to inoculum ratio, the effect of agitation speed as gentle (35 times per minute) or minimal mixing (10 min mixing before feeding) was advantageous compared to vigorous mixing (110 times per minute). However, under low substrate to inoculums ratio, gentle mixing was the best. Besides, Kaparaju *et al.* (2007) also compared the type of agitation that will affect the bacteria performance. In minimal mixing, it is found to be sufficient to distribute the feed adequately and stimulate the formation of new initiation centres for autocatalysis reaction whereas vigorous continuous mixing is shown to disrupt the structure of microbial flocks. Nevertheless, intermediate mixing appears to be the most optimal for substrate conversion.

2.9.2 Temperature

Temperature is an important environmental and operating factor in biological processes, because it can affect the microbial activity by influencing the activity of some essential enzymes (Wang & Wan, 2008). Moreover, temperature can affect biochemical reactions in many ways, such as reaction rate, the reaction pathway, microorganism yields, and rate of death. Anaerobic processes, including dark fermentation, are readily affected by temperature changes because anaerobes are sensitive to the operating temperature. The reactions can be operated at different temperatures which are mesophilic (25-40℃), thermophilic (40-65℃), extreme thermophilic (65-80℃) or hyperthermophilic (>80℃) (Liu, 2008). Microbial activity was low at mesophilic range (30-40 ℃) but was rather efficient and high at thermophilic range (50-55℃) (Lin et al., 2008). Thermophilic conditions can take advantage of high temperature of wastewater discharge from various industries like palm oil mill, food processing plants and distilleries (Prasertsan *et al.*, 2009). But Liu (2008) has indicated that the extreme thermophilic process is more efficient compared with the mesophilic and thermophilic. It is due to the production will be higher, has better pathogenic destruction for digested residues and can minimize contamination by bacteria with proper parameter control.

The dominant organisms might vary when fermentation was operated at different operating temperature ranges because the organisms responsible for anaerobic digestion are quite sensitive with temperature changes and have narrow temperature range for optimal growth. However, Wang and Wan (2008) stated that in the appropriate range, temperature can enhance the ability of mixed cultures to degrade substrate with increasing temperature from 20-40℃ and decrease with further increasing temperature from 40-55℃. It is also found by Wang and Wan (2008) that due to the fermentation, the final pH in batch tests decreased with increasing temperature from 20-35℃, but it increased with further increasing temperature from 35-55℃.

2.9.3 Reaction time

Reaction time is another important factor which significantly affects the treatment process of POME (Chou *et al*., 2010). Normally, in anaerobic processes, pH and reaction time are interacting to each other and it is the effective ways to increase the microbial activity at mesophilic and thermophilic conditions; short reaction time results in low pH (Liu, 2008). Prasertsan *et al*. (2009) observed that microbial activity rate decreased as reaction time decreased from 4 to 1 day. More carbohydrates in POME were degraded at longer reaction time. However, most of the researchers were done with a reaction time less or equal to 24 h. If it is extended for more than 42 hr, the microbial activity will decrease (Chou *et al*., 2010). The pH of solution become more alkaline with time when microbial is inoculated in samples (Miles & Moy, 1979).

2.10 Optimization of biological pH treatment

Design of experiment (DOE) is a structured method by which certain factors are selected and designedly varied in a controlled manner to obtain their effects on the output of a process, often followed by the analysis of the experimental results (Nath & Das, 2011). It should be predict the important parameters and the relationship between them. The experimental design can be broadly classified into two categories: one-factor-at-a-time design and factorial design (FD) according to the number of the factors to be investigated at a time (Wang & Wan, 2009).

2.10.1 Response Surface Methodology (RSM)

RSM has an important application in the process design and optimization as well as the improvement of existing design (Zinatizadeh *et al*., 2006). Typically, the experimenter attempts to find the optimal setting for the input variables that maximizes (or minimizes) the predicted responses. Otherwise, RSM has been employed extensively in the field of engineering and manufacturing where many parameters are involved in a process. In the last few years, RSM has been applied to optimize and evaluate interactive effects of independent factors in numerous chemical and biochemical processes (Zinatizadeh *et al*., 2006). A common and powerful DOE approach is afforded by FD combined with RSM (Nath & Das, 2011).

2.10.2Factorial Design (FD)

Factorial designs are widely used in experiments and it is able to study the effects of more than one factor at two or more levels. It can be classified into two categories which are full factorial design and fractional factorial design. In a full factorial design, every combination of each factor level is tested. When the number of runs for a full factorial design is relatively large, the desired information can often be obtained by performing only a fraction of the full factorial design, which is often referred to as fractional factorial design to distinguish it from the full factorial design. With a fractional factorial design, the effects of certain factors on a response can be studied under economical and practical conditions (Wang & Wan, 2009). Taguchi design, Plackett-Burman design, central composite design and Box-Behnken design are some types of fractional factorial designs.

Central composite design (CCD) and Box-Behnken design (BBD) are widely used experimental designs for RSM to estimate a second-order polynomial approximation to a response in that region. CCD is a five-level fractional factorial design developed by Box and Wilson. The design usually consists of a 2^n full factorial design, 2 x *n* axial designs and *m* central designs. Whereas, BBD is a three-level fractional factorial design developed by Box and Behnken. The design can be thought of as a combination of a two-level factorial design with an incomplete block design. In each block, a certain number of factors are put through all combinations for factorial design, while other factors are kept at the central levels. It usually includes some central designs.

2.10.3 Design Expert Software

With the aim of maximum response, Y, the optimum condition (within the experimental range) can be obtained by using the numerical optimization in Design Expert Software in any version. Only the factors considered in model building were varied for prediction, other insignificant variables were maintained at constant values ('0' coded level).

The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R^2. The model terms were selected or rejected based on the *p*-value with 95% confidence level. The responses were completely analyzed using analysis of variance (ANOVA). The simultaneous interaction of the independent variables was investigated by constructing the response surface plots and contour plots based on the effects of the levels of the corresponding factors (Chou *et al*., 2010). The optimum values of selected variables were obtained by solving the regression equation and also by analyzing the response surface contour plots (Ahmad *et al*., 2010).

3 MATERIALS AND METHODS

3.1 Flow chart process

During conducting this experiment, there are several methods that need to be considered. In this experiment, POME has being used as substrate whereas soil mixed culture and POME mixture as inoculums. It is started with substrate and inoculums are being collected and prepared for doing some batch tests. The preliminary is conducted first for determining the best condition between the factors that has been selected. After that, the experiment is optimized to be run and data is collected to get the final results. The results are obtained by doing analysis for each test.

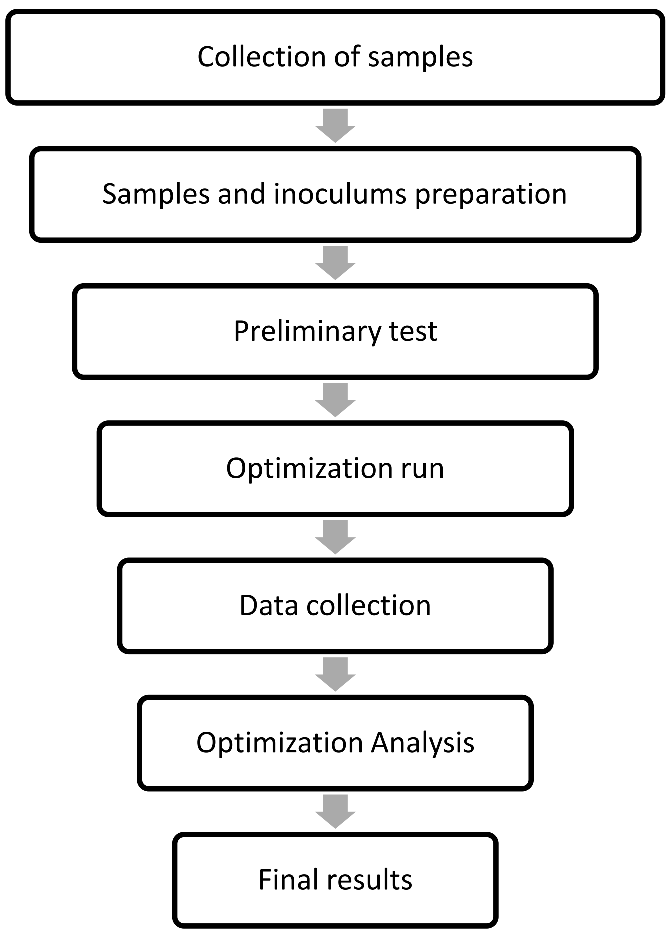

Figure 3.1: Flow chart process of experiment

3.2 Palm oil mill effluent (POME)

POME was obtained from Sri Senggora Kilang Kelapa Sawit Sdn. Bhd. located at Maran, Pahang, Malaysia. The POME was collected in a carboy container then brought to the laboratory and kept in a freezer at 4℃ until it was used to avoid its degradation. The initial pH measured was 4.96.

3.3 Soil mixed culture and preparation of inoculums

Soil mixed culture was taken behind Kolej Kediaman 1, Universiti Malaysia Pahang (UMP) which the bacteria is grow at the area around the root tips. The soil was transported to a laboratory, sealed in bags and being stored at ambient temperature. By referring Miles & Moy (1979), the mixed culture inoculants were obtained by mixing 100 g sandy loam soil with 200 mL distilled water. After allowing 10 seconds for the sand to settle, 20 mL of the supernatant liquid were withdrawn and added to each incubation flask containing the nutrient and substrate, stored at 20℃. In this study, the soil and water are mixed together to give the soil mixed culture solution in the ratio 1:1 (100 mL soil and 100 mL water). Next, the supernatant liquid of soil solution will be added to the substrate which is palm oil mill effluent (POME) to be use as inoculums. The culture was acclimatized with the ratio of 1:3 (50 mL soil solution to 150 mL POME). Regarding Lin *et al*. (2008), an acclimatization process in a biological process will enhances the ability of the microbes to degrade organics. The purpose of acclimatization is to familiarize the culture with the condition of the test and allow for significant increase of microbial population. The culture then was shaken continuously at 150 rpm in the incubation shaker. The acclimatization period is for 10 days at 30℃ before being used in the batch experiment.

3.4 Preliminary study of biological pH treatment

A preliminary study was done to investigate the experimental factors and to narrow the corresponding ranges before the application of statistical design (Chou *et al*., 2010). In this preliminary study, the purpose of doing it is to determine the best reaction time to conduct the experiment with other variables. One test was carried out with 150 mL of POME as a control test. The control test is to confirm that there was no change in pH resulting from degradation of the organic matter in the substrate itself. The other one is a mixture between POME and soil mixed culture solution in the ratio of 1:3 (soil solution to POME). The batch tests were conducted in incubator shaker (Infors HT Ecotron) and the pH was checked for every 24 hours by using pH meter (Mettler Toledo).

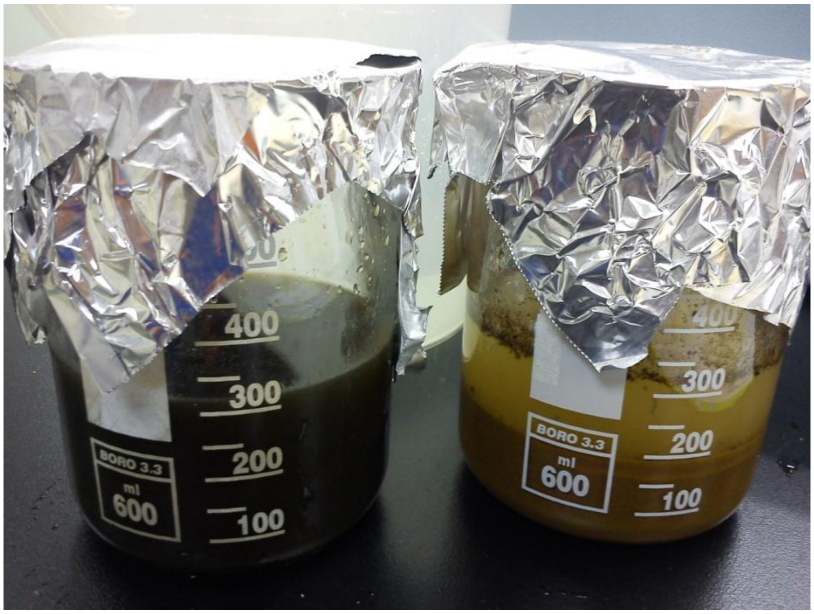

Figure 3.2: Preparation of POME and soil mixed culture solution

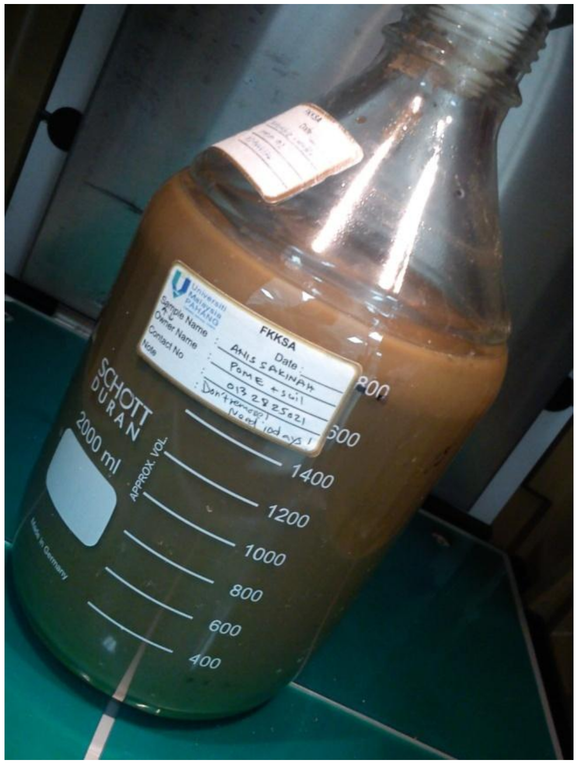

Figure 3.3: Preparations of inoculums

Figure 3.4: The samples is incubated in incubation shaker

3.5 Experimental setup and procedure

The experiment was run under unsterilized batch operation. After the preliminary study, batch tests were conducted in 250 mL conical flasks with working volume to head space of four-fifths (200 mL working volume and 50 mL head space). Next, the solution that contains 50 mL of inoculums was added to 150 mL of POME by using ratio 1 to 3 for inoculums to POME for each conical flask. Then, each conical flask is closed with a gauze and cotton to make them in anaerobic condition. The batch tests were incubated in an incubation shaker (Model Infors HT Ecotron). The incubations of mixed cultures is aims to allow the soil microorganisms to operate concomitantly, by dominating with synergism or antagonism, and to perhaps obtain a real picture of what may occur in the natural environment rather than being not incubated (Miles & Moy, 1979).

3.6 Set-up for optimization process

RSM is a mathematical and statistical technique for building models, evaluating relative significance of several independent variables (i.e., environmental factors), and for determining optimum conditions for desirable responses (Rasdi *et al.*, 2009) was employed in this study. RSM with factorial CCD using the software package Design Expert Version 6.0 was used for pH optimization.

The experiment is being conducted where the agitation speed varied from 100 to 200 rpm with a central value of 150 rpm, the temperature varied from 25 to 35℃ with a central value of 30℃ and the reaction time varied from 7 to 8 days with a central value of 7.5 days. To describe the interactive effects of three variables; agitation speed, reaction time and temperature based on the previous report and their interaction, the batches were subjected to a fractional factorial central composite design as shown in Appendix A.1.

3.7 *Analytical method*

The analysis method only consists of pH analysis. The pH in the solution after experiment was measured using pH meter (Model Mettler Toledo) for every 24 hours until at the end of the experiment.

Figure 3.5: The samples is analyzed by pH meter (POME control and mixed POME and soil)

4 RESULTS AND DISCUSSION

4.1 Preliminary studies

Reaction time controls the microbial growth rate and therefore should constantly be greater than the maximum growth rate of the organism(s) (Nath & Das, 2011). Thus, this preliminary is to determine the best reaction to conduct the experiment with other variables concerned whereas to identify the maximum growth rate of microorganisms. The changes of pH were measured with variable time conducted and presented in Figure 4.1 and Figure 4.2. The experimental results for preliminary studies were shown in Table 4.1. These preliminary tests show us that pH increase earlier than expected. The pH of POME increased rapidly started after the Day 3.

Table 4.1: pH of POME in terms of reaction time

Reaction Time (Day)	pH (initial pH=4.96)	
	1st	2nd
1	5.15	5.14
2	5.16	5.22
3	5.17	5.40
4	7.91	6.94
5	8.26	8.34

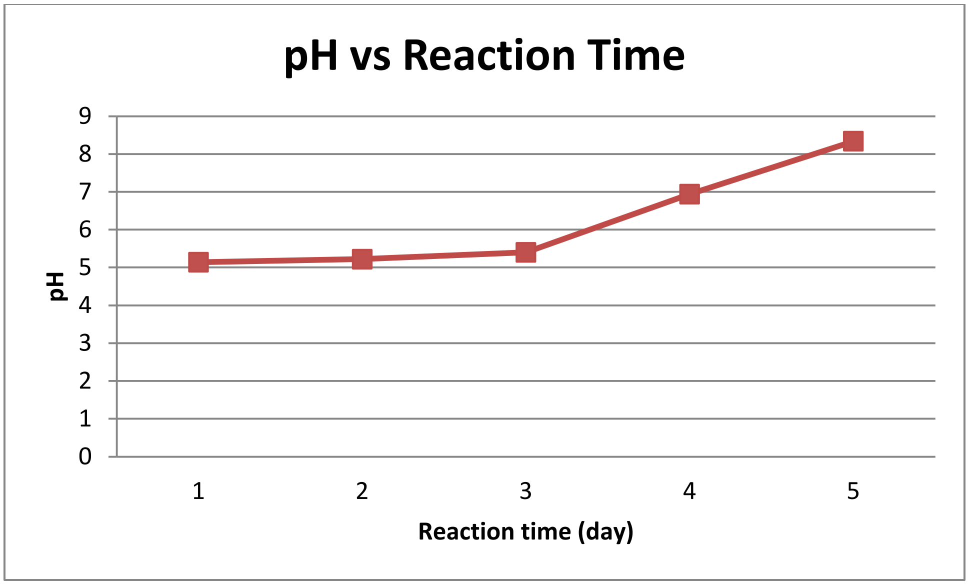

Figure 4.1: pH versus Reaction Time (day) for 1st time

In order to reconfirm the result, another preliminary study was carried out under the fixed condition which is at temperature of 30°C and 150 rpm. The same result was obtained which is at the Day 3; the pH will start to increase rapidly (Table 4.1). Besides, the longer reaction time, the pH increases gradually based on the result.

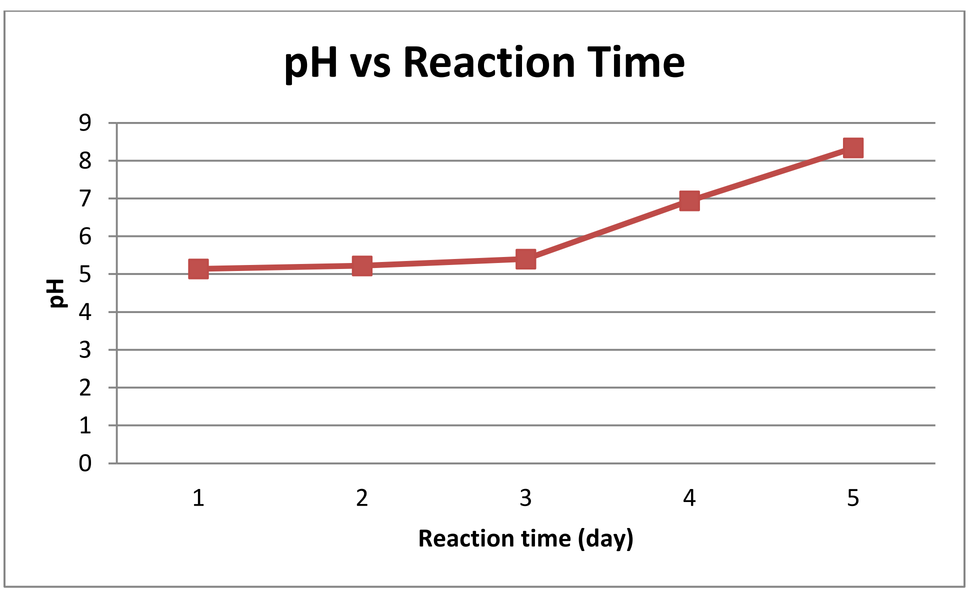

Figure 4.2: pH versus Reaction time (day) for 2nd time

Most of the researchers were done with a reaction time less or equal to 24 h. If it is extended for more than 42 hr, the microbial activity will decrease (Chou *et al*., 2010). It is reported that reaction time affects the microbial community to a certain extent depend on which types of microbial population exists in the process (Badiei *et al*., 2011). Refer to the past results by several researchers tabulated by Wu et al. (2009); a researcher who using *Bacillus thuringiensis* H-14 (Suwandi, 1991) was obtained 3 days for maximum results. Other than that, Mashitah et al., 2002 obtained 45 h and 51 h for using *Trichoderma harzianum* and *Aspergillus niger*, respectively. However, we need to consider mixed culture to ensure the exact comparison with past researchers. By using mixed culture, the maximum of microbial activity obtained by Yee *et al*. (2003) is about 3.5 days. Moreover, Badiei *et al*. (2011) has concluded that the highest microbial activity is achieved in 3 days while in longer and shorter than that will affected on the efficiency of microbial degradation. It is might due to the lower amount of substrate consumed by the bacteria.

Nonetheless, Badiei *et al*. (2011) also has mentioned that the longer reaction time might result in solid accumulation from substrate content (Figure 4.3). It will provide the changes in the microbial population shift and growth which can prevent the culture from effectively utilizing the substrate. Also, pH is started increase rapidly during 3^{rd} days possibly correlates to existing appropriate condition to activate spore formed bacteria (Figure 4.4) and could utilize the substrate more efficiently. Hence, the reaction time is no longer being considered as a factor and the fixed condition for reaction time is 3^{rd} – 4^{th} days in further experiment.

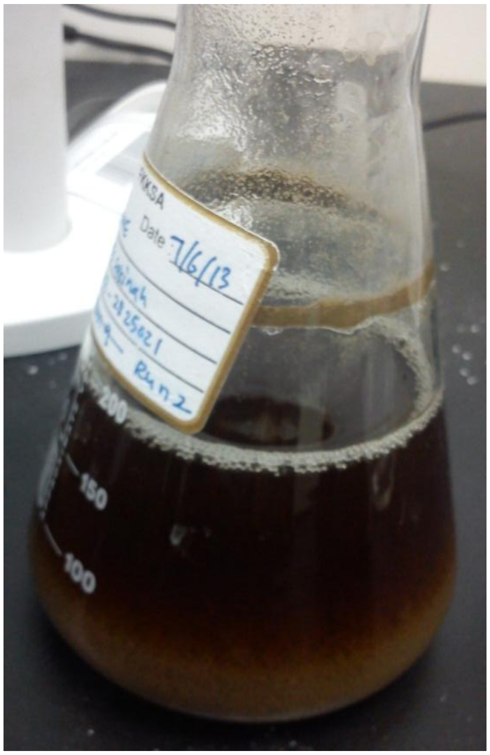

Figure 4.3: Solid accumulation in POME treatment

Figure 4.4: Spore formed bacteria in POME treatment

4.2 *Experimental results*

The optimum levels of key factors and the effect of their interactions on pH treatment via biological process were determined by the CCD. CCD has been used more widely in biological production processes. By considering as there are no reaction time involved as a factor related, again, CCD experiment was designed to optimize the agitation speed and temperature to maximize the response and determined the potential interaction (Appendix A.2).

In this study, the biological process itself occurs in the route of hydrogen production. By comparing with other research, Wang *et al*. (2005) concluded that pH had a similar influence on total volatile fatty acids (VFA), butyrate and hydrogen yields, resulting in an identical optimum pH value for total VFA, butyrate and hydrogen yields. It was reported that the effect of pH is due to the change of ionization state of the components in enzymatic reactions (Jo *et al*., 2007). Based on the above statement, agitation speed and temperature were among the factors that being considered to increase the pH by affecting microbial activity.

The fact is, temperature affects the maximum specific growth, substrate utilization rate and metabolic pathway of microorganisms, resulting in a shift of by-product compositions (Jo *et al*., 2007), then may lead to the pH changes. Also, the agitation speed can enhance the mass transfer between solid wastes as well as increase the decomposition and degradation rate.

4.3 *Optimization for pH treatment using RSM*

The result of second-order response surface model in the form of analysis of variance (ANOVA) for pH treatment is shown in Table 4.2. From the analysis, the reduced quadratic model demonstrates that the model was significant as the *F*-value = 7.53 with a low probability value (P = 0.0097). There is only 0.97% chance that *F*-value could occur due to noise.

Besides, the lack of fit test is designed to determine whether the selected model is adequate to describe the observed data, or whether a more complicated model should be used. If the model does not fit the data well, this value will be significant; if the variances are close to the same, the ratio will be close to one and it is less likely that lack of fit is significant (Chong *et al*., 2009). In this study, the the lack of fit was highly significant (P <0.0001), meaning that the model could not be used to perform predictions and the model does not fit the data well. Although the models cannot be used for prediction, an analysis of factor effects can be made. As stated by Mirhosseini *et al.* (2008), it can be concluded that the factors considered might be incompatible with the ranges. Additionally, Assarzadeh and Ghoreishi (2013) noted that it might be from insignificant factor exists in the model.

The multiple correlation coefficient, R^2 and adjusted R^2 were evaluated. R^2 (0.8433) indicated that the models could fit the responses and only about 15.67% of the total variation could not be explained by this model. Moreover, the adjusted R^2 measures the amount of variation about the mean explained by the model. It is adjusted for number of model parameters relative to the number of points in the design. The adjusted R^2 of 73.13% for pH is attributed to the independent variables which do not differ appreciably from the unadjusted values implied that the models adjust well to the experimental data. In addition, the closer the value of R (0.9183) to 1, the better the correlation between the experimental and predicted values (Feng *et al*., 2010). However, "Pred R-Squared" of -0.4073 was not reasonable agreement with the "Adj R-Squared" of 0.7313.

Furthermore, an adequate precision was used to measure the ratio of signal to noise, which is usually desired to be greater than 4 (Feng *et al*., 2010). In this study, the value of the ratio (7.574) suggested that the polynomial quadratic model was an adequate signal, and could be used to navigate the design space. Other than that, the coefficient of variation % (CV%) is a measure of residual variation of the data relative to the size of the mean. Usually, the higher the value of CV, the lower is the reliability of experiment (Shabbiri *et al*., 2012). In this study, a low coefficient variation (CV = 2.23) showed the reliability of the experiments conducted.

Apart from that, ANOVA analysis showed the significant terms (P<0.05) which comprised of the second order effect of temperature (A^2) and the second order effect of agitation (B^2). Hence, the agitation (P = 0.0010) produce largest effect on pH changes followed by the temperature (P = 0.0071). However, the independent factors, main effects of temperature (A) , agitation (B) and the interative effects of temperature and agitation were insignificant (P > 0.05) indicating that these terms had little impact on pH changes in POME treatment. The mathematical model relating the effects of independent variables of agitation speed and temperature on pH changes is given in the quadratic regression as follows:

$$\boldsymbol{Response\ 1\ (pH) = -27.03466 + 1.59543A + 0.14727B - 0.022379A^2 - (3.23793 \times 10^{-4})B^2 - (1.6 \times 10^{-3})AB} \qquad \textbf{equation (1)}$$

where A and B are coded values of independent variables. Positive sign in front of the terms indicates interactive effect, while negative sign indicates incompatible effect (Ahmad *et al.*, 2010).

Table 4.2: Analysis of variance table (ANOVA) for pH treatment

Source		**Sum of Squares**	**DF**	**Mean Square**	**F Value**	**Prob>F**
Model (significant)		1.19	5	0.24	7.53	0.0097
A		0.012	1	0.012	0.38	0.5573
B		0.034	1	0.034	1.08	0.3339
A^2		0.45	1	0.45	14.14	0.0071
B^2		0.94	1	0.94	29.60	0.0010
AB		0.040	1	0.040	1.26	0.2983
Residual		0.22	7	0.032		
Lack of Fit		0.22	3	0.074	272.60	<0.0001 (significant)
Pure Error		1.080×10^{-3}	4	2.700×10^{-4}		
Cor Total	1.42	12				
R-Squared	0.8433					
Adj R-Squared	0.7313					
Pred R-Squared	-0.4073					
Adeq Precision	7.574					
Std Dev	0.18					
Mean	7.98					
C.V.	2.23					
PRESS	1.99					

4.4 *Interactive effect of the temperature and agitation speed on pH treatment*

The optimum level of each variable and the effect of their interactions on the Response 1 were studied by plotting three dimensional (3D) response surfaces and two dimensional (2D) contour lines (Figure 4.5).

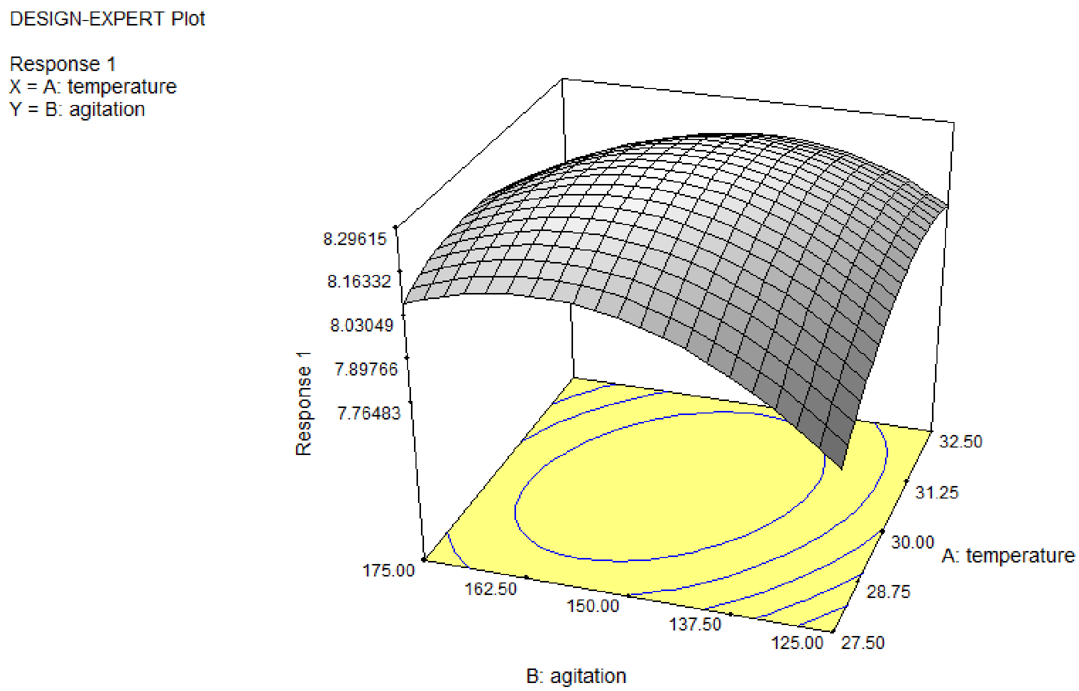

Figure 4.5: The 3D-plot and 2D-projection (contour plot) showing the interaction between temperature and agitation on response

In the Figure 4.5 and 4.6 depicts the effects of temperature and agitation on the response (pH), while the reaction time was fixed at its optimal condition. The optimum conditions for maximizing Response 1 were calculated based on equation (1) as agitation of 150 rpm and temperature of 30℃. Under this condition, the highest of pH obtained lead to the maximum of microbial activity was estimated at 8.3. It can be clearly seen in the Figure 4.5. Figure 4.5 illustrates the three-dimensional (3D) response surfaces based on equation (1), by making two variables within the experimental range. The response surface of Response 1 (pH) displayed a clear optimum point which fell inside of the boundary range. From the examination of contour plot, there was a slight elongation sloping downward. pH increased with increasing agitation and temperature to optimum condition, then decreased with a further increase. This result shows that agitation and temperature had an individual significant influence on pH.

In the Yuan *et al*. (2011) has been observed that significant microbial activity increase when temperature was raised from 10 to 30℃. Lowering operational temperature usually leads to a decrease in the maximum growth of microbial activity (Mu *et al*., 2006). Based on the Figure 4.2, the pH is increases during the temperature between 27.50 to 30.00 ℃ and started decreases further increasing of temperature. The final pH from Wang and Wan (2008) study was starting to decrease sharply at 30 to 35 ℃. One possible reason for this may be that mixed culture used was responsible for fermentation, has a high conversion rate of carbohydrate to products and metabolites, and the high concentrations of metabolites may cause the pH to drop. Besides, the results obtained from Jamil *et al*. (2009) indicated that the microbial activity increases with gradually increasing value of agitation rate which is in adequate mixing condition. Agitation speed significantly affected the optimal pH condition (Chou *et al*., 2008). The purpose of mixing is to facilitate the distribution of the cells within the culture; aiding the homogenous exposure of the microorganisms to the light and substrate.

Otherwise, the 2D presented a clear elongated running diagonally on plot, suggesting that agitation and temperature was interdependent, or that there was a significant interaction on pH treatment. It can be further explained through Figure 4.6.

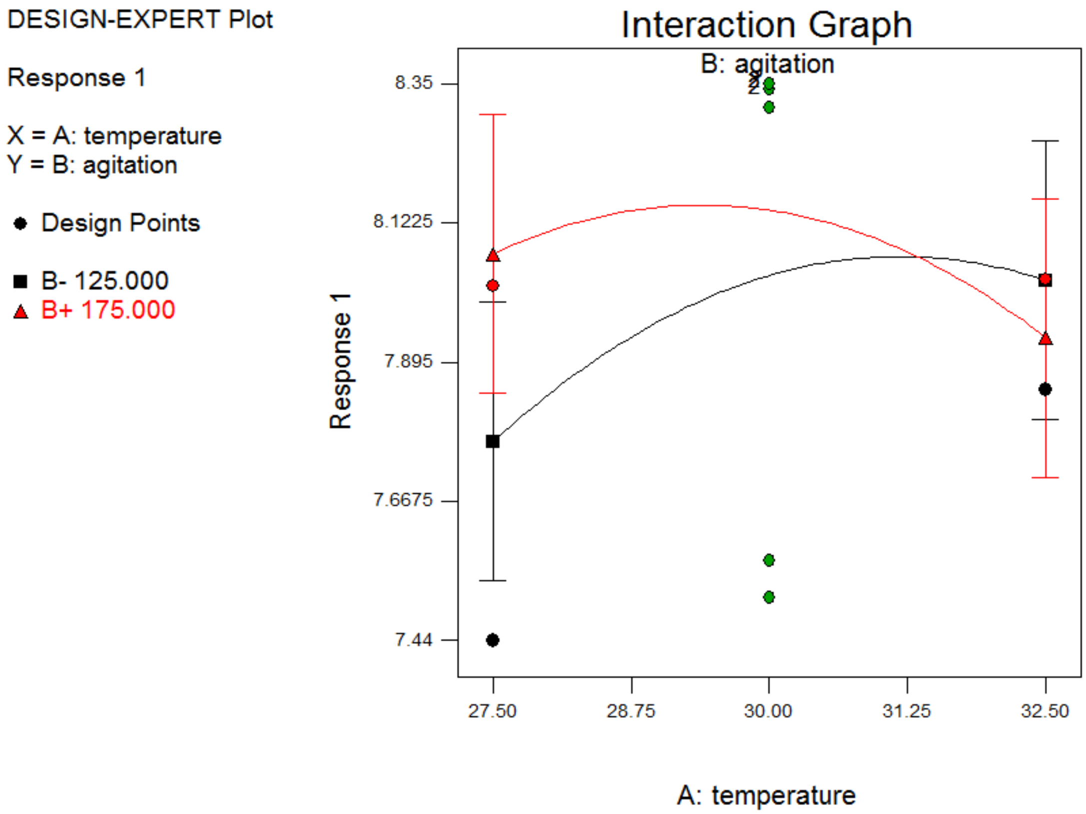

Figure 4.6: Interaction graph between the factors (agitation and temperature) and response

Figure 4.6 shows the evidence that pH will increase in the range between 7.67 to 8.35 upon increasing of agitation between 125 to 175 rpm and temperature between 27.50 to 32.50 ℃ to optimum conditions, but declined with further increases of theses factors. The pH will keep increasing at 125 rpm as temperature increase but decrease a bit in the range between 31.25 to 32.50 ℃. However, the optimum value of pH can be obtained by running the agitation at 175 rpm on the range of temperature in between 28.75 to 30.00 ℃, then decrease pH value as increasing the temperature. These pointed that pH and temperature were interdependent according to the Mu *et al*. (2006) study and slightly interdependent (Jo *et al*., 2007) and their interactive effects were insignificant while agitation speed has individual influence factors proved by Jamil *et al*. (2009).

Yuan *et al*., 2011 made their hypothesis that higher temperature and more intense mixing (or increased contact opportunity) are necessary for completion of the biological process. Whereas the lower value of agitation speed (125 rpm) was having a lower value of final pH at first but increase pH value at high temperature compared to the agitation speed of 175 rpm. Lack of mixing drastically reduced the amount of product generated due to the less microbial activity, particularly at low temperatures (Yuan *et al*., 2011). It is may due to the unsuitable agitation speed during the stages of digestion. When agitation is inefficient, overall rate of process will be affected the medium at different stages of digestion whereby every stage has a different pH and temperature (Poh & Chong, 2009).

4.5 Optimum conditions of temperature and agitation speed

The targeted pH for the experimental studies is 7.5. Within the temperature between 27.5 and 32.5 and also, agitation of 125 to 175, the pH was obtained in the range between 7.44 and 8.35. Refer to the Table 4.4, the optimum condition was related with the desirability among them. It is found to be temperature of 32.5 ℃ and agitation of 125 rpm was chosen and suggested by software. The expected pH value of 8.03 may obtained. Many operators prefer to operate in mesophilic temperature due to better process stability (Poh & Chong, 2009). It may contributed to the low cost of pH treatment because less energy is required.

Ingesson *et al*. (2001) stated that adequate mixing is required to ensure sufficient contact between the substrate and microbes and to promote heat and mass transfer while excessive mixing can deactivate the microbes and reduce the conversion yields. Their result has showed that the high speed shaking produced the highest initial rate and final conversion yield. It could be explained by the greater amount of bound microbe during the first few hours of the reaction under fast shaking conditions rather than low speed shaking regime. To compare between 125 and 175 rpm, it was determined that in between their range, 125 rpm having higher value of pH since it might increase microbial activity rapidly and having less reaction time to obtain targeted pH value. Meanwhile, Poh and Chong (2009) had stated that the optimum pH for microbial growth is between 6.8 and 7.2 while pH lower than 4 and higher than 9.5 are not tolerable. It is because the microbial community had their specific sensitivity on pH changes and generally on that ranges. Thus, their activity will decrease when pH is deviates from the optimum value. The maximum response value for pH was estimated as 8.03; are close enough to the targeted pH. Thus, these conditions are quite enough to the third solution number in Table 4.3. was chosen.

Table 4.3: The optimum condition of agitation and temperature on optimum pH from software

Solutions number	Temperature (℃)	Agitation (rpm)	Response	Desirability
1	27.50	125.00	7.76483	0.688
2	32.50	175.00	7.93483	0.488
3	32.50	125.00	8.02824	0.379
4	27.50	175.00	8.07149	0.328
5	27.54	175.00	8.07449	0.324

4.6 Validation of the optimal condition of the experiment

Another experiment was carried out to validate the optimal condition identified by RSM. Referred to Table 4.4, data was tabulated from the validation run where the experimental error can be calculated as shown in equation (2). The calculation was shown in A.3. The experimental response variables were very close to those predicted by RSM, indicating that RSM was a useful tool to optimize the pH treatment. Besides, the error generated was 2.293% indicated that the data was being acceptable in point of engineering discipline (Assarzadeh & Ghoreishi, 2013) since the result does not excess 10%. Therefore, these optimal conditions value can be accepted.

$$(\%)error = \frac{|predicted - experimental|}{experimental} \times 100$$ **equation (2)**

(Wu *et al.*, 1995)

Table 4.4: Experimental design for validating the optimal condition identified by RSM

	Temperature	Agitation Speed	Response 1 (pH)
Experimental	32.50	125.00	7.85
Predicted	32.50	125.00	8.03

4.7 Comparison with the other researchers

The comparison of the optimal condition in this study with other previous studies can be refer to the Table 4.5. However, the differences between these studies might be from types of culture used. For instance, Chong *et al*. (2009) were used *Clostridium butyricum EB6* as their microbe and suggesting at the temperature of 36 ℃, the pH for maximum growth of microbes was estimated at 6.05 . The result is proven by Wang *et al*. (2005) where the optimum condition occurs at temperature of 35.1℃ and pH of 5.5. It is due to the sludge possessed a high microbes in optimum conditions. In other study by Jo *et al*. (2007) established that the temperature at 38 ℃ greatly used for indicating pH at 6.13 by using *Enterobacter aerogenes*. The change of metabolic pathway from acids production to non-acids production is the characteristics of genus *Enterobacter* if the initial condition did not inhibit bacterial growth or residence.

Besides, Lira *et al*. (2010) predicted that the microbial activity at maximum of pH at 4.8 was affected by the temperature of 41 ℃ using thermophilic *bacillus sp*. It was found out that the maximum activity of Bacillus sp. was identified with temperature values lower than 53℃ and pH lower than 6.0. Otherwise, the minimum and maximum growth temperature of *Bacillus sp.* studied was 30 and 65℃. Nonetheless, the optimum condition in this study were very similar with Wang and Wan (2008) as using mixed culture in the experiment. The changes in pH with increasing temperature may result from the metabolic pathway shift induced by the different bacteria that were dominant at each temperature (Wang & Wan, 2008).

Otherwise, Chou *et al*. (2008) had described that their optimum condition for agitation speed was 120 rpm for pH of 6.0. It was achieving by using laminar and turbulent flows of low speed and high speed, respectively. This result indicated that the microbes could shift their metabolisms when agitation speed was changed from laminar flow to turbulent flow. But, our study did not change its speed from lower to higher speeds. Ingesson *et al*. (2001) also has same opinion, where intermittent regime that consists of low and high speed shaking intervals will be efficient to increase microbial growth nearly as continuous shaking. Besides, they also noted that at excessively high mixing speeds (>200 rpm) could lower the substrate conversion, and higher mixing speeds (340-510 rpm) will only increased the rate of reaction and not the final conversion yield while moderate mixing speeds (100-200 rpm) provide a good combination of initial microbial rates and high conversion yields. But mostly on this comparison, they did not consider agitation speed as their factor of increasing microbial activity in order to enhance pH value. After all, we could not overrule their effect similar to this study due to the difference in agitation effect and types of culture used. Therefore, this study contributed much on study the interaction between agitation speed and temperature towards pH treatment.

Table 4.5: Comparison of the optimal condition for pH obtained in this study and those in other studies

Range examined		Response Variable (optimum pH)	Optimal condition		References
Agitation speed (rpm)	Temperature (°C)		Agitation speed (rpm)	Temperature (°C)	
125-175	27.5-32.5	8.03	125	32.50	This study
20-150	40	6.0	120	40	Chou *et al*. (2008)
150 strokes/min	31.6-48.4	7.2	150 strokes/min	38.6	Wang & Wan (2008)
120	25-40	5.5	120	35.1	Wang *et al*. (2005)
200	32-42	6.05	200	36	Chong *et al*. (2009)
150	33-43	5.5	150	38	Jo *et al*. (2007)
-	30-70	4.8	-	41	Lira *et al*. (2010)

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusions

This study was designed to optimize the factors that affecting an increasing pH of acidic POME. In conclusion, the objective of this experiment was achieved. In this study, soil mixed culture was used to increase the pH of acidic POME meanwhile optimization on factors based on research surface methodology (RSM) with factorial central composite design (CCD) using the software package Design Expert Version 6.0.

The most significant finding in this study is to optimize the condition for each factor considered which are agitation speed, temperature and reaction time. However, preliminary studies were conducted to determine the best reaction that affected maximum growth of microorganisms. Hence, the reaction time was fixed in 3^{rd} to 4^{th} days before conducting the experiment.

Besides, the experiment was conducted to determine the optimum condition between agitation speed and temperature showed that pH will increase upon increasing of agitation and temperature between to optimum conditions, but declined with further increases of these factors. From these optimum condition, it can be concluded that agitation and temperature had an individual significant influence on pH. Yet, the agitation and temperature was interdependent or having significant interaction on pH treatment while pH and temperature were interdependent according to the study and slightly interdependent and their interactive effects were insignificant. Based on the results, the predicted optimum condition suggested by software is temperature of 32.50℃ and agitation speed of 125 rpm. The expected pH value of 8.03 may obtained. Thus, the experimental response variables were very close to those predicted by RSM, indicating that RSM was a useful tool to optimize the pH treatment. Besides, the error generated was 2.293% indicated that the data being acceptable in point of engineering discipline since the result does not excess 10%.

Based on the comparison with other researcher, it can be concluded that the results might differ from the type of cultures used with their suitability of temperature and agitation effect used. Therefore, this study contributed much on study the interaction between agitation speed and temperature towards pH treatment.

5.2 Recommendations

Generally, the purpose of optimization is to find an alternative with the most cost effective or highest achievable performance under the given constraints, by maximizing desired factors and minimizing undesired ones. It can be recommended that ones can improve the model by adjusting model fitting capabilities. To improve model fiiting capabilities, it has been suggested by Assarzadeh and Ghoreishi (2013) to eliminate insignificant terms by backward elimination procedure and then repeating ANOVA to test for the lack of fit of the new reduced polynomial regression equation.

Therefore, it can be recommended that these results may be useful in considering agitation speed and temperature to treat the POME in terms of pH and can be applied in industrial application. For instance, the popular method applied nowadays is ponding system because of its low price, low maintenance costs and simple to construct. In the ponding system, the mixer can be installed in the earlier pond which is anaerobic pond to control the pH of POME by using suitable temperature operated . In other method, the POME can be treated by using anaerobic suspended growth processes and various of anaerobic digesters such as open steel steel tank digesters, enclosed steel tank digesters, and anaerobic contact digesters where mixing and temperature can be applied.

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APPENDIX

Table A.1: CCD for response surface analysis of 3 factors influencing pH treatment of POME

Run	Factor 1 A: Temperature	Factor 2 B: Time	Factor 3 C: Agitation Speed
1	25.00	7.50	150.00
2	30.00	7.50	200.00
3	27.50	7.75	125.00
4	35.00	7.50	150.00
5	30.00	7.50	150.00
6	32.50	7.75	175.00
7	30.00	7.50	150.00
8	30.00	7.50	150.00
9	30.00	8.00	150.00
10	30.00	7.00	150.00
11	30.00	7.50	100.00
12	30.00	7.50	150.00
13	27.50	7.25	125.00
14	32.50	7.75	125.00
15	27.50	7.75	175.00
16	27.50	7.25	175.00
17	30.00	7.50	150.00
18	32.50	7.25	125.00
19	30.00	7.50	150.00
20	32.50	7.25	175.00

Table A.2: CCD for response surface analysis of 2 factors influencing pH treatment of POME

Run	Factor 1 (A: Temperature)	Factor 2 (B: Agitation Speed)	Response 1
1	35.00	150.00	7.78
2	30.00	150.00	8.35
3	30.00	100.00	7.51
4	30.00	150.00	8.34
5	30.00	150.00	8.31
6	32.50	125.00	7.85
7	30.00	100.00	7.57
8	30.00	150.00	8.34
9	27.50	125.00	7.44
10	25.00	150.00	7.80
11	30.00	150.00	8.35
12	27.50	175.00	8.02
13	32.50	175.00	8.03

A.3: Calculations to check the accuracy of the model

From data in Table 4.4:

$$\frac{|8.03 - 7.85|}{7.85} \times 100 = 2.293\%$$