BIOLOGICAL TREATMENT OF ACIDIC PALM OIL MILL EFFLUENT (POME) BY USING SOIL MIXED CULTURE (SMC)

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BIOLOGICAL TREATMENT OF ACIDIC PALM OIL MILL EFFLUENT (POME) BY USING SOIL MIXED CULTURE (SMC)TITLE

SITI MAZLIFAH BINTI ISMAIL

Thesis submitted in fulfillment of the requirements
for the award of the degree of
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DEDICATION

This work is dedicated to:

My parents and parent in law,

who have been supporting me,

my siblings,

who always make my day,

my supervisor,

who I adore so much,

my friends,

who have helped me in many ways and,

my husband and daughter,

who has always been there when I needed,

Thank you for everything.

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In the name of Allah, the most gracious and merciful;

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ABSTRAK

Kilang minyak sawit menghasilkan hampir 50 juta tan sisa kilang minyak sawit (POME) berasid setiap tahun. Walaupun sumbangannya terhadap pertumbuhan ekonomi, ia juga menghasilkan sejumlah besar air kumbahan yang perlu dirawat. Peningkatan minat terhadap rawatan POME berasid menarik perhatian ramai penyelidik terutamanya dalam proses rawatan secara biologikal. Kajian ini memberi tumpuan kepada rawatan biologikal POME berasid dengan menggunakan kultur tanah campuran (SMC). Dalam kajian ini SMC telah diaklimitasi selama 10 hari (30 °C dan 150 rpm) dengan POME berasid dan digunakan sebagai inokulum. Terdapat tiga objektif dalam kajian ini. Objektif pertama adalah menentukan ciri POME berasid dan tanah. Pencirian POME berasid melibatkan penentuan nilai pH, suhu, keperluan oksigen biokimia (BOD), keperluan oksigen kimia (COD), jumlah pepejal terampai (TSS), jumlah pepejal (TS), minyak dan gris, dan nitrogen ammonia. Untuk tanah, pencirian melibatkan penentuan pH, tekstur, kandungan kelembapan, kekonduksian, kandungan nitrogen, kandungan karbon organik, fosforus yang ada dan kapasiti pertukaran kation. Objektif kedua adalah untuk menganalisis faktor yang mempengaruhi rawatan biologikal POME berasid. Terdapat lima faktor dipilih untuk analisis faktor. Faktor-faktor tersebut adalah masa tindak balas, suhu, kelajuan pengadukkan, nisbah tanah kepada air dan jenis tanah. Perisian Design Expert (Versi 6.0) telah digunakan untuk rekabentuk eksperimen. Ujikaji faktorial dua peringkat digunakan untuk menganalisis faktor. Analisis varians (ANOVA) membuktikan kestabilan model ini dengan nilai koefisien penentuan (R^2) pada 0.8301 (rawatan pH), 0.8239 (pengurangan BOD) dan 0.9397 (pengurangan COD). Masa tindak balas memberikan sumbangan tertinggi dalam rawatan pH (29.84%), pengurangan BOD (58.49%) dan pengurangan COD (38.64%). Ia kemudian diikuti dengan kelajuan pengadukkan pada 9.29% (rawatan pH), 7.54% (pengurangan BOD) dan 14.90% (pengurangan COD). Kesan interaksi antara masa tindak balas dan kelajuan pengadukkan memberikan sumbangan tertinggi iaitu 17.21% (rawatan pH), 16.65% (pengurangan BOD) dan 5.54% (pengurangan COD). Objektif ketiga ialah untuk mengoptimumkan rawatan biologikal POME berasid. Bagi proses pengoptimuman, rekabentuk eksperimen dibina dengan menggunakan rekabentuk komposit berpusat (CCD). Terdapat dua faktor yang telah dipilih daripada analisis faktoran iaitu masa tindak balas dan kelajuan pengadukkan. Dari pengoptimuman, ANOVA menunjukkan nilai R^2 pada 0.8326 (rawatan pH), 0.8991 (pengurangan BOD) dan 0.8278 (pengurangan COD). Ia membuktikan bahawa model ini sesuai untuk regresi. Keadaan optimum yang dicadangkan semasa proses pengoptimuman disahkan dengan menjalankan ekperimen pada masa tindak balas pada 5 hari dan kelajuan pengadukan pada 150 rpm Hasil yang didapati menyimpulkan nilai pH optimum (8.14), pengurangan BOD (99.16%) dan pengurangan COD (81.69%) diperolehi dari keadaan yang dicadangkan. Oleh itu, aplikasi perisian Pakar Reka bentuk yang mampu mendapatkan keadaan optimum. Pencirian telah dilakukan untuk memahami sifat substrat dan inokulum untuk meningkatkan proses rawatan. Hasil dari pemeriksaan dan pengoptimuman menunjukkan bahawa penggunaan SMC adalah sesuai untuk rawatan POME berasid.

ABSTRACT

Palm oil mill generates at about 50 million tons of acidic palm oil mill effluent (POME) annually. Despite of its contribution towards the economic growth, it also produces large amount of wastewater that need to be treated. The increasing interest in acidic POME treatment attract the attention of many researchers especially in biological treatment process. This study focused on biological treatment of acidic POME by using soil mixed culture (SMC). In this study SMC was acclimatized for 10 days (30°C and 150 rpm) with acidic POME and used as inoculum. There were three objectives in this study. The first objective was to characterize the acidic POME and soil. The characterization of acidic POME involves the determination of pH value, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total solid (TS), oil and grease, and ammoniacal nitrogen. For the soil, the characterization involves the determination of pH, texture, moisture content, conductivity, nitrogen content, organic carbon content, available phosphorus and cation-exchange capacity. The second objective was to analyze factors affecting biological treatment of acidic POME. There were five factors selected for factorial analysis. The factors were reaction time, temperature, agitation speed, soil to water ratio and soil types. Design Expert software (Version 6.0) was used for the experimental design. Two-level factorial design was applied for the factorial analysis. The analysis of variance (ANOVA) proved the stability of this model with the coefficient of determination (R^2) value at 0.8301 (pH treatment), 0.8239 (BOD removal) and 0.9397 (COD removal). Reaction time gave the highest contribution in pH treatment (29.84%), BOD removal (58.49%) and COD removal (38.64%). It then followed by agitation speed at 9.29% (pH treatment), 7.54% (BOD removal) and 14.90% (COD removal). The interaction effect between reaction time and agitation speed gave highest contribution which was at 17.21% (pH treatment), 16.65% (BOD removal) and 5.54% (COD removal). The third objective was to optimize the biological treatment of acidic POME. The experimental table for the optimization was constructed by using central composite design (CCD). There were two factors chosen from the factorial analysis which were reaction time and agitation speed. From the optimization, the ANOVA showed R^2 value was 0.8326 (pH treatment), 0.8991 (BOD removal) and 0.8278 (COD removal). It proved that the model was fit for regression. The suggested optimum conditions that obtained during optimization were validated by using validation experiment at reaction time of 5 days and agitation speed of 150 rpm. The result that was obtained concluded that the optimum pH value (8.14), BOD removal (99.16 %) and COD removal (81.69 %), was obtained at the suggested conditions. Therefore, the applications of Design Expert software capable in obtaining the optimum conditions. Characterization was done to understanding the substrate and inoculum properties in order to improve the treatment process. The results from the screening and optimization showed that the used of SMC was a suitable for acidic POME treatment.

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α	Alpha
°C	Degree celcius
%	Percent

UMP

LIST OF ABBREVIATIONS

3D	Three-Dimensional
ANOVA	Analysis of Variance
AS	Alluvium Soil
BOD	Biochemical Oxygen Demand
C	Carbon
CCD	Central Composite Design
CEC	Cation Exchange Capacity
cmol/kg	Centimoles per kilogram
COD	Chemical Oxygen Demand
CPO	Crude Palm Oil
CPOK	Crude Palm Oil Kernel
CSTR	Continuous Stirred Tank Reactor
DS	Dissolved Solid
EFB	Empty Fruit Bunch
FFA	Free Fatty Acid
FFB	Fresh Fruit Bunch
IAAB	Integrated Anaerobic-Aerobic Bioreactor
MC	Moisture Content
mg/L	Miligram per Liter
MRE	Mixed Raw Effluent
N	Nitrogen
POME	Palm Oil Mill Effluent
ppm	Part per Million
PS	Peat Soil
rpm	Rotate per Minute
RSM	Response Surface Method
s/w	Soil to Water
SMC	Soil Mixed Cuture
SS	Suspended Solid
TFD	Two-Level Factorial Design
TN	Total Nitrogen
TSS	Total Suspended Solid
UASB	Up-flow Anaerobic Sludge Blanket
UASFF	Up-flow Anaerobic Sludge Fixed-Film

CHAPTER 1

INTRODUCTION

1.1 Background

The palm oil industry is one of the major agro-industries in Malaysia that contributes towards economic development in Malaysia. The production of palm oil results in the generation of large quantities of polluted wastewater commonly referred to as palm oil mill effluent (POME). POME is generated from three major sources which are sterilizer condensate, hydrocyclone waste and separator sludge (Borja and Banks, 1994). Oil palm cultivation and processing like other agricultural and industrial activities, raises environmental issues such as water pollution and air pollution.

It is a mandatory requirement for all palm oil mills to treat their waste water on site to an acceptable level before it is allowed to be discharged into the water courses (Ma, 1999). Over the past decades, several cost-effective treatment technologies comprising anaerobic, aerobic and facultative processes have been developed for the treatment of acidic POME. More than 85% of palm oil mills use solely ponding systems due to their low costs (Yeoh, 2004).

Biological treatment processes, in an effort to minimize cost, utilize microbial communities of varying degrees of diversity that interact in a multitude of ways to mediate a myriad of biological reactions (Wise, 1987). The high organic content, mainly oil and fatty acids, enables POME to support bacterial growth that reduces its polluting strength. The used of mixed culture provides several advantages compared to pure culture. The mixed culture can better adapt for changing conditions during growth (Nor Habibah, 2006).

The main focus of this research is to reduce the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) as well as to increase the pH value of acidic POME by using soil mixed culture. The stabilization or organic matter accomplished biologically using a variety of microorganism. The microorganisms convert the colloidal and dissolved carbonaceous organic matter into various gases and protoplasm. Protoplasm need to be removed as it measured as BOD in the effluent.

1.2 Problem Statement

One of the liquid wastes and by-products that are produced from palm oil mill is acidic palm oil mill effluent (POME). The production of highly polluting acidic POME has resulted in serious environmental hazards. Acidic POME is a highly polluting wastewater that pollutes the environment if discharged directly into rivers without a proper treatment (Mohammed and Chong, 2014). This pollutant is due to high chemical oxygen demand (COD), biochemical oxygen demand (BOD), phenol, and color concentrations that exist in acidic POME. Thus, the treatment of acidic POME has gained much interest from other researchers due to the wastes generated in the mills. This treatment is an important issue in minimization the water pollution issue.

Many technologies have been studied and applied for treating acidic POME such as biological digestion (Chotwattanasak and Puetpaiboon, 2011), coagulation and flocculation (Saifuddin and Dinara, 2011) and membrane technology (Ahmad et al., 2005). However, these treatments have advantages and disadvantages. For example, the membrane technology has the high removal efficiency in COD but the treatment is very costly. In order to overcome the time constrains and costing issue, a biological treatment studied is being raised up.

In wastewater treatment, soil can acts as a filter, exchanger and absorber. Microbes that exist in soil help to degrade the organic matter in the wastewater and increasing the wastewater treatment capacity. Application of soil in biological treatment have low environmental impact and less cost compared to the chemical and physical treatment. Thus, a biological treatment using soil mixed culture was selected in treating acidic POME. Optimization and screening the significant parameters in the biological acidic POME treatment using the classical method is very challenging. It involves the changing of one variable at a time while fixing all other variables at one level and

studying the effect of the variable on the response. This is time-consuming, expensive and complicated process for a multi-variable system. To overcome this difficulty, response surface method (RSM) was applied in the experimental design.

In this research, biological treatment using soil mixed culture was used to increase the pH value and reduce BOD and COD of the acidic POME. RSM was used to screen and optimize the selected variables and the Design Expert software (Version 6.0) was applied in analyzing the experimental results..

1.3 Objective

The objectives of this research are:

1. To characterize the acidic palm oil mill effluent and soil content.
2. To analyze the factor that effecting biological treatment of acidic palm oil mill effluent.
3. To optimize the process of biological treatment of acidic palm oil mill effluent.

1.4 Scopes of Study

In order to achieve the objectives of the research, the following actions were carried out:

i. To characterize the properties of acidic POME before and soils used in biological acidic POME treatment. The characterization of acidic POME involves the determination of pH value, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total solid (TS), oil and grease, and ammoniacal nitrogen. The characterization of soil involves the determination of pH, texture, moisture content, conductivity, nitrogen content, organic carbon content, available phosphorus and cation-exchange capacity.

ii. To conduct biological treatment of acidic POME using soil mixed culture.

iii. To utilize the response surface method (RSM) in factorial analysis and optimization process on biological acidic POME treatment.

iv. To screen and analyze the following factors effecting biological POME treatment by using two-level factorial design.

   a. Reaction time

   b. Temperature

   c. Agitation speed

   d. Soil to water ratio

   e. Types of soil

v. To optimize the factors affecting biological POME treatment by using central composite design (CCD) and conduct experimental validation at suggested optimum condition.

1.5 Thesis Overview

This thesis consists of five chapters. A brief introduction about the development of palm oil industry in Malaysia, environmental regulations and current POME treatment systems were given in Chapter 1 (Introduction). This chapter also includes problem statements that gave some basis and directions to be followed in this study. Then, the specific objectives of the present study were elaborated together with the scopes of the study to be covered.

Chapter 2 (Literature review) explains the palm oil mill processing for producing POME from crude palm oil (CPO), the characteristics of POME, and the Enactment of Environmental Quality Act for discharging POME. It also reviews about the biological method involved in the treatment process; inoculums used and factor that affecting biological POME treatment. In this chapter it also explains and discusses about screening and optimization using RSM.

Chapter 3 (Methodology) presents the detail of the materials and chemicals used in the present study. Then, the overall experimental flowchart is presented. Detail of the

experimental set-up is then elaborated in this chapter. This followed by the detail experimental procedures, experimental design table and experimental data analysis method.

Chapter 4 (Results and discussion) which is the main part of this thesis is outlined by three main studies. In first section, characteristics of acidic POME and soil content are analyzed in detail. Then, screening the factors affecting biological treatment of POME is discussed in the second section. Optimization of biological POME treatment is discussed in third section. Detail information on the factors affecting biological POME treatment are also studied and presented in last two sections of this chapter.

Chapter 5 (Conclusions) concludes the findings from the current studies as well as recommendations for future studies in the related field made from the understanding and information generated in the present study. These recommendations are given due to their significance and importance to be further investigated and explored by future research work in this area.

CHAPTER 2

LITERATURE REVIEW

2.1 Palm Oil Mill Processing

Palm oil processing is carried out in palm oil mills, where oil is extracted from matured mesocarp of the oil palm fruits. There are two types of oil produced from oil palm which is crude palm oil (CPO) and crude palm oil kernel (CPOK). CPO produced from the fibrous mesocarp while CPOK produced from the palm oil kernel. The oil differs in terms of their chemical composition and nutritional content. Palm oil has a balanced ratio of saturated and unsaturated fatty acids while palm kernel oil has mainly saturated fatty acids.

Despite of its contribution towards economic development to the country, the palm oil industry also generates large amounts of waste. According to Azhari et al., (2010), the palm oil wastes is categorized in the form of empty fruit bunch (EFB) (23%), mesocarp fiber (12%), shell (5%), and palm oil mill effluent (POME) (60%) for every ton of fresh fruit bunch (FFB) processed. The most common palm oil extracting processing in Malaysia from FFB is the wet process (Ahmed et al., 2015). Enormous volumes of water and steam are necessary for removing the dirt and sterilizing in palm oil extracting process.

Figure 2.1 shows a flow diagram in palm oil extraction process. From Figure 2.1, the FFB are generally dropped onto a ramp and brought to sterilizer cages. The FFB must be handled properly so that the fruit bunches are not damaged. The damaged of palm oil fruits give results to poor-quality palm oil due to the growing of free fatty acid (FFA) content in the palm oil fruits. After loading into the sterilizer cages, the FFB is exposed to the steam-heat in horizontal sterilizers for 75 to 90 minutes (140°C, 293.84kPa). This step prevents the formation of free fatty acids by the action of

enzymes. It also minimizes the kernel breakage during the pressing and nut cracking process. The sterilized fruits then are fed into a stripper where the fruits are separated from the bunch stalks. When the stripper rotates, the fruit bunches are lifted up and then dropped constantly along the stripper.

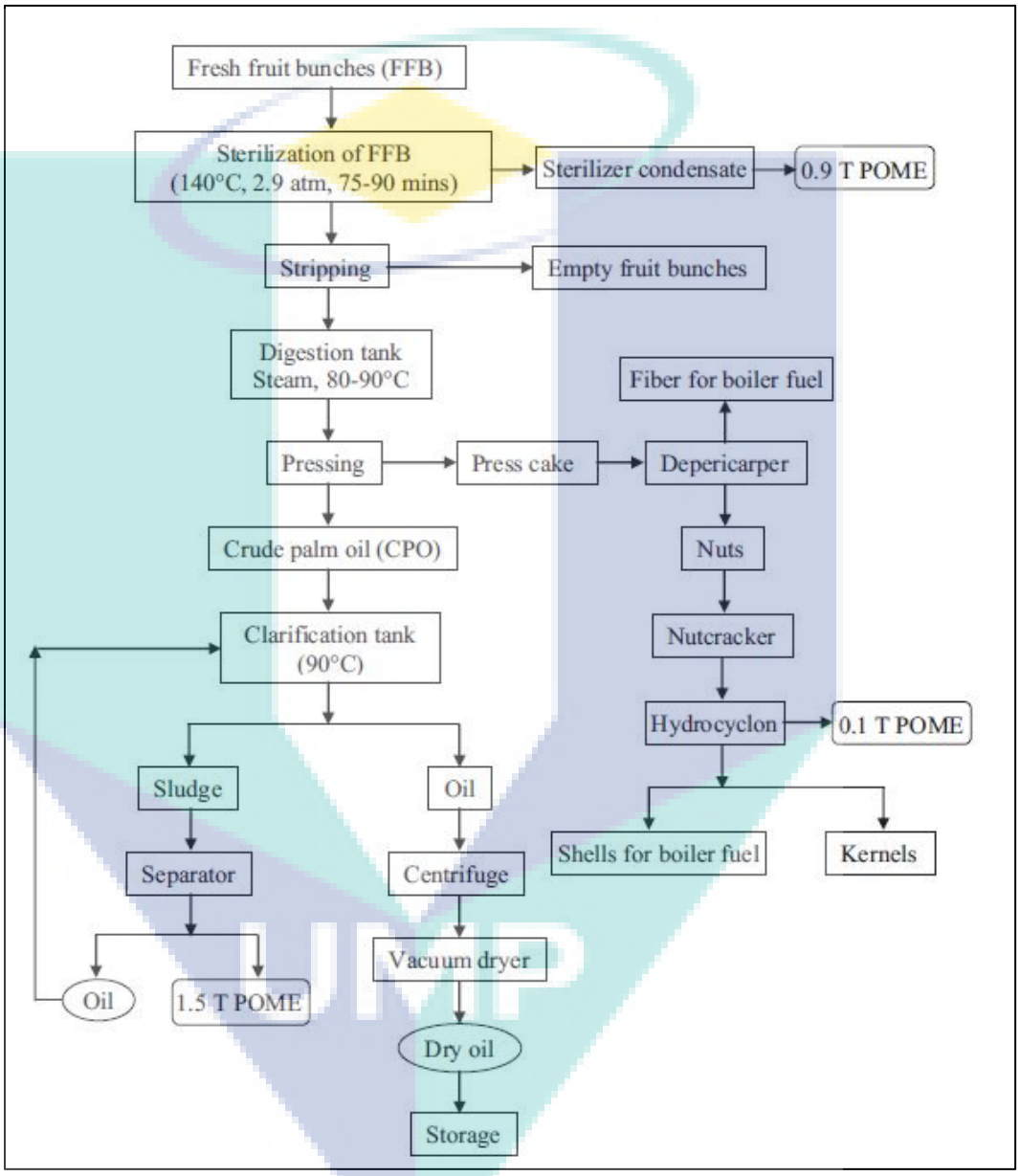

Figure 2.1 Flow diagram for palm oil extraction

Source: Ahmed et al., (2015)

The separated fruits then are passed into a digester followed by a screen and a bucket conveyor. The fruits then are softened and mashed under steam heated conditions (80°C to 90°C). The oil-bearing cells of the mesocarp are broke at this stage and the mechanical twin screw machine is used to press out the CPO with the addition of hot water in order to increase the oil flow. The digested CPO contains 35% to 45% of palm oil, 45% to 55% of water and the rest are the fibrous materials. The digested CPO is pumped into a clarification tank to separate the oil and temperature is maintained at 90°C to enrich the oil separation. At the bottom phase of the clarification tank, it contains some oil and it is brought through the sludge separator.

From Figure 2.1, POME is dispersed from three major sources which are sterilizer condensate, clarification tank and hydrocyclone tank. The characteristic of the dispersed POME from different sources in palm oil processing is shows in Table 2.1. From Table 2.1, POME that is dispersed from clarification tank is more acidic compared with POME that is dispersed from sterilizer condensate. High BOD and COD concentration also can be found in POME that is dispersed from clarification tank. This is due to the large amount of POME that has been discharged in clarification tank.

Table 2.1 Characterization of dispersed POME in Palm oil mill

Parameter	Unit	Sterilizer Condensate	Clarification tank	Hydrocyclone tank
Chemical oxygen demand (COD)	mg /L	47000	64000	15000
Biochemical oxygen demand (BOD_3, 30° C)	mg /L	23000	29000	5000
Dissolved solid (DS)	mg /L	34000	22000	100
Suspended solid (SS)	mg /L	5000	23000	7000
Total nitrogen (TN)	mg /L	500	1200	100
Ammoniacal nitrogen	mg /L	20	40	-
Oil and grease	mg /L	4000	7000	300
pH		5.0	4.5	-

Source: Ahmed et al., (2015)

2.2 Palm Oil Mill Effluent (POME

The enormous amounts of water are required in order to extract the crude palm oil (CPO). This situation leads to the generation of huge volume of palm oil mill effluent (POME). According to Ahmad et al., (2003), the amount of POME produced during the extraction of palm oil is about 1.5 tonnes. POME is a highly polluting wastewater that pollutes the environment if discharged directly into the water sources without a proper treatment. The pollutant is due to the high chemical oxygen demand (COD), biochemical oxygen demand (BOD), phenol, and color concentrations in POME (Zahrim et al., 2009). POME is a combination of wastes from the three sources in palm oil extraction process which are clarification wastewater (60%), sterilizer condensate (36%) and hydrocyclone wastewater (4%) (Ahmed et al., 2015).

2.2.1 Characterization of Acidic Palm Oil Mill Effluent (POME)

The raw palm oil mill effluent (POME) is an acidic liquid waste. It is a thick brownish, viscous and contains voluminous colloidal matter. The acidic POME is non-toxic effluent but has been identified as one of the major sources of aquatic pollution in Malaysia. The acidic POME contains about 95 % to 96 % of water, 4 % to 5 % total solids, 2 % to 4 % suspended solids and 0.6 % to 0.7 % of oil and grease. According to Ahmed et al., (2015), the raw acidic POME is discharged at a temperature ranging between 80 ° C to 90 ° C. The acidic POME have unpleasant odor with high biochemical oxygen demand (BOD) and chemical oxygen demand (COD) concentrations. Acidic POME also contains a large amount of amino acids, inorganic nutrients (Na, K, Ca, Mg, Mn, Fe, Zn, Cu, Co and Cd), short fibers, nitrogenous compounds, free organic acids and carbohydrates (Santosa et al., 2008). The characteristic of acidic POME from previous researcher data are shown in Table 2.2.

From Table 2.2, the acidic POME is featured with low pH value (3.4 to 4.7), high BOD concentration (22700 mg/L to 25545 mg/L), high COD concentration (44300 mg/L to 70900 mg/L), high total solid content (45000 mg/L to 45500 mg/L), high suspended solid content (18000 mg/L to 25800 mg/L), high oil and grease content (4000 mg/L to 8020 mg/L) and high total nitrogen content (711 mg/L to 750 mg/L. The high and low value of acidic POME parameters is compared with the Malaysian Palm Oil Board (MPOB) value. High BOD and COD value shows that the effluent contain

high organic and inorganic matter. The organic and inorganic matter presents in the effluent contributed in the high amount of total solid, suspended solid, total nitrogen and oil and grease content. The characteristics of acidic POME may change substantially for different batches, days and factories operation. It also depends on the processing techniques, the age and type of fruit, the discharge limit of the factory, climate and condition of the palm oil processing (Wu et al., 2010).

UMP

Table 2.2 Measured parameters of discharged raw acidic POME in Malaysia

Parameter	Unit	Malaysian Palm Oil Board (MPOB)	Zinatizadeh et al., (2006)	Ahmad et al., (2003)	Wu et al., (2007)	Vijayaraghavan et al., (2007)
Biochemical oxygen demand, (BOD_5)	mg/L	25000	22700	25000	-	25545
Chemical oxygen demand (COD)	mg/L	50000	44300	50000	70900	55775
Total solid (TS)	mg/L	45000	-	45500	-	-
Suspended solid (SS)	mg/L	18000	19780	18000	25800	18479
Oil and grease	mg/L	4000	4850	4000	-	8020
Total nitrogen (TN)	mg/L	750	-	750	-	711
pH		4.7	4.05	4.7	4.52	3.4-3.6
Temperature	°C	-	-	-	-	83-85

2.2.2 Acidic Palm Oil Mill Effluent (POME) Treatment

The palm oil mills in Malaysia faced the challenge in balancing the environmental protection, economic viability and sustainable development after the Department of Environment enforced the regulation for the discharge of effluent from the CPO industry. The discharged regulation are under the Environmental Quality (Prescribed Premises) (Crude Palm Oil) Order and Regulations, 1977. Several studies and research has been done by the government, private sector and educational institute to find the most effective techniques to treat acidic POME. The acidic POME treatment requires an efficient system in facing the current challenges such as costing issue and long retention times for the treatment.

The common biological processes that being used in treating the raw acidic POME is aerobic treatment process and anaerobic treatment process. Biological treatment processes are cost effective processes. It utilizes the microbial communities and usage in decomposing the organic matter present in wastewater. However, the biological treatment requires a proper maintenance and monitoring during the treatment process. This is due to the treatment process that depends on microorganisms to break down the pollutants (Ahmad et al., 2005). The microorganisms used in acidic POME treatment are sensitive towards the changes in the environment. Thus the proper monitoring has to be taken to provide a conducive environment for the microorganisms to growth.

Aerobic treatment process and anaerobic treatment process is the common methods used in treating the raw acidic POME. However, the anaerobic treatment process is more suitable for acidic POME treatment compared with aerobic treatment process (Poh and Chong, 2009). Lower energy consumption while producing methane gas as valuable end product makes anaerobic treatment process is better compared with aerobic treatment process.

The most common anaerobic treatment process in treating raw acidic POME is ponding system. About 85% of the palm oil mill used ponding system in treating acidic POME (Poh and Chong, 2009). The ponding system consists of de-oiling tank, acidification ponds, anaerobic pond and facultative or aerobic ponds (Chan and Chooi, 1984). The number of ponds used in the treatment process is depends on the capacity of

the palm oil mill. According to Chan and Chooi, (1984), the anaerobic ponds in the ponding system have the longest retention time which is around 20 day to 200 day. The open digested tanks are used in treating acidic POME when there is limited land area available for ponding system. In this study, a batch lab scale experiments were carried out by applying anaerobic process method towards the acidic POME treatment.

Table 2.3 shows the advantages and disadvantages of various anaerobic treatment methods in the acidic POME treatment. From Table 2.3 the conventional methods (ponding system) have disadvantages in terms of treatment time, area required for treatment and facilities to capture biogas. However, this method is economically viable and has the capacity to tolerate a wider range of organic loading rate. High-rate bioreactors are more effective in biodegradation as shorter retention times are needed in producing higher methane yield. However, the operating cost and maintenance has been the major issues (i.e. power requirement for bed fluidization, support media and investment on control systems).

Table 2.3 The advantages and disadvantages of anaerobic treatment methods

	Advantages	Disadvantages	References
Conventional anaerobic digestion (pond and digester)	• Low capital cost • Low operating and maintenance cost • Required simple design • Required minimal energy • Small sludge production • Low nutrient requirement • Recovered sludge can be used as fertilizer. • Digested POME could be used for algae culture	• Required large areas of land for application • Long retention times • There is no facilities to capture biogas • Required post treatment to remove remaining organic matter	Chan and Chooi (1984)
Anaerobic filtration	• Smaller reactor volume needed • Construction, operation and maintenance costs are low • High removal efficiency of COD, BOD and suspended solids • Able to handle high volume of loads • Retains high biomass concentration in the packing	• High media maintenance and support cost • Requires a constant source of water • Unsuitable for high suspended solid • Removal of pathogens and nutrients are low • Long start up time • Effluent require post treatment and/or appropriate discharge	Borja and Banks (1994b, 1995b)

Table 2.3 Continued

Fluidized bed reactor	• Most compact of all high-rate processes • Small areas are required • No channelling, plugging and gas hold up • Large surface area for mass transfer and biomass attachment • Used to treat high strength wastewater at both ambient and elevated temperature	• High power requirements for bed fluidization • High cost of carrier media • Not suitable for high suspended solid wastewaters • Unable to capture produced biogas	Leslie Grady et al. (1999)
Upflow anaerobic sludge blanket (UASB) reactor	• Useful for treatment of high suspended solid wastewater • Producing high quality effluent • No media required (less cost) • High COD removal efficiency and methane emission rate • High concentration of biomass retained in the reactor	• Long start-up period if granulated seed sludge is not used • Performance depends on sludge settle ability • Poor separation between treated effluent and biomass	Lettinga (1995), Kalyuzhnyi et al. (1998), Goodwin et al. (1992)
Up-flow anaerobic sludge fixed-film (UASFF) reactor	• Achieved higher organic loading compare UASB and anaerobic filtration. • Higher biomass retention • More stable operation • Ability to tolerate shock loadings • Suitable for diluted wastewater • Reduce the clogging	• Poor separation between treated effluent and biomass • Stability and efficiency of the reactor depend on internal packing, effluent recycling ratio, rate of feed flow and up-flow velocity	Ayati and Ganjidoust (2006)

Table 2.3 Continued

Continuous stirred tank reactor (CSTR)	• Provides more contact of wastewater with biomass through mixing • Increased gas production compared to conventional method • Relatively easy to clean and maintenances • Low operating cost due to low amount of electrical energy required	• Less efficient gas production at high treatment volume • Less biomass retention	
Anaerobic contact process	• Reaches steady state quickly • Short hydraulic retention time • Produces relatively high effluent quality	• Less stable due to oxygen transfer in digesting tank • Settle ability of biomass is critical to successful performance	Hamdi and Garcia (1991)

2.3 Factors Affecting Biological Treatment of Acidic Palm Oil Mill Effluent (POME)

The acidic The acidic palm oil mill effluent (POME) is generated mainly from oil extraction, washing and cleaning processes in the palm oil mill industry. The acidic POME contains cellulosic material, fat, oil and grease that need to be treated before discharged (Parveen et al., 2010). There are several factors that need to be considered in treating acidic POME especially in treating pH, BOD removal and COD removal of acidic POME.

Reaction time has been reported as important factors that contributed in acidic POME treatment (Chou et al., 2010). According to Ahmad et al., (2005), the biological treatment of acidic POME depends solely on the microbes to break down the waste properties. The microbes required essential time to growth and degraded the organic matter present in wastewater. According to Zinatizadeh (2006), the organic matter present in wastewater is degraded into methane and carbon dioxide. The reaction time provides the time for microbes to multiply and decompose the organic matter. The increasing in reaction time increased the microbial activities and its performance. The study conducted by Prasertsan et al., (2009) observed that the microbial activity rate decreased as reaction time decreased. This shows microbial activities is directly proportional with the reaction time.

Temperature has been reported as important factors that contributed in treating acidic POME. It has been reported that the temperature can affect the microbial activity by influencing the activity of some microbes or enzyme (Wang and Wan, 2008). The temperature also can affect the biochemical reactions such as reaction rate, reaction pathway, microorganism yields and death rate of microbes. Anaerobic processes are affected by temperature changes because anaerobes are sensitive towards operating temperature. The study conducted by Yu et al., (2002) found that the substrate degradation rate and biogas production rate at 55° C (thermophilic) is higher than the operation at temperature of 37° C (mesophilic). However, failure to control the temperature resulted in having accumulation of volatile fatty acid due to inhibition of methanogenesis. At the high temperatures, production of volatile fatty acid is higher compared to mesophilic temperature range (Yu et al., 2002).

The microbial activities and its performance can be improved by agitation. Agitation is another factor that contributed in treating the acidic POME. According to Lamed et al., (1988), the microbial activities increased in stirred condition. It was supported by Clark, et al., (2012) that mixing is a possible option to speed up the microbial activity. Mixing provides good contact between the microbes and substrates. It reduced the resistance of mass-transfer and minimizes the build-up of inhibitory intermediates (Leslie Grady et al., 1999). Mixing can be accomplished through mechanical mixing, biogas recirculation or through slurry recirculation (Karim et al., 2005a).

The soil to water ratio also known as soil concentration has been reported as important factors that affecting microbial growth (Rasdi et al., 2009). High soil concentration contain large amount of soil microbes that can be used in acclimatization and treatment process. Soil microbes play an important role in organic matter degradation and removal of nitrogen, bacteria and viruses. The soil microbe populations can take benefit from the additions of nutrients, organic matter and septic microbes present in septic tank effluent because these materials serve as a food source.

The type of soil used is another factor that contributed in acidic POME treatment. Different types of soil give different properties such as moisture content, organic content and soil structure. This lead to the different amount of microorganism exists in the soil. Soils contain naturally-occurring bacteria, fungi, and protozoa and are responsible for many biological wastewater treatment processes (George W. Loomis, 1999). According to Akhtar and Malik (2000) the organic matter amendments to soil have been shown to have significant effects on soil nutrients, soil physical conditions and soil biological activity. This contributed towards the biological treatment of acidic POME.

2.4 Mixed Culture

A mixed culture is a microbial culture that contains two or more different strains of organisms. The use of mixed culture provides several advantages over a pure culture. The mixed culture can better adapt to changing conditions during growth (Nor Habibah, 2006). According to Bailey and Ollis (1986), natural occurring mixed cultures are particularly efficient means for utilization of substrate mixtures in the context of wastewater treatment. A facultative anaerobe capable of metabolizing glucose known as *Citrobacter* has yielded acetate, formate, ethanol and lactate as its products. The lactate produced can be used as substrate to form methane by other methanogens. This shows that the mixed culture provides more alternatives or process pathway for the formation of anaerobic digestion to produce methane and carbon dioxide.

The microbes in a mixed culture can break down the waste properties. According to Lin et al. (2008), mixed culture is useful in degrading the organic waste. Processes using mixed cultures are more practical than processes using pure cultures because the mixed cultures are simpler to operate, easier to control and have a broader source of feedstock or sources (Wang and Wan, 2008). Mixed culture can adapt variety of changes in the system such as temperature and pressure changing due to it capability and ability to change according to nature. For future industrial applications, the use of mixed cultures for treatment of organic wastes might have more advantages compared with the pure cultures as pure cultures can easily contaminated.

The study conducted by Oswal et al. (2002) used treated mixed culture (*Yarrowia lipolytica*) in treating POME. The result from the study shows the reduction in COD value at 95%. This shows the usage of mixed culture in POME treatment process. The study conducted by Ismail et al. (2009) used suspended mixed culture in producing biohydrogen from POME. This shows a wide application of mixed culture in the industrial purpose.

The study conducted by Kimura and Ito (2001) shows another application of mixed culture in wastewater treatment. The mixed culture used in the study is suitable for wastewater treatment that contains high concentration of terephthalic acid. This is due to the mixed culture used in that study contains microbes that can degrade terephthalic acid. Another application of mixed culture is from Ibn Abubakar et al.

(2012), where it used mixed culture to removed pyrene from soil slurry bioreactor. The mixed culture used in the study contains pyrene-degrading microbes. There is also a study conducted by Moussa et al. (2003), where it study the activity of ammonia and nitrite oxidisers in mixed bacterial cultures. The study conducted by Pramanik et al., (2011) investigates the effects of microbial diversity of mixed cultures in biological wastewater treatment.

The used of mixed culture in different wastewater treatment shows a variety of application of mixed culture in degrading organic matter thus it increasing the capability and ability of mixed culture in adapting any changes. Different researcher used different types of mixed culture that suitable with the treatment process. This shows a wide usage of mixed culture.

2.5 Response Surface Method (RSM)

Response surface method (RSM) is a collection of mathematical and statistical techniques for empirical model building. An experiment is a series of tests in which changes are made in the input variables in order to identify the reasons for changes in the output response. The RSM have several classes of designs, with their own properties and characteristics. Central composite design (CCD), box-Behnken design and three-level factorial design are the most popular designs applied by the researchers. The CCD was used to study the effects of the variables towards their responses and subsequently in the optimization studies (Bhatia et al., 2007).

Besides analyzing the independent variables effects, this experimental methodology also generates a mathematical model. The relationship between the responses and the inputs is given in Equation 2.1:

$$Y = f(x_1,x_2,x_3...x_n) \pm \varepsilon \qquad 2.1$$

where Y is the response, *f* is the unknown function of response, x_1, x_2, x_3,⋯ x_n were the input variables that can affect the response, n is the number of the independent variables and ε is the statistical error that represents other sources of variability not accounted for by *f* (Bhatia et al., 2007).

2.5.1 Factorial Analysis

A fractional factorial design analysis used to describe variability among observed, correlated variables in terms of a potentially lower number of unobserved variables called factors (Srinivasan and Viraraghavan, 2010). A very important special case of the factorial design is that where each of the k factors of interest has only two levels. Because each replicate of such a design has exactly 2^k experimental trials or runs, these designs are usually called *2^k factorial designs.* The 2^k factorial designs are very important in response surface work. The application of factorial design include as a start for RSM study when screening experiments should be performed to identify the important process or system variables and as a basic building block used to create other response surface designs.

According to Lee et al., (2012), the factorial design is applied in the experiments where the interaction effect on factors on the response is taking in account. A 2^k factorial design is particularly applied in the earlier stages of experimental work when there are several factors need to be investigated. However, as the number of factors in a 2^k factorial design increases, the number of runs required for a complete replicate of design also increased. Thus, fractional factorial designs have been introduced to minimize the experimental run.

The factorial design is used to screen the selected factors and the most critical factors that contributed towards the process are selected for further study. The study conducted by Martin-Lara et al., (2011) used factorial design to screen the variables that affecting the process. Factorial design also used to estimate the main effects and interaction effects of different variables as well as to develop an empirical model for the process. The study conducted by Lee et al., (2012) used factorial analysis to investigate the effect of the independent factors and the interaction factors. Results from the study shows that fractional factorial design is suitable in investigating the effect of large number factors with a minimum number of experiments.

2.5.2 Optimization

Optimization techniques are useful in finding the optimum production or unconstrained maxima or minima of continuous and differentiable functions. According to Zinatizadeh et al. (2010), RSM has an important application in the process design and optimization as well as the improvement of existing design.

For application of RSM on optimization, some stages need to be followed such as the selection of the most important independent variables through screening studies. The choice of the experimental design and the mathematic - statistical treatment of the obtained experimental data also need to be considered. The evaluation of the model's fitness and the obtaining of optimum values for each variable also considered.

Central Composite Design (CCD) is an experimental design that is useful in RSM. The used of CCD is to build a second order (quadratic) model for the response variable without completing three-level factorial experiment. There are two major types of central composite designs which are the spherical central composite design and the rotatable central composite design. The spherical central composite design is where the star points are the same distance from the central as the corner points. The rotatable central composite design is where the star points are shifted or placed such that the variances of the predicted values of the responses are all equal. CCD was very efficient and flexible design that provides necessary information on experiment variable effects and overall experimental error in a minimal number of required runs (Nayak et al., 2014).

The CCD in RSM is utilized to illustrate the output of the response in the designed experiment and to explicate the optimization level of the independent variables. According to the study conducted by Ahmad et al., (2005), the used of CCD in experimental design helps in obtaining the optimum values of the process parameters. Ahmad et al., (2005) used CCD to study the interaction effects of the selected factors in POME treatment. The study conducted by Zinatizadeh et al., (2006) used CCD to study the interactive effects of feed flow rate and up-flow velocity on the performance of an up-flow anaerobic sludge fixed film (UASFF) reactor in treating POME. The result from the study shows that CCD is useful to identify the most significant operating factors and optimum levels with minimum effort and time.

The study conducted by Chan et al., (2013) used RSM for optimization study on the simultaneous anaerobic and aerobic processes in an integrated anaerobic- aerobic bioreactor (IAAB) treatment system for POME. Results from the study shows that application of RSM in conjunction with CCD improved the experimental design in obtaining the optimum value.

CHAPTER 3

METHODOLOGY

3.1 Process Flow of Methodology

The process flow chart of the research methodology was shown in Figure 3.1. The methodology was divided into three main contents in order to achieve the three objectives. In this experiment, acidic palm oil mill effluent (POME) was used as substrate whereas soil mixed culture (SMC) as inoculum. The experiment started with the collection of substrate and soils. Then characterizations were conducted in order to determine the physical and chemical properties of the samples. The characterization of acidic POME involves the determination of pH value, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total solid (TS), oil and grease, and ammoniacal nitrogen. The characterization of soil involves the determination of pH, texture, moisture content, conductivity, nitrogen content, organic carbon content, available phosphorus and cation-exchange capacity. Soil water was prepared by mixing the soil with distilled water. SMC was prepared by acclimatizing the soil water with acidic POME. The factors affecting biological acidic POME treatment was selected.

The most important parts of this research were factorial analysis and optimization process. Five factors were selected which is reaction time, temperature, agitation speed, soil to water ratio and types of soil used. Factorial analysis was carried out to screen the most affecting factors in biological acidic POME treatment before continued with optimization process. The response surface method (RSM) was applied in factorial analysis and optimization process. The experimental design for factorial analysis was constructed by using two-level factorial design. Central composite design (CCD) was used in constructing experimental design for optimization process. The

experimental results were analyzed by using Design Expert software (Version 6.0). Experimental validation was performed at the suggested optimum condition that was obtained from the analysis.

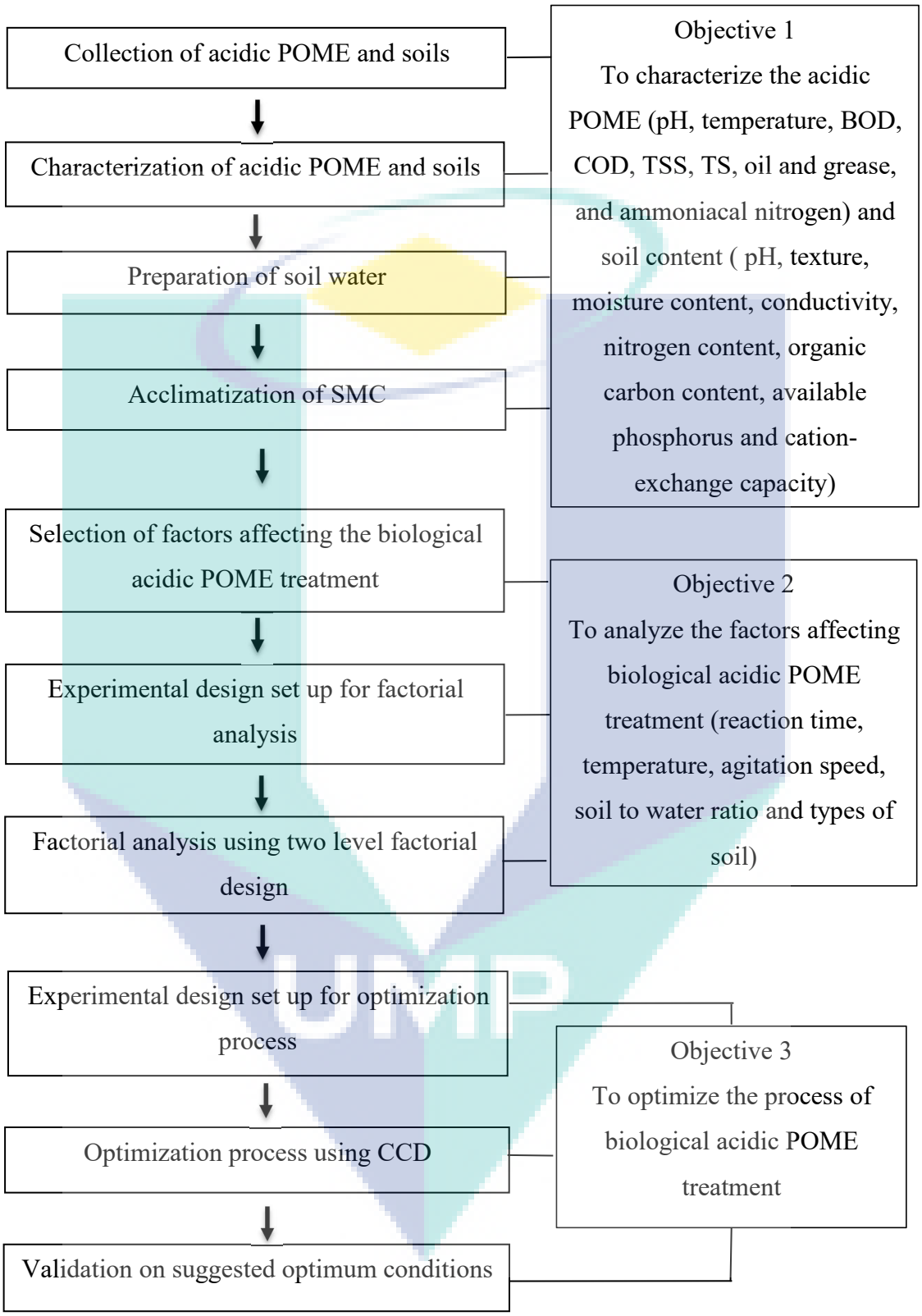

Figure 3.1 Process flow for methodology

3.2 Acidic Palm Oil Mill Effluent (POME)

3.2.1 Collection of Acidic Palm Oil Mill Effluent (POME)

The acidic POME was collected from the mixed raw effluent (MRE) of a palm oil mill in Kuantan, Pahang. The acidic POME was placed in a polyethylene container and was kept in a freezer at 4°C to avoid its degradation (Chan et al., 2010).

3.2.2 Characterization of Acidic Palm Oil Mill Effluent (POME)

The characterization of acidic POME involves pH, temperature, biochemical oxygen demand (BOD), chemical oxygen demand (COD), total suspended solids (TSS), total solid (TS), oil and grease, and ammoniacal nitrogen. The tests used APHA, (2005), standard methods for the examination of water and wastewater. Table 3.1 shows the characterization parameters and testing method for characterization of acidic POME.

The pH was a measure of the acidic or alkaline nature of a liquid. The pH value was measured using pH meter (Mettler Toledo). Temperature is important parameter in environmental and water quality. Temperature influences the types of aquatic life and regulates the maximum dissolved oxygen concentration of the water (Ibrahim et al., 2012). The organisms within the ecosystem had preferred temperature regimes that change as a function of season, organism age or life stage, and other environmental factors. In term of chemical and biological reactions, the higher the water temperature the higher the rate of chemical and metabolic reactions. In this research, temperature was measured using glass thermometer (SAMA Precision Mercury Lab Thermometer).

Biochemical oxygen demand (BOD) test measures the ability of naturally occurring microorganisms to digest organic matter. It usually takes in 5 days incubation at 20°C by analyzing the depletion of oxygen. Determination of BOD_5 involves the measuring of oxygen demand in both organic matter and organism exists in the acidic POME (Ibrahim et al., 2012). In this research, BOD_5 was conducted by dilution method, Standard Method 5210 B (Appendix A1).

Chemical oxygen demand (COD) test measures the oxygen equivalent of the organic material in wastewater (Sehar et al., 2011). The mg/L COD results were defined as the mg of O_2 consumed per liter of sample. Wastewater sample was heated for two

hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion ($Cr_2O7_2^-$) to green chromic ion (Cr_3^+). In this research, COD was conducted by using Standard Method APHA (Appendix A2).

The suspended solids parameter was used to measure the quality of wastewater influent, monitor several treatment processes and measure the quality of the effluent. A well-mixed measured sample was filtered through a weighed standard glass-fiber filter and the residue retained on the filter was dried to a constant weight at 104 °C ± 1 °C. The increasing weight of the filter represents the total suspended solids (TSS). In this research, TSS was conducted by using Standard Method APHA 2540 D (Appendix A3).

Total solids (TS) represent of all solids in a wastewater sample. It includes the total suspended solids, total dissolved solids and volatile suspended solids (Ibrahim et al., 2012). In this research, TS was measured by using Standard Method APHA 2540 (Appendix A4).

Oil and grease have poor solubility in water. Thus, oil and grease had an important consideration in handling and treatment of the material for disposal. In this research, ammoniacal nitrogen was measured by using Standard Method APHA 5520 B (Appendix A5).

In ammonia-nitrogen test, ammonia compounds combine with chlorine to form monochloride. Monochloride reacts with salicylate to form 5-aminosalicylate. The 5-aminosalicylate was oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color was masked by the yellow color from the excess reagent present to give a final green-colored solution. In this research, ammoniacal nitrogen was conducted by using Standard Method APHA (Appendix A6).

Table 3.1 Characterization parameters for acidic POME

Parameter	Unit	Test method (Appendix A)
pH	-	Standard method, APHA (2005)
Temperature	°C	Standard method, APHA (2005)
Biochemical oxygen demand (BOD)	mg /L	Standard method, APHA (2005)method 5210 B
Chemical oxygen demand (COD)	mg /L	Sandard method, APHA (2005) metod 5220 C
Total suspended solid (TSS)	mg /L	Standard method, APHA (2005) method 2540 D
Total solid (TS)	mg /L	Standard method, APHA (2005) method 2540
Oil and grease	mg /L	Standard method, APHA (2005) method 5520 B
Ammoniacal nitrogen	mg /L	Standard method, APHA (2005)

3.3 Soil Samples

3.3.1 Collection of Soils

Two soil samples were collected namely peat soil (PS) and alluvium soil (AS). All soils were taken 15cm from the surface layer. The PS was collected at the palm oil mill. The AS was collected near the palm oil tree root system. The soil samples were placed in bulk plastic containers and stored at 4 °C prior to use (Che at al., 2014).

3.3.2 Characterization of Soils

The characterization of peat and alluvium soil involve the determination of pH, texture, moisture content, conductivity, nitrogen content, organic carbon content, available phosphorus and cation-exchange capacity. Soil characterization was important to determine the soil properties and its behavior. Table 3.2 shows the characterization parameters and testing method for soil samples.

The soil pH determined whether the soil was acidic, neutral or alkaline. The acidity, neutrality or alkalinity of a soil was measured in terms of hydrogen ion activity of the soil water system. The pH value was measured using pH meter (Mettler Toledo).

The soil texture measured the percentage of sand (fine and coarse), silt and clay exist in the soil. Differences in soil texture gave impacts on organic matter levels. In

this research soil texture analysis was conducted by using standard method in determination of soil texture (Appendix B1).

The soil moisture content indicated the amount of water present in the soil. It measured the quantity of the water exist the soil. In this research the moisture content analysis was measured by using moisture content and loss of ignition method (Appendix B2).

The conductivity indicated the total ion and the ability of a material to transmit electrical current (Barbosa and Overstreet, 2011). Soil conductivity measured the soil water ability in carry an electrical current. In this research the soil conductivity analysis was conducted by using standard method in determination of conductivity in soil (Appendix B3).

The nitrogen analysis measured the amount of nitrogen present in the soil. Nitrogen in soils was a complex mixture of chemical and biological processes. In this research the nitrogen analysis was conducted by using Kjedahl Method (Appendix B4).

The carbon analysis indicated the amount of organic carbon matter presented in the soils. It measured the percentage of carbon that exists in the soils. In this research the soil carbon analysis was conducted using standard method in determination of a carbon (Appendix B5).

The available phosphorus in soils indicated the amount of phosphorus presented in the soils. Phosphorus is one of the three nutrients generally added to soils in fertilizers. One of the main roles of phosphorus in living organisms is in the transfer of energy. In this research the soil available phosphorus analysis was conducted using standard method in determination of available phosphorus in soil (Appendix B6).

The cation-exchange capacity (CEC) of a soil measured the quantity of negatively charged sites on soil surfaces that can retain positively charged ions (cations) by electrostatic forces. Soil with higher CEC has a greater capacity to maintain adequate quantities of Ca^{2+}, Mg^{2+} and K^{+} than a soil with low CEC. The CEC of soil samples were measured using the distillation method (Appendix B7).

Table 3.2 Parameters in soil characterization

Parameter	Unit	Test method (Appendix B)
pH	-	Standard method
Nitrogen (N)	%	Determination of total nitrogen by microKjedahl method
Moisture content (MC)	%	Moisture content and loss of ignition
Carbon (C)	%	Determination of carbon (Walkley – Black method)
Conductivity	-	Determination of conductivity in soil
Available phosphorus	ppm	Available phosphorus in soil
Cation exchange capacity (CEC)	cmol/kg	Determination of exchange cations by ICP & CEC by distillation method
Coarse sand	%	Determination of soil texture
Fine sand	%	Determination of soil texture
Slit	%	Determination of soil texture
Clay	%	Determination of soil texture

3.4 Preparation of Soil Mixed Culture (SMC)

Soil mixed culture (SMC) was prepared by acclimatizing the soil water with acidic palm oil mill effluent (POME). Soil water was prepared by mixing the soil with distilled water. There were two types of soils used in this study namely peat soil (PS) and alluvium soil (AS). The PS and distilled water were mixed together to give the soil to water (s/w) ratio of 1:1 (100 g soil and 100 mL distilled water) and 1:3 (100 g soil and 300mL distilled water). The same procedure was done for AS. The supernatant liquid of soil water was added to the acidic POME in ratio 1:3 (50 mL s/w and 150 mL POME). The mixture was acclimatized for 10 days (30°C and 150 rpm) and called SMC. Acclimatization process in a biological process enhanced the ability of the microbes to degrade organics (Lin et al, 2008). The purpose of acclimatization process was to familiarize the culture with the experimental condition and increase the microbial population.

3.5 Preliminary Study of Biological Acidic Palm Oil Mill Effluent (POME) Treatment

A preliminary study was conducted to investigate the experimental factors ranges before the application of statistical design. The preliminary study was carried out

with 150 mL of acidic POME used as control test. The control test was used to confirm that there was no change in pH, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) resulting from degradation of the organic matter in the substrate itself. The preliminary was conducted by mixing the acidic POME with soil mixed culture in a ratio of 1:1 to 1:3 and the agitation speed was at 130 rpm to 200 rpm. The batch tests were conducted in incubator shaker and the pH was checked for every 24 hours by using pH meter (Mettler Toledo).

3.6 Experimental Design for Factorial Analysis

In this research, factorial analysis was conducted by using two-level factorial design. The two-level factorial design was used to screening the selected factors which were reaction time, temperature, agitation speed, soil to water ratio and types of soil. Factorial analysis was performed to study the interaction effect of factors on the response (Lee et al., 2012).

3.6.1 Experimental Set Up For Factorial Analysis

The experimental table for factorial analysis was designed and constructed using two-level factorial in response surface method (RSM). The Design Expert software (Version 6.0) was used in constructing the experimental table. The same software was used in analyzing the experimental results. The ranges of the factors for factorial analysis were shown in Table 3.3. The experiments were carried out by varying the factors according to the selected ranges. Experiments were performed by mixing the SMC with acidic POME at ratio 1:3 (50 mL SMC and 150 mL acidic POME). Then the mixture was placed in incubator shaker. The experiments were carried out under anaerobic condition. Experiments were performed according to the experimental design table (Table 3.4).

Table 3.3 Experimental factors and ranges for factorial analysis

Factors	Unit	Model Symbol	Type	Low (-1)	High (+1)
Reaction time	day	A	Numeric	3	5
Temperature	°C	B	Numeric	25	30
Agitation speed	rpm	C	Numeric	150	180
Soil to water ratio	-	D	Categoric	1:3	1:1
Types of soil	-	E	Categori	Peat	Alluviu

Table 3.4 Experimental design table for factorial analysis

Run	Reaction time (day)	Temperature (°C)	Agitation speed (rpm)	Soil to water ratio	Types of soil
1	3	25	150	1:3	Alluvium
2	5	25	150	1:3	Peat
3	3	30	150	1:3	Peat
4	5	30	150	1:3	Alluvium
5	3	25	180	1:3	Peat
6	5	25	180	1:3	Alluvium
7	3	30	180	1:3	Alluvium
8	5	30	180	1:3	Peat
9	3	25	150	1:1	Peat
10	5	25	150	1:1	Alluvium
11	3	30	150	1:1	Alluvium
12	5	30	150	1:1	Peat
13	3	25	180	1:1	Alluvium
14	5	25	180	1:1	Peat
15	3	30	180	1:1	Peat
16	5	30	180	1:1	Alluvium
17	4	27.5	165	1:3	Peat
18	4	27.5	165	1:1	Peat
19	4	27.5	165	1:3	Alluvium
20	4	27.5	165	1:1	Alluvium

3.7 Experimental Design for Optimization Process

In this research, optimization process was conducted by using central composite design (CCD). CCD was used to identify the relationship existing between the response function, process variables and the optimum condition. The CCD method provides sufficient information on the effects of variables and overall experimental performance with a minimum number of experiments (Ahmad et al., 2005).

3.7.1 Experimental Set Up for Optimization Process

The experimental design for optimization process was performed by using CCD in RSM. The Design Expert software (Version 6.0) was used in constructing the experimental table. The same software was used in analyzing the experimental results. Based on the result from factorial analysis, two most affecting factors were selected for optimization. The selected parameters were the reaction time and agitation speed as shows in Table 3.5. The reaction time varied from 4 to 6 days and agitation speed

varied from 130 rpm to 170 rpm. Thirteen randomized experiments, including five replicates at the center points were assigned.

Three factors from factorial analysis were fixed at temperature (30°C), soil to water ratio (1:1) and soil types (alluvium). Experiments were performed by mixing the inoculum with acidic POME in ratio 1:3 (50 mL inoculum and 150 mL acidic POME). Then the mixture was placed in incubator shaker. The experiments were carried out under anaerobic condition. Experiments were carried out according to the experimental design table (Table 3.6).

Table 3.5 Ranges of factors for optimization process

Factor	Symbol	Unit	Level				
			-α	-1	0	+1	+α
Reaction time	A	day	4	4.5	5	5.5	6
Agitation speed	B	rpm	13 0	140	15 0	160	170

Table 3.6 Experimental design for optimization process

Run	Factors	
	Reaction Time, day	Agitation Speed, rpm
1	5	130
2	6	150
3	4	150
4	5	150
5	4.5	140
6	5	150
7	4.5	160
8	5.5	140
9	5	170
10	5	150
11	5	150
12	5	150
13	5	160

3.8 Analysis for Biological Acidic POME Treatment

The response studied in both factorial analysis and optimization process were pH, biochemical oxygen demand (BOD) removal and chemical oxygen demand (COD) removal. The pH value determined the acidity or alkalinity of an aqueous solution. The pH value was measured using pH meter (Mettler Toledo). BOD is the amount of oxygen used by bacteria to oxidize the organic pollutants. It measured the concentration of organic substances in wastewater that can be oxidized by bacteria. BOD test was conducted at a temperature of 20ºC for 5 days. BOD was measured using Dissolved Oxygen Meter (YSI 5100). COD serves to determine the amount of oxygen required to oxidize organic matter with the oxidizing agent in acidic conditions. The COD vials were placed in COD digestion reactor (HACH DRB200) for two hours. Then the COD value was measured using spectrophotometer (HACH DR2800). The calculation for BOD and COD removal was shown in Equation 3.1 and 3.2.

$$\text{BOD removal}\,(\%) = \left(\frac{\text{initial BOD value - final BOD value}}{\text{initial BOD value}}\right) x100\% \qquad 3.1$$

$$\text{COD removal}\,(\%) = \left(\frac{\text{initial COD value - final COD value}}{\text{initial COD value}}\right) x100\% \qquad 3.2$$

3.9 Validation of Optimum Condition

Experimental validation was conducted based on the suggested optimum condition proposed by the Design Expert software (Version 6.0). Suggested optimum condition with predicted value for pH, BOD and COD were listed in Table 3.7. The experimental and predicted values were compared in order to determine the validity of the model. The validity of the model depends on the error obtained from predicted and experimental value. The percentage error was calculated using Equation 3.3.

$$\text{Error}\,(\%) = \left(\frac{\text{Predicted value - Experimental value}}{\text{Predicted value}}\right) x100\% \qquad 3.3$$

Table 3.7 Suggested optimum condition for validation process

Run	Reaction time, day	Agitation speed, rpm	Predicted value		
			pH	BOD removal (%)	COD removal (%)
1	5	150	8.14	99.16	81.69
2	5.18	150	8.14	99.20	81.61

UMP

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characterization

4.1.1 Characterization of Acidic Palm Oil Mill Effluent (POME)

Palm oil industry produced large amount of acidic palm oil mill effluent (POME) due to the oil extraction process. The physical properties of acidic POME were a thick brownish liquid which contained a mixture composed of water, oil, and suspended solids. The characteristic of acidic POME obtained from this study was presented in Table 4.1. In this study, the pH value for acidic POME was 4.00. The pH value of acidic POME was more acidic compared with the pH value from Malaysian Palm Oil Board (MPOB) which was at 4.7. The pH was a measure of the acidic or alkaline nature of a liquid. It measured the concentration of the hydrogen ion [H+] activity in a liquid.

A determination of biochemical oxygen demand (BOD) test was involved in measuring the oxygen demand of both the organic matter and organism in the acidic POME. It was used to measure the approximate amount of oxygen required by bacteria and other microorganisms while stabilizing the decomposable the organic matter present (Ibrahim et al., 2012). In this study, the BOD_5 value of acidic POME was 23300 mg/l. The most common value was 25000 mg/l recorded by Malaysian Palm Oil Berhad (MPOB). Low concentration of BOD_5 shows that the effluent has less organic matter. The microorganisms will consume less oxygen to decompose the organic matter in the effluent.

Chemical oxygen demand (COD) was used to measure the oxygen equivalent of the organic material in wastewater. It was useful on measuring the water quality. The most common application of COD was determined the amount of organic pollutants

found in water (Sehar et al., 2011). In this study, the COD value of acidic POME was 42100 mg/l. The COD value in this study was considered low when compared with MPOB standard which was at 50000 mg/l. Low value of COD in wastewater indicates that the wastewater contain low organic and inorganic waste.

Total solids represent of all solids in wastewater sample. It included the total suspended solids, total dissolved solids, and volatile suspended solids. In this study, the total solid (TS) of acidic POME was 55240 mg/l. The total suspended solid (TSS) of acidic POME was 18690 mg/l. The high value of TS and TSS indicates that the wastewater contain high amount of dissolved substances.

In this study the ammoniacal nitrogen value of acidic POME was 39.2 mg/l. Total nitrogen was the sum of organic nitrogen, ammonia (NH^3), and ammonium (NH^{4+}) in the chemical analysis of soil, water, or wastewater. Nitrogen was an essential ingredient for cell growth but excessive amount of nitrogen can cause problem to environment. Excess nitrogen discharged into water sources can contributes to become rich in dissolved nutrients. It can contribute to massive algae blooms leading to oxygen depletion in water. Oil and grease were poor solubility in water. In this study, the value of oil and grease of acidic POME was 538 mg/l. The high concentration of oil and grease inside the sewer treatment system can cause the sewer to clog than can lead towards overflow.

Temperature was an essential water quality and environmental parameter because it influences the kinds and types of aquatic life. It also regulates the maximum dissolved oxygen concentration of the water. The organisms within the ecosystem had preferred temperature regimes that change as a function of season, organism age or life stage, and other environmental factors. In term of chemical and biological reactions, the higher the water temperature the higher the rate of chemical and metabolic reactions (Ibrahim et al., 2012). In this study, the temperature for fresh acidic POME was 75°C. From the previous study by other researcher, the temperature for fresh acidic POME ranging from 80°C to 90°C (Ma and Ong, 1985). The difference in temperature was due to vary of different batches, day and factories, depending on the processing techniques and age or type of fruits and condition of palm oil processing (Sehar et al., 2011).

The present of organic and inorganic matter in acidic POME had increased the pH value, BOD and COD concentration. According to Mohammad et al., (2014), concentrations of naturally dissolved organic acids such as tannins and lignins increased the pH value. Organic matter that exists in acidic POME required oxygen for decomposition. BOD was used to measure the amount of oxygen needed for decomposition. Therefore, low organic content exist in wastewater resulted in low BOD value. The organic and inorganic content also referred to the total solids that present in the wastewater.

Table 4.1 Characterization of raw acidic palm oil mill effluent (POME)

Parameter	Value
pH	4.00
Biochemical Oxygen Demand (BOD)	23300mg/l
Chemical Oxygen Demand (COD)	42100mg/l
Ammoniacal Nitrogen	39.2mg/l
Total Solids (TS)	55240mg/l
Total Suspended Solid (TSS)	18690mg/l
Oil and Grease	538mg/l
Temperature	75°C

4.1.2 Characterization of Soils

Table 4.2 shows the result on soil characterization. The peat and alluvium soil were analyzed to determine their pH, texture, moisture content, conductivity, nitrogen content, organic carbon content, available phosphorus and cation-exchange capacity. Soil characterization was important to determine the soil properties and its behavior. Bacteria that exist in soil were used as a source of inoculum.

From the result, alluvium soil had higher pH value compared to the peat soil. Soil with lower pH value tends to release magnesium and ferum ions. This situation leads to the production of phosphorus in soil (Bond, et al., 1998). This can be shown by higher available phosphorus exist in the peat soil (Table 4.2).

Soil moisture content depends on the type of soil and its texture. Percentage of coarse sand, fine sand, slit and clay contributes towards its moisture content. Saturated coarse, sandy soil can hold less water than saturated heavy silt clay. This can be shown by higher moisture content exist in peat soil compared to the alluvium soil (Table 4.2). Salt concentration that exists in the soil was directly proportional with soil conductivity.

Soil conductivity restricts the water intake in the soil thus increase its moisture content. This can be shown by low moisture content exist in the alluvium soil that contain low soil conductivity (Abd Rahim et al., 2008).

Cation-exchange capacity depends on organic carbon that exists in soil. High organic carbon content shows that the soil had higher cation-exchange capacity. This can be shown by high cation-exchange capacity exist in peat soil that has high organic carbon content. The cation-exchange capacity of a soil represents the total amount of exchangeable cations that the soil can absorb. The lower the cation-exchange capacity, the higher the ammonia-nitrogen removal in the biological treatment.

Conductivity of a soil indicated the total ion and the ability of the soil to transmit electrical current (Barbosa and Overstreet, 2011). Higher conductivity that exists in soil would result in better biological treatment because ions would remove the organic matter by binding with it. Conductivity of a soil depends on the soil particle size and its texture. Higher sand percentage would results in higher soil conductivity. This can be shown by higher soil conductivity exists in peat soil.

Table 4.2 Soils characterization

Soil type	**Alluvium**	**Peat**
pH	4.3	3.5
Nitrogen (%)	0.05	0.37
Moisture content (%)	17.18	46.16
Organic Carbon (%)	0.55	11.40
Conductivity	45.65	1039
Avail Phosphorus (ppm)	7.59	2747
Coarse sand (%)	12	51
Fine Sand (%)	37	20
Slit (%)	18	6
Clay (%)	38	18

4.2 Factorial Analysis on Biological Acidic Palm Oil Mill Effluent (POME) Treatment

4.2.1 Analysis on Biological Acidic POME Treatment

The experimental design for factorial analysis on biological treatment of acidic palm oil mill effluent (POME) was done by using the Design Expert software (Version 6.0). The two-level factorial design analysis was used in analyzing the selected factors. These factors were reaction time (A), temperature (B), agitation speed (C), soil to water ratio (D) and soil types (E). The purpose of this analysis was to determine the contribution factors and the interaction effect between the factors. The experimental responses in this study were pH, biochemical oxygen demand (BOD) removal and chemical oxygen demand (COD) removal.

BOD was defined as the difference between the initial dissolved oxygen concentration and the dissolved oxygen concentration after specific incubation time (Simon et al., 2011). It was a useful parameter for assessing the biodegradability of dissolved organic matter that exists in wastewater. COD analysis was defined as the measurement of the oxygen-depletion capacity of a water sample contaminated with organic waste matter. It measures the equivalent amount of oxygen required to chemically oxidize the organic compounds in water. The COD analysis was commonly utilized parameters in monitoring the water quality. The COD value for wastewater will be greater than BOD value. This was due to the more organic compounds can be oxidized chemically rather than biologically.

The experimental result for biological treatment of acidic POME was showed in Table 4.3. From the table, the pH value was found within the range from 7.22 to 8.20. The significant effect of each factor on the pH value was evaluated by analysis of variance (ANOVA). Results from ANOVA in Table 4.4 shows that the regression model for biological pH treatment was significant. The coefficient of determination (R^2) value of the pH model was 0.8311.

From ANOVA, reaction time gave highest contribution which was at 32.27%. This followed by agitation speed (10.05%), soil to water ratio (7.17%), soil types (2.48%) and temperature (0.50%). Interaction effect between reaction time and agitation speed gave highest contribution which was at 18.63%. The equations for the

pH model were showed in Equation 4.1 to 4.4. Factors D (soil to water ratio) and E (soil types) were categoric factor and not included in the equation.

Soil to water ratio: 1:3

Soil types: Peat

$$pH = 8.66075 + 0.88375A - 0.3135B + 4.16667x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad 4.1$$

Soil to water ratio: 1:1

Soil types: Peat

$$pH = 10.09425 + 0.81375A - 0.3135B - 2.0x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad 4.2$$

Soil to water ratio: 1:3

Soil types: Alluvium

$$pH = 8.74045 + 0.88375A - 0.3135B + 4.16667x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad 4.3$$

Soil to water ratio: 1:1

Soil types: Alluvium

$$pH = 10.17425 + 0.81375A - 0.3135B - 2.0x10^{-3}C + 0.024AB - 8.16667x10^{-3}AC + 1.36667x10^{-3}BC \quad 4.4$$

Where A = reaction time, B = temperature and C = agitation speed.

The experimental result for BOD removal of acidic POME was showed in Table 4.3. The initial BOD value of acidic POME before the treatment was 23300 mg/L. From the Table 4.6, the BOD removal was found within the range from 87.70% to 98.98%. The significant effect of each factor on the BOD removal was evaluated by analysis of variance (ANOVA). Results from ANOVA in Table 4.5 shows that the regression model for BOD removal was significant. The coefficient of determination (R^2) value of the BOD removal model was 0.8239.

From ANOVA, reaction time gave the highest contribution which was at 58.49%. This followed by agitation speed (7.54%), temperature (2.45%), soil to water ratio (1.80%) and soil types (0.13%). Interaction effect between reaction time and agitation speed gave highest contribution which was at 5.54%. The equations for the BOD removal model were showed in Equation 4.5 to 4.8. Factors D (soil to water ratio) and E (soil types) were categoric factors and not included in the equations.

Soil to water ratio: 1:3
Soil types: Peat

$$\%\,\text{BOD removal} = 187.0755 - 1.4925A - 1.7B - 0.85117C - 0.241AB + 0.069833AC + 0.017833BC \quad 4.5$$

Soil to water ratio: 1:1
Soil types: Peat

$$\%\,\text{BOD removal} = 189.3395 - 1.4925A - 1.7B - 0.85117C - 0.241AB + 0.069833AC + 0.017833BC \quad 4.6$$

Soil to water ratio: 1:3
Soil types: Alluvium

$$\%\,\text{BOD removal} = 187.9855 - 1.4925A - 1.7B - 0.85117C - 0.241AB + 0.069833AC + 0.017833BC \quad 4.7$$

Soil to water ratio: 1:1

Soil types: Alluvium

$$\%\,\text{BOD removal} = 187.8589 - 1.4925A - 1.7B - 0.85117C - 0.241AB + 0.069833AC + 0.017833BC \quad (4.8)$$

Where A was reaction time, B was temperature and C was agitation speed.

The experimental result for COD removal from acidic POME was showed in Table 4.3. The initial COD value of acidic POME before the treatment was 42100 mg/L. From the Table 4.3, the COD removal was found within the range from 46.75% to 94.42%. The significant effect of each factor on the COD removal was evaluated by analysis of variance (ANOVA). Results from ANOVA in Table 4.6 shows that the regression model for COD removal was significant. The coefficient of determination (R^2) value of the COD removal model was 0.9397.

From ANOVA, reaction time gave the highest contribution which was at 38.64%. This followed by agitation speed (14.90%), soil types (1.24%), temperature (1.21%) and soil to water ratio (0.18%). Interaction effect between reaction time and agitation speed gave highest contribution which was at 16.65%. The equations for the COD removal model were showed in Equation 4.9 to 4.12. Factors D (soil to water ratio) and E (soil type) were categoric factors and not included in the equations.

Soil to water ratio: 1:3

Soil types: Peat

$$\%\,\text{COD removal} = -383.3025 + 95.88A + 5.89225B + 1.67567C - 1.34325AB - 0.32063AC \quad (4.9)$$

Soil to water ratio: 1:1

Soil types: Peat

$$\%\,\text{COD removal} = -399.3895 + 98.45624A + 5.89225B + 1.67567C - 1.3425AB - 0.32063AC \quad (4.10)$$

Soil to water ratio: 1:3

Soil types: Alluvium

$$\%\,COD\,removal = -357.96975 + 95.88A + 5.89225B + 1.67567C - 1.34325AB - 0.32063AC \quad 4.11$$

Soil to water ratio: 1:1

Soil types: Alluvium

$$\%\,COD\,removal = -360.71075 + 98.45624A + 5.89225B + 1.67567C - 1.3425AB - 0.32063AC \quad 4.12$$

Where A was reaction time, B was temperature and C was agitation speed.

Table 4.3 Experimental result on biological pH treatment of acidic palm oil mill effluent (POME)

Run	Factors						Response	
	Reaction Time, day (A)	Temperature, °C (B)	Agitation speed, rpm (C)	Soil to water ratio (D)	Soil types (E)	pH	Percentage BOD removal (%)	Percentage COD removal (%)
1	3	25	150	1:3	Alluvium	7.43	95.18	55.58
2	5	25	150	1:3	Peat	7.95	98.86	86.98
3	3	30	150	1:3	Peat	7.22	95.63	70.76
4	5	30	150	1:3	Alluvium	8.05	98.52	80.86
5	3	25	180	1:3	Peat	7.91	87.70	80.78
6	5	25	180	1:3	Alluvium	7.89	98.53	81.57
7	3	30	180	1:3	Alluvium	7.85	91.91	80.36
8	5	30	180	1:3	Peat	8.09	98.91	86.37
9	3	25	150	1:1	Peat	7.61	93.36	46.75
10	5	25	150	1:1	Alluvium	8.06	98.98	94.42
11	3	30	150	1:1	Alluvium	7.53	92.90	70.78
12	5	30	150	1:1	Peat	8.00	99.55	78.69
13	3	25	180	1:1	Alluvium	7.85	86.56	79.57
14	5	25	180	1:1	Peat	7.71	98.47	85.18
15	3	30	180	1:1	Peat	7.88	92.75	78.03
16	5	30	180	1:1	Alluvium	8.11	98.61	85.75
17	4	27.5	165	1:3	Peat	7.50	90.62	70.03
18	4	27.5	165	1:1	Peat	7.90	98.91	77.36
19	4	27.5	165	1:3	Alluvium	7.60	92.13	74.92
20	4	27.5	165	1:1	Alluvium	8.20	98.59	80.59

Table 4.4 ANOVA for biological pH treatment of acidic POME

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	1.07	10	0.11	3.94	0.0321
A -Reaction time	0.42	1	0.42	15.3	0.0045
B -Temperature	6.4 x 10^{-3}	1	6.4 x10^{-3}	0.24	0.6406
C -Agitation speed	0.13	1	0.13	4.77	0.0606
D -Soil to water ratio	0.092	1	0.092	3.4	0.1024
E -Soil types	0.032	1	0.032	1.18	0.3096
AB	0.058	1	0.058	2.12	0.1836
AC	0.24	1	0.24	8.83	0.0178
AD	0.02	1	0.02	0.72	0.4205
BC	0.042	1	0.042	1.55	0.249
CD	0.034	1	0.034	1.26	0.2944
Residual	0.22	8	0.027		
Cor Total	1.29	19			
Std. Dev.	0.16		R-Squared		0.8311
Mean	7.82		Adj R-Squared		0.6199
C.V. %	2.11		Pred R-Squared		0.2990
PRESS	0.90		Adeq Precision		7.367

Table 4.5 ANOVA for BOD removal from acidic POME

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	260.63	9	28.96	4.68	0.0156
A -Reaction time	185.23	1	185.23	29.93	0.0004
B -Temperature	7.76	1	7.76	1.25	0.2919
C -Agitation speed	23.86	1	23.86	3.86	0.0812
D -Soil to water ratio	5.71	1	5.71	0.92	0.3617
E -Inoculum types	0.41	1	0.41	0.07	0.8036
AB	5.81	1	5.81	0.94	0.3580
AC	17.56	1	17.56	2.84	0.1264
BC	7.16	1	7.16	1.16	0.3102
DE	7.14	1	7.14	1.15	0.3107
Curvature	0.37	1	0.37	0.06	0.8130
Residual	55.69	9	6.19		
Cor Total	316.69	19			
Std. Dev.	2.49		R-Squared		0.8239
Mean	95.33		Adj R-Squared		0.6479
C.V. %	2.61		Pred R-Squared		0.3640
PRESS	201.42		Adeq Precision		6.995

Table 4.6 ANOVA for COD removal from acidic POME

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob > F
Model	2077.04	10	207.7	12.47	0.0008
A -Reaction time	858.64	1	858.64	51.53	< 0.0001
B -Temperature	26.96	1	26.96	1.62	0.2391
C -Agitation speed	331.15	1	331.15	19.87	0.0021
D -Soil to water ratio	3.97	1	3.97	0.24	0.6386
E -Inoculum types	27.54	1	27.54	1.65	0.2345
AB	180.43	1	180.43	10.83	0.0110
AC	370.08	1	370.08	22.21	0.0015
AD	26.55	1	26.55	1.59	0.2424
CE	29.08	1	29.08	1.75	0.2230
DE	222.64	1	222.64	13.36	0.0064
Curvature	11.88	1	11.88	0.71	0.4230
Residual	133.3	8	16.66		
Cor Total	2222.22	19			
Std. Dev.	4.08		R-Squared		0.9397
Mean	77.27		Adj R-Squared		0.8643
C.V. %	5.28		Pred R-Squared		0.7301
PRESS	599.73		Adeq Precision		14.320

4.2.2 Main Effect Analysis on Biological Treatment of Acidic POME

The contribution for each factor on biological treatment of acidic POME was presented in Table 4.7 (pH), Table 4.8 (BOD removal) and Table 4.9 (COD removal). Reaction time gave highest contribution which were 32.27% (pH treatment), 58.49% (BOD removal) and 38.64% (COD removal). Experiments were carried out by varying the reaction time from 3 days to 5 days. Low pH value was detected at a short reaction time. It had been observed that pH, BOD removal and COD removal were changing during the reaction time. The changing of pH value was affected by population growth of microbes in the treatment process (Yan et al., 2010). The changing in BOD removal and COD removal were affected by biodegradation capabilities and degradation rates of microbes during the treatment (Chan, et al., 2010). The growth of microbes in the treatment was related with the reaction time. Reaction time gave essential time for the

microbes to multiply during the treatment process thus increase its growth rate. Microbes in inoculum can bind enzymes and organisms thus affecting the movement of cells through the inoculum and the breakdown of organic matter (Parraga et al., 1998).

Agitation speed gave contribution at about 10.05% (pH treatment), 7.54% (BOD removal) and 14.90% (COD removal). Experiments were carried out by varying the agitation speed from 150 rpm and 180 rpm. Agitation speed plays an important role in biological POME treatment. It ensures a proper mixing of substrate and inoculum. According to the Lamed et al., (1988) microbial activities increased in a stirred culture thus increased the biodegradation capabilities of microbes in BOD removal. Biodegradation was the process in which organic substances were broken down into smaller compounds.

Temperature gave the lowest contribution in pH treatment which was 0.50%. In this study experiments were carried out at temperature of 25 °C and 30 °C (in range of mesophilic temperature). According to Lin et al. (2008), the microbial activity was low at mesophilic range (30-40 °C) but was efficient and high at thermophilic range (50-55 °C). However, the used of temperature in mesophilic range were conducted in this study to adjusting the treatment system near to ambient temperature so that the operation cost can be reduced. This affected the microbial performance thus reducing the microbial capability in acidic POME treatment. This condition makes temperature gave less contribution in pH treatment of acidic POME. Soil types gave the lowest contribution in BOD removal which was 0.13%. This showed soil types gave less impact toward BOD removal from acidic POME. According to George (1999), soil texture and structure gave impact towards BOD removal. Soil texture consists of clay, silt, fine and coarse sand. Finely textures soils (those having more clay and silt) help in removing the incoming pollutants. By referring to the Table 4.2, the smaller differences in soil texture can be seen. This makes soil types gave less impact towards BOD removal. Soil to water ratio gave the lowest contribution in COD removal which was 0.18%. According to Rasdi et al., (2009), soil to water ratio or soil concentration affected microbial growth rate. In this study experiments were carried out by using two different soils to water ratio which were 1:1 and 1:3. The small differences in the ratio gave similar effect on microbial growth rate. This condition makes the soil to water ratio gave less contribution in reducing the COD value.

Table 4.7 The percentage contribution of each factors and their interaction in pH treatment

Term	Effect	Sum Sqr	% Contribution
A-Reaction time	0.323	0.416	32.27
B-Temperature	0.040	0.006	0.50
C-Agitation speed	0.180	0.130	10.05
D-Soil to water ratio	0.152	0.092	7.17
E-Soil types	0.089	0.032	2.48
AB	0.120	0.058	4.47
AC	-0.245	0.240	18.63
AD	-0.070	0.020	1.52
BC	0.103	0.042	3.26
CD	-0.093	0.034	2.66

Table 4.8 The percentage contribution of each factors and their interaction in BOD removal

Term	Effect	Sum Sqr	% Contribution
A-Reaction time	6.81	185.23	58.49
B-Temperature	1.39	7.76	2.45
C-Agitation speed	-2.44	23.86	7.54
D-Soil to water ratio	1.20	5.71	1.80
E-Soil types	-0.32	0.41	0.13
AB	-1.21	5.81	1.83
AC	2.09	17.56	5.54
BC	1.34	7.16	2.26
DE	-1.34	7.14	2.25

Table 4.9 The percentage contribution of each factors and their interaction in COD removal

Term	Effect	Sum Sqr	% Contribution
A-Reaction time	14.65	858.64	38.64
B-Temperature	2.60	26.96	1.21
C-Agitation speed	9.10	331.15	14.90
D-Soil to water ratio	1.00	3.97	0.18
E-Soil types	2.62	27.54	1.24
AB	-6.72	180.43	8.12
AC	-9.62	370.08	16.65
AD	2.58	26.55	1.19
CE	-2.70	29.08	1.31
DE	7.46	222.64	10.02

4.2.3 Interaction Effects in pH Treatment

The interaction effect between reaction time and agitation speed gave highest contribution which was 18.63%. The interaction graph between reaction time and agitation speed was presented in Figure 4.1. From Figure 4.1, it shows that the pH value was directly proportional with reaction time and agitation speed. In anaerobic process, pH and reaction time were interacting to each other. It was an effective method to increase the microbial performance (Liu et al., 2008) due to the microbes that performed in anaerobic condition. According to Prasertan et al., (2009), microbial performance was decreased as reaction time decreased. This was due to the microbial growth rate during the treatment process. The microbial activities were increased in the stirred culture thus it also directly proportional with agitation speed (Lamed et al., 1988). Thus at higher agitation speed the pH value was increased as the microbial activities increased the treatment performance.

At reaction time of day three, the pH value was high at agitation speed of 180 rpm compared to the agitation speed at 150 rpm. Agitation helps to speed up the activity of microorganism (Clark et al., 2012). Thus higher agitation speed provides a better mixing to the treatment. Figure 4.1 also shows the interaction of reaction time and agitation speed at day five. The similar pH value could be observed at agitation speed 180 rpm and 150 rpm. This was due to population of microbes that achieved its maximum rate at reaction time of five days and complete reaction was done at five day of reaction time.

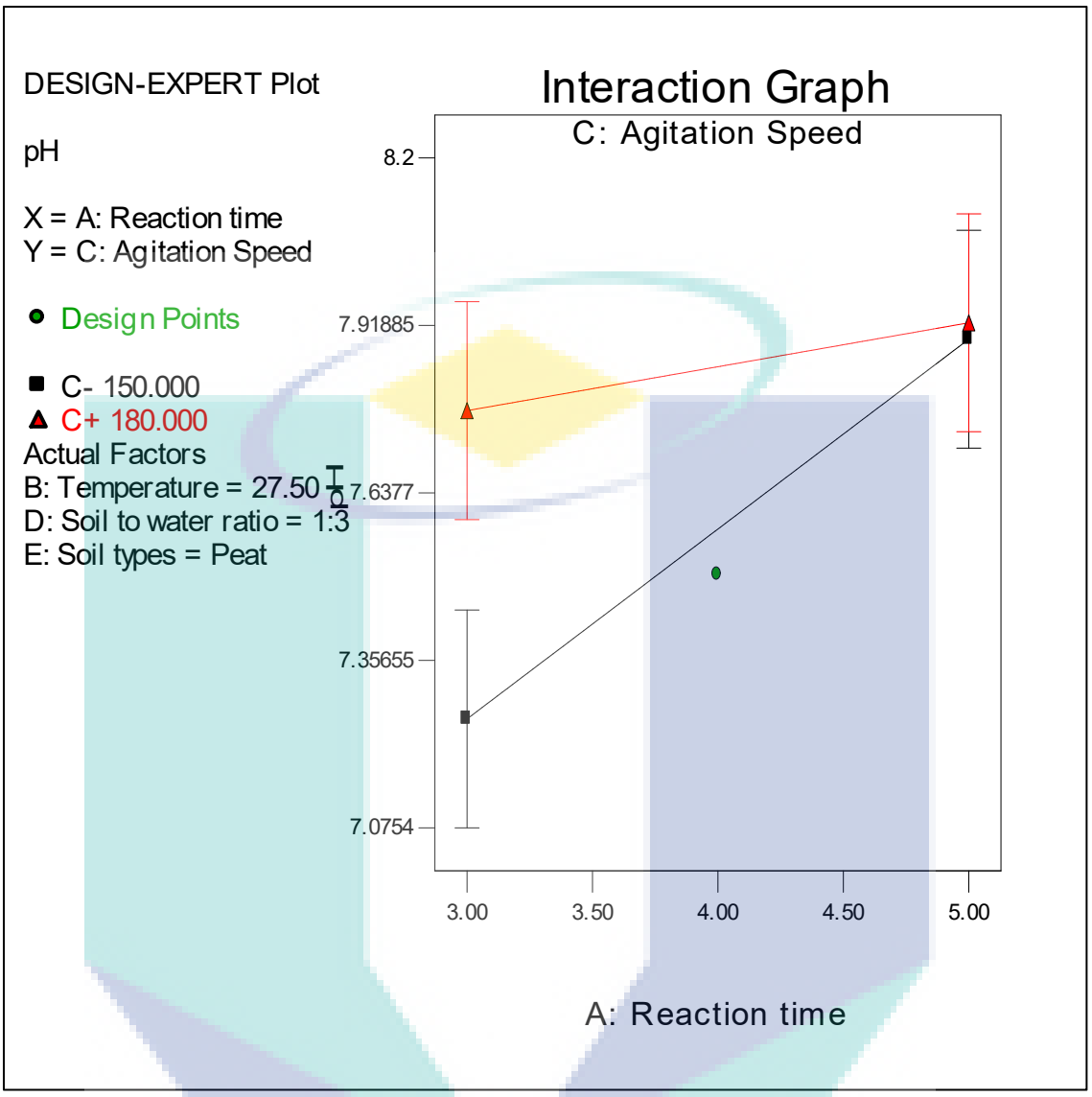

Figure 4.1 Interaction graph between reaction time and agitation speed for biological pH treatment

The effect of interaction between temperature and agitation speed for biological pH treatment was presented in Figure 4.2. From Figure 4.2, it shows that pH value was directly proportional to the temperature and agitation speed. The pH value was increased gradually with temperature at agitation speed of 180 rpm. At agitation speed of 150 rpm, the pH value was decreased gradually with addition of temperature.

According to Wang et al., (2008), pH value was decreased with increasing of temperature but with addition of agitation it increase the final pH value. This situation can be seen in Figure 4.2 where pH value was increased at agitation speed of 180 rpm and decreased at agitation speed of 150 rpm. Differences in agitation speed provide significant impact on microbial performance during the biological pH treatment. This was confirmed by Kaparaju et al., (2009) that different agitation speed used affects the microbial performance. In order to treat wastewater biologically, temperature plays an important rules where higher temperature was not suitable for microbial growth (Wang et al., 2008). Agitation was required in microbial growth where it speeds up the microbial activities and increased its performance.

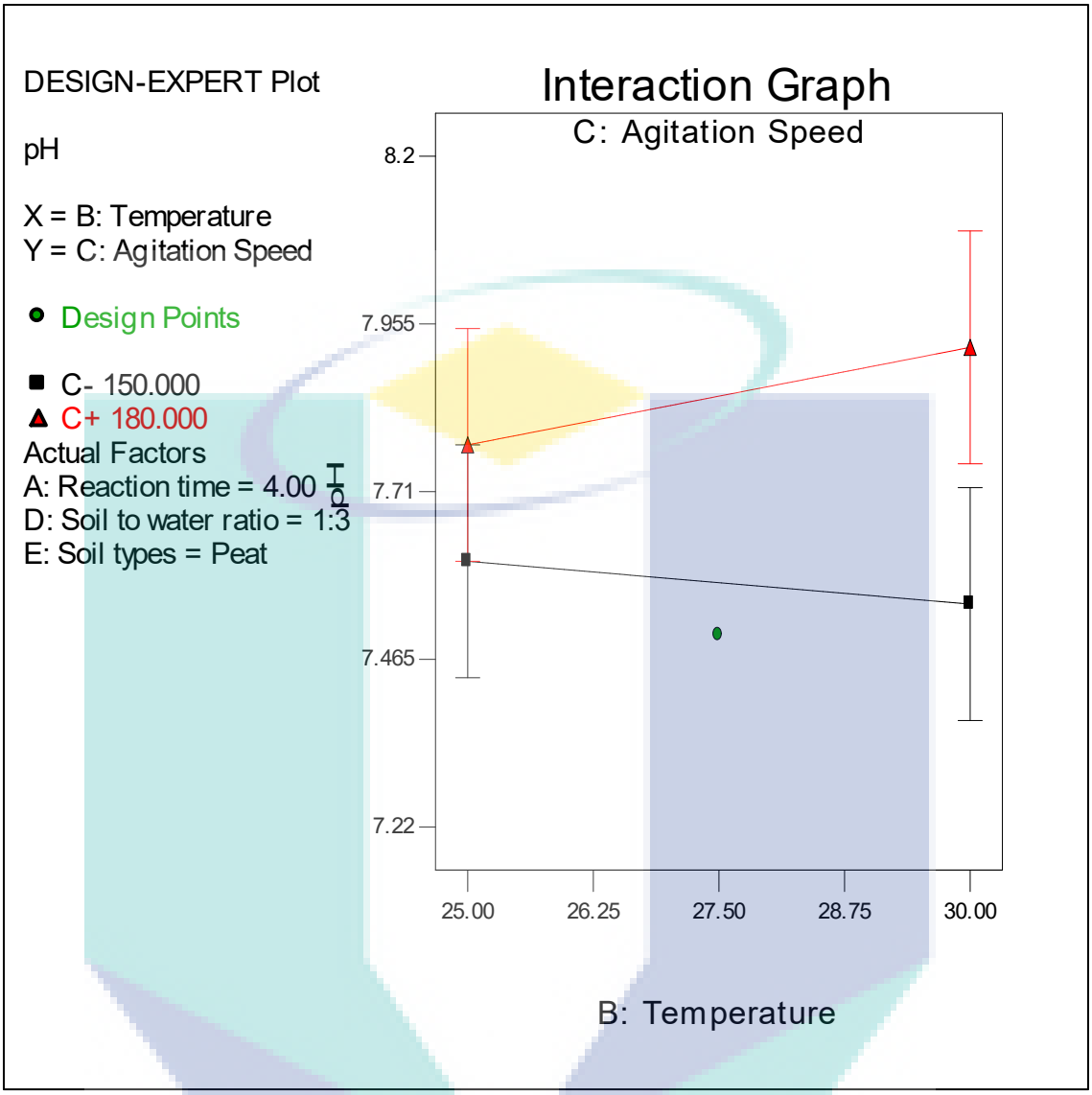

Figure 4.2 Interaction graph between temperature and agitation speed for biological pH treatment

The effect of interaction between agitation speed and soil to water ratio (s/w) for biological pH treatment was presented in Figure 4.3. From Figure 4.3, it shows that pH value was directly proportional with agitation speed and s/w. The s/w also can be known as soil concentration. Higher soil concentration contain higher amount of microbes. The addition of agitation increased the microbial performance during the treatment process (Lamed et al., 1988) by increasing the microbial activities and microbial growth rate.

At agitation speed of 150 rpm the pH value was high at s/w 1:1 compared to s/w 1:3. This situation happens due to large amount of microbes that exists in the s/w 1:1. At s/w 1:1, the soil concentration was higher compared to the s/w at 1:3. It carried large amount of microbes that was used in the treatment (Rasdi et al., 2009). These microbes were used in biological pH treatment to break down the waste water properties. It was observed that at agitation speed of 180 rpm, the pH value was almost same for both s/w. It was expected that the reaction between microbes in inoculum and substrate had been completed.

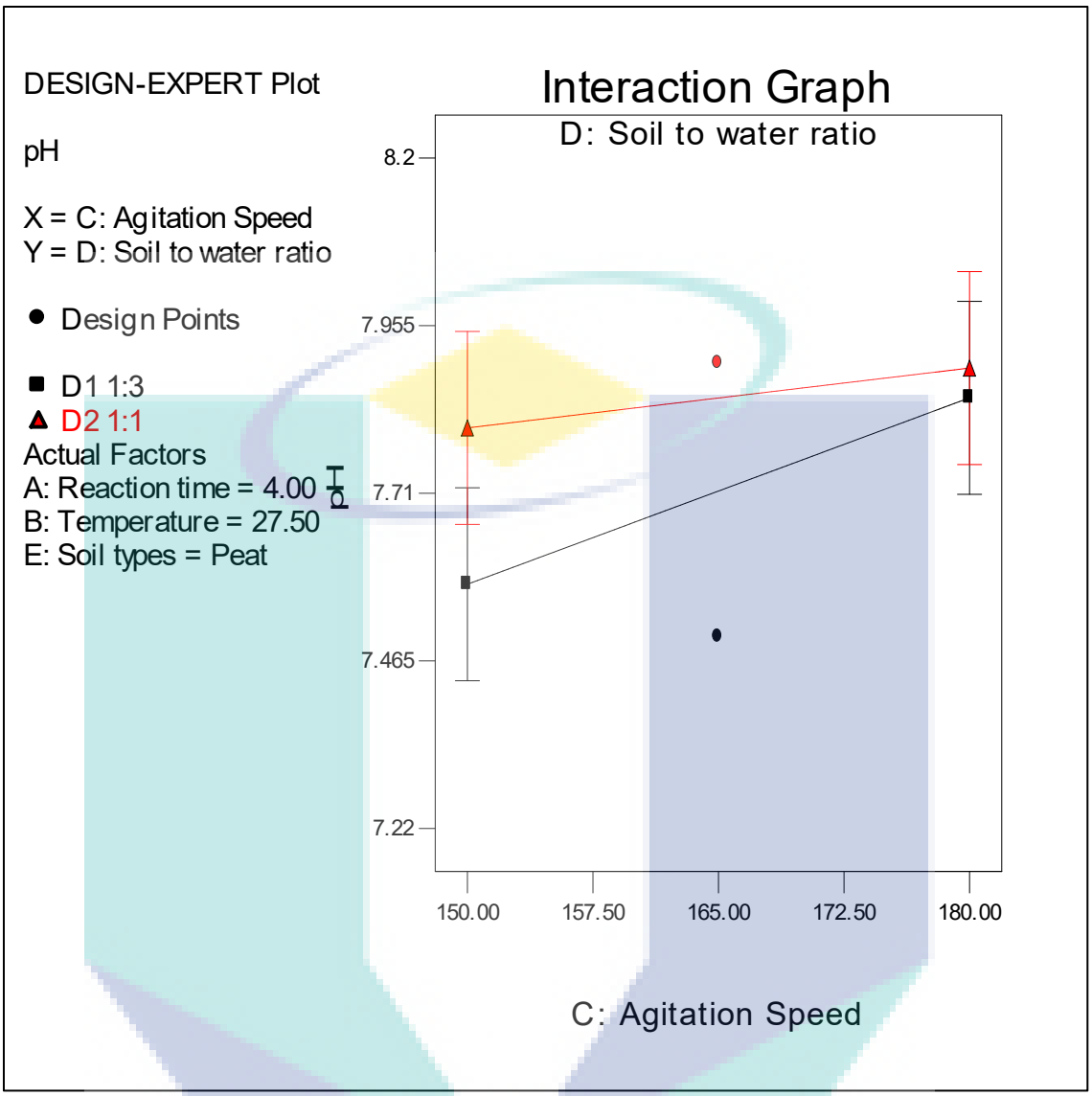

Figure 4.3 Interaction graph between agitation speed and soil to water ratio for biological pH treatment

4.2.4 Interaction Effects in Biochemical Oxygen Demand (BOD) Removal

The interaction effect between reaction time and agitation speed gave the highest contribution which was at 58.49%. The interaction graph for reaction time and agitation speed was presented in Figure 4.4. From Figure 4.4, it shows that the BOD removal was directly proportional with reaction time and agitation speed. The BOD value depends on the dissolved organic matter in wastewater. The microbes use organic matter as the sources of food through oxidation in which oxygen was consumed. The demand of oxygen to degrade this organic matter was based on the amount of organic matter present in the wastewater. Reaction time gave essential time for microbes to utilize the organic matter that presents in wastewater.

At low reaction time (3 days), the BOD removal was higher at agitation speed of 150 rpm compared to the agitation speed of 180 rpm. Addition of agitation reduces the BOD removal in acidic POME treatment. According to Devi and Dahiya (2008), at higher agitation speed the microbes began to lose their bonding to each other. This makes the microbial activities in wastewater decreased thus reduces the BOD removal. Figure 4.4 also shows the interaction of reaction time and agitation speed at day five. The almost similar BOD removal could be observed at agitation speed of 180 rpm and 150 rpm. At high reaction time (5 days), addition of agitation does not affect the BOD removal. This showed the microbes in wastewater achieved its maximum capabilities in degrading the organic matter (Agamuthu et al., 2013).

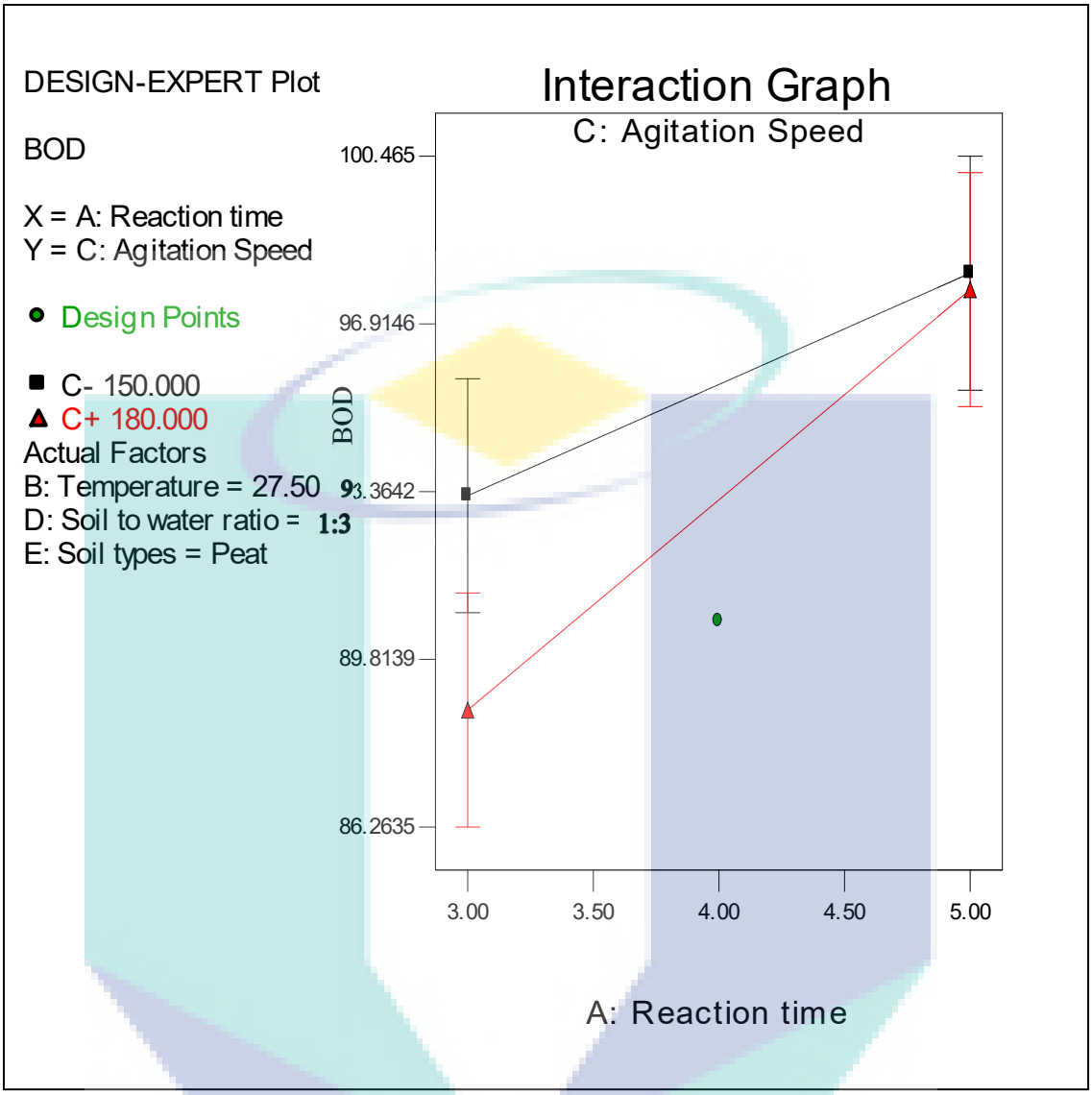

Figure 4.4 Interaction graph between reaction time and agitation speed for BOD removal

The effect of interaction between temperature and agitation speed for biological pH treatment was presented in Figure 4.5. From Figure 4.5, it shows that BOD removal was directly proportional with the temperature and agitation speed. The BOD removal was gradually increased with temperature at agitation speed of 180 rpm and 150 rpm. Most biological process speed up as the temperature increases and slow down as the temperature drops. Oxygen utilization was caused by the metabolism of microbes and it was similarly affected by temperature (Mackenzie and David, 2008).

It was reported from previous study that BOD removal was decreased with increasing of agitation (Devi and Dahiya, 2008). The situation can be seen in Figure 4.5 where BOD removal was higher at agitation speed of 150 rpm compared with the agitation speed of 180 rpm. Agitation provided proper mixing between substrate and inoculum. According to Mukataka et al., (1983), the excessively high mixing speed reduced the interaction between substrate and inoculum. The reduction in interaction affects the microbial activities in degrading the organic matter. This condition gave less impact towards BOD removal.

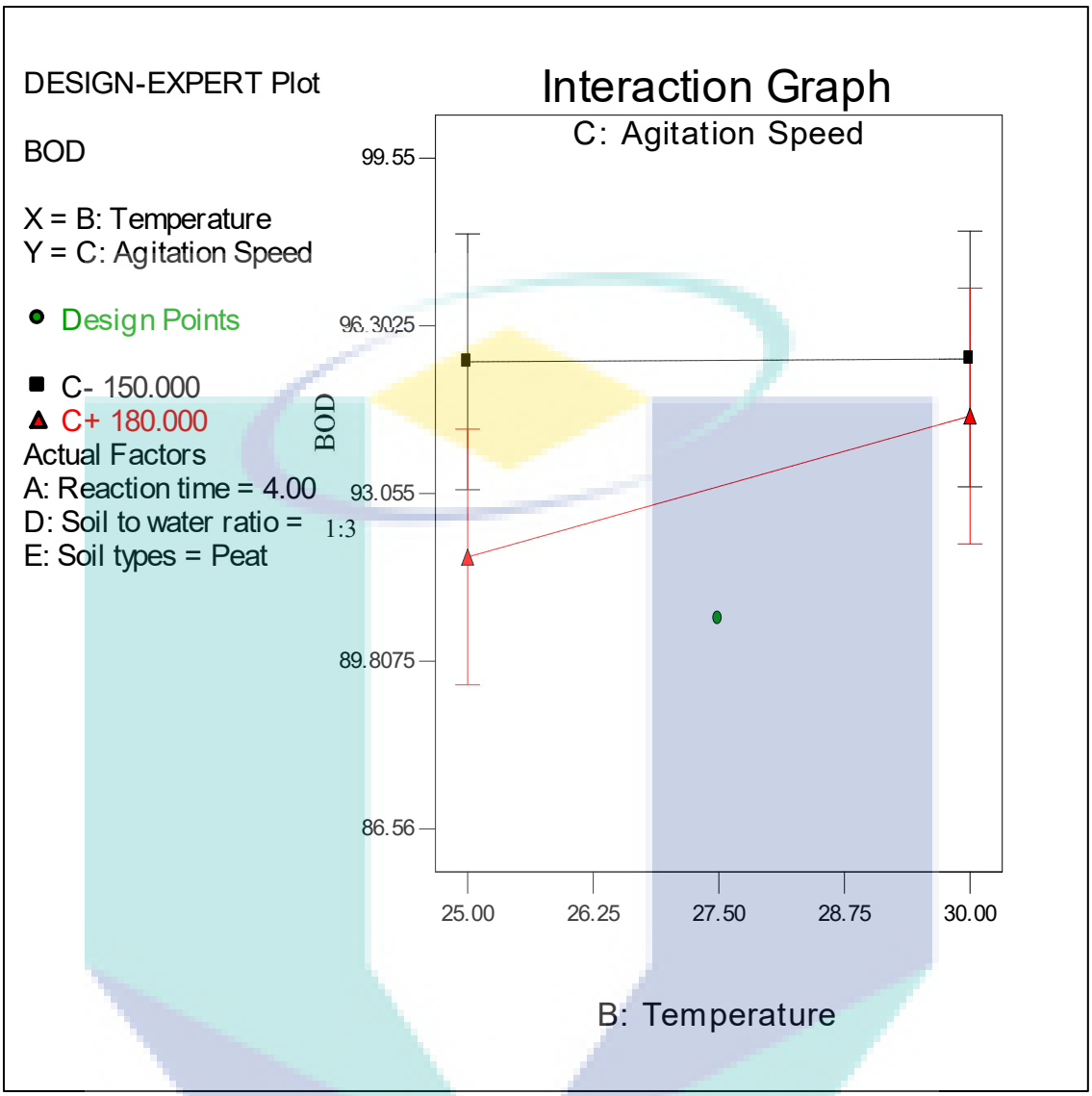

Figure 4.5 Interaction graph between temperature and agitation speed for BOD removal

4.2.5 Interaction Effects in Chemical Oxygen Demand (COD) Removal

The interaction effect between reaction time and agitation speed gave the highest contribution which was at 16.65%. The interaction graph for reaction time and agitation speed was presented in Figure 4.6. From Figure 4.6, it shows that the COD removal was directly proportional with reaction time and agitation speed. The COD value depends on the decomposition of organic matter and the oxidation of inorganic chemicals. Thus the COD value will be greater than BOD value. In this study, reaction time gave essential time for the microbes to utilize the both organic and inorganic matter that presents in wastewater. According to Prasertsan et al., (2009), microbial activity rate decreased as reaction time decreased. At higher reaction time microbial activity rate increased and improve the treatment process.

At low reaction time (3 days), the COD removal was higher at agitation speed of 180 rpm compared to the agitation speed of 150 rpm. According to Clark et al., (2002), mixing was the possible option to speed up the microbial activities. The addition of agitation provides essential mixing for the substrate and inoculum. The increasing in microbial activities contributed towards the capabilities and ability of microbes to decompose the organic matter and oxidation of inorganic matter. This condition contributed on the COD reduction. Figure 4.6 also shows the interaction of reaction time and agitation speed at day five. The almost similar COD removal could be observed at agitation speed of 180 rpm and 150 rpm. At high reaction time (5 days), addition of agitation gave less impact in the COD removal. Agitation enhanced the interaction between substrate and inoculum (Noorshamsiana et al., 2013). At high reaction time, the complete reaction between substrate and inoculum can be seen. Therefore, agitation did not give significant impact toward the process. This was confirmed by similar COD removal achieved at high reaction time. This showed the microbes in wastewater achieved its maximum capabilities in decomposing the organic and inorganic matter (Agamuthu et al., 2013).

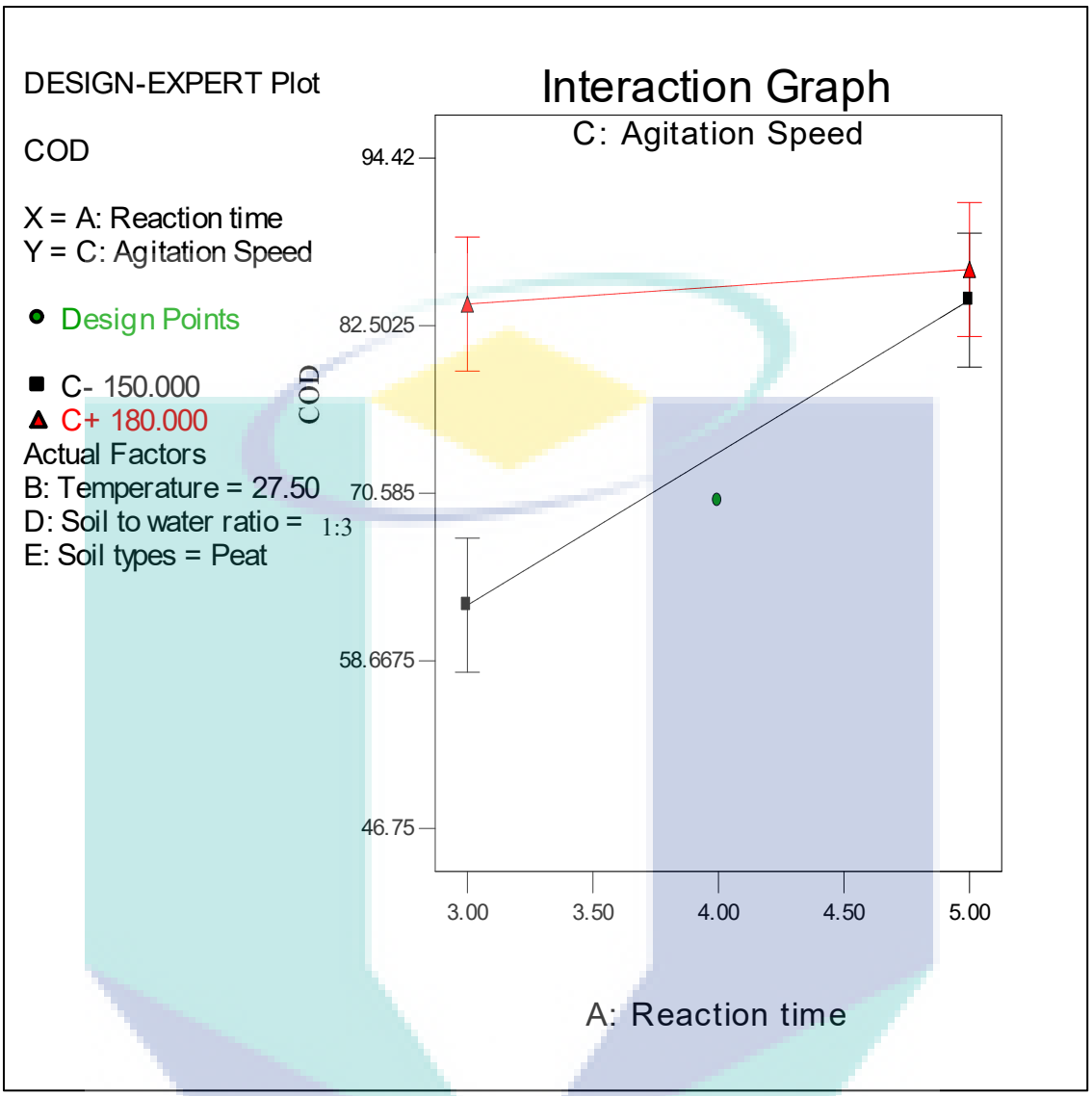

Figure 4.6 Interaction graph between reaction time and agitation speed for COD removal

The effect of interaction between reaction time and soil to water ratio for COD removal was presented in Figure 4.7. From Figure 4.7, it shows that COD removal was directly proportional with the reaction time and soil to water ratio. At low soil to water ratio (1:3), higher COD removal could be observed. Soil to water ratio also known as soil concentration. At low soil concentration, the soil microbes tend to consumed more organic matter. This contributed in increasing the microbial activities. This can affect the microbial performance in reducing the COD value.

It was reported from previous study that COD removal was increased with increasing of reaction time (Prasertsan et al., 2009). Reaction time gave essential time for the microbes to decompose the organic matter and oxidized the inorganic matter. At high reaction time (5 days) the almost similar COD removal can be observed at soil to water ratio 1:1 and 1:3.

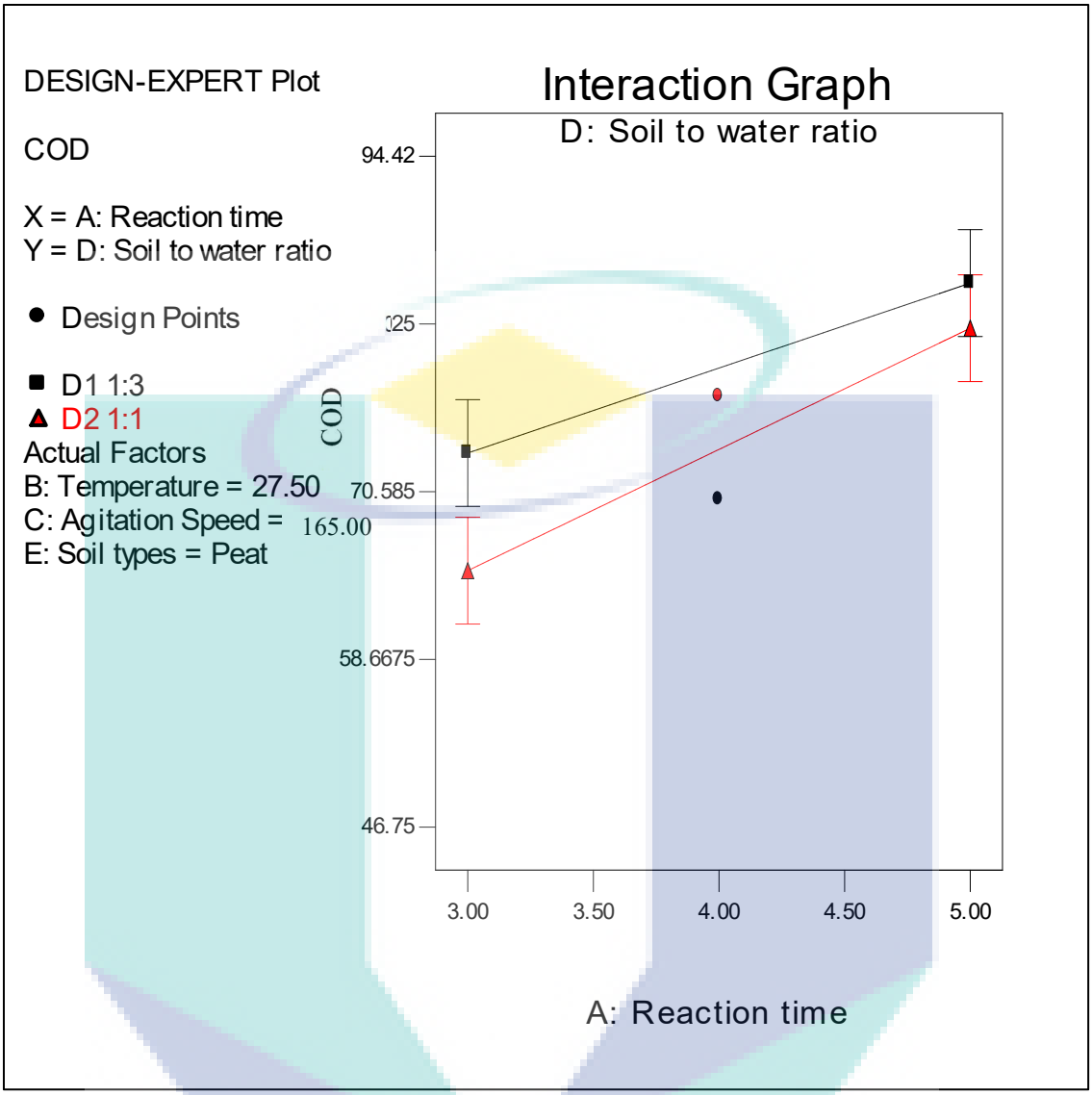

Figure 4.7 Interaction graph between reaction time and soil to water ratio for COD removal

4.3 Optimization Process on Biological Acidic Palm Oil Mill Effluent (POME) Treatment

4.3.1 Analysis on Biological Acidic POME Treatment

The experimental design for optimization process on biological treatment of acidic palm oil mill effluent (POME) was performed by using central composite design (CCD). The Design Expert software (Version 6.0) was used in analyzing the selected factors. These factors were reaction time (A) and agitation speed (B). Three factors from factorial analysis were fixed at temperature (30 °C), soil to water ratio (1:1) and soil types (alluvium). The optimization process was performed to determine the optimum condition of the process. The experimental responses in this study were pH, biochemical oxygen demand (BOD) removal and chemical oxygen demand (COD) removal.

The experimental result for biological pH treatment of acidic POME was showed in Table 4.10. From the table, the pH value was found within the range from 7.54 to 8.14. High pH value was obtained at the center point level. The significant effect of each factor on the pH value was evaluated by analysis of variance (ANOVA). Results from ANOVA in Table 4.11 shows that the regression model for biological pH treatment was significant. The coefficient of determination (R^2) value of the pH model was 0.8326. The equation for the pH model was showed in Equation 4.13.

$$pH = -15.5713 + 1.40661A + 0.25933B - 0.12233A^2 - 8.18319x10^{-4}B^2 - 1.0x10^{-3}AB \quad 4.13$$

Where A was reaction time and B was agitation speed. A and B were referred as the main effect while AB was the interaction involved in pH treatment. Quadratic effects were presented by A^2 and B^2 to indicate the presence of the curvature in the model.

The experimental result for BOD removal from acidic POME was showed in Table 4.10. From the table, the BOD removal was found within the range from 98.44% to 99.22%. The significant effect of each factor on the BOD removal was evaluated by analysis of variance (ANOVA). Results from ANOVA in Table 4.12 shows that the regression model for BOD removal was significant. The coefficient of determination

(R^2) value of the BOD removal model was 0.8991. The equation for the BOD removal model was showed in Equation 4.14.

$$\text{BOD removal}(\%) = 61.78858 + 2.70477A + 0.39061B - 0.30181A^2 - 1.34203x10^{-3}B^2 + 4.0x10^{-3}AB \quad 4.14$$

Where A was reaction time and B was agitation speed. A and B were referred as the main effect while AB was the interaction involved in BOD removal. Quadratic effects were presented by A^2 and B^2 to indicate the presence of the curvature in the model.

The experimental result for COD removal from acidic POME was showed in Table 4.10. From the table, the COD removal was found within the range from 77.55% to 81.90%. The significant effect of each factor on the COD removal was evaluated by analysis of variance (ANOVA). Results from ANOVA in Table 4.13 shows that the regression model for COD removal was significant. The coefficient of determination (R^2) value of the COD removal model was 0.8278. The equation for the COD removal model was showed in Equation 4.15.

$$\text{COD removal}(\%) = -268.2501 + 50.05943A + 2.96396B - 3.10328A^2 - 7.68319x10^{-3}B^2 - 0.126AB \quad 4.15$$

Where A was reaction time and B was agitation speed. A and B were referred as the main effect while AB was the interaction involved in BOD removal. Quadratic effects were presented by A^2 and B^2 to indicate the presence of the curvature in the model.

Table 4.10 Experimental result on biological acidic POME treatment

Run	Factors			Response	
	Reaction Timeday	Agitation Speed, rpm	pH	BOD removal (%)	COD removal (%)
1	5	130	7.54	98.44	77.55
2	6	150	8.00	99.22	78.15
3	4	150	7.99	98.44	79.22
4	5	150	8.12	99.14	81.83
5	4.5	140	8.01	98.91	78.88
6	5	150	8.13	99.14	81.81
7	4.5	160	8.05	99.04	79.55
8	5.5	140	8.11	98.95	81.59
9	5	170	8.04	98.75	79.88
10	5	150	8.14	99.15	81.90
11	5	150	8.12	99.13	81.71
12	5	150	8.10	99.13	81.59
13	5	160	8.13	99.16	79.74

Table 4.11 ANOVA for pH treatment of acidic POME

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.25	5	0.050	6.96	0.0121
A -Reaction time	$3.33x10^{-3}$	1	$3.33x10^{-3}$	0.46	0.5190
B -Agitation speed	0.094	1	0.094	12.95	0.0088
A^2	0.021	1	0.021	2.96	0.1289
B^2	0.15	1	0.15	21.22	0.0025
AB	$1.0x10^{-4}$	1	$1.0x10^{-4}$	0.014	0.9097
Residual	0.051	7	$7.232x10^{-3}$		
Lack of Fit	0.050	3	0.017	75.37	0.0006
Pure Error	$8.8x10^{-4}$	4	$2.2x10^{-4}$		
Cor total	0.30	12			
Std. Dev.	0.085		R-Squared		0.8326
Mean	8.04		Adj R-Squared		0.7131
C.V. %	1.06		Pred R-Squared		-0.5378
PRESS	0.47		Adeq Precision		8.723

Table 4.12 ANOVA for BOD removal from acidic POME

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	0.77	5	0.15	12.47	0.0022
A -Reaction time	0.25	1	0.25	19.90	0.0029
B -Agitation speed	0.077	1	0.077	6.20	0.0416
A^2	0.13	1	0.13	10.53	0.0142
B^2	0.41	1	0.41	33.31	0.0007
AB	1.6 x 10^{-3}	1	1.6 x 10^{-3}	0.13	0.7299
Residual	0.087	7	0.012		
Lack of Fit	0.086	3	0.029	411.61	< 0.0001
Pure Error	2.8 x 10^{-4}	4	7.0 x 10^{-5}		
Cor total	0.86	12			
Std. Dev.	0.11		R-Squared		0.8991
Mean	98.97		Adj R-Squared		0.8270
C.V. %	0.11		Pred R-Squared		0.0622
PRESS	0.81		Adeq Precision		9.661

Table 4.13 ANOVA for COD removal from acidic POME

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	23.92	5	4.78	6.73	0.0133
A -Reaction time	0.048	1	0.048	0.068	0.8022
B -Agitation speed	1.01	1	1.01	1.42	0.2723
A^2	13.79	1	13.79	19.40	0.0031
B^2	13.53	1	13.53	19.03	0.0033
AB	1.59	1	1.59	2.23	0.1787
Residual	4.98	7	0.71		
Lack of Fit	4.92	3	1.64	112.92	0.0003
Pure Error	0.058	4	0.015		
Cor total	28.90	12			
Std. Dev.	0.84		R-Squared		0.8278
Mean	80.26		Adj R-Squared		0.7048
C.V. %	1.05		Pred R-Squared		-0.7081
PRESS	49.36		Adeq Precision		6.378

4.3.2 Response Surface Plot in Biological Acidic POME Treatment

Figure 4.8 and 4.9 showed the contour and three-dimensional response surface plots for biological pH treatment. From the response surface plot, the pH value reached the maximum level at 5 days of reaction time and agitation speed of 150 rpm. The pH value was increased as the reaction time increased and decreased when complete reaction occurs in the process. According to Yan et al., 2010, the changing in pH value was affected by growth of microbes in the treatment process. The growth of microbes in the treatment was related with the reaction time. Reaction time gave essential time for the microbes to multiply during the treatment process thus increase its growth rate. According to Parraga et al., 1998, microbes that exist in inoculum can bind organisms thus affecting the movement of cells through the inoculum and the breakdown of organic matter. The microbial movement activity increases the microbial population growth and its performance in rising up the pH value.

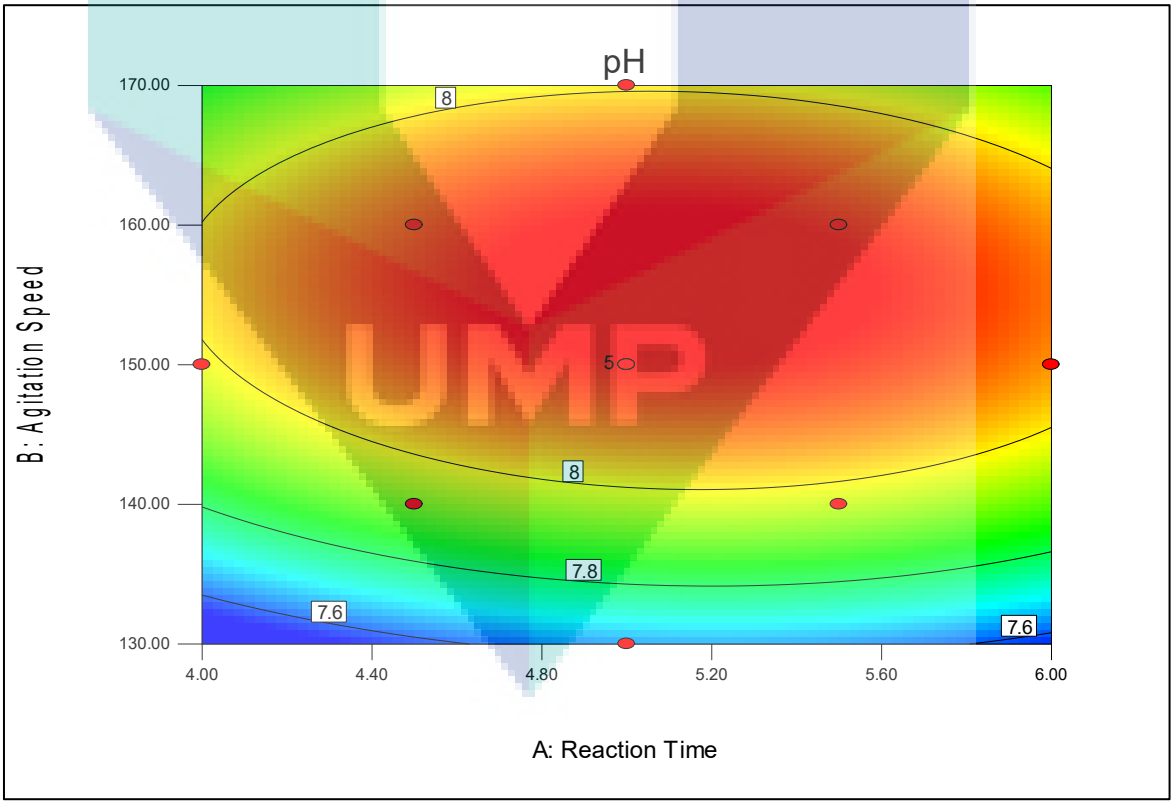

Figure 4.8 Contour plot for pH treatment

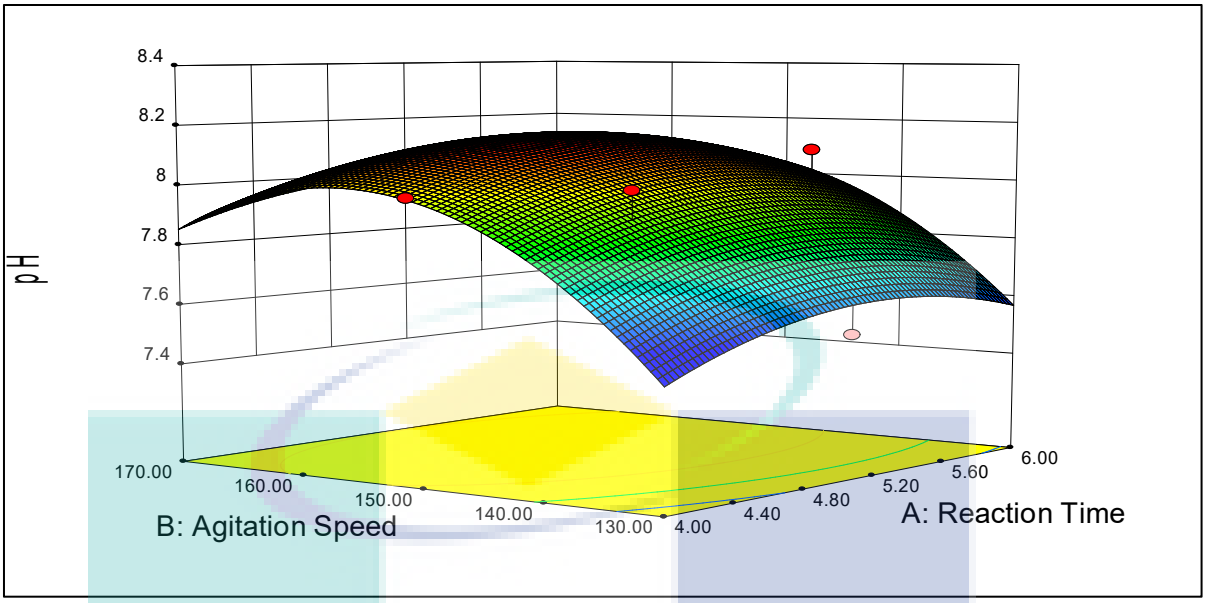

Figure 4.9 3D response surface plot for pH treatment

Figure 4.10 and 4.11 showed the contour and three-dimensional response surface plots for BOD removal. From the response surface plot, the BOD removal reached the constant value at 5 days of reaction time and agitation speed of 150 rpm. In this study, the BOD removal was changing during the reaction time. It had been observed that BOD removal was increased with increasing of reaction time. According to Chan et al., 2010, the biodegradation capabilities of microbes affect the BOD removal. Thus high reaction time needed to degrade the organic matter exists in the wastewater.

In this study, the experiments were carried out by varying the agitation speed from 130 to 170 rpm. From the Figure 4.11, the BOD removal increased with increasing of agitation speed and slightly decreased when agitation reached 170 rpm. The microbial activities increased in a stirred condition (Lamed et al., 1988). The increasing in microbial activities helps in increasing the biodegradation capabilities of microbes in the BOD removal process. In this study, the addition of agitation reduces the BOD removal. According to Devi et al., (2008), at high agitation speed the microbes started to lose their interaction to each other. This makes the microbial activities in wastewater decreased thus reduces the BOD removal.

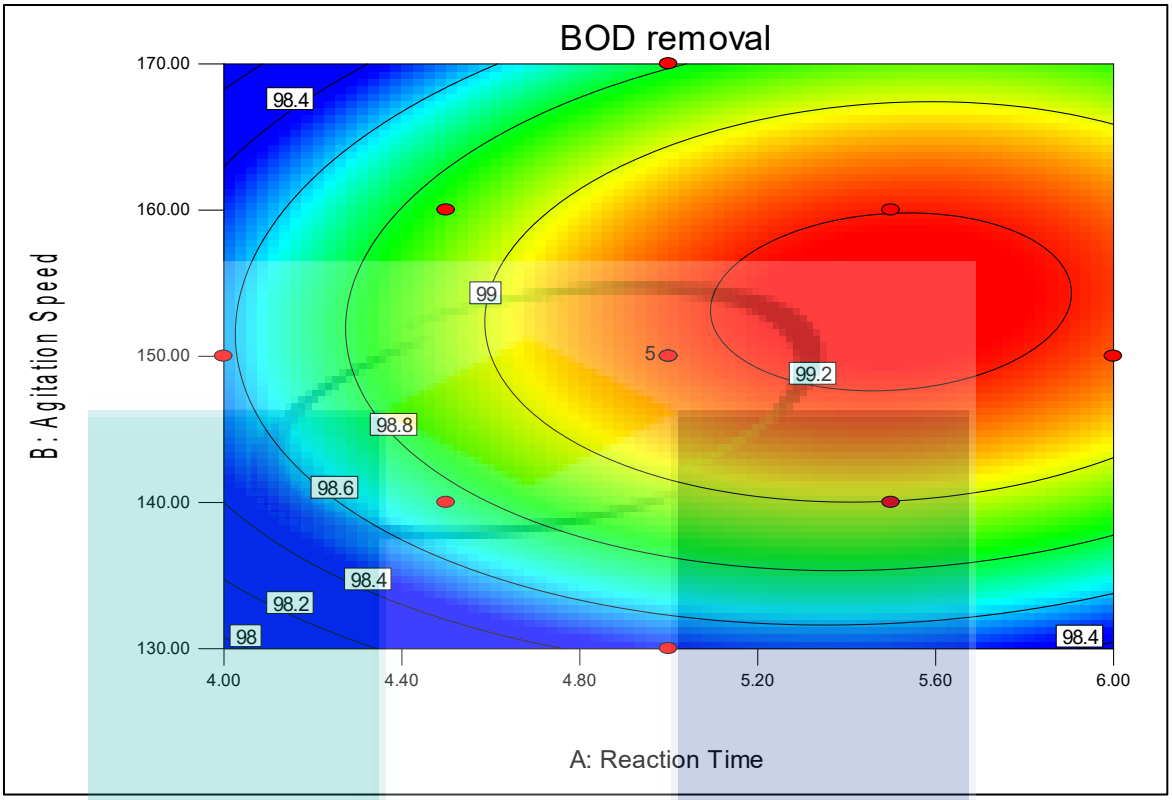

Figure 4.10 Contour plot for BOD removal from acidic POME

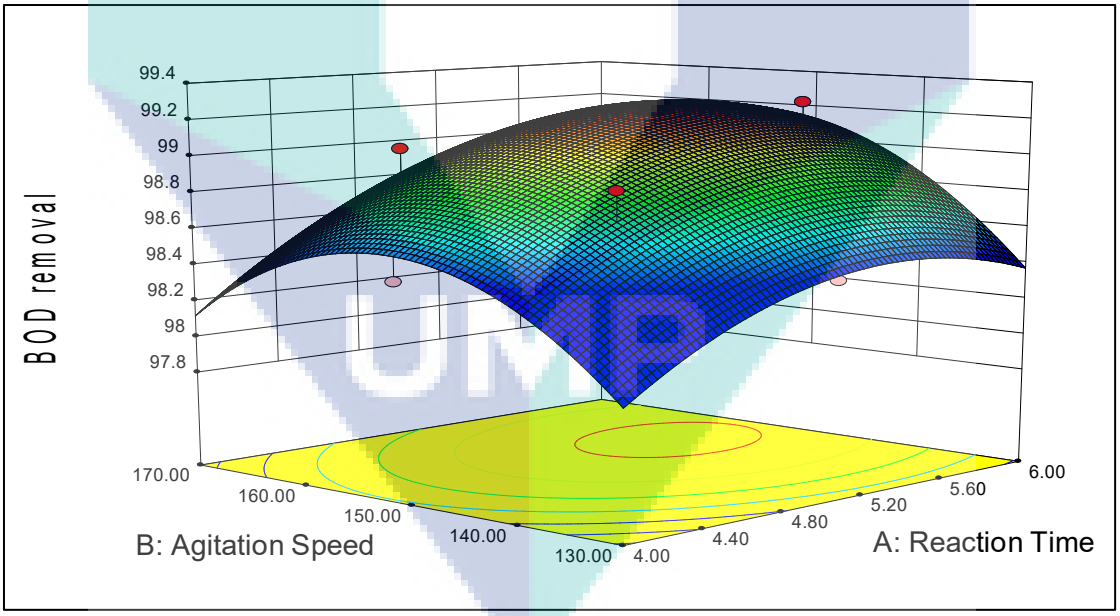

Figure 4.11 3D response surface plot for BOD removal from acidic POME

Figure 4.12 and 4.13 showed the contour and three-dimensional response surface plots for COD removal. From the response surface plot, the COD removal reached the constant value at 5 days of reaction time and agitation speed of 150 rpm. In this study, the experiments were carried out by varying the reaction time from 4 to 6 day. It had been observed that COD removal increased with increasing of reaction time and slightly decreased when reaction time reached 6 day. The changing in COD removal value was affected by microbial growth rate in inoculum (Chan et al., 2010). Reaction time gave essential time for the microbes to growth and decomposes the organic matter. The decomposition of organic matter contributed in reducing the COD value. It had been observed that reaction was complete at five day of reaction time. This was confirmed by low COD removal achieved at reaction time of 6 day.

In this study, the experiments were carried out by varying the agitation speed from 130 to 170 rpm. From the Figure 4.13, the COD removal increased with increasing of agitation speed and slightly decreased when agitation reached 160 rpm. According to Lamed et al., 1988, agitation helps in speed up the microbial activities thus increasing the capabilities of microbes to decompose organic matter. The decomposition of organic matter increased treatment process by reducing the present of organic matter. This condition contributed towards the reduction in COD removal. Complete reaction can be seen at agitation of 150 rpm as COD removal decreased when agitation reached 160 rpm.

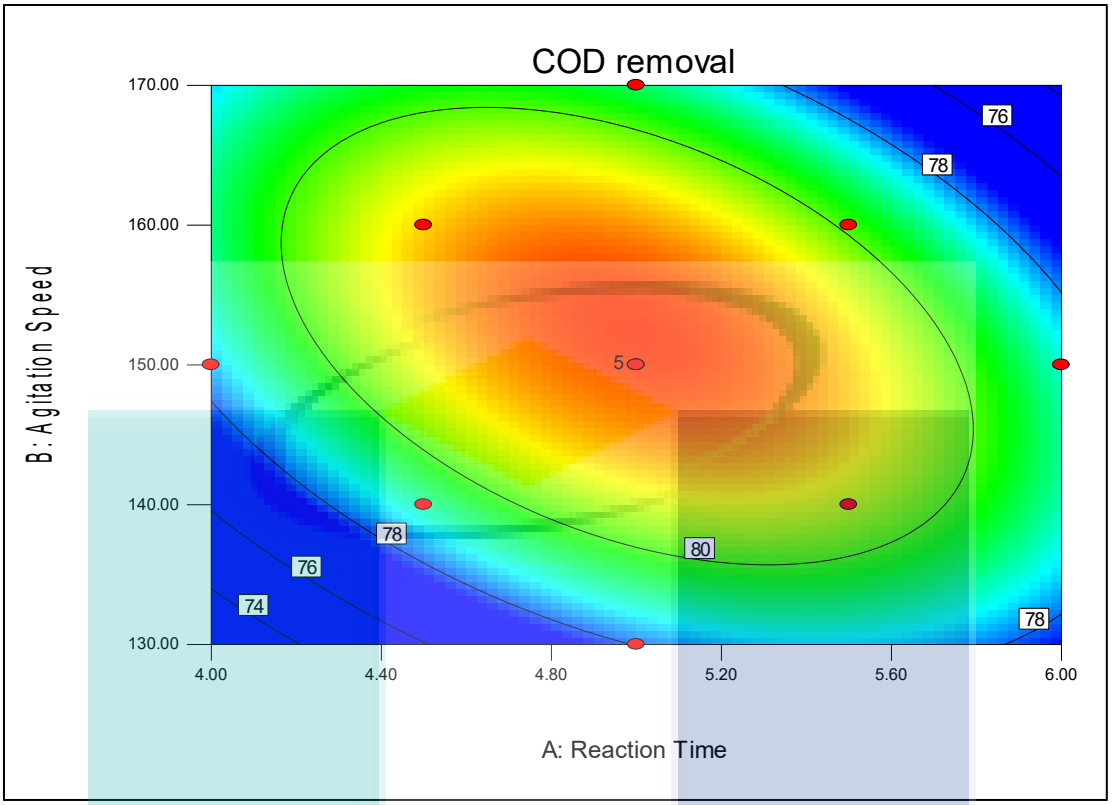

Figure 4.12 Contour plot for COD removal from acidic POME

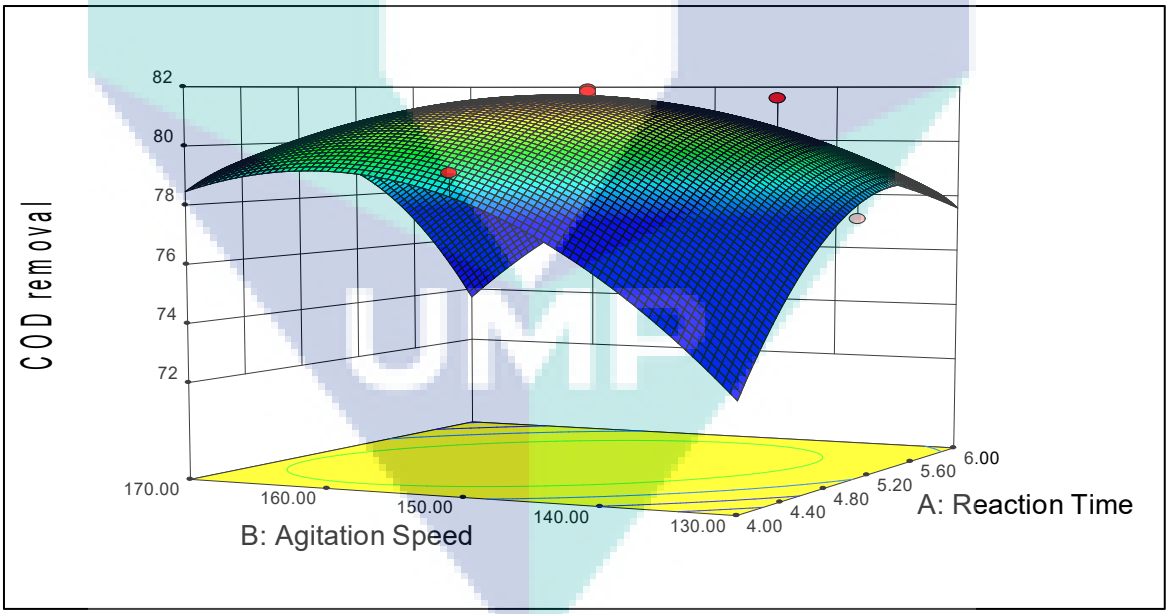

Figure 4.13 3D response surface plot for COD removal from acidic POME

4.4 Validation on Suggested Optimum Condition

Experimental validation was conducted based on the suggested optimum condition proposed by the Design Expert software (Version 6.0). Table 4.14, Table 4.15 and Table 4.16 showed the suggested optimum condition with predicted values and experimental values for pH, BOD removal and COD removal. The focuses of this study were to maximize the pH, BOD removal and COD removal value.

From the experimental validation results, the experimental values were closed to the predicted values. This showed the validity of the models. The percentage error for pH treatment was 2.47%, 0.33% for BOD removal and 3.32% for COD removal.

Table 4.14 Experimental and predicted value at optimum condition for pH treatment

Run	Reaction time (day)	Agitation speed (rpm)	Predicted value	Experimental value	Experimental error (%)
1	5	150	8.14069	7.94	2.47
2	5.18	150	8.14278	7.92	2.74

Table 4.15 Experimental and predicted value at optimum condition for BOD removal

Run	Reaction time (day)	Agitation speed (rpm)	Predicted value (%)	Experimental value (%)	Experimental error (%)
1	5	150	99.1628	98.84	0.33
2	5.18	150	99.2037	98.85	0.36

Table 4.16 Experimental and predicted value at optimum condition for COD removal

Run	Reaction time (day)	Agitation speed (rpm)	Predicted value (%)	Experimental value (%)	Experimental error (%)
1	5	150	81.6869	85.46	4.62
2	5.18	150	81.614	84.32	3.32

4.5 Comparison with Other Researcher

The palm oil mill effluent (POME) was generated from three major sources, which were sterilizer condensate, hydrocyclone waste and separator sludge (Borja and Banks, 1994). There were various effluent treatments which was currently used by the Malaysian palm oil industry such as anaerobic and facultative ponds, tank digestion and mechanical aeration, tank digestion and facultative ponds, decanter and facultative ponds, physicochemical and biological treatment.

In this study, the biological treatment of acidic POME has been statistically improved through the optimization studies by using central composite design (CCD). This study shows the improvement in biological POME treatment where the final pH value was found at 8.14. The BOD removal was found at 99.15% reduction and the COD removal was found at 81.90% reduction within 5 days of reaction time. Table 4.17 shows the comparison in pH value, BOD removal and COD removal with others researcher.

From Table 4.17, higher pH value can be observed by the study conducted by Vijayaraghavan et al., (2007) compared to this study. The study conducted by Vijayaraghavan et al., (2007) use diluted raw POME with high pH value (7.8 ± 0.2) for the treatment using activated sludge reactor. This resulted in obtaining high pH value after the treatment process. This study used raw POME with low pH value (4.0) for the biological POME treatment. The high pH value (8.14) obtained after the treatment process can be observed. Even though the high pH value can be obtained from study conducted by Vijayaraghavan et al., (2007), longer reaction time (30 days) was needed for the treatment compared to this study (5 days). From the study conducted by Oswal et al., (2002), the pH value was found within the range of 6.0 to 7.0. The pH value obtained in this study was higher compared with Oswal et al., (2002). The study conducted by Oswal et al., (2002) used raw POME and was initially treated with marine *Yarrowia* strain. The used of treated mixed culture in the treatment by Oswal et al., (2002) helps in increasing the microbial growth rate and speed up the microbial activities. This situation resulted in having high pH value in short reaction time (2 days). This study used soil mixed culture in treating raw POME. Higher pH value (8.14) can be observed compared to Oswal et al., (2002) (6 – 7).

The study conducted by Najafpour et al., (2005) gave BOD removal efficiency at 91% by using rotating biological contactor (RBC). From the Table 4.17, the BOD removal in this study was higher compared with Najafpour et al., (2005). The study conducted by Najfpour et al., (2005) used continuous treatment on POME by using attached growth microbial film on a biological contactor (RBC). This study used batch treatment on POME by using soil mixed culture. Higher BOD removal (99.15 %) can be seen in this study compared with Najafpour et al., (2005) at same reaction time (5 days). From the study by Chan et al., (2010), BOD removal efficiency was found within the range of 97% to 98%. The study conducted by Chan et al., (2010) gave the highest BOD removal efficiency compared to other researchers. Chan et al., (2010) used anaerobically digested POME in its treatment using sequencing batch reactor. The anaerobically digested POME contains lower POME concentration compared with raw POME. This situation contributed in obtaining higher BOD removal as the substrate contain lower BOD value compared with raw POME. However this method take longer retention time (30 days) compared with this study (5 days).

From the study by Chan et al., (2010), COD removal efficiency was found within the range of 95% to 96% by using sequencing batch reactor. The COD removal was higher compared to this study. The study conducted by Chan et al., (2010) used treated POME with low COD value while this study used raw POME with high COD concentration. This resulted in getting higher COD removal compared with this study. From the study by Oswal et al., (2002), the COD removal was found at 91% by using treated mixed culture (tropical marine yeast). The COD removal was higher compared with this study. The treated mixed culture contain microorganism that specifically used in treating POME. This contributed in getting higher COD removal compared with this study.

There were different methods used in acidic POME treatment. Vijayaraghavan et al., (2007), Chan et al., (2010) and Najafpour et al., (2005) used chemical and mechanical approach in treating acidic POME. This type of treatment required nutrient supplementation for relatively resistant organic wastes to improve the treatment of wastewater (Prasertsan et al., 2009).

Table 4.17 Comparison with other research using POME as substrate

Method	pH range	BOD removal range (%)	COD removal range (%)	Reaction time	Research
Mixed culture	8.14	99.15	81.90	5 days	This research
Activated sludge reactor	7.0 – 8.5	-	-	30 days	Vijayaraghavan et al., (2007)
Sequencing batch reactor	7.3 – 7.5	97 - 98	95 - 96	30 days	Chan et al., (2010)
Mixed culture treated	6.0 – 7.0	-	95	2 days	Oswal et al., (2002)
Rotating biological contactor (RBC)	-	91	-	5 days	Najafpour et al., (2005)

CHAPTER 5

CONCLUSION

5.1 Conclusion

This chapter was designed to conclude the whole thesis based on the objectives of this study. In this study, it consisted of three objectives and each of the objectives was divided into several scopes. These scopes were specified in order to achieve all the objectives.

5.1.1 Characterization of Acidic Palm Oil Mill Effluent (POME) and Soil

In this study, the first objective was to characterize the acidic POME and soil. Results from this study shows that acidic POME had low pH value with high temperature, biochemical oxygen demand (BOD) and chemical oxygen demand COD. In this study the presence of organic and in organic matter was high due to the high value of total solid (TS) and total suspended solid (TSS).

The characterization of the soil were done with alluvium soil had higher pH value compared with peat soil. The nitrogen content in peat soil was higher compared with alluvium soil. Soil moisture content depends on the type of soil and its texture. Percentage of coarse sand, fine sand, slit and clay contributes towards its moisture content. In this study, the moisture content of peat soil was 46.16% with high coarse sand followed by fine sand, clay and slit. The moisture content for alluvium soil was 17.18% with high clay content followed by fine sand, slit and coarse sand. In this study, the organic content in peat soil was higher compared with alluvium soil. Cation-exchange capacity depends on organic carbon that exists in soil. High organic carbon content shows that the soil had higher cation-exchange capacity. In this study, peat soil contain high cation-exchange capacity compared with alluvium soil. In this study the

conductivity of peat soil was higher compared with alluvium soil. The available phosphorus in peat soil was higher compared with alluvium soil.

5.1.2 Factors Affecting Biological Acidic Palm Oil Mill Effluent (POME) Treatment

The second objective of this study was to analyze factors affecting biological treatment of acidic POME. Five factors were analyzed in factorial analysis which was reaction time, temperature, agitation speed, soil to water ratio and soil types. These factors were studied for their effect and contribution on biological treatment process. The pH value was measured using pH meter (Mettler Toledo). The BOD was measured using Dissolved Oxygen Meter (YSI 5100) and the COD value was measured using spectrophotometer (HACH DR2800). In factorial analysis, Design Expert software was used to analyze the contribution of the factors. From the result, reaction time gave the highest contribution towards pH treatment, BOD removal and COD removal. In term of the interaction, reaction time and agitation speed gave the highest contribution which was at towards pH treatment, BOD removal and COD removal.

5.1.3 Optimization of Reaction Time and Agitation Speed on Biological Acidic Palm Oil Mill Effluent (POME) Treatment.

The third objective was to optimize the biological acidic POME treatment. Two factors from the factorial analysis were selected to study in optimization by using central composite design (CCD). The suggested optimum condition for reaction time was 5 day and agitation speed of 150 rpm. From ANOVA, the R2 for this model was 0.8326 (pH treatment), 0.8991 (BOD removal) and 0.8278 (COD removal). The pH value, BOD removal and COD removal obtained in optimization was higher than factorial analysis. Validation test was conducted to justify this optimum condition with pH value (8.14), BOD removal (99.16%) and COD removal (81.69%). The final pH value, BOD removal and COD removal were at 7.94, 98.84% and 85.46%. It showed an error of 2.47% (pH treatment), 0.33% (BOD removal) and 4.62% (COD removal).

5.2 Recommendation

Several recommendations were proposed in this chapter in order to improve the biological treatment of acidic POME. The recommendations are listed below.

5.2.1 Kinetic Study of Biological Acidic Palm Oil Mill Effluent (POME) Treatment

Kinetic study is one of the methods that use to clarify the reaction mechanism of a process. It is commonly apply after the optimization study. Kinetic study consists of a series elementary process which explains the overall reaction process. In this study, it will develop the mathematical model that can be used to study the influence of the several factors to the reaction rate and the process rate. The determination of kinetic parameters would allow the application of the biological treatment at another level especially in scale up process.

5.2.2 Chemical Stability and Degradation Mechanism of Biological Acidic Palm Oil Mill Effluent (POME) Treatment

The study of the chemical stability and degradation mechanism are important in order to develop the analytical method for biological acidic POME treatment. In mechanism study, the role of each factor that contributed towards the biological treatment of acidic POME can be better analyzed. Understanding the mechanism of biological treatment process would improve the optimization study and the optimum value obtain.

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APPENDIX A1

Determination of biological oxygen demand (BOD)

Apparatus:

1. Incubation bottles: 300 mL bottles
2. BOD incubator
3. Volumetric flask, 1 L
4. Beaker, 500 mL
5. Dissolved oxygen meter

Reagent:

Reagents were prepared in advanced but discard if there is any sign of precipitation or biological growth in the stock bottles. Use reagents grade or better for all chemicals and use distilled or equivalent water.

1. Phosphate buffer solution
   Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4CI in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment.
2. Magnesium sulfate solution
   Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.
3. Calcium chloride solution
   Dissolve 27.5 g $CaCI_2$ in distilled water and dilute to 1 L.
4. Ferric chloride solution
   Dissolve 0.25 g $FeCI_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.
5. Acid and alkali solutions, 1 N for neutralization of caustic or acidic waste samples.
   i. Acid-Slowly and while stirring, add 28 mL concentrated sulfuric acid to distilled water. Dilute to 1 L.
   ii. Alkali-Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

Procedure:

1. Preparation of dilution water: 1 mL each of phosphate buffer, magnesium sulfate, calcium chloride, ferric chloride solution was added into 1 L volumetric flask. Distilled water was added to 1 L.
2. 10 mL wastewater sample was added into a 500mL beaker.
3. Dilution water was added up to 300mL into the same beaker.
4. pH value was adjusted to 6.5 to 7.5 by adding acid/alkali.
5. 300 mL dilution water was prepared as control in another 500mL beaker.
6. All prepared samples and control was inserted in 300 mL-incubation bottle each.
7. The dissolved oxygen (DO) concentration for each sample was measured and recorded using Dissolved Oxygen Meter.
8. Water was added to the flared mouth of bottle and cover with an aluminum foil.
9. All the bottles were placed in BOD Incubator for five days. Set the temperature at 20°C.
10. Final DO value was measured after five days.

Calculate BOD_5 according to the formula below;

$$BOD_5, mg/L = (D_1 - D_2) / P$$

Where;

D_1 = DO value in initial sample

D_2 = DO value in final sample

P = Decimal volumetric fraction of sample used

Or;

$$BOD_5, mg/L = (D_1 - D_2) \times \text{Dilution factor}$$

Dilution factor = Bottle volume (300mL) / Sample volume

APPENDIX A2

Determination of chemical oxygen demand (COD)

Apparatus:

1. COD Digestion Reactor
2. Spectrophotometer, HACH DR/2400 @ DR/2800
3. COD Digestion Reagent Vial LR @ HR
4. COD rack
5. Volumetric pipette, 2 mL
6. Paper towel/Tissue

Procedure:

1. 100 mL of sample was homogenized for 30 seconds in a blender. * For samples containing large amounts of solids, the homogenization time was increased.
2. For the 200-15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, the homogenized sample was poured into a 250-mL beaker and gently stirred with a magnetic stir plate. *If the sample does not contain suspended solids, omit step 1 and step 2.
3. The COD reactor was turn on and preheated to 150°C. The safety shield was placed in front of the reactor.
4. The caps were removed from two COD Digestion Reagent Vials. *Be sure to use vials for the appropriate range.
5. One vial was hold at a 45-degree angle. The clean volumetric pipette was used to add 2.00 mL of sample to the vial. This was the prepared sample.
6. The second vial was hold at a 45-degree angle. The clean volumetric pipette was used to add 2.00 mL de-ionized water to the vial. This was the blank sample.
7. The vials cap was tightly and rinsed with de-ionized water and wiped with a clean paper towel.

8. The vials were hold by the cap over a sink. The sample was gently inverted for several times. The vials were placed in the preheated COD Reactor. *The sample vials will become very hot during mixing.
9. The vials were heated for two hours.
10. The reactor was turned off. The vials were left for about 20 minutes to cool to 120°C or less.
11. Each vial was inverted several times while still warm. The vials were placed into a rack and cool to room temperature.
12. "Hach Programs" was touched. The program 430 COD LR (Low Range) or 435 COD HR (High Range/High Range Plus) was selected and touched "Start".
13. The outside of the vials were cleaned with a damp towel followed by a dry one to removed fingerprints or other marks.
14. The 16-mm adapter was installed. The blank sample was placed into the adapter.
15. Touched "Zero". The display will show: 0 mg/L COD.
16. When the timer beeps, the sample vial was placed into the adapter. Touched "Read". Results will appear in mg/L COD.

APPENDIX A3

Determination of total suspended solid

Apparatus:

1. Glass fiber filter disk, 47 mm @ 70 mm – pre dry in the oven
2. Measuring cylinder, 100 mL
3. Pipette, 10 mL
4. Analytical balance
5. Oven, preheated to 103°C to 105°C
6. Desiccator
7. Buchner flask and funnel
8. Vacuum pump
9. Aluminum weighing dishes / crucible dish

Procedure:

1. The filter disk was dried in the oven at 103°C to 105°C for one hour, cooled in a desiccator and weighed.
2. Filtering apparatus was assembled and suction began. The filter was wet with a small volume of distilled water to seat it.
3. 50 mL of water sample (mixed to ensure homogeneity) was pipette onto center of the filter disk in a Buchner flask, using gentle suction (under vacuum).
4. Filter was washed with three successive 10 mL volumes of distilled water, allowing complete drainage between washings and suction was continued for about 3 minute after filtration completed.
5. Filter was carefully removed from filtration apparatus and transferred to aluminum weighing dish / crucible dish as a support.
6. The sample was dried at least one hour at 103°C to 105°C in an oven, cooled in desiccator to balance temperature and weighed.
7. The cycle of drying, cooling, desiccating and weighing was repeated until a constant weigh was obtained.

Calculation:

$$Total\ suspended\ solid\ (TSS), \frac{mg}{L} = \frac{((A-B)x\ 1000)}{sample\ volume, mL}$$

Where:

A = weigh of filter and dried residue, mg

B = weigh of filter, mg

APPENDIX A4

Determination of total solid

Apparatus:

1. Oven
2. Crucible
3. Desiccators
4. Analytical balance
5. Dish tongs
6. Magnetic stirrer
7. Wash bottle

Procedure:

1. A clean porcelain dish which had been washed and dried in a hot air oven at 105°C for one hour was taken. The empty evaporating dish was weighed in analytical balance. The weigh measured was denoted as W1.
2. 75 mL of unfiltered sample was transferred using pipette in porcelain dish.
3. The oven was switched on and allowed to reach 105°C.
4. The oven and furnace temperature was checked and regulated frequently to maintain the desired temperature range.
5. The sample was dried to get the constant mass. Drying for long duration usually one to two hours was done to eliminate necessity of checking for constant mass.
6. The container was cooled in desiccator.
7. The cooled dish was weighed and denoted as W2.
8. Calculation:

$$Total\ solid\ (TS), mg/L = \frac{W2 - W1}{sample\ volume}$$

Where:

W1 = initial weigh of the crucible

W2 = weigh of the crucible and sample

UMP

APPENDIX A5

Determination of oil and grease (Hexane Extractable Gravimetric Method)

Apparatus:

1. pH paper varies
2. Silica gel with indicator (for desiccator) varies
3. Adapter, vacuum connector/gas inlet
4. Aspirator, vacuum pump
5. Analytical balance
6. Boiling chips, silicon carbide
7. Clamp
8. Clamp holder
9. Clamp, pinch
10. Condenser, reflux, with ground glass joints
11. Cylinder, graduated, 500 mL
12. Cylinder, graduated, 50 mL
13. Desiccator
14. Desiccator plate
15. Filter funnel, 65-mm
16. Filter paper, 12.5-cm, folded, pore size 8 to 12 µm 1
17. Flask, Erlenmeyer, 125 mL
18. Flask, Erlenmeyer, 125 mL, with ground glass joint
19. Funnel, separatory, 500 mL
20. Marker, laboratory
21. Oven
22. Pipette filler, safety bulb
23. Pipette, serological, 5 mL
24. Ring support, 4-inch 1
25. Rod, glass
26. Steam bath, 8-inch, 5-ring
27. Hot plate, 7 inch x 7 inch, digital

28. Stir bar, 22.2 x 7.9 mm
29. Support, ring stand, 5-inch x 8-inch base
30. Tongs, crucible, 9-inch
31. Tube, connecting, J-shaped, with ground glass joint
32. Tubing, rubber, 7.9 mm x 2.4 mm

Reagents:

1. Hydrochloric Acid Solution, 6.0 N (1:1)
2. Hexane, ACS grade
3. Sodium sulfate
4. Silica gel, 100–200 mesh 1–30 g

Procedure:

1. 350 mL of sample was collected in a clean 500 mL separatory funnel.
2. The pipette and pipette filler were used to add 4 mL of 1:1 hydrochloric acid solution to the separatory funnel. The sample was mixed well. The pH must be 2 or less to hydrolyze oils and grease and prevent sodium sulfate interference.
3. The glass rod and a pH paper were used to measure the sample pH after the acid addition.
4. The 125 mL distillation flask that contains 3–5 boiling chips was cleaned and dried. Analytical balance was used to weigh the flask to the nearest 0.1 mg. The weight of the flask was recorded.
5. 20 mL of n-hexane was added to the separatory funnel. If the sample was collected in a separate container, rinse the collecting vessel which contained the sample with 20 mL of n-hexane.
6. The stopper was inserted and the separatory funnel was inverted.
7. The gases were released through the stopcock. To release gases from the separatory funnel, invert it and shake it once very hard. Make sure to hold the stopper. Under a hood, point the delivery tube in a safe direction. Slowly open the stopcock to release all of the gas. Close the stopcock. Do this procedure until the release of gas is not heard.

8. Vigorously shake the separatory funnel for 2 minutes.
9. The separatory funnel was placed in the stand. The separatory funnel or the stand was not moved for a minimum of 10 minutes to let the separation of the lower water layer and the upper solvent layer. If the solvent layer is brown, the sample can have oil with color on it. If this step is done again (for a third time), and the water layer is cloudy, do not move the separatory funnel for 20 minutes to make sure of the separation of the water and solvent layers.
10. The lower water layer from the separatory funnel was drained slowly into the initial sample container or a 500 mL volumetric flask. The drain must take approximately 3 to 4 minutes. The water layer was kept for use in step 13. To make sure that water is not used in step 12, let some drops of solvent layer drain into the water layer until the solvent layer is visible on top of the water. If the water layer drains too quickly, there will be too much water in the solvent layer. This causes sodium sulfate and water interference.
11. The filtering funnel was set up. The glass funnel was placed in the neck of the distillation flask. The folded 12.5 cm filter paper was placed in the funnel. 10 g of anhydrous sodium sulfate was added to the filter paper. The sodium sulfate was rinsed with a small amount of n-hexane. The n-hexane was discarded correctly. For the second and third extractions, the same filter, funnel and sodium sulfate was used. Between extractions, remove the large, hard sodium sulphate chunks to decrease sodium sulfate contamination.
12. The solvent layer was drip-drained into the pre-weighed boiling flask through a funnel that contains filter paper and 10 g anhydrous sodium sulfate. The sodium sulfate was stirred carefully with a glass rod while the solvent layer drains. Be careful and do not damage the filter paper. Any spillage will cause inaccurate results. To reduce spillage, the glass rod was used to route the sample solution into the filter.
13. The water layer was returned to the separatory funnel. The same glass funnel was used for the second and third extraction. To reduce spillage, second funnel was used to pour the water layer into the separatory funnel.
14. Steps 5 through 13 were repeated again two more times. After the third extraction, the water layer was discarded.

15. The separatory funnel was rinsed with three different 5 mL aliquots of fresh n-hexane to remove oil film that stayed on the funnel walls. Each aliquot was drained through the funnel that contains the sodium sulphate into the distillation flask.
16. The tip of the glass funnel was rinsed with 5 mL of n-hexane while removing it from the distillation flask.
17. The distillation flask was examined for sodium sulphate contamination. Sodium sulphate contamination will show as cubic crystals at the bottom of the distillation flask. If there is sodium sulphate contamination, the solvent layer was filtered again through filter paper without sodium sulfate.
18. Distillation was completed when there were no boiling bubbles or the distillation flask was dried. Steam bath or a hot plate was used to keep a water bath at the correct temperature for the distillation.
19. The condenser/connector portion of the distillation assembly was disconnected at the pinch clamp. The distillation flask was removed from the heat source with tongs or a lint-free cloth. The distilled n-hexane was applicable for future HEM extractions.
20. The vacuum connector/gas inlet adapter was attached to remove the remaining solvent vapors from the distillation flask. The vacuum was applied for 1 to 2 minutes or until all n-hexane solvent vapors were removed.
21. The distillation flask was examined for sodium sulphate contamination. Sodium sulphate contamination will show as cubic crystals at the bottom of the distillation flask. If there was sodium sulphate contamination, the extract was dissolved again in n-hexane, filter into another preweighed flask and steps 18 to 20 was repeated.
22. The flask was placed in a desiccator for 30 minutes (or longer if necessary) until the flask temperature decreases to room temperature. If the silica gel indicator changes to red, the silica gel was replaced.
23. Analytical balance was used to weigh the flask to the nearest 0.1 mg. The weight was recorded.

Calculation:

$$HEM\ \left(\frac{mg}{L}\right) = \frac{(A - B)}{sample\ volume}\ x\ 1000$$

Where:

A = Weight (mg) of residue

B = Weight (mg) of flask with boiling chips (step 4)

APPENDIX A6

Determination of ammoniacal nitrogen

Apparatus:

1. HACH spectrophotometer DR/500
2. Rounded / square sample cell, 10 mL
3. Measuring cylinder, 25 mL
4. Beaker, 50 mL

Reagent:

1. Ammonia Cyanurate reagent powder pillows – 2
2. Ammonia Salicylate reagent powder pillows – 2
3. NitraVer 5 Nitrate reagent powder pillows – 1

Procedure:

1. "HACH programs" was touched. Program "385 N, Ammonia, Salic" was selected. "Start" was touched.
2. A round sample cell was filled to the 10 mL mark with sample.
3. Another round cell was filled to the 10 mL mark with deionized water (the blank).
4. The contents of one Ammonia Salicylate Powder Pillow were added to each cell. Stopper and shaker were applied to dissolve the powder.
5. The timer icon was touched "OK". The three minute reaction period began.
6. The contents of one Ammonia Cyanurate Powder Pillow were added when the timer beeps. Stopper and shaker were applied to dissolve the powder.
7. The timer icon was touched "OK". The 15-minute reaction period began. * A green color will develop if ammonia - nitrogen is present.
8. The blank was placed into the cell holder when the timer beeps.
9. "Zero" Touched. The display showed: "0.00 mg/L NH_3-N"

10. The sample was wiped and placed into the cell holder.
11. ‘Read” touched. Results appeared in mg/L NH_3-N

APPENDIX B1

Determination of soil texture

Coarse sand

1. Soil samples were transferred to a plastic tray for air-drying.
2. Proper labeling was done to avoid identification errors during transfer process. Large cods were break up to speed up drying.
3. Large plant residues were removed. Avoid placing in direct sunlight. After drying, total weight was weighed.
4. Then, the soils were sieved through a 2 mm sieve. Clods, not passing through the sieve were carefully crushed by pestle and mortar and sieved again. Gravel, rock fragments etc. not passing through the sieve, after removal of any adhering finer particles, was weighed and their content was reported as fraction of the whole.
5. Coarse sand was picked out as quantitatively as possible and the content was determined separately at fraction > 2 mm. the fraction < 2 mm (oven-dry soil) was homogenized and constituted the sample subjected to the usual laboratory procedures.

Fine sand

1. The soil was passed through a 50 µm sieve which was placed in a funnel positioned above a sedimentation cylinder with a stand and clamp.
2. This was making to 1 L mark with water.
3. The sand fraction in the remaining on the sieve was washed, evaporates on water bath and dried at 105°C for at least an hour.
4. Sand friction was weighed.

Slit (fraction < 50 µm)

1. After adding material < 50 µm possibly collected during sieving, the sedimentation was closed with a rubber stopper and shake well.
2. The cylinder was placed on the table, stopper was removed and 20 mL was immediately pipette from the center of the cylinder.

3. The aliquot was transferred to a tared moisture tin, evaporated in water bath and dried overnight at 105°C.
4. The tin was removed from drying oven and closed with lid and cooled in desiccators.
5. Slit fraction was weighed.

Clay (fraction < 2 µm)

1. A cylinder was placed on a vibration-free table under the pipette assembly.
2. Exactly after five and half an hour, 20 mL was pipette and transferred to a tared moisture tin, evaporate on water bath and dry overnight at 105°C.
3. The tin was removed from drying oven and closed with lid and cooled in desiccators.
4. Clay fraction was weighed.

Calculation

The basis of calculation was obtained by summation of the individual fractions.

APPENDIX B2

Determination of moisture content and loss of ignition

Apparatus:

1. Moisture tins or flasks with fitting lid
2. Drying oven

Procedure:

1. Approximately of 5 g sample was transferred to a tared moisture tin and weighed (A gram).
2. The sample was dried overnight at 105°C (lid removed)
3. The tin was removed from the oven and closed with lid and cooled in desiccator and weight (B gram)
4. Moisture content in wt% was obtained by calculation:

$$Mosture\ content\ (\%) = \frac{A - B}{B - tare\ tin}\ x\ 100$$

APPENDIX B3

Determination of conductivity in soil

Apparatus:

1. Conductivity meter with dip cell and pipette cell
2. 10 mL, 50 mL and 100 mL beaker
3. 1 L volumetric flask

Reagent:

1. Standard potassium chloride solution, 0.1 M
2. Standard analytical concentrate ampoule of 0.1 M KCl was diluted according to instruction
3. 10 mL standard 0.1 M KCl solution was pipette into a 100 mL volumetric flask and make to volume with water. Alternatively, dissolve 0.7456 g of oven-dried (105°C) in water in a 1 L volumetric flask and make to volume with water

Procedure:

1. About 30 mL standard 0.01 M KCl solution was added to a 50 mL beaker and the temperature was measured.
2. Pipette cell was rinsed and filled with standard KCl solution or cell was directly dipped in the solution.
3. Compensation dial was set at measured temperature and reading of the meter was adjusted to 1.412 mS/cm with cell constant-dial (This is the specific conductivity of the standard 0.01 M KCl solution at 25°C)
4. The temperature of the extract was measured and compensation dial was set at this temperature. The reading automatically corrected to 25°C.
5. Pipette cell was filled with extract or insert dip cell into extract and conductivity was read.

APPENDIX B4

Determination of total nitrogen by Micro Kjedahl method

Apparatus:

1. Digester (Kjedahl digestion tubes in heating block)
2. Steam distillation unit (fitted to accept digestion tubes)
3. Burette 25 mL

Reagent:

1. Sulphuric acid-selenium digestion mixture
2. Hydrogen peroxide, 30%
3. Sodium hydroxide solution (NaOH), 38%
4. Mixed indicator solution
5. Baric acid indicator solution, 1%
6. Hydrochloric acid (HCl), 0.010M standard

Procedure:

Digestion

1. Approximately 5 g of fine particle to pass a 0.25 mm sieve.
2. The 1 g of this material was weighed into digestion tube. In each batch, two blanks and a control sample were included.
3. The 2.5 mL of digestion mixture was added.
4. Three aliquots of 1 mL hydrogen peroxide. The next aliquot was added when frothing has subsided. If frothing excessive, the tube was cooled in water.
5. The tubes were placed on the heater and heated for about 1 hour at moderate temperature (200°C).
6. The temperature was turned up to approximately 330°C and heating was continued until mixture became transparent.

7. The tubes were removed from the heater and were allowed to cool. The approximately of 10 mL of water was added to wash the bottle while swirling.

Distillation

1. 20 mL boric acid indicator solution was added to 250 mL beaker and was placed on stand beneath the condenser tip.
2. 20 mL of sodium hydroxide solution was added to digestion tube and distilled for about 7 minutes.
3. The beaker was removed from distiller, condenser tip was rinsed and distillate was titrated with 0.01 M hydrochloric acid until color changes.

Calculation

$$\% N = \frac{a-b}{s} \, x \, M \, x \, 1.4 \, x \, mcf$$

Where

a = mL of HCl required for sample titration

b = ml of HCl required for blank titration

s = air-dry sample weight in gram

M = molarity of HCl

1.4 = 1.4 x 10^{-3} x 100% (14 = atomic weight of nitrogen)

mcf = moisture correction factor

APPENDIX B5

Determination of carbon (Walkley-Black method)

Apparatus:

1. 500-mL Erlenmeyer flask
2. 10-mL pipette
3. 10-and 20-mL dispensers
4. 50-mL burette
5. Analytical balance
6. Magnetic stirrer
7. Incandescent lamp

Reagent:

1. H_3PO_4, 85%
2. H_2SO_4, concentrated (96%)
3. NaF, solid
4. Standard 0.167*M* $K_2Cr_2O_7$: Dissolve 49.04 g of dried (105°C) $K_2Cr_2O_7$ in water and diluted to 1 L
5. 0.5*M* Fe^{2+} solution: Dissolve 196.1 g of $Fe(NH_4)_2(SO_4)\cdot 6H_2O$ in 800 mL of water containing 20 mL of concentrated H_2SO_4 and dilute to 1 L. The Fe^{2+} in this solution oxidizes slowly on exposure to air so it must be standardized against the dichromate daily
6. Ferroin indicator: Slowly dissolve 3.71 g of o-phenanthroline and 1.74 g of $FeSO_4\cdot 7H_2O$ in 250 mL of water

Procedure:

1. 0.10 to 2.00 g dried soil (ground to <60 mesh) was weighed and transferred to a 500-mL Erlenmeyer flask. The sample contains 10 to 25 mg of organic C (17 to

43 mg organic matter). Two gram of sample was used for light coloured soils and 0.1 g for organic soils.

2. 10 mL of 0.167 M $K_2Cr_2O_7$ was added by means of a pipette.
3. 20 mL of concentrated H_2SO_4 was added by means of dispenser and was swirled gently to mix. Avoid excessive swirling. This result in organic particles adhering to the sides of the flask out of the solution.
4. The flask was allowed to stand for 30 minutes. The flask was placed on an insulation pad to avoid rapid heat loss.
5. The suspension was diluted with 200 mL of water to provide a clearer suspension for viewing the endpoint.
6. 10 mL of 85% H_3PO_4 and 0.2 g of NaF were added. The H_3PO_4 and NaF were added to complex Fe^{3+} which interferes with the titration endpoint.
7. 10 drops of ferroin indicator was added. The indicator was added prior to titration to avoid deactivation by adsorption onto clay surfaces.
8. 0.5 M Fe^{2+} was titrated to a burgundy endpoint. The colour of the solution at the beginning was yellow-orange to dark green, depending on the amount of unreacted $Cr_2O_7^{2-}$ remaining. The colour was shifted to a turbid gray before the endpoint and then changed sharply to a wine red at the endpoint. Magnetic stirrer and incandescent light were used to make the endpoint easier to seen in the turbid system (fluorescent lighting gives a different endpoint color).
9. Reagent blank was done using the above procedure without soil. The blank was used to standardize the Fe^{2+} solution daily.
10. Calculate %C and % organic matter:

a. % Easily Oxidizable Organic C

$$\%C = \frac{(B - S)x\ M\ of\ Fe^{2+} x\ 12\ x\ 100}{g\ of\ soil\ x\ 4000}$$

Where:

B = mL of Fe2+ solution used to titrate blank

S = mL of Fe2+ solution used to titrate sample

12/4000 = milliequivalent weight of C in g.

Easily oxidizable organic C was converted to total C by divided with 0.77 (or multiply by 1.30) or other experimentally determined correction factor.

b. % Organic Matter

$$\% \, OM = \frac{\% \, total \, C \, x \, 1.72}{0.58}$$

UMP

APPENDIX B6

Determination of available phosphorus in soil

Apparatus:

1. Soil scoop calibrated to hold 1.5 g of light-colored silt loam soil.
2. Erlenmeyer flasks (50-ml)
3. Pipette banks (3-ml)
4. Time-controlled oscillating shaker set at 160 excursions per minute
5. Filter paper
6. Funnel tubes (15-ml)
7. Matched colorimetric tubes (10-ml)
8. UV-Vis spectrophotometer

Reagent:

1. Stock P-A solution (1.25 N HCl, 1.5 N NH_4F): 54 ml of 48% HF was added to 700 ml of deionized water. The pH was neutralised to 7.0 with NH_4OH. 108 ml of concentrated HCl (11.6 N) was added and diluted to 1 L.
2. Diluted P-A solution (0.025 N HCl, 0.03 N NH_4F): 20 ml of stock P-A solution was diluted to 1 L with deionized water.
3. P-B solution (0.87 N HCl, 0.38% ammonium molybdate, 0.5%H_3BO_3): 3.8 g of ammonium molybdate, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ was dissolved in 300 ml of deionized water at 60°C. The solution then was cooled to room temperature. 5.0 g boric acid, H_3BO_3 was dissolved in 500 ml of deionized water and 75 ml concentrated HCl (11.6 N) was added. The molybdate solution was added and dilute to 1 L with deionized water.
4. P-C powder: 2.5 g 1-amino-2- napthol-4 sulfonic acid fine powder, 5.0 g sodium sulfite (Na_2SO_3), and 146 g of sodium metabisulfite ($Na_2S_2O_5$) were mixed and grinded thoroughly.
5. P-C solution: 8 g of dry P-C powder was dissolved in 50 ml of warm deionized water.

Procedure:

1. 1.5 g scoop of soil was placed into a 50-ml Erlenmeyer flask.
2. 15 mL of P-A solution was added.
3. The suspension on oscillating shaker was shaken for 5 minutes.
4. The sample was filtered through filter paper into a 15-mL funnel tube.
5. 3.0-mL aliquot of filtrate was pipetted with constant suction pipette apparatus and transferred to a 10-mL colorimeter tube.
6. 3.0 mL of P-B solution was added with the same pipette apparatus and mixed well.
7. 3 drops of P-C solution was added, and mixed immediately.
8. The color was readable after 15 min with a UV-Vis spectrophotometer.
   Note: UV – Vis spectrophotometer should be set at 645 nm.

APPENDIX B7

Determination of cation-exchange capacity in soil

Apparatus:

2. 250 mL beaker
3. Balance to weigh to the nearest 0.01 g
4. 7.0 cm Buchner funnel
5. Filter paper
6. 250 mL suction flask connected to vacuum pump
7. 250 mL volumetric flasks
8. Balance, stir plate, stir bars and container for reagents
9. Apparatus and instrumentation for NH_4^+ analysis.

Reagent:

2. 1 M NH_4OAc at pH 7: The solution was prepared in fume hood to avoid breathing vapors of ammonia and acetic acid. 580 mL of glacial acetic acid (99.5%) was added to 5 L of water. 680 mL of concentrated ammonium hydroxide (58% NH_4OH) was added. Water was added to yield a volume approximately 1900 mL. The pH was adjusted to 7. The solution was diluted to 10 L.
3. Ethyl alcohol (95%)
4. 1 M KCl: 745 g KCl was dissolved in 8 L of water. The solution was diluted to 10 L.

Procedure:

1. 10 grams of air-dried soil ground was weighed and placed into a 250 ml beaker.
2. 25 mL NH4OAc was added to the soil. The solution was covered and let overnight.
3. A 7 cm Buchner funnel was prepared by fitting it with a filter paper. The filter was wetted with a minimum amount of NH4OAc. The funnel was inserted into a 250 mL suction flask. The vacuum pump was turned on to seat the moistened filter. The soil- NH4OAc mixture was stirred and transferred into the filter.

4. 75 mL NH4OAc was measured for each sample into a plastic squirt bottle with one bottle for each sample. 10 mL of NH4OAc in the bottle was used to transfer all the soil to the Buchner funnel.
5. The soil was covered with filter paper to keep the soil moist between leaching.
6. The soil was leached five to seven times with 10 to 15 increments of NH_4OAc.
7. The leachate was transferred into a 250 mL volumetric flask and brings to volume with 1 M NH_4OAc.
8. The soil was leached with ethanol to remove excess NH4OAc. The soil was leached with 25 mL portions of ethanol five to six times for a total volume of about 150 mL.
9. The soil was leached with 1 M KCl to remove adsorbed NH_4. The soil was leached with 25 mL portions of 1 M KCl four to five times for a total volume of about 125 mL.
10. The leachate was transferred to a 250 mL volumetric flask and brings to volume using 1 M KCl. The solution for NH_4 concentration was analysed using calorimetry, distillation or ion- selective electrode potentiometry.

Calculation:

1. If mg/L of NH_4-N is quantified in the leachate, use the following to calculate CEC.

   $$\text{CEC } (cmol_c/kg) =$$

   $$(\text{mg } NH_4\text{-N / L})\ (0.25 \text{ L / 10 g soil})\ (1 \text{ meq } NH_4\text{-N / 14 mg } NH_4\text{-N}) \times 100$$

   If mg/L of NH_4 is quantified in the leachate use 18 mg NH_4 instead of 14 mg NH_4-N.

APPENDIX C

List of Publication

Zainol, M. and Ismail, S.M. (2015). The study on Biological pH Treatment of Acidic Palm Oil Mill Effluent. *Jurnal Teknologi. (Sciences and Engineering) 76*, (1) 139-146.

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