THE EFFECT OF WATER CONTENT AND NUTRIENT ON SOIL MICROBIAL BEHAVIOUR

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ABSTRACT

Palm oil mill effluent (POME) is one of the major wastes from palm oil mill industry and it has the problematic environmental pollution potential among the palm oil mill wastes. Mostly, factories in Malaysia have been existed since 70^{th} century. In most cases, palm oil mill effluent was discharged in ponds near the mills. In the cases of ponds without proper liner system, POME will tend to seep into deep ground. If the inflitrated POME is not treated, it will inevitably pollute groundwater reservoir. The aim of this study was to demonstrate the optimum water content in which soil microbe can survive effectively to remove contaminants in contaminated soils. The sample of soil will be used in this research study is taken from palm oil mill plantation which is located in Tawau, Sabah. Two samples were prepared in different condition and there are soil-water mixture and soil-POME mixture. The both conditions of samples were prepared by mixing the air dried soil powders with deionised water and POME respectively at target water content equals to 1.2 times with the respective liquid limits of the test soils. Soil-water mixture and soil-POME mixture was placed in the desiccators with different suction pressure generated by different types of salt solutions. Both samples were placed in the desiccator until the stable water content reading were recorded. There are consists five types of salt solutions used in this test which are K_2SO_4, K_2CO_3, Nacl, KNO_3 and KCl. The different salt solution generated different values of suction pressure and water contents. The suction pressure value of 10.58 Mpa is the most effective to generate the optimum water content corresponding to the least value of total nitrogen produced in soil-POME mixture sample. The reduction of total nitrogen in soil-POME mixture was decreased the initial value of total nitrogen from 4.5 mg/l to 0.4 mg/l after undergoing the treatment process through vapor equilibrium technique. The optimum water content for microbe to survive is the most effective at a water content of 83.5%. The identification of microorganisms was carried out by using the spread plating and an isolation plating technique. Two types of bacteria were successfully identified by the central laboratory and there are *Trichoderma atroviridae* and *Paecilomyces lilacinus*. The implication of these findings is that the bacteria found in soil can be useful in the rehabilitation of POME polluted soil and possibly other oil polluted sites.

ABSTRAK

Efluen kilang ladang kelapa sawit (EKLKS) adalah salah satu daripada bahan buangan utama dari industri kilang minyak sawit dan ia mempunyai potensi pencemaran alam sekitar yang bermasalah di kalangan buangan kilang minyak sawit. Kebanyakannya, kilang-kilang di Malaysia telah wujud sejak abad ke-70. Dalam kebanyakan kes, efluen kilang minyak sawit telah dilepaskan dalam kolam berhampiran kilang-kilang. Dalam kes-kes kolam tanpa sistem pelapik yang betul, EKLKS akan cenderung untuk meresap ke dalam tanah. Jika EKLKS yang menyusup tidak dirawat, ia akan mencemarkan takungan air bawah tanah. Tujuan kajian ini adalah untuk menunjukkan kandungan air optimum di mana mikrob tanah boleh hidup dengan berkesan untuk menghapuskan bahan cemar dalam tanah tercemar. Sampel tanah akan digunakan dalam kajian penyelidikan ini diambil daripada kilang perladangan minyak sawit yang terletak di Tawau, Sabah. Dua sampel telah disediakan dalam keadaan yang berbeza dan terdapat campuran tanah-air dan campuran tanah-EKLKS. Keadaan kedua-dua sampel yang telah disediakan dengan mencampurkan udara kering serbuk tanah dengan air deionised dan EKLKS masing-masing pada kandungan air sasaran bersamaan dengan 1.2 kali dengan had cecair masing-masing tanah ujian. Campuran tanah-air dan campuran tanah-EKLKS diletakkan dalam desiccators dengan tekanan sedutan yang berbeza yang dihasilkan oleh pelbagai jenis penyelesaian garam. Kedua-dua sampel diletakkan di desiccator sehingga membaca kandungan air yang stabil telah direkodkan. Terdapat terdiri lima jenis penyelesaian garam yang digunakan dalam ujian ini yang K2SO4, K2CO3, NaCl, KNO3 dan KCl. Penyelesaian garam yang berbeza dihasilkan nilai yang berbeza tekanan sedutan dan kandungan air. Nilai tekanan sedutan daripada 10.58 Mpa adalah yang paling berkesan untuk menjana kandungan air optimum yang bersamaan dengan nilai-kurangnya daripada jumlah nitrogen yang dihasilkan di dalam campuran sampel tanah-POME. Pengurangan jumlah nitrogen di dalam tanah - campuran EKLKS telah menurun nilai awal daripada jumlah nitrogen daripada 4.5 mg / l hingga 0.4 mg / l selepas menjalani proses rawatan melalui teknik keseimbangan wap. Kandungan air yang optimum bagi mikrob untuk terus hidup adalah yang paling berkesan pada kandungan air 83.5%. Pengenalpastian mikroorganisma telah dijalankan dengan menggunakan saduran penyebaran dan teknik plating pengasingan. Dua jenis bakteria telah berjaya dikenal pasti oleh makmal pusat dan terdapat *Trichoderma atroviridae* dan *Paecilomyces lilacinus*. Implikasi daripada ini adalah bahawa bakteria yang terdapat di dalam tanah boleh menjadi berguna dalam pemulihan EKLKS tanah tercemar dan kemungkinan tapak tercemar dengan minyak.

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%	Percentage
SL	Shrinkage Limit
LL	Liquid Limit
PL	Plastic Limit
V	Volume of specimen
V_d	Volume of dry soil
ρ_w	Density of water
M_S	Summation
W_W	Weight of water (g)
W_S	Weight of soil (g)

LIST OF ABBREVIATIONS

POME	Palm Oil Mill Effluent
SWCCs	Suction-water content characteritics
SWCC	Drying suction-water content characteristics
VET	Vapour equilibrium technique
TN	Total Nitrogen

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Raw palm oil mill effluent (POME) consisting of complex vegetative matter is a thick, brownish, colloidal slurry of water, oil and solids including about 2% suspended solids originating mainly from cellulose fruit debris, that is palm fruit mesocarp. The raw or partially treated POME ususally has extremely high content of degradable organic matter and has been reported to alter the physicochemical properties of soil, pollution of waterways and significantly alter microbial numbers in POME polluted soil. Mostly, factories in Malaysia have been existed since 70^{th} century. Normally, the POME generated is poured away into available pieces of lands or ponds located near the mill. POME discharged from an oil mill could pollute streams and surrounding land.

The amount of POME store in soil depends on the soil suction and the permeability of soil. Soil suction is a major factor affecting the behaviour of unsaturated soils and it is defined as the water potential in a soil-water system. Generally, in geotechnical enginnering, the soil water potential is referred to as negative pore water pressure. The negative pore water pressure as quantified in term of relative humidity in soil and is commonly call total suction. POME which consists of water and nutrients seep into the deep ground will cause pollution in soil and the environment. POME pollutant are degraded slowly in soil. The properties of POME which include long chain hydrocarbons make it expedient to remedy the polluted soil to hasten the period of recovery of the soil.

The implication of improper discharge of POME from the oil mill will cause pollution to environment. Therefore, the researchers in the past have shown that, the bioremediation has been successfully used in reducing the POME contaminants in soil. Bioremediation is a process that uses microorganisms to reduce the amount of contaminants in soil. Microorganisms alter and break down the POME into other substances such as carbon dioxide, water and simpler compounds that do not effect the environment. The ability of microorganisms found in soil significantly can reduce the contaminantion in POME polluted sites.

1.2 Problem Statement

In most cases, POME will be discharged in ponds near the mills. In the cases of ponds without proper liner system, POME will tend to seep into deep ground. If the infiltrated POME is not treated, it will inevitably pollute groundwater reservoir. Hence, it is necessary to treat the infiltrated POME. Researchers,in the past have shown that. Soil microbe can be used to reduce the POME polluted soils. However, no attempt has been made to identify the optimum suction in which soil microbe can survive and effectively remove contaminants in contaminated soils.

1.3 Objectives of Study

The main objectives of this researches are to identify the optimum suction in which soil microbe can survive and effectively remove contaminants in contaminated soils, while the sub-objectives of this research are:

1. To determine the optimum water content for microbe to survive.
2. To investigate the types of microbe present in raw soil sample.
3. To check the applicability of soil microbe to treat POME contaminated soil.

1.4 Scopes of Study

The preparation of the soil sample in this research study will categorized into two condition. Two condition were considered are the preparation of soil slurry sample and soil contaminated by POME. The optimum water content of survivality of soil microbe and effectively remove contaminants in contaminated soil can be observed and studied.

The sample of soil will be used in this experiment is taken from the palm oil plantation which is located in Tawau, Sabah and the palm oil mill effluent (POME) is taken from Jabor. The contaminated soil will generically prepared by mixing POME with soil. In this research study, only one type of contaminant will be considered which is Total Nitrogen. In addition, the single type in the same use of soil microbe will be considered in this study.

1.5 Research Significance

The study will be a significance endeavour in provided the information for the future researches. From this research, the optimum water content of soil microbe effectively remove the contaminants in contaminated soil can be determined based on the least value of total nitrogen produced in the contaminated soil samples at several applied suction. The output for this research can be a guideline to improvise the condition of POME polluted site. In addition, this research is very significance to reduce the pollution in soil and environment.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

This chapter presents a comprehensive review on the characteristics of palm oil mill effluent (POME) and nutrients required by microorganisms. Additionally, a brief review of concept soil suction and suction control techniques are presented. Review of literature on drying suction-water content SWCCs. The final section of this chapter deals with review of literature on types of microorganisms in soil and application of microbe in soil.

2.2 Palm Oil Mill Effluent

Palm oil mill effluent (POME) is the residual liquid waste product obtained after extraction of oil from the fruits of the oil palm (Orji *et al*., 2006). The raw partially treated of POME usually has an extremely high content of degradable organic matter (Okwute *et al*., 2014). (Hashim and Zaharah, 1994) reported that, POME contains a high nutrient value and major plant nutrients. The nutrient composition of POME consists of nitrogen (N), phosphorus (P), pottasium (K), magnesium (Mg) and calcium (Ca). A higher nitrogen (N) elements were utilized from POME.

POME is one of the major wastes from the palm oil industry and it has the most problematic environmental pollution potential among the palm oil mill waste (Okechi *et al.*, 2014). Normally, POME is discharged indiscriminately into the environment, particularly on farmlands (Ogboghodo *et al.*, 2001, 2003, 2006 and Okechi *et al*, 2014).The discharged of POME from an oil mill is objectionable and could pollute streams, rivers, or surrounding land. The POME polluted soil may be treated by microorganisms and the microorganisms singnifically reduce organic compounds in POME polluted soil thereby bringing about bioremediation (Okwute *et al.*, 2014).

2.2.1 Nutrient

Nutrients are required by microorganisms for cellular material as well as for energy source. The **Table 2.1** indicates the common types of nutrients for bacterial growth.

Table 2.1. Common Nutrients for Bacterial Growth (after Mitchell and Santamarina, 2005).

Nutritional requirements		Nutrients Sources and Compounds
Elements needed to form molecules in soil	Cellular Carbon	CO_2, HCO_3^-, and organic compounds
	Minerals	N, P, K, Mg, S, Fe, Ca, Mn, Zn, Cu
The energy needed to sustain life		Organic compounds, inorganic compounds and sunlight.
Other growth-conductive factors		Amino acids, Vitamins, etc.

2.3 Soil Suction

Soil suction is defined as the water potential in a soil-water system (Richards 1974). Generally in the geotechnical engineering, the soil water potential is referred to as suction (Laikram, 2007).The suction in an unsaturated soil is controlled by three components, namely capillarity, adsorption of water on the surface of soil minerals, and osmotic phenomena (Blatz *et al*., 2008). Soil suction is referred to as the free energy state of soil-water, which can be measured in terms of its partial vapor pressure (Ng and Menzies, 2007). A change of total suction is generally caused by the change of relative humidity in the soil. Generally in engineering studies, total suction has two components which are matric suction and osmotic suction (Ng and Menzies, 2007).

2.3.1 Matric suction

Matric suction is defined as the different between pore water pressure (μ_w) and the pore-air pressure (μ_a) acting on the air-water interface (Laikram, 2007). (Fredlund and Xing, 1994) reported that the structure of the soil influences suction as a function of the particle packing, with smaller pore sizes producing larger suctions. Two techniques have been used to control soil matric suction during application of matric suction, namely axis translation and osmotic techniques (Cui *et al*., 2010).

2.3.2 Osmotic Suction

Osmotic suction is one of the important components influencing the behavior of soils. Osmotic suction is a function of the amount of dissolved salts in the pore fluid and is expressed in terms of pressure (Ng and Menzies, 2007). Osmotic solution can be altered by either changing the mass of the water or amount of ions in solution (Laikram, 2007). (Arifin and Schanz, 2009) reported that at given water content, the volumes of specimen prepare with the sodium chloride giving higher osmotic suction are smaller than those of the specimen prepared with distilled water.

2.4 Suction Control Method

There are several methods can be used for controlling matric and total suction of soil specimens. **Table 2.2** indicates the approximate measurement ranges and time for controlling of soil suction.

Table 2.2. Approximate measurement ranges and time for control of soil suction (Murray and Sivakumar, 2010)

Instrument	Suction measured	Measurement range (kPa)	Equilibration time
Axis translation technique	Matric	0-1,500	Several hours to days
Osmotic technique	Matric	0-10,000	up to 2 months
Vapour equilibrium technique	Total	4,000-600,000	1-2 months

2.4.1 Vapour Equilibrium Technique

Vapour equilibrium technique (VET) is where the environment of constant suction can be created in sealed containers using the osmotic potential of chemical solution (Blatz *et al*., 2008).This technique is for controlling total suction was again developed by soil scientists (Murray and Sivakumar, 2010). There are two types of osmotic solutions used to generate constant suction conditions which are saturated salt solutions and acid solutions (Blatz *et al*., 2008). (Blatz et al., 2008) reported that, the mixtures of sodium chloride and pottasium chloride are often achieve desired target suction values and the salt solutions are also limited to a lower range of suction values (0-10Mpa range). A range of salt solutions and the relative humidity levels that can be generated are available in many published works and chemistry handbooks (Tang and Cui., 2005, Bruno and Svoronos, 2003 and Blatz *et al*., 2008).

The much higher suction can be imposed by using the acid solutions (Blatz *et al*., 2008). (Tang and Cui, 2005) reported that, the method of VET has the ability to induce total suction up to 1000 Mpa. Many applications where the VET is used for controlling suction such as tensiometer, relative humidity sensor and psychrometer to verify the target suction achieved (Agus and Schanz, 2004 and Blatz *et al*., 2008).The limitations of using VET is that long time periods are required to achieve equilibrium conditions due to the diffusion process that controls transfer of water vapour between the specimens and the vapour in the dessicator head space, the strict control of temperatures is the requirement in this suction method and losses of water component due to evaporation (Blatz *et al*., 2008). **Figure 2.1** indicates a glass dessicator with porous disk over the solution which suspends soil specimens in the vapour environment above the chemical solutions.

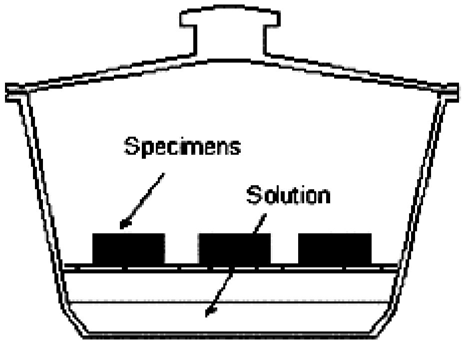

Figure 2.1 Soil specimens in constant suction environment (after Tang et al., 1998)

2.5 Soil-Water Content Characteristics

The soil-water characteristics curve (SWCC) for a soil is the relationship between the water content and the soil suction (Murray and Sivakumar, 2010). The water content can be either gravimetric water content,volumetric water content or degree of saturation (Agus *et al*., 2001). SWCCs can be measured in the laboratory or predicted using a grain-size distribution curve taking into account such factors as dry density, porosity, and void ratio (Aubertin *et al*., 2003, Fredlund *et al*., 1997, Tyler and Wheatcraft, 1989, Gupta and Larson, 1979, Agus *et al*., 2001). In SWCC, there are two paths are obtained, corresponding to the compression and swelling lines of the consolidation curve, namely drying (i.e. desorption) and wetting (i.e. adsorption) paths (Agus *et al*., 2001). In this study, the SWCC were obtained following the drying path.

2.5.1 Drying Suction-Water Content (SWCC)

The water content of a soil decrease as its suction increases following a drying path process (Agus *et al*., 2001). **Figure 2.2** indicates the typical characteristics shapes of SWCC for drying and wetting condition. The drying (desorption) of the SWCCs of most soils causes hysteretic behaviour (Haines, 1930, Hillel, 1998, Pham *et al*., 2005, and Uchimura *et al*., 2013). The soil can retain more water in the drying process than in the wetting processes for the same suction value (Uchimura *et al*., 2013).

The drying process involves loading the matric suction on the soil sample to expel pore water (Song, 2014). (Song, 2014) reported that, the mass of water outflow increased with increasing matric suction during the drying process. The duration of wetting process is relatively longer than the drying process due to the flow resistance induced by entrapped air in pores during the drying and wetting processes (Song, 2014). In addition, the volumetric water content of the drying process is higher than that of the wetting process at the same matric suction.

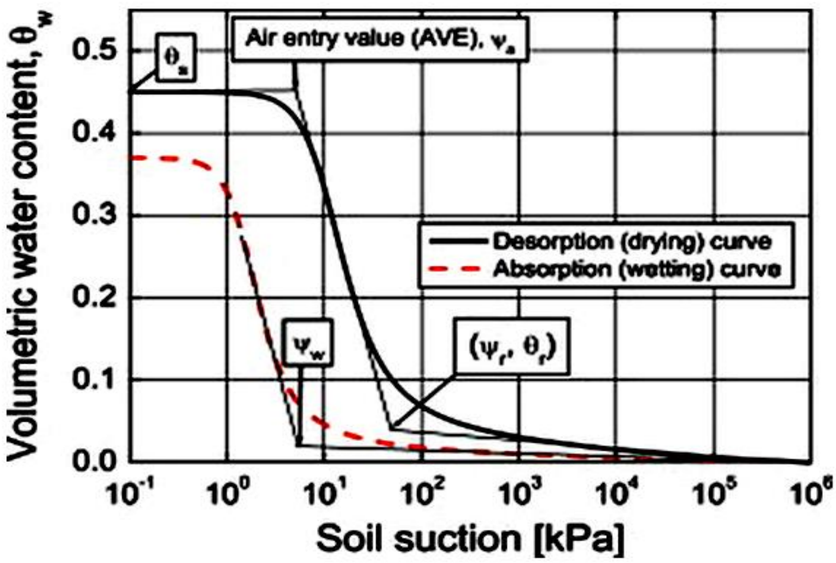

Figure 2.2: Typical Soil- Water Characteristics Curve (Uchimura *et al*., 2013)

2.6 Microorganisms in Soil

Microorganisms found in the soil include bacteria, algae, fungi, actinomycetes, protozoa and viruses (Ibe at el., 2014). The microorganisms in soil can be grouped according to the classes (bacteria, archea and eukarya) and characteristics. (Mitchell and Santamarina, 2005) reported that, bacteria and archea have a simple cell structure with no membrane enclosed nucleus and there are distinguished by their chemical composition rather than by their structures. In addition, these cells can live under extreme pH, temperature, and salt concentration, therefore they are present in most subsurface environments (Mitchell and Santamarina, 2005).

2.6.1 Application of Microbe in Soil

Microorganisms in soil perform important functions in soil and there are some of applications of microbe in soil such as decomposer of sugars and carbon compounds, pathogens to the plants and also degradation of pollutants in soil (Hoorman, 2011). Bioremediation is the process that uses microorganisms to return the natural environment altered by contaminants to its original condition (Khan, 2011 and Okwute *et al*., 2014). Microbes in soil are the key to carbon and nitrogen recycling. *Trichoderma atrividae* species are fungi commonly presents in soil (Singh *et al*., 2012). (Rosikon *et al*., 2014) stated that, the application of *Trichoderma atrividae* has positive effect in reducing on soil parameters (C, N and P). Bioremediation of pollutants using *Trichoderma atrividae* such as Phytoextraction in Cd- and Ni- contaminated soils (Cao *et al*., 2008 and Singh *et al*., 2012) and heavy metal from sludge (Errasquin and Vazquez, 2003 and Singh *et al*., 2012). *Paecilomyces lilacinus* is another type of soil fungus and is commonly presents in soils (Gortari *et al*., 2008).

CHAPTER 3

METHODOLOGY

3.1 Introduction

This chapter presents the experimental methods and several devices used in this study. The physical soil properties of sample used and the laboratory methods used in the determination of the soil physical properties. Additionally, this chapter brief about the selection of material, preparation of soil samples and determination of soil microbe characteristics by using the spread plating method and isolation plating method. The method and devices will be used in determining the suction-water content characteristics (SWCCs). Lastly, in this chapter brief about the method used in determining the palm oil mill effluents (POME) characteristics in polluted soil samples which is focuses on the total nitrogen elements as the parameter to be measured.

3.2 Selection of Material

The undisturbed soil sample used in this research study is taken from the palm oil plantation which is located in Tawau, Sabah. Soil sample were collected using auger at the sampling depth of 5 metres. After complete collection of soil sample, the soil sample was transported to laboratory. In laboratory, the soil sample was crushed and sieved with only passing 63 micron was considered. Then, the soil sample sealed in dry and clean plastic bags with proper labelling.

3.3 Soil Sample Preparation

The soil samples were prepared in a saturated slurry condition.The saturated slurry condition of soil samples were prepared for establishing the drying suction- water content SWCCs. Two soil samples were prepared in the experimental work on determining the drying suction-water content and there are soil-water mixture and soil-POME mixture. The soil-water mixture samples were prepared by mixing the air dried soil powders with deionised water at target water content equals to 1.2 times with the respective liquid limits of the test soil. The soil-POME mixture samples were prepared by mixing the air dried soil powders with palm oil mill effluent (POME) at target water content equals to 1.2 times with the respective liquid limits of the test soil.The soil-water and soil- POME mixtures were then stored in sealed plastic bags and kept in air tight containers to allow for water equilibration to take place for about seven days prior to being tested.