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EFFECT OF SOIL SUCTION ON SOIL MICROBIAL ACTIVITY

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Report submitted in partial fulfilment of the requirements for the award of the

Degree of Bachelor of Civil Engineering

Faculty of Civil Engineering & Earth Resources

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JULY 2015

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

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Specially dedicated to:

My parents

Haji Zamanuddin bin Haji Abdul Rahman & Hajjah Soraya binti Haji Salim

My Siblings

Saidatina, Zharif & Syafiqah

And

To All of My Lecturers and Friends.

Thanks for Everything

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ABSTRACT

In Malaysia, the total oil palm acreage rises from year 1970-2000. Palm oil mill effluent (POME) is a residual liquid waste product obtained after extraction of soil from the fruits of oil palm and has high content of nutrients, total solids, total suspended solids that causes pollution. Palm oil mill generated huge POME with an average of 53 million m3 and is poured away into available land near the mill. By using soil microbial, it is able to treat the contaminated soil that is caused by POME. However, soil bacteria can only survive in an optimum condition, thus, the water content in the soil should be determined. Therefore, two samples of soil, (i) uncontaminated soil (ii) contaminated, are tested with different suctions by using Vapour Equilibrium Technique to find the optimum water content and suction for the soil microbial to survive. In order to check the reduction of contaminants which is Carbon (C) in the contaminated soil, the initial and final total organic carbon (TOC) of contaminated soil is recorded. The identification of the soil bacteria is by using Spread Plate Method. The test is conducted on the uncontaminated soil to identify the fungi and bacteria that digested the carbon in the soil. Based on the results, there is a reduction of the contaminant in the polluted soil by 3.884 mg/L. The optimum water content and suction for the soil bacteria to make it able to reduce the contaminant is 83.49% and 10.58 MPa respectively. Therefore, the soil microbial is able to treat contaminated soil caused by POME with an applied suction of 10.58 MPa and an optimum water content of 83.49%.

ABSTRAK

Di Malaysia, jumlah keluasan kelapa sawit meningkat dari tahun 1970-2000. Efluen kilang minyak kelapa sawit (POME) adalah sisa bahan buangan cecair yang diperolehi selepas pengekstrakan tanah dari buah kelapa sawit dan mempunyai kandungan yang tinggi nutrien, jumlah pepejal, jumlah pepejal terampai yang menyebabkan pencemaran. Kilang minyak kelapa sawit menghasilkan POME yang tinggi dengan purata 53 juta m3 dan dibuang ke dalam tanah yang ada berhampiran kilang. Dengan menggunakan mikrob tanah, ia mampu untuk merawat tanah yang tercemar yang disebabkan oleh POME. Walau bagaimanapun, bakteria tanah hanya boleh hidup dalam keadaan yang optimum, dengan itu, kandungan air di dalam tanah hendaklah ditentukan. Oleh itu, dua sampel tanah, (i) tanah yang tidak tercemar (ii) tercemar, diuji dengan sedutan air yang berbeza dengan menggunakan teknik wap keseimbangan untuk mencari kandungan air yang optimum dan daya sedutan untuk mikrob tanah untuk terus hidup. Dalam usaha untuk memeriksa pengurangan bahan cemar dimana ia adalah Karbon (C) di dalam tanah, jumlah karbon organik (TOC) dalam tanah yang tercemar yang awal dan akhir ditentukan. Pengenalpastian bakteria tanah adalah dengan menggunakan Kaedah Penyebaran Plat. Ujian ini dijalankan di atas tanah yang tidak tercemar untuk mengenal pasti kulat dan bakteria yang dicerna karbon di dalam tanah. Berdasarkan kepada keputusan, terdapat pengurangan bahan cemar di dalam tanah yang tercemar oleh 3,884 mg / L. Kandungan air yang optimum dan daya sedutan yang optimum untuk bakteria tanah mampu mengurangkan pencemaran adalah 83,49% dan 10,58 MPa masing-masing. Oleh kerana itu, mikrob tanah mampu merawat tanah yang tercemar yang disebabkan oleh POME dengan meletakkan daya sedutan 10.58 MPa dan kandungan air optimum 83.49% dalam tanah.

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NA	Nutrient Agar
PDA	Potato Dextrose Agar
SWCC	Soil-Water Characteristic Curve
TOC	Total Organic Carbon

Chapter 1

INTRODUCTION

1.1 INTRODUCTION

Palm oil is one of the most important vegetable oils in the world's oil and fats market. The development of oil palm in Malaysia has been acknowledged by many. In 1920, the hectarage of palm oil plantation is 400 and it increases to 5,000,109 hectares in 2011. Palm oil mill generated huge POME (Rupani, *et al*., 2010) with an average of 53 million m3 (Madaki and Seng, 2013). A rapid increase in both downstream and upstream activities imposed an impact on environment.

Wastewater is discharged in the process of extracting the oil from the palm oil. This wastewater is called as palm oil mill effluent (POME). POME is a residual liquid waste product obtained after extraction of oil from the fruits of oil palm and has high content of nutrients, total solids, total suspended solids that causes pollution (Rupani, *et al*., 2010). POME is disposed into available land, waterways or non-concrete lining pond and cause pollution to the soil, ground water and surrounding environment.

The capability of POME to pollute soil and groundwater is due to soil suction that is induced in the soil. Soil suction is an ability of a soil to store water. According to Murray and Sivakumar (2010), soil suction is an ability of a soil to absorb additional water, whether it is fully saturated or unsaturated.

However, polluted soil can be cleaned up by the use of microorganisms that is available in soil. In soils, there are numerous living organisms which includes bacteria, fungi, algae, protozoa and viruses. These organisms live together as one community and very important for passive and aggressive treatment in subsurface regime (Yong, *et al*., 2007). There are many researchers have done in remediating soil by using soil microbes such as Ojonoma and Udeme (2014).

1.2 PROBLEM STATEMENT

Palm oil mills has generated to extremely high polluting waste-water, known as Palm Oil Mill Effluent (POME). POME that is produced due to the processing of palm oil is often discarded to an unlined concrete pond. Due to soil suction, this problem leads to the leaching of contaminants that pollute the soil and groundwater. Apart from that, POME releases methane gas to atmosphere. POME has extremely high content of degradable organic matter (carbon) and nutrients such as nitrogen, phosphorus and calcium (Ojonoma & Udeme, 2014). POME also contains a very high biochemical oxygen demand (BOD) and chemical oxygen demand (COD), which is 100 times more than the municipal sewage.

POME is a not a toxic material, since there is no chemical added during the oil extraction process. However, it is reported that POME has caused environmental issues due to the reduction of oxygen. These oxygen is needed by aquatic life, unfortunately, the depletion of oxygen is due to the organic and nutrient contents.

Due to high contents of nutrients in POME it is necessary to treat the POME infiltrated into the ground. Literature suggested that, soil microbe could be beneficial in treating the POME contaminated soils. This study is conducted to identify the optimum suction for microbial activity to take place as well as to determine the effectiveness of soil microbe in treating the contaminated soil.

1.3 OBJECTIVE

The main objective of this study:

a) To obtain the optimum suction and water content of a soil for soil microbial to survive.
b) To identify the type of microbe present in soil.
c) To check the effectiveness of soil microbe to treat the POME contaminated soil.

1.4 SCOPE OF STUDY

The main purpose of this research is to obtain optimum suction and water content of a soil for soil microbial to survive, to identify the type of microbe that is available in soil and lastly is to check the effectiveness of soil to treat POME contaminated soil.

The test will be conducted in three laboratory, known as, geotechnical laboratory, environmental laboratory and biotechnology laboratory. The soil and POME which is obtained from palm oil mill in Jabor, Pahang are considered in this study. The samples are generically produced to imitate POME contaminated soil.

An establishment of soil-water characteristic curve is to be studied in order to achieve the first objective which is obtaining optimum suction and water content for the soil microbial to survive. The establishment is done with drying test by using vapour equilibrium technique to induce suction in soil in the range from 3 MPa to 300 MPa. Only one contaminant is considered, namely, Carbon (C) to assess the ability of soil microbe to treat the POME contaminated soil.

Chapter 2

LITERATURE REVIEW

2.1 INTRODUCTION

This chapter presents a brief reviews on palm oil mill effluent (POME), soil suction, soil-water characteristic curve (SWCC) and the application of soil microbiology. In addition, few techniques to control suction and measure suction is comprehensively explained in this chapter such as axis translation technique, osmotic technique, vapour equilibrium technique and chilled mirror dew point technique.

2.2 PALM OIL MILL EFFLUENT (POME)

In Malaysia, the acreage of palm oil has increased from 320 to 3,338 hectares from the year 1970 to 2000 (Rupani, *et al*., 2010). According to Reeves *et al*. (1999), palm oil is an edible plant oil derived from the pulp of oil palm *Elaeis gyuineensis* (Ibe, *et al*., 2014). Table 2.1 shows the agriculture acreage in Malaysia from year 1970 to 2000.

Table 2.1 Malaysia – Agriculture Acreage 1970-2000 [1000 Hectares] (Rupani, *et al*., 2010)

Crops	**1970**	**1985**	**1990**	**1995**	**2000**
Oil palm	320	1,482	2,030	2,540	3,338
Rubber	2,182	1,949	1,837	1,679	1,590
Crops	1970	1985	1990	1995	2000
Rice	533	655	681	673	692
Coconut	349	334	316	249	116

Palm oil is extracted by using huge amount of water. According to Yacob *et al*. (2005), palm oil mills in Malaysia in 2004 generated an average of 30 million tonnes of POME while, Variappan and Yen (2008) recorded that 66.8 million tonnes of POME were produced in 2005 (Madaki & Seng, 2013). The production of POME is increasing over the years due to the high growth rate of palm oil production.

2.2.1 Composition of POME

Based on Madaki and Seng (2013), POME is a thick brownish colloidal mixture of water, oil and fine suspended solids when it is fresh. POME consists of water, cellulosic material, fat, oil, suspended solids and total dissolved solids (Rupani, *et al*., 2010) and very high degradable organic matter (Ojonoma & Udeme, 2014). The characterisitics of POME is said to be relied upon the quality of the raw material and the production process of palm oil. POME also possessed 4.5 pH value due to the existence of organic acids and contains very high Biochemical Oxygen Demand (BOD).

Solids that is found in POME known as palm oil mill sludges (POMS) are leaves, seed shells, decanter cake, fibre and empty fruit branches. These solids comprises in POME in the range of 18,000 mg L^{-1} up tp 40,000 mg L^{-1} as shown in Table 2.2.

Table: 2.2 Characterisic of raw POME and the regulatory discharge limits (Rupani, *et al*. ,2010)

Parameters *	**Value**	**Regulatory discharge limits**
Temperature	80-90	45
pH	4.7	5.0 – 9.0
Biochemical Oxygen Demand	25,000	100 (50)
Chemical Oxygen Demand	50,000	-
Total Solids (T.S)	40,500	-
Total Suspended Solids (T.S.S)	18,000	400
Total Volatile Solids (T.V.S)	34,000	-
Oil and Grease (O&G)	4,000	50
Ammonia-Nitrate (NH3-N)	35	150
Total Kjeldahl nitrogen (TKN)	750	200

*All values are in mg/L except for pH

2.2.2 POME as pollutant

POME has been reported as a source of pollutant to the soil and groundwater as it could alter the physicochemical properties of soil, reduce the growth of oil palm seedlings, oxygen depletion in water body and disturb the bio-mass in POME polluted soil (Ojonoma & Udeme, 2014). The high concentration of BOD in raw POME is caused by the residual oil. Before the oil can be completely decomposed, the microbes in water require or demand high level of dissolved oxygen. These microbes take dissolved oxygen faster than atmospheric oxygen can dissolve in water in order to digest the organic matter. Hence, these phenomenon could result in oxygen depletion in water way as well as the death and reduction of aquatic life. Bek Nielsen, *et al*. (1999) found out that POME has caused problem in water quality (Okwute, *et al*., 2007).

According to Orji, *et al* (2006), the pollution due to palm oil has become a serious problem because of the rapid growing rate of the palm oil mill industries (Ibe, *et*

al., 2014). POME is normally discharged on farmland (Ibe, *et al*., 2014) near the mills. In Malaysia, the most recognised method to treat POME is utilised by open pond system due to its low capital and operating cost (Baharuddin, *et al*., 2010) that will cause the effluent to seep into the ground, therefore, the pollution will occur.

2.3 SOIL SUCTION

Soil suction is defined by Richards (1974) as the water potential in a soil-water system. It is the state of soil water (Fredlund & Rahardjo, 1993) or ability of a soil to absorb additional water, whether it is fully saturated or unsaturated (Murray & Sivakumar, 2010). The free energy of the soil water can be measured in terms of the partial vapour pressure of the soil water (Fredlund & Rahardjo, 1993).

Soil suction shows a relation with relative humidity(%). According to (Fredlund & Rahardjo, 1993), when the relative humidity is equal to 100% in a soil, it would indicate the suction in the soil, (i.e soil suction = 0). However, if the relative humidity value is less than 100%, then there is a presence of suction in the soil. It is useful to know the relationship between the suction value of a soil and its water content in order to know the behaviour of volume changes and shear strength in the soil (Barbour, 1998).

Total suction is calculated as:

$$h_t = h_m + h_\pi \qquad \text{Eq. 2.1}$$

where h_t is the total suction of the soil, h_m is the matric suction, and h_π is the osmotic suction.

Total suction can be written using Kelvin's equation associated with relative humidity:

$$h_t = \frac{RT}{V} \ln\left(\frac{P}{P_o}\right) \qquad \text{Eq. 2.2}$$

where h_t is total suction, R is universal gas constant, T is absolute temperature, V is molecular volume of water, P / P_o is relative humidity, P is partial pressure of pore

water vapor, and P_o is saturation pressure of water vapor over a flat surface of pure water at the same temperature.

Due to soil suction that occurs in soil, POME which is mainly constitutes of 97% of water could infiltrate the soil and inevitably seep into the ground and eventually contaminate groundwater as described by Bek Nielsen (1999).

2.4 SUCTION CONTROL TECHNIQUES

Several techniques have been performed in controlling suction of soils such as axis translation technique, osmotic technique, vapour equilibrium technique and chilled mirror dew point technique. Each technique induces different suction values as well as each of it has its own unique way to implement the test.

2.4.1 Axis Translation Technique

This technique has been used by many researchers and only allowed for a measuring a matric suction in the soils (Fredlund & Rahardjo, 1993). The soil specimen is placed on top of a saturated high entry disk in an air pressure chamber. According to Olson and Langfender (1965), in order to make sure that there is a good contact between soil specimen and the disk, a 1kg mass is put on top of the soil specimen. The setting of this technique including the soil specimen to be put on the disk has to be performed rapidly of approximately 30s. The water pressure compartment below the disk has to be maintained nearly zero pressure by increasing the air pressure in the chamber. The pressure transducer connected to the water is used as null indicator.

The matric suction of the soil specimen is measured by the difference between the air pressure and negative water pressure ($u_a - u_w$) in kPa at equilibrium (Fredlund & Rahardjo, 1993). There is signification relationship between the decreasing of water content and increasing of matric suction (Fredlund & Rahardjo, 1993).

Generally, axis translation technique can be used to measure negative pore-water pressure wth a maximum high air entry disk value up to 1500 kPa (Fredlund & Rahardjo, 1993).

2.4.2 Osmotic Technique

Osmotic technique is developed by a biologist then adopted by soil scientists and geotechnical researchers due to limitations in axis-translation technique (Ng & Menzies, 2007). Osmotic technique control matric suction of a soil (Delage, *et al*., 2008). The soil specimen is placed in contact with a semi-permeable membrane behind an aqueous solution containing large sized polyethyleneglycol (PEG) molecules that is circulated. The membrane is permeable to water and ions in the soil but it is not permeable to large solute molecules and soil particles (Ng & Menzies, 2007). PEG molecules cannot pass through the membrane while water molecules can, an osmotic suction is applied to the sample through the membrane. According to Blatz, *et al*. (2008), suction value can obtained in the range of 0 to 10 MPa.

The limitation of this technique is the membrane is sensitive to microbial attack. Due to microbial attack, the PEG solution can be easily infiltrated into the membrane and suction is no longer controlled (Delage, *et al*., 2008). Therefore, it is important to use penicillin in the solution before starting the test in order to prevent from bacteria attack.

2.4.3 Vapour Equilibrium Technique

Vapour equilibrium technique is done by controlling the relative humidity in a closed system (Delage, *et al*., 2008). In this technique, soil specimens is placed on top of a porous disk over a salt solution in a glass desiccator (see Figure 2.1) and generally, the time taken for the soil to achieve an equilibrium state is observed (Tang & Cui, 2005).

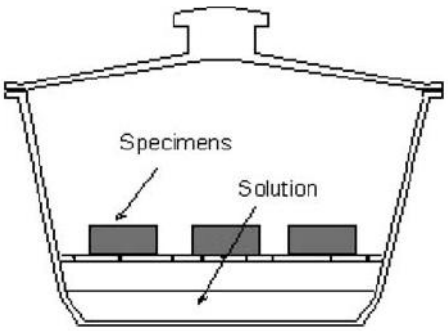

Figure 2.1: Vapour equilibrium technique

Suction is produced in a soil specimen due to an event of a net water exchange between liquid and vapour phases in a desiccator (Delage, *et al*., 2008). The net water exchange between liquid and vapour phases has to be at equilibrium in order for the suction value to be taken. However, the primary disadvantage in this technique is that the time to reach moisture equilisation is very long (two to three months) because of the vapour transfer depends on the diffusion process that controls the transfer of water vapour (Blatz, *et al*., 2008). Secondly, a small changes in temperature will effect the soil suction, therefore there should be a very tight control on temperature (Blatz, *et al*., 2008).

Two types of osmotic solution used in this technique which is (1) saturated salt solutions and (2) unsaturated acid solutions (Blatz, *et al*., 2008). The advantage of using saturated salt solutions is that the concentration of osmotic solutions does not vary though there is an exchange of water between soil specimen and vapour environment and due to safety concern (Blatz, *et al*., 2008). The drawbacks of using salt solution is that each type of salt will control the suction in the specimen based on the purity and specific behaviour and properties of the chemical component (Blatz, *et al*., 2008). The range of suction in vapour equilibrium technique ranging from 3 MPa to 1000 MPa (Ng & Menzies, 2007). The second osmotic solution which is the unsaturated acid solution, it imposed a higher suction than the saturated salt solution. However, the concentration of the solution varies in the event of water exchange, thus, it effects the targeted suction value.

2.4.4 Chilled-Mirror Dew-Point Technique

Chilled-mirror sensing technology has been used since the 1950s for determination of dew-point temperature in a closed, humid environment (Juca, *et al*., 2004). This approach allows the total suction to be determined with an upper limit of 60 MPa with an equilibrium time of around five minutes (Murray & Sivakumar, 2010). Humidity measurement involves thermoelectric chilling for a reflective surface, usually a metallic mirror, to a temperature at which condensation from ambient water vapour in the closed chamber forms on the mirror surface (Juca, *et al*., 2004). A beam of light, typically from a light-emitting diode (LED), is directed to the mirror and reflected to a photodetector (Juca, *et al*., 2004). When condensation occurs as the mirror is cooled to the dew-point temperature, the light reflected from the mirror is scattered and the intensity detected by the photodetector is consequently reduced (Murray & Sivakumar, 2010). The dew-point temperature is maintained contant by a microprocessor circuit and measured by a resistance thermometer embedded in the mirror. The total suction can be calculated using Kelvin's law.

2.5 SOIL – WATER CHARACTERISTIC CURVE (SWCC)

SWCC is discussed because there is a dinstinctive relationship between water content and soil suction. There are many terms used to refer the relationship between soil suction and water content. For instance, soil-water characteristic curve, water-suction relationship, retention curves, and moisture retention curves.

However, the term "soil-water characteristic curve" is used to describe the water content of the soil that is measured. The term characteristic is used to explain the character or behaviour of the unsaturated soil such as the permeability functions, water storage functions, shear strength functions and thermal property functions (Fredlund, *et al*., 2011). SWCC is identified as the key soil information required for the analysis of seepage, shear strength, and volume change problems involving unsaturated soils (Juca, *et al*., 2004).

2.6 MICROORGANISMS IN SOILS

Biological properties of soils are very important factors in the passive and aggressive treatment and management of organic chemical in the subsurface soil regime (Yong, *et al*., 2007). According to Lipman (1931), Microorganisms that live in soil consist of bacteria, ray fungi, algae, protozoa, round worms, Rotarians, and larvae of insects. These microorganisms are very small and cannot be seen by naked eye except for certain type of fungi, and insects. Microorganisms in soils find soil as their medium in performing their activities such as degradation of organic matter, disease suppression, disease and nutrient (Jenkins, 2005). Apart from that, microorganisms obtain an enough supply of energy and nutrient from soil for their growth and production.

2.6.1 Bacteria

Bacteria are the most ample microorganisms in soil. And it has a size range of 5 to 8 microns (Lipman, 1931). Due to its size, the quantity of bacteria in soil per unit area is numerous. Bacteria activity is affected by the presence of oxygen, moisture, inorganic compound, or any change in temperature of the surrounding (Lipman, 1931). Soil bacteria is allocated into three groups in which the first group is cocci, second is bacilli and the third is spirilla.

The optimum temperature for most of the bacteria to grow is in between 20 to 30^{o}c (Jenkins, 2005). The sufficient amount of moisture and oxygen is required for the bacteria to survive. There are numerous processes brought by soil bacteria in order for it to survive if any lack of sources. These organisms is mutually supporting with one another.

2.6.2 Fungi

Fungi is a microscopic plant-like cell that grows as long strands called as hyphae (Hoorman, 2011). Fungi acts as a recycle bin and it reabsorbs nutrients in soil (Yong, *et al*., 2007). The size of fungi is 0.001 inch in diameter (Hoorman, 2011). Mushroom is one type of all fungi and its features are spores, gills and fruiting bodies. Due to its size, fungi dominates the soil biomass although it has lower number of individuals in healthy soils (Hoorman, 2011). Fungi grow more slowly in acidic pH range than bacteria and it is sensitive to changes in moisture levels (Yong, *et al*., 2007).

Fungi plays a very important role in improving the soil structure and increasing nutrient uptakes (Hoorman, 2011). Fungi also acts as decomposer in soil web which the soil web is meant by the community of organisms that live in the soil and how it interacts with environment, plants and animals (Lipman, 1931). Soil is dominated by a population of fungi although its number is lesser than the number of bacteria (Jenkins, 2005). Fungi has the ability to store and recycle carbon (C) because it is a carbon use efficiency of 40-50% and it is also noticed that fungi consists of much higher of carbon (C) content than nitrogen (N) in their cells compared to bacteria (Hoorman, 2011). Many plants develop and use a certain types of fungus and bacteria to raise up the nutrient extraction from soil (Hoorman, 2011).

2.6.3 Microorganism as an agent in treating contaminated soil

Bacteria are becoming increasingly important in bioremediation. Bacteria are capable of filtering and degrading a large variety of human-made pollutants in the soil and groundwater. Case studies showed that microorganisms resided in cow dung & chicken droppings is able to reduce levels of POME in soil (Ojonoma & Udeme, 2014). The microorganisms that present is cow dung is of the genera *Pseudomonas* (Ojonoma & Udeme, 2014). *Pseudomonas aeruginosa* is bacteria that is able to utilize oil as carbon source & could be useful in bioremediation of highly contaminated soil (Das & Mukherji, 2013).

Other than bacteria, fungi are also the choice in bioremediation. Fungi are known to degrade hydrocarbon (Omokaro, 2009). According to Obire (1988), there are several species oil-degrading fungi found in petroleum-producing regions of Nigeria, namely, *Aspergillus niger*, *Penicillium glabrum* and *Trichoderma harzianum* (Omokaro, 2009). While, *Paecilomyces inflatus* is relevant in degrading lignocelluloses in nature, especially soil (Beata, 2007)

Chapter 3

RESEARCH METHODOLOGY

3.1 INTRODUCTION

This chapter presents the methodologies of the experiments to achieve the objectives. The determination of physical characteristics of soil were done according to BS:1337:Part 2:1990 standards. The biological characteristic of soil was done by using the spread plate method. Next, the establishment of SWCCs were done by using vapour equilibrium technique and the determination of carbon contaminant in contaminated soil was done by using total organic carbon (TOC) test.

3.2 SAMPLE SELECTION

Undisturbed soil was collected at palm oil mill at Tawau, Sabah by using sample auger at five metre depths. The sample was sealed in a bag and transported to the laboratory. The sample was crushed and sieved, and only the soil that passed through sieve size 63 micron was considered in this study.

3.3 SAMPLE PREPARATION

Bentonite clay soil that was taken in Tawau, Sabah was used in this experiment. The soil specimens were prepared in slurry conditions which is (i) uncontaminated soil and (ii) contaminated soil. Both of the uncontaminated and contamimated soil was used in the drying tests to establish the suction water content

curve (SWCC). Next, the uncontaminated soil was used to determine the soil microbe that is present in the soil by spread plate method meanwhile the contaminated soil was used for checking of contaminants concentration by using TOC test.

3.3.1 Uncontaminated Soil

Uncontaminated soil specimen was prepared by mixing dried powder soils with distilled water equal to 1.2 liquid limits of soil. The soil specimen then was sealed in plastic bags and stored in air tight containers for the mixture of soil-water to be in equilibrium condition. The storage of the soil specimens took place for seven days in a room with a room temperature.

3.3.2 Contaminated Soil

Contaminated soil specimen was prepared by mixing dried powder soils with palm oil mill effluent to 1.2 liquid limits of soil. The soil specimen was then stored in a sealed plastic bags and kept in air tight container for the mixture of soil-water to be in equilibrium condition. The storage of this soil also took place for seven days in a room with a room temperature.

3.4 DETERMINATION OF PHYSICAL CHARACTERISTICS OF SOIL

3.4.1 Particle Size Distribution

This test is performed to determine the percentage of different grain sizes contained within a soil. Sieve analysis is done to determine the distribution of the large and coarse particles. Fine analysis is used to determine the distribution of finer particles. This experiment is based on the reference of BS1377 – standard test method for particle – size analysis of soils.

3.4.2 Specific Gravity

This test is done to obtain the specific gravity of a soil by using a density bottle. Specific gravity is the ratio of the mass of unit volume of soil at a stated temperature to the mass of the same volume of gas-free distilled water at a stated temperature. This experiment is based on the reference of BS1377: Part 2:1990:8.3 – Standard test of Specific Gravity for fine grained soil using density bottle.

3.4.3 Atterberg Limit

This test is done in order to determine the plastic and liquid limits of a fine grained soil. The liquid limit (LL) is based on measurement on penetration into the soil of a standardized cone of specific mass. At the liquid limit, the cone penetration is 20mm. The plastic limit (PL) is the water content, in percent, at which a soil can no longer be deformed by rolling into 3.2mm (1/8in.) diameter threads without crumbling. This experiment is based on the reference of BS1377: Part 2: 1990:4.Wa3.

3.4.4 Water Content

This test is done to determine the water (moisture) content of soils. The water content is the ratio, expressed as a percentage, of the mass of "pore" or "free" water in a given mass of soil to the mass of the dry soil solids. This experiment is based on the reference of BS1377:1990 – standard test method for laboratory determination of water content of soil, rock, and soil-aggregate mixtures.

3.5 DETERMINATION OF BIOLOGICAL CHARACTERISTICS OF SOIL

10 g of soil sample was suspended in one mole of saline solution for 24 hours. Sodium chloride, NaCl was considered in this test. The solution was prepared in one litre bottle by mixing NaCl salt and deionised water. Soil is then added into the solution. After that, 500 mL was pipette from the bulk solution by spread plate method.

The following is the step to prepare nutrient agar. 11.5 g of powdered nutrient agar (NA) was weighed and put into a glass bottle that contained 500 mL of distilled water. The solutions were swirled for one minute. Then, a magnetic bar was put into the solutions and the solutions were placed on the magnetic stirrer and is heated until the colour of the solutions turned colourless as shown in Figure 3.1.

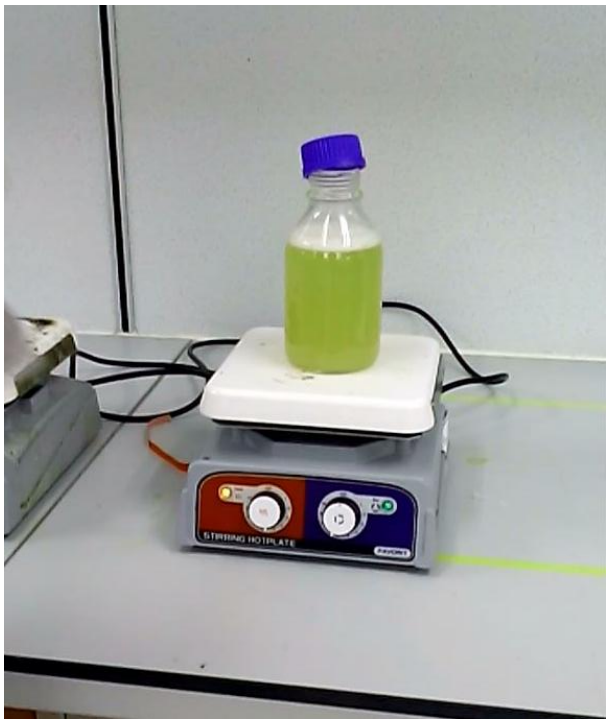

Figure 3.1 The nutrient agar solutions is stirred and heated until the colour turns colourless

In the meantime, the plates for placing the NA can be sterilised in the laminar flow cabinet for 15 to 20 minutes. After the colour of NA solutions have turned colourless, the NA solutions were then placed in the autoclave for sterilisation of agar medium for one and a half hour.

The plates that was sterilised in the laminar flow cabinet was kept reimaned in the cabinet while the NA solution was taken out from the autoclave machine. The NA solutions were immediately placed inside the laminar flow cabinet. The NA solutions were then poured on to the plates with half of full plates and kept it aside for the solution to cool down and the texture turned jelly-like as shown in Figure 3.2.

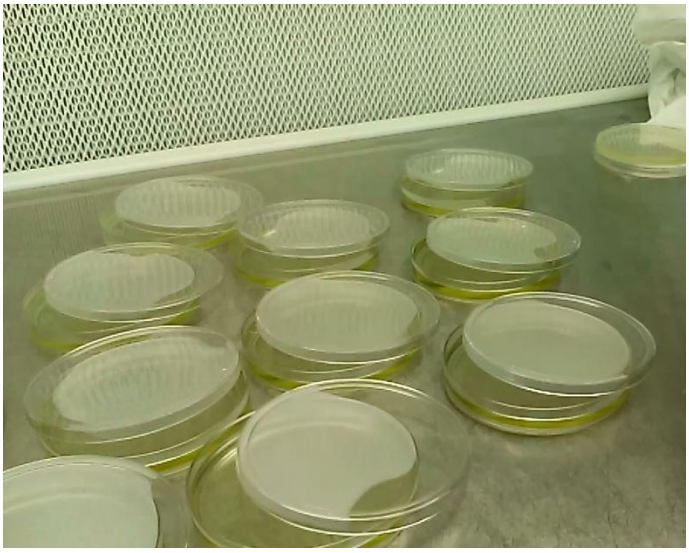

Figure 3.2 The nutrient agar solutions were placed in the plate and set aside for cooling down and changing of textures

After that, the plates were closed with its lids, then it was placed upside down for five minutes. The NA solutions were then kept for storage in air-conditioned room.

The determination of bacteria and fungi in the soil samples was implemented by using Spread Plate Method. 500 microlitres of uncontaminated soil samples were taken and were placed on the NA plates. The lids of the plates were lifted as little as possible and the soil samples were transferred onto the plates. The lids were then placed back on the plates. Then, a sterile spreader was used to spread the soil samples on the plates. The soil samples were spreaded onto all the edges at the end of the plate and the spreading process was stopped when there was a slight resistance when spreading. For potato dextrose agar (PDA), the same steps were repeated, however, different mass of the powder PDA were used.

3.6 DETERMINATION OF SOIL-WATER CHARACTERISTIC CURVE (SWCC)

3.6.1 Drying Test

Drying test of soils was done by using the vapour equilibrium technique. Vapour equilibrium technique was implemented to control total suction of a soil. The test was carried out in closed-lid desiccators by using five types of saturated salt solutions, namely, Potassium Sulphate ($K_2SO_{4)}$, Potassium Chloride (KCl), Potassium Dichromate ($K_2CO_{3)}$, Potassium Nitrate ($KNO_{3)}$, Sodium Chloride (NaCl).

The preparation of saturated salt solutions were by using Chilled-Mirror Dew Point Technique where, firstly, the salts were weighed and it was put in the desiccators with 250 mL of distilled water in it. The solutions were mixed and stirred using magnetic stirrer until the solutions can no longer be dissolved. Next, the saturated salt solutions were used to measure the suction that was induced by the salts by using Chilled-Mirror Dew Point technique.

Next, the soil samples was placed on the porous disk in each desiccators. The mass of the soil samples were then weighed in every three days until no further changes in the mass of soil. Figure 3.3 (a) shows the setup of drying test by using the vapour equilibrium technique and Figure 3.3 (b) shows the compacted soils which consists of uncontaminated and contaminated soil within the desiccators.

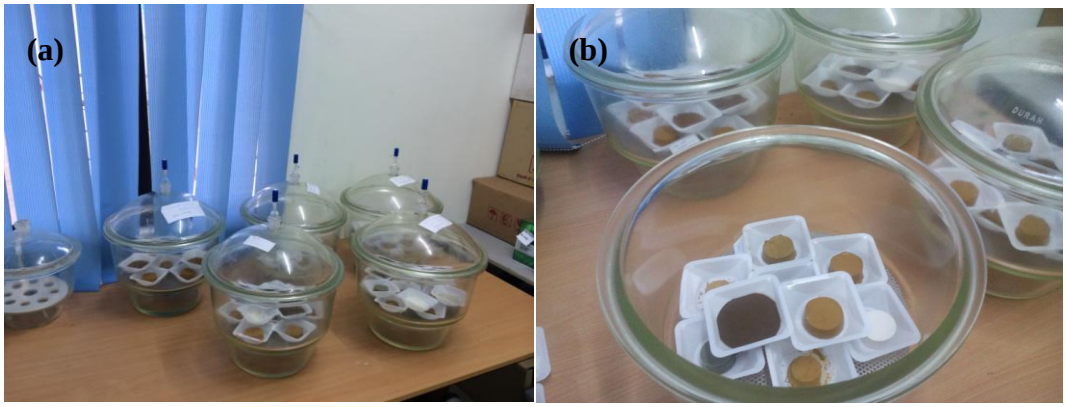

Figure 3.3 Drying Test by using Vapour Equilibrium Technique, (a) Desiccator test setup and (b) Compacted soils within the desiccator

3.7 DETERMINATION OF TOTAL ORGANIC CARBON (TOC)

2 g of the contaminated soil that is under treatment in the desiccator tests were taken in each desiccator. The contaminated soil was taken by a spatula then it was weighed for 2 g. Then, the 2 g contaminated soil was oven dried for a day. After it has been oven dried, the dried contaminated soil was then crushed until it passed through 0.42 mm sieve. The contaminated soil has to be extracted from solid form into liquid form. The extraction method is based on the reference of Soil Survey Standard Test Method : Organic Carbon.

Soil sample from each suction is being extracted into liquid form. 10 mL of potassium dichromate, $K_2Cr_2O_7$ were added in the flask which contains 2 g of dried soil. The flasks were swirled gently to disperse the soil in the solution. 20 mL of concentrated Suphuric Acid, H_2SO_4 were added into the solution and the flasks were immediately swirled.The solutions were then heated until the temperature reaches 135^{o}C by using hot plate equipment . The solution is set aside for it to cool slowly. After the solutions were cooled to room temperature, 200 mL of distilled water is

added in the solution. Figure 3.4 shows a total of six extracted soils including one untreated contaminated soil for the determination of TOC concentration level before treatment.

Figure 3.4 Soil extracted following the soil survey standard test method : organic carbon

The solutions were filtered for 50 mL through a funnel in order to segregate the liquid solutions from the solids that is in the solutions. Then, the same solutions were filtered once again by using syringe filter and was placed in the 50 mL centrifuge tubes. Figure 3.5 shows the extracted soil that has been filtered and kept in the 50 mL centrifuge tubes.

Figure 3.5 Extracted soils in 50 mL centrifuge tubes

A tube was placed in a beaker that contained distilled water for it to act as a blank. The TOC reading was given by the machine through the computer. The computer notified if the process for creating blank is done. The tube was then placed in the extracted soil's centrifuge tube, the reading was given by the machine through the computer. This process was being completed in 30 minutes and once the test was finished, the computer will be notified and the TOC reading will be shown in the computer. The whole process was being repeated for a new sample. Figure 3.6 shows a TOC Analyser based on the reference ASTM D7573-09, Standard Test Method for Total Carbon and Organic Carbon in Water used in this study. Shimadzu TOC-Vcpn was used in this purpose.

Figure 3.6 Total Organic Carbon (TOC) Analyser used in this study

Chapter 4

RESULTS & DISCUSSION

4.1 INTRODUCTION

This chapter presents the findings of the experiments that were done to achieve the objectives. The determination of physical characteristics of soil were done according to BS:1337:Part 2:1990 standards. The biological characteristic of soil was done by using the spread plate method. Next, the establishment of SWCCs were done by using vapour equilibrium technique and the determination of carbon contaminant in contaminated soil was done by using total organic carbon (TOC) test. All results and justifications were elaborated in this chapter.

4.2 PHYSICAL CHARACTERISITICS OF SOIL

The experiments were done to determine the basic properties of the soil based on the basic laboratory test. It was done at Soil Mechanics and Geotechnical Laboratory. The basic properties of the uncontaminated soil is tabulated in Table 4.1.

Table 4.1 Basic properties of the uncontaminated soil

Properties	**Uncontaminated Soil**
Liquid Limit, w_L (%)	129.30
Plastic Limit, W_P(%)	46.12
Shrinkage Limit, W_s(%)	34.00
Initial Water Content, W_i (%)	57.39
Hydroscopic Water Content, W_i (%)	6.43
Organic Matter	17.98
Swelling Potential	40.00
External specific surface area – BET(m^2/g)	34.79
Cation Exchange Capacity,B (meq/100g)	42.77

4.3 BIOLOGICAL CHARACTERISTICS OF SOIL

The identification of soil microbe resided in the soil is based on the spread plate method. After five days, it is found that there are numerous of bacteria and fungi resided in the soil. However, only two fungi were determined in the soil which are Paecilomyces Lilacinus and Trichoderma Atrovirido. Figure 4.1 shows the fungi that resided in the uncontaminated soil. The fungi resided in the soil is Paecilomyces Lilacinus and Trichoderma Atrovirido in reducing the contaminants in contaminated soil.

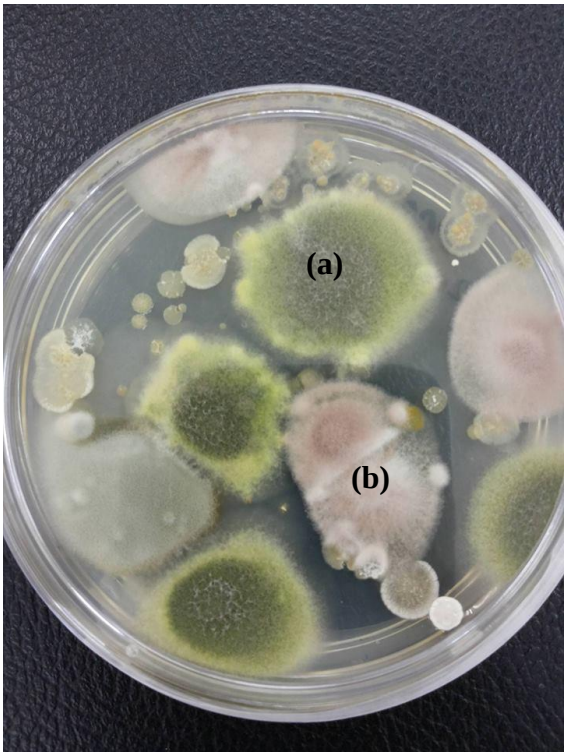

Figure 4.1 Fungi that were found in uncontaminated soil based on spread plate method, (a) *Trichoderma Atroviridо* and (b) *Paecilomyces Lilacinus*

These fungi is a carbon degrading fungi and it plays an important role to the soil as it is able to use carbon as a source of nutrients, therefore, it will contribute to the reduction in the carbon contaminants in the soil.

4.4 SUCTIONS CALIBRATION

Table 4.2 shows the suctions that were induced by the saturated solutions based on Chilled-Mirror Dew Point Technique. Different salts induced different suctions as shown in table below.

Table 4.2 Suctions calibrated by saturared salt solutions

Saturated Salt Solutions	Suctions (MPa)
K_2SO_4	3.60
KNO_3	10.58
KC	23.58
NaCl	39.38
K_2CO_3	111.77

4.5 TIME EQUILIBRATION PLOT

The equilibrium time graph of the unpolluted soil and POME contaminated soil is shown in this sub-chapter. The results is determined from the drying test through vapour equilibrium technique. The water content is calculated through the given mass.

4.5.1 Uncontaminated Soil

Figure 4.2 shows the graph pattern for the drying curves at different suction values. The graph is plotted based on the water content that was measured twice a week until the mass of the soils contant. Water content of the uncontaminated soil is seen to be decreasing as time goes by. The initial water content of the uncontaminated soils at all suctions are at 129.30%. However, as the time passed by, the water content for each suctions decreased with different values. Water was dragged out from the soils along with time. These phenomenon was caused by the suctions that is applied in the systems or desiccators. Based on the figure below, the water content decreases rapidly when the suction is high.

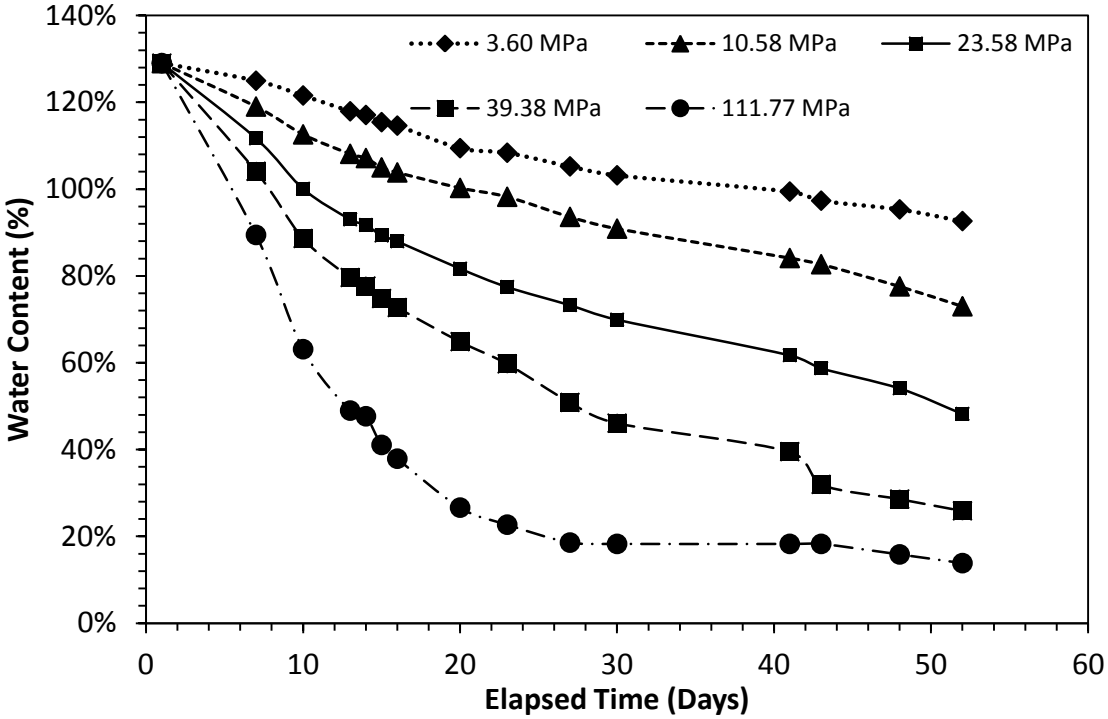

Figure 4.2 Equilibration time in drying tests at various applied suctions for uncontaminated soil

Figure 4.3 shows the comparison of the highest and the lowest water contents of the uncontaminated soil. At an applied suction of 3.60 MPa, the water content is seen to be decreasing slightly. Meanwhile, at an applied suction of 111.77 MPa, the water content is seen to be reducing rapidly starting from day one. However, at day 20 the water content is found that stabilisation is quickly attained and the water content has started to become constant. The wide gap between both suctions shows that at higher suction, the water in the soil is quickly drawn out as compared to the lower suction.

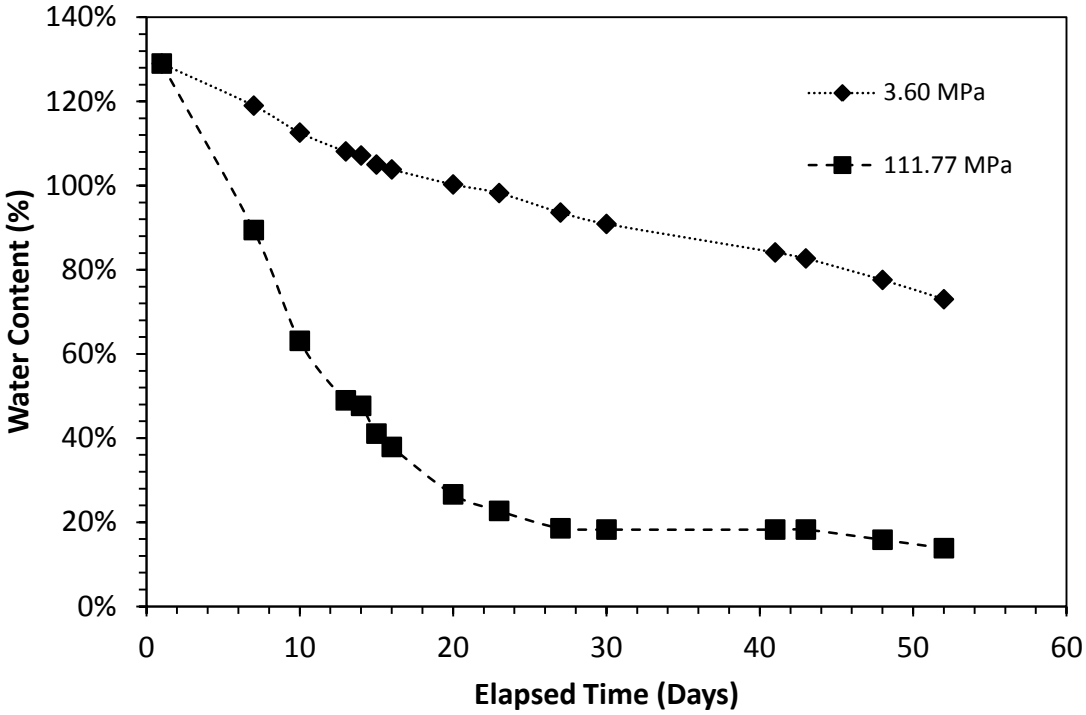

Figure 4.3 Equilibration time in drying tests at applied suctions of 3.60 MPa and 111.77 MPa for uncontaminated soil

4.5.2 Contaminated Soil

Figure 4.4 shows the pattern of the graph for the drying curves at different suction values. The graph is plotted based on the water content that was measured twice a week until the mass of the soils contant. Water content of the contaminated soil is seen to be decreasing as from time to time. The initial water content of the contaminated soils at all suctions are at 105%. However, as the time passed by, the water content for each suctions decreased with different values. Water was dragged out from the soils along with time. These phenomenon was caused by the suctions that is applied in the systems or desiccators. Based on the figure below, the water content decreases rapidly when the suction is high. At day 35, the water content of the soils are found to be reduced drastically. This is due to the experiment that was done for the checking of contaminants, where 2 g of contaminated soils were taken from each desiccators, which resulted in huge difference in the water content at day 35 (see Chapter 3.5).

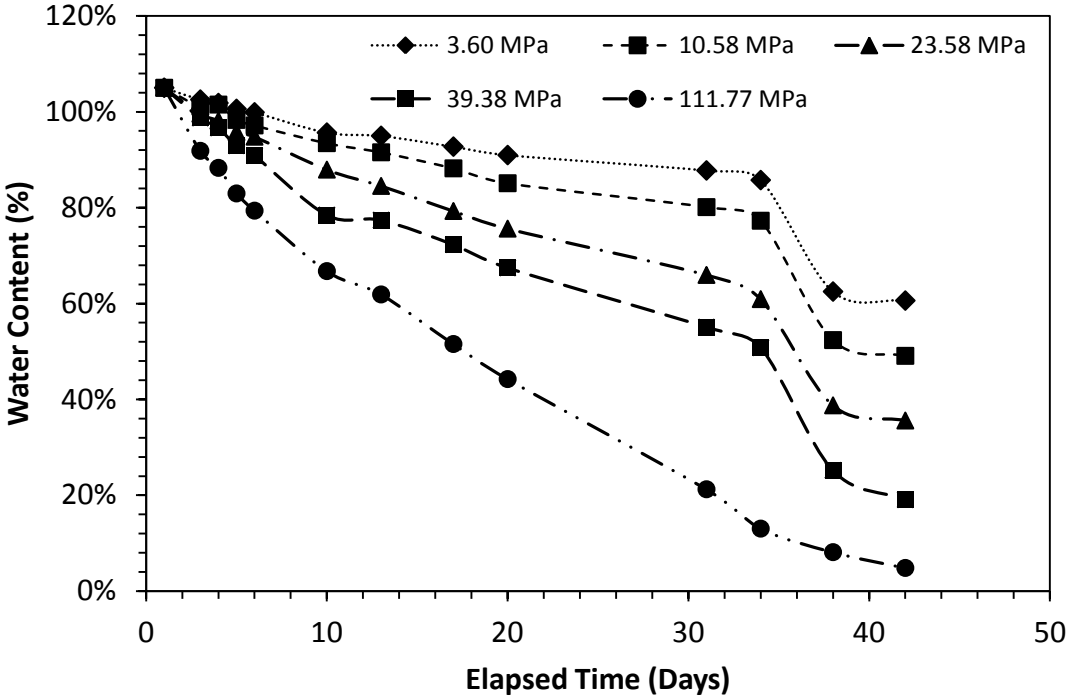

Figure 4.4 Equilibration time in drying tests at various applied suctions for contaminated soil

Figure 4.5 shows the comparison of the highest and the lowest water contents of the contaminated soil. The pattern is the same as uncontaminated soil, at an applied suction of 3.60 MPa, the water content is found to be having a slight decrease. As compared to the applied suction of 111.77 MPa, the water content is seen to be decreasing rapidly starting from day one. The wide gap between both suctions shows that at higher suction, the water in the soil is quickly drawn out as compared to the lower suction.

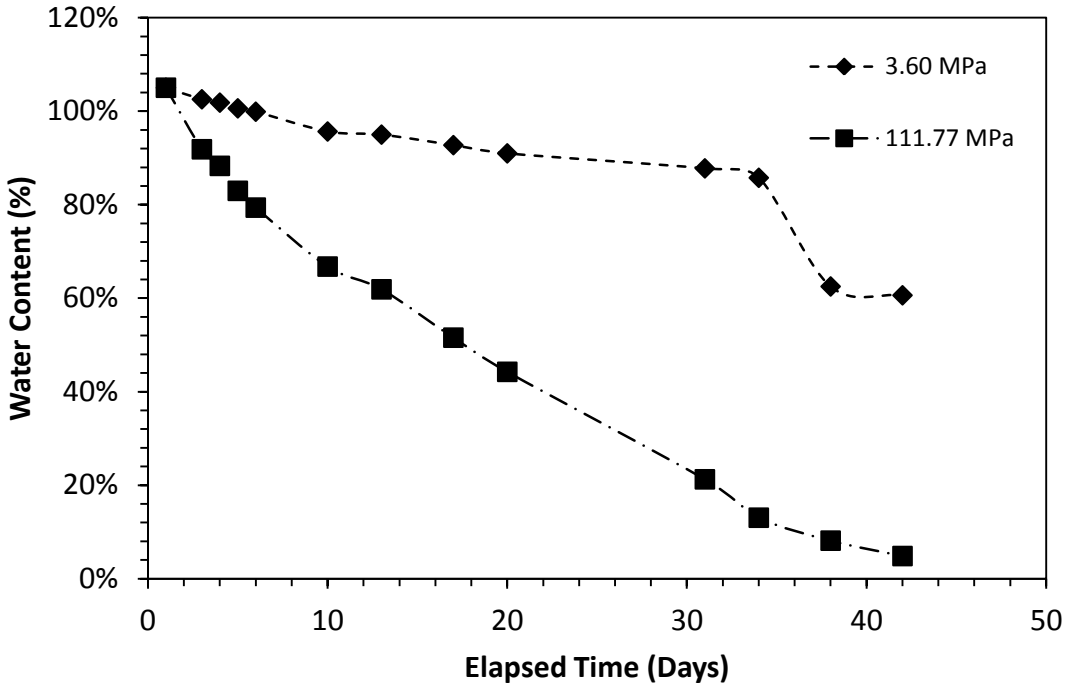

Figure 4.5 Equilibration time in drying tests at applied suctions of 3.60 MPa and 111.77 MPa for contaminated soil

4.6 SOIL-WATER CHARACTERISTIC CURVE (SWCC)

Figure 4.6 shows the comparison of the drying curves of water contents over various suctions between uncontaminated and contaminated soil. The graph is plotted based on the water content obtained at the end of the experiment over the suctions applied to the soil in the desiccator tests. Suctions that were induced to the soils are 3.60, 10.58, 23.58, 39.38 and 111.77 MPa.

Based on the figure below, the water content for both soils are seen to be at different value. This is due to the difference in liquid limit for both soils where for uncontaminated soil and contaminated soils are 129.30% and 87% respectively. However, the pattern for both soils are found to be decreasing in the same way. Thus, the relationship of water content of the soils and suctions that were induced in the desiccators is that whenever the suctions is high, the water contents will be reduced

rapidly. It can be seen in the figure for uncontaminated soil where at an applied suction of 3.60 MPa, the water content is at peak, meanwhile, as the suction increases, the water content decreases. The same pattern can be seen for contaminated soil.

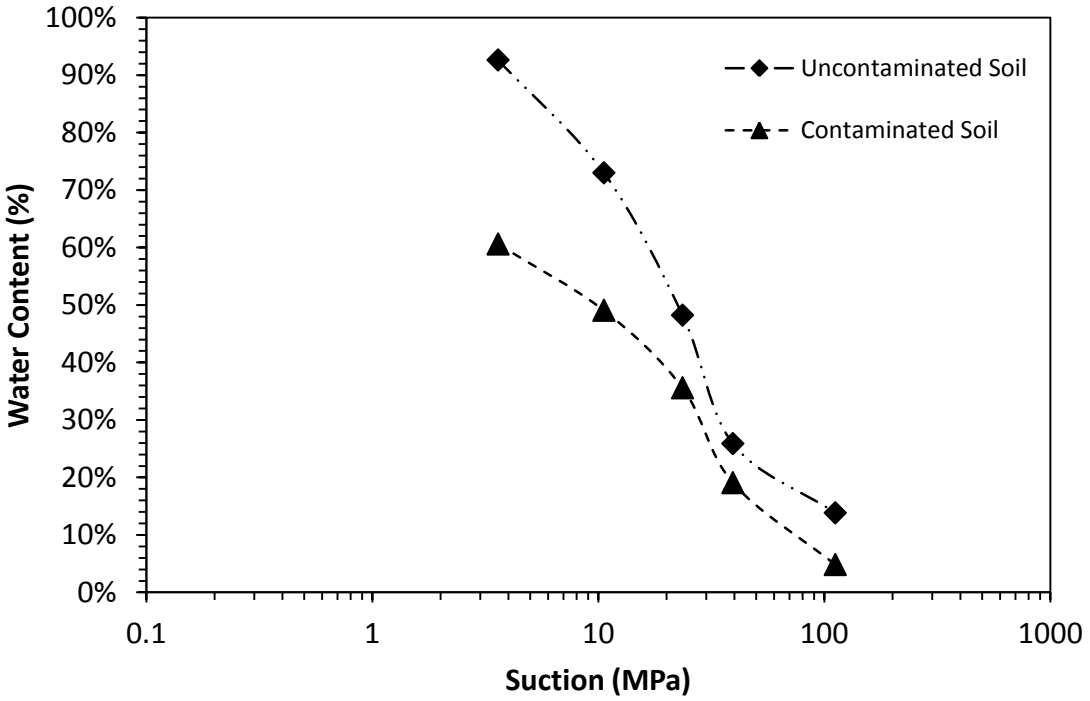

Figure 4.6 Soil-Water characteristic curve of the uncontaminated soil and contaminated soil.

4.7 TOTAL ORGANIC CARBON (TOC) IN SOIL

Figure 4.7 shows the TOC concentration level of the contaminated soils over the suctions that were imposed to the soils. The contaminated soil was placed in the desiccators for treatment by soil microbial, and the TOC concentration level is monitored based on the suctions applied to the soils at the end of the experiment. Initially, the TOC concentration level of the contaminated soil was found to be 8.036 mg/L. After the contaminated soil was placed in the desiccator for seven weeks, the TOC concentration of the soil in each desiccators is found to be reducing compared to the reading before the soil was placed in the desiccators. Based on Figure 4.7, the

lowest TOC concentration is seen to be at an applied suction of 10.58 MPa with 4.152 mg/L, whereas, at suction 39.38 MPa and 111.77 MPa, the TOC concentration is high and have only a slight reduction as compared to the initial TOC concentration (8.036 mg/L). This shows that the optimum suction should be imposed to the soil for better reduction in TOC concentration level is at 10.58 MPa.

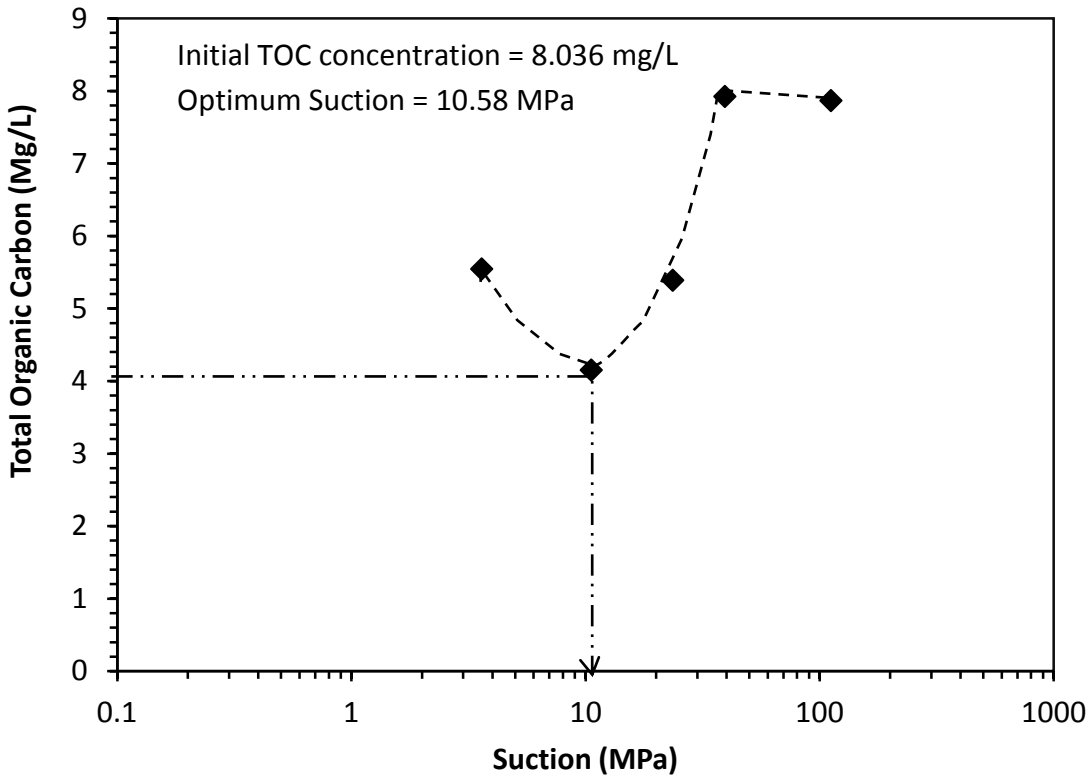

Figure 4.7 Relationship of suction and total organic carbon of POME contaminated soil

In Figure 4.7, the optimum suction for the soil microbial to remove most contaminants carbon is at 10.58 MPa, therefore, the relationship of the water content and suction based on Figure 4.6 is 83.49%. This is determined based on the lowest TOC concentration after the contaminated soil being treated in the desiccator test. When the water content in the soil is 83.49%, it is found that the microorganisms resided in the soil is able to survive, thus, the microorganisms were able to degrade the highest contaminant carbon compared to all other condition of water content.

The microorganisms that were able to reduce the TOC concentration in soils are namely, (i) *Paecilomyces Lilacinus* and (ii) *Trichoderma Atroviride* as stated in Chapter 4.2 . The ability of fungi to decompose the organic compounds such as carbon makes it useful in bioremediation. As mentioned by Omokaro (2009), *Trichoderma harzianum* is able to degrade hydrocarbon. In addition to that, *Paecilomyces inflatus* is relevant in degrading carbon especially in soils (Beata, 2007).

CHAPTER 5

CONCLUSION

5.1 CONCLUSIONS

The conclusions can be made based on the findings of this study:

1. Vapour equilibrium technique can be used to establish SWCC of soils. The equilibration time for both uncontaminated and contaminated soils are similar.
2. The drying curve for SWCC of both uncontaminated and contaminated soils show that high suctions resulted in high water released from soil. Therefore, the water content in soil at higher suctions is lower than lower suctions.
3. The lowest TOC concentration after the contaminated soil were placed in desiccators via vapour equilibrium technique test is 4.152 mg/L compared to the initial concentration of TOC which is 8.036 mg/L.
4. Fungi found in the soil were *Paecilomyces Lilacinus* and *Trichoderma Atroviridо.* Both of these fungi were responsible to reduce the carbon contaminants in the contaminated soils as it is able to use up oil as carbon source.
5. Contaminated soils can be treated by using soil microbe with the optimum suction of 10.58 MPa and the optimum water content of 83.49 %.

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