

MICROBIAL COMMUNITY ANALYSIS IN  
UPFLOW ANAEROBIC SLUDGE BLANKET  
FOR THE TREATMENT OF PALM OIL MILL EFFLUENT

SIM LIN LIN

A project report submitted in fulfilment of the  
requirement for the award of the degree of  
Master of Engineering  
(Civil – Environmental Management)

Faculty of Civil Engineering  
Universiti Teknologi Malaysia

NOVEMBER 2005

Dedicated to Jesus Christ,  
My personal Lord and Savior,  
And  
To my beloved parents and family.

## **ACKNOWLEDGEMENT**

This study would not have been completed without the assistance and support of those who guided me in the course of my masters project. First and foremost, I would like to thank God for His grace and mercy throughout this study. It is by His hands and wisdom in guiding me to finish my work within the study period.

Second, I would like to extend my thankfulness to my honorable supervisor, Prof. Dr. Zaini Ujang, for his support, encouragement and academic guidance during the course of my study. I would like to specially thank his patience and tolerance towards me, in which he always trusts me that I am able to do it. His diligence, dedication and working attitudes are good examples for me to follow.

Third, I would also like to thank my housemates and church members who gave me support unceasingly, who accompanied me through many sleepless nights while I was writing my thesis.

Last but not least, I am grateful to my family members for their love, care, support and encouragement.

## **ABSTRACT**

Palm oil mill effluent (POME) has been treated using various technologies, yet not many of them provide a solution for waste degradation. Microbial community structure of the waste was not fully understood. In this study, the characteristics of POME were determined. The relation of pH, total suspended solids, total volatile solids and chemical oxygen demand of POME were studied. BLASTN and RDPII online-database were used to assess microbial community analysis. Acid-formers, methane-formers and fungal isolates were expected to be contributing to the treatment of POME. The relationship among microbial community members was studied. None of a single microbe was able to perform an effective POME treatment. A mixed culture of archaea, bacteria and fungus were essential to degrade the POME to an acceptable standard.

## ABSTRAK

Efluen daripada kilang kelapa sawit telah dirawat dengan pelbagai teknologi, tetapi tidak banyak daripada teknologi itu membekalkan satu cara yang baik dalam degradasi sisa. Struktur komuniti mikroorganisma dalam sisa kelapa sawit belum pernah difaham sepenuhnya. Dalam kajian ini, ciri-ciri efluen kelapa sawit telah dikaji. Hubungan antara pH, *total suspended solids*, *total volatile solids* dan *chemical oxygen demand* just dikaji. Pangakalan data BLASTN dan RDPII digunakan untuk mengkaji analisis komuniti mikroorganisma. Pembentuk asid, pembentuk metana dan fungi just dipercayai menyumbang kepada rawatan sisa daripada kilang kelapa sawit. Hubungan antara komuniti mikroorganisma juga dikaji. Tiada satu mikroorganisma sendiri yang boleh merawat efluen kelapa sawit dengan efektif. Satu campuran arkea, bakteria dan fungi adalah perlu untuk mendegradasikan efluen kelapa sawit kepada suatu piawai yang diterima.

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## LIST OF SYMBOLS AND ABBREVIATIONS

BOD	-	Biochemical oxygen demand
COD	-	Chemical oxygen demand
DGGE	-	Denaturing gradient gel electrophoresis
$f_e$	-	$e^-$ mole of substrate oxidized per $e^-$ mole of substrate used
$f_s$	-	$e^-$ mole of substrate used for cell synthesis per $e^-$ mole of substrate used
FISH	-	Fluorescent <i>in situ</i> hybridization
PCR	-	Polymerase chain reaction
POME	-	Palm oil mill effluent
$R_a$	-	Reactions for electron acceptors
$R_d$	-	Reactions for electron donors
sCOD	-	Soluble chemical oxygen demand
SMP	-	Soluble microbial products
TS	-	Total solids
TSS	-	Total suspended solids
UASB	-	Upflow anaerobic sludge blanket
VSS	-	Volatile suspended solids
$Y$	-	Yield
$\Delta G_R$	-	Energy released from oxidation reduction reactions
$\Delta G_s$	-	Energy used for cell growth

## CHAPTER 1

### INTRODUCTION

#### 1.1 The Palm

Palm oil originates from Africa. It is not a native plant in Malaysia. Nevertheless, after crossing over the oceans and finally landed on our land over 100 years ago, it was discovered that our soil and ambiance gave a conducive environment for it to grow. Today, palm oil trees have become significantly important crop in our country and are now the most important national crop. The most common type of palm oil species grown in Malaysia is the cross-breed of *Dura* and *Pisifera* species, which produced the *Tenera* species.

The palm oil was introduced to Malaysia at 1870s as an ornamental plant. It started to develop the commercial value as a crop in 1917. The modern expansion of the industry can be traced back to the 1960s when the Malaysian Government embarked on a massive programme of agriculture diversification. Today, palm oil is the leading agricultural crop in Malaysia, covering about two million hectares or a third of the total cultivated area.

There are over 500 million palm oil trees in Malaysia today. Everyday, research and experiments are being conducted to produce quality seeds that can yield more and better quality oil. Only the finest seeds are germinated under controlled conditions. The germinated seeds are planted into poly-bags and kept in nurseries to grow for 12 to 15 months. When they are ready, the young palm seedlings are transferred into a well-irrigated field. The seedlings are left to grow for about 32 months or nearly 3 years. Fertilizers are added from time to time to ensure that the growing trees have sufficient nutrients.

Mature trees will sprout oil palm fruits which are called Fresh Fruit Bunches (FFB). Each tree will continue to produce about 12 FFB each year for about 20 to 30 years. Oil palm fruits turn into yellowish red when ripe. They must be harvested at just the right time when the amount and the quality of oil are best. Each FFB can weigh until 20 to 30 kg. Care must be taken to prevent the fruits from getting bruised or spoilt. Rough handling can reduce the quality of the oil obtained eventually.

Harvested FFB are loaded into a lorry or rail cage to be transported to the mills for processing as soon as possible. Most mills are usually located in or near the oil palm plantations.

## **1.2 The Oil**

### **1.2.1 Types of Oil**

There are different types of oils that can be extracted from the palm oil fruits, for instance palm oil, palm olein, palm stearin, palm kernel oil, palm kernel olein and palm kernel stearin.

#### **1.2.1.1 Palm Oil**

Palm oil is derived from the flesh of the fruit of the oil palm species *E. guineensis*. In its virgin form the oil is bright orange-red due to high content of carotene.

Palm oil is semisolid in room temperature; a characteristic brought about by its approximate 50% saturation level. Palm oil and its products have good resistance to oxidation and heat at prolonged elevated temperatures; hence making palm oil an ideal ingredient for frying oil blends. Manufacturers and end users around the world incorporate high percentage of palm oil in their frying oil blends for both performance and economic reasons. In fact, in many instances, palm oil has been used as 100% replacement for traditional hydrogenated seed oils such as soybeans oil and canola. Products fried in palm oil include potato chips, French fries, doughnuts, ramen noodles and nuts.

Another positive attribute of palm oil as a frying oil is that it imparts longer shelf life to the fried products; generally attributed to the comfortable level of unsaturation, absence of linolenic acid and presence of natural antioxidants in the oil.

Palm oil is suited to be used in high percentages in vegetable oil shortenings, biscuit fats and bakery fats because of its natural solid-liquid content. In margarine production, palm oil is highly suited as a component as it imparts the desirable beta prime crystalline tendency in the fat blend. Palm oil's natural semi-solid consistency means need for no or little hydrogenation.

#### **1.2.1.2 Palm Olein**

Palm olein is the liquid fraction obtained by fractionation of palm oil after crystallization at controlled temperatures. The physical characteristics of palm olein differ from those of palm oil for it is fully liquid in warm climate and has a narrow range of glycerides.

In addition to finding uses as in the case of palm oil, palm olein is widely used as cooking oil. It also blends perfectly with other popular vegetable oils that are traditionally used in many parts of the world; prompting a nickname "blending partner" for palm olein. For instance, in Japan, refined palm olein is blended with rice bran and in Malaysia, it is blended with groundnut oil.

Like palm oil, palm olein is also widely used as frying oil and much of its popularity is due to its good resistance to oxidation and formation of breakdown products at frying temperatures and longer shelf life of finished products. In fact,



palm olein is considered the gold standard in frying and is perhaps, on its own, the most widely used frying oil in the world.

### **1.2.1.3 Palm Stearin**

Palm olein is the more solid fraction obtained by fractionation of palm oil after crystallization at controlled temperatures. It is thus a co-product of palm olein. It is always traded at a discount to palm oil and palm olein; making it a cost effective ingredient in several applications.

The physical characteristics of palm stearin differ significantly from those of palm oil and it is available in a wider range of melting points and iodine values. Palm stearin is a very useful source of fully natural hard fat component for products such as shortening and pastry and bakery margarines.

In addition to palm olein and stearin, there are easily a dozen other fractions, obtained from palm oil including various grades of double fractionated palm olein (*aka superolein*) and palm mid fractions. Where pourability and clarity can be issues for palm olein, especially in temperate countries, superolein finds uses as frying oil and cooking oil, usually in blends with seed oils. Palm mid fraction is commonly used as a highly versatile natural ingredient in the manufacture of tub margarine.

#### **1.2.1.4 Palm Kernel Stearin and Other Oils**

Palm kernel stearin is the more solid fractions of palm kernel oil obtained from fractionation. Palm kernel oil is obtained from the kernel of the oil palm fruit. The oil composition is very different from that of palm oil. Palm kernel olein is the liquid component of palm kernel oil obtained from fractionation.

Palm kernel oil, palm kernel stearin, and palm kernel olein find uses in margarine, confectionaries, coffee whitener, filled milk, biscuit cream and coating fats; with little or no further processing. There is a growing trend to use palm kernel oil products as an ingredient in the production of non-hydrogenated trans fat free margarine.

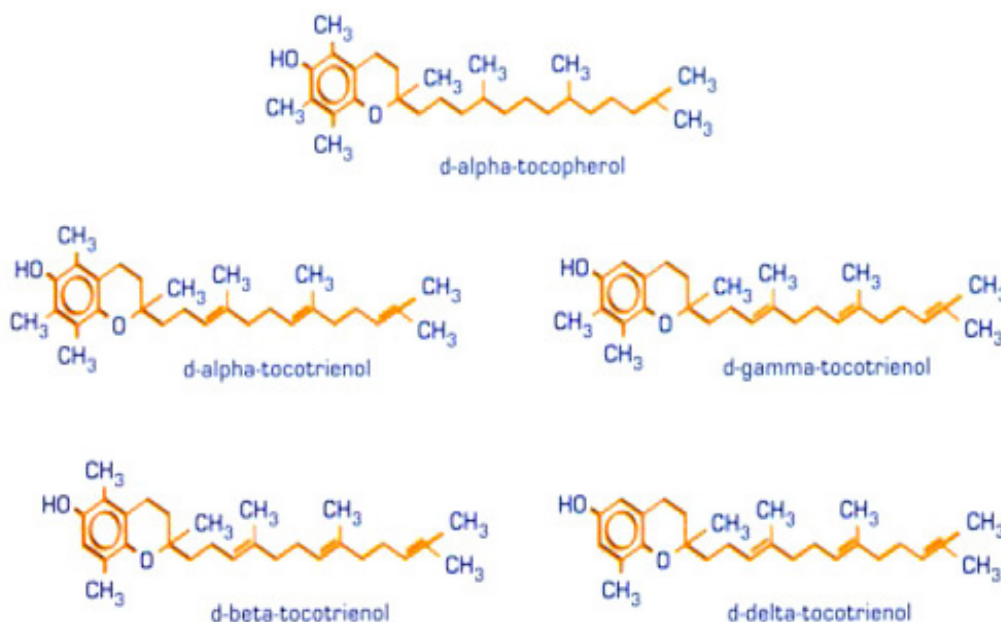
Palm kernel stearin is widely used to substitute for the more expensive cocoa butter in many of its traditional applications. In some examples, particularly when hydrogenated, palm kernel stearin exhibits performance superior to that of cocoa butter. Apart from their excellent melting properties, hydrogenated palm kernel oil products generally have good resistance to fat bloom and shows good resistance to oxidation stress.

#### **1.2.2 Chemical Composition of Palm Oils**

Crude palm oil is the richest natural source of Tocotrienols. The Malaysian Palm Oil Board (MPOB) has developed a special patented technology for extraction. The latest technology to purify *Tocotrienols* from the fruits of oil palm (*Elais guineensis*) was developed to produce a superior quality and purity.

### 1.2.2.1 Tocotrienols

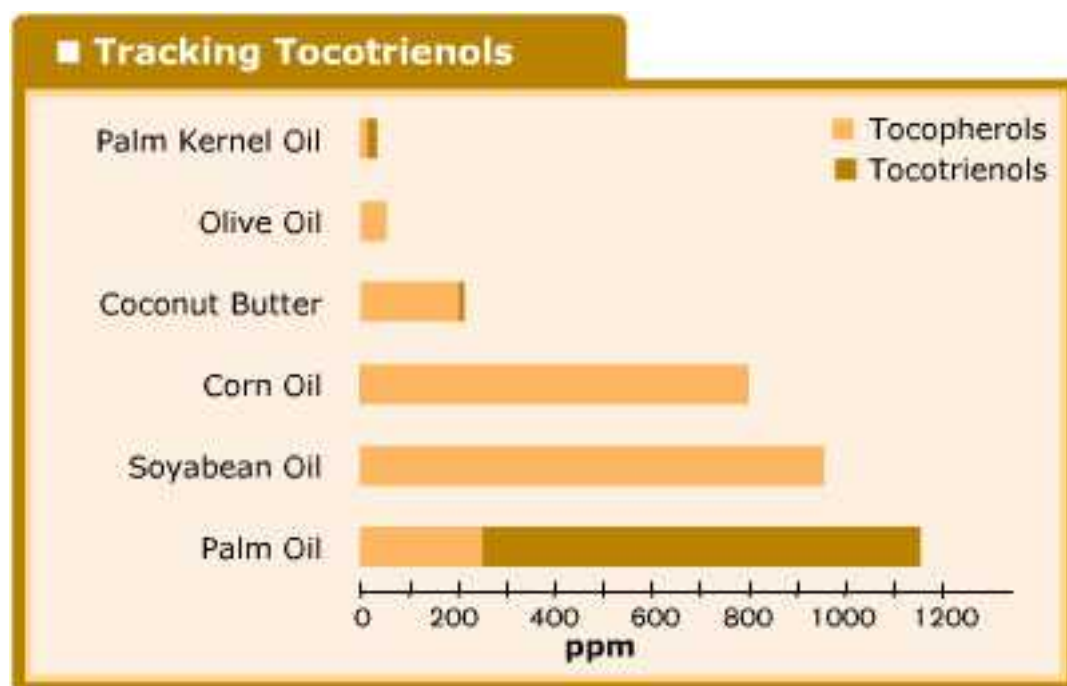
Tocotrienols are members of the Vitamin E family comprising of Tocotrienols and Tocopherols. Tocotrienols differ from Tocopherols in that they contain three double bonds in the side chain (Figure 1.1). Tocotrienols Isoprenoid side chain has three double bonds as compared to Tocopherols' saturated side chain. In total there are four types of Tocopherols namely alpha, beta, gamma and delta and four corresponding of Tocotrienols isomers.



**Figure 1.1:** Molecular structure of Tocotrienols

Alpha Tocotrienols is 40-60 times more potent than normal Tocopherols, making it one of the most powerful lipid-soluble anti-oxidants available. Among vegetables oils, palm oil is the richest natural source of Tocotrienols. Tocotrienols are surprisingly not found in any other vegetable oils like soybean oil, canola oil, rape seed oil and sunflower oil. Tocotrienols can be found naturally but in much lesser quantities in rice barn, barley, wheat gem and oats.

Palm Tocotrienols complex contains significant amount of D-Alpha-Tocopherol, D-Alpha-Tocotrienol, D-Gamma-Tocotrienol and D-Delta-Tocotrienol as compared to those other naturally found vitamin E, which are mostly Tocopherols.



**Figure 1.2:** Tracking Tocotrienols in different oils

### 1.3 The palm oil industry

#### 1.3.1 Market statistics and prices

Malaysia currently accounts for 51% of world palm oil production and 62% of world exports, and therefore also for 8% and 22% of the world's total production and exports of oils and fats (MPOPC, 2005). As the largest producer and exporter of palm oil and palm oil products, Malaysia has an important role to play in fulfilling the growing global needs for oils and fats in general.

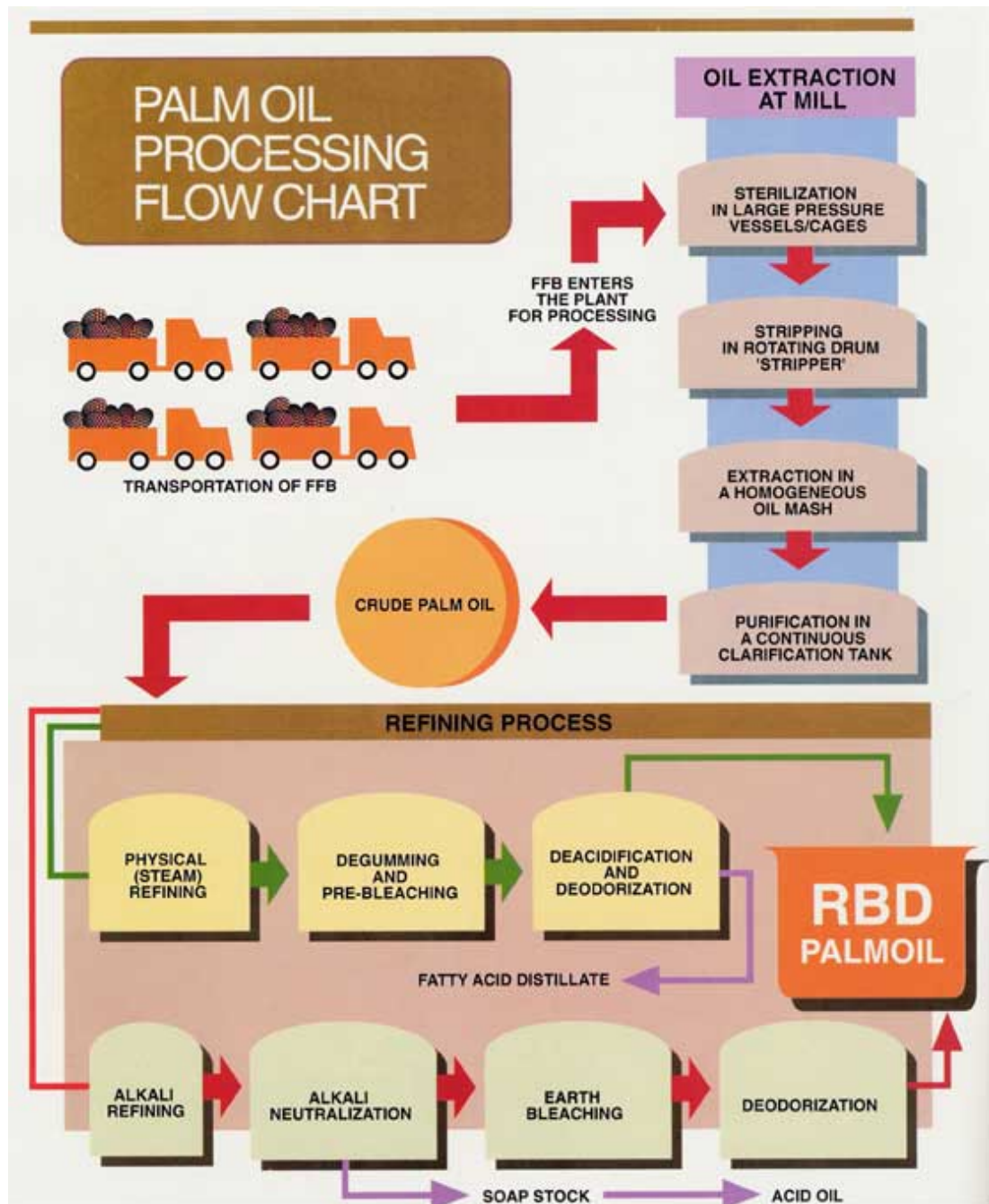
**Table 1.1:** Table below shows the summary of the Malaysian palm oil industry 2005

	Dec '04	Jan '05	Feb '05	Mac '05
<b>Production (Tonnes)</b>				
Crude palm oil	1,313,679	1,153,177	1,048,180	1,215,939
Palm kernel	342,869	313,609	288,320	335,838
Palm kernel oil	162,982	151,413	130,944	161,230
Palm kernel cake	187,677	174,404	150,233	182,884
<b>Closing stock (Tonnes)</b>				
Palm oil	1,489,788	1,571,222	1,540,170	1,446,768
Palm kernel	167,476	149,446	149,176	134,545
Palm kernel oil	194,934	169,018	171,272	192,879
Palm kernel cake	238,417	238,932	233,222	229,410
<b>Export (Tonnes)</b>				
Palm Oil	1,002,778	1,002,778	935,323	1,190,547
Palm kernel oil	84,778	84,778	51,941	79,529
Palm kernel cake	141,195	141,195	144,681	212,113
<b>Import (Tonnes)</b>				
Crude palm oil	30,500	29,400	54,267	34,656
Processed palm oil	2,855	9,244	2,581	10,185
<b>Price</b>				
Fresh fruit bunches (1% equivalent)	14.85	14.01	13.67	15.14

### 1.3.2 The processing of palm oil

A unique feature of the oil palm is that it produces two types of oils – palm oil from the flesh of the fruit, and palm kernel oil from the seed or the kernel. For every 10 tonnes of palm oil, about 1 tonne of palm kernel oil is also obtained.

Several processing operations are used to produce the finished palm oil that meets the users' requirements. The first step of processing is at the mill, where the crude palm oil is extracted from the fruit. The various steps in extraction are shown in Figure 1.3 below.



**Figure 1.3:** Steps in palm oil extraction

The crude palm oil may be further refined during the second stage of processing to get a wide range of palm products of specified quality. The partly and wholly processed grades require little further treatment before use, offering end users a saving in processing cost.

As mentioned previously, palm oil can also be fractionated, using simple crystallization and separation processes to obtain solid (stearin) and liquid (olein) fractions of various melting characteristics. The different properties of the fractions make them suitable for a variety of food as well as non-food product.

#### **1.4 Problem Statement**

Palm oil mill effluent (POME) has been treated using various technologies, yet not many of them provide a solution for waste degradation. Microbial degradation plays an important role in treating the wastewater from the mill, yet microbial community structure of the waste was not fully understood. This study designed to understand the structure of microbial community in UASB and their phylogenetic relation.

## **1.5 Objectives**

The main objectives of this study were as follows:

- (i) To elucidate species that constituted the community.
- (ii) To assess community structure and its variation.
- (iii) To determine phylogenetic affiliation of the community members.
- (iv) To determine the genetic diversity of natural microbial communities.
- (v) To evaluate the UASB performance in relation to upflow velocity and influent pH.

## **1.6 Scope of Investigation**

This study was confined to the following scopes:

- (i) The study focused on introducing laboratory-scale UASB for POME treatment.
- (ii) This study concentrated on the raw POME taken from Palm Oil Mill in Bukit Besar, Kulai.



## **1.7 Hypotheses**

The implementation of UASB treatment process by focusing on microbial community structure gives a better knowledge about microbial degradation, and subsequently improves the performance of the UASB system.

## **1.8 Limitations**

In this study, the application of UASB system in POME treatment were limited to the nature of the UASB process (long duration), time constraint and availability of certain resources needed in carrying out laboratory work.

## **1.9 Thesis Outline**

This thesis consists five chapters. Presentation of the findings of the present investigation begins in Chapter II with a literature review, describing the background of this study. The characteristics of POME, different treatment technologies applied in POME degradation, the existing UASB system, microbial community structure and molecular techniques used in this assessment. Consequently, the overview concept and theory involved in UASB system were discussed.

Chapter III explains the methodology used in UASB process and microbial community structure assessment. In addition, this chapter describes the software

used in assessing the microbial community. Some simple manual methods according to APHA were discussed.

Chapter IV presents the results obtained from both laboratory measurements and literature findings. Findings were combined and discussed together as a whole in discussion.

Chapter V, conclusion of the finding of this thesis was mentioned. Future work on this topic was also being suggested.

### **1.10 Significance of Research**

The main aim of this study was to assess the microbial community in the UASB system. Some potential POME-degrading microbes were identified and microbiology as well as mathematical approaches using software were the main approaches involved in conducting this research. The relationship of these identified microorganisms was studied to give a clearer blueprint of their contribution towards POME treatment in UASB system.

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