BIOREMEDIATION OF OIL CONTAMINATED WASTEWATER USING MIXED CULTURE

HUSNI BIN BASHARUDIN

UNIVERSITI MALAYSIA PAHANG

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BORANG PENGESAHAN STATUS TESIS

JUDUL **BIOREMEDIATION OF OIL CONTAMINATED WASTEWATER USING MIXED CULTURE**

SESI PENGAJIAN: **2007/2008**

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HUSNI BIN BASHARUDIN

A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2008

I declare that this thesis entitled “bioremediation of oil contaminated wastewater using mixed culture” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree

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Special Dedication to my family members that always love me,
My friends, my fellow colleague
and all faculty members

For all your Care, Support and Believe in me

ACKNOWLEDGEMENT

Bismillahirrahmanirahim,

I am so thankful to Allah S.W.T for giving me patient and spirit throughout this project and the research is successfully complete. With the mercifulness from Allah therefore I can produces a lot of useful idea to this project.

I am indebted to my supervisor, Mrs Nasratun binti Masngut the lecturer from the Faculty of Chemical Engineering and Natural Resources for his advice, insightful comments and genours support. Thank for your guide and without your guide this research will not complete and well organized. Thank you for your support and brilliant ideas that you gave to me. I would like to dedicate my appreciation to all the lecturers that involve in this subject/project for their invaluable time, guidance and advice. Without your cooperation and sacrifices this research will not able to complete and published.

To my beloved father and mother, Basharudin bin Abu Bakar and Jamaliah bt Mohamed. I am grateful to have both of you in my life and giving me full of support to through this life. I pray and wish to both of you are always in a good health and in Allah mercy. You are the precious gift from Allah to me. Thank you very much.

ABSTRACT

The objective of this research is to study the effect of the oil degradation based on the different temperature and different oil concentration using mixed culture from domestic wastewater. The evaluation is based on microorganism activities by the rate of oil degradation. The inoculum used was a mixed culture containing oil-degrading microorganism isolates from the sewage system located at Perak. For the wastewater used was artificially made by using palm oil as carbon substrate and medium for the microorganism to study the effect of temperature and oil concentration. Wastewater was treated by using the inoculum of the mixed culture for 20 days of incubation time and temperature range from 10^{o}C to 60^{o}C. From the experiment, it was observed that, the rate of oil degradation ~~is~~ was high at mesophilic condition which was at temperature of 30^{o}C resulted of 4.722 g from 10 g of oil have been degraded during the incubation time. The result also showed that, oil concentration at high value can limit the rate of oil degradation. It showed that rate of oil degradation was inversely proportional to the concentration of oil. From this study, it is shown that the effectiveness of oil degradation is increased by increasing in temperature and the optimum temperature in this study was 30^{o}C at low oil concentration.

ABSTRAK

Objektif kajian ini adalah untuk mengkaji perubahan dalam–penguraian minyak berdasarkan perbezaaan suhu dan perbezaan kepekatan minyak dengan menggunakan kultur campuran daripada air buangan domestik. Penilaian adalah berdasarkan kadar penguraian minyak daripada aktiviti mikroorganisma. Inokulum yang digunakan adalah kultur campuran yang mengandungi mikroorganisma pengurai minyak yang diasingkan daripada air buangan domestik yang terletak di Perak. Untuk air buangan yang digunakan, ia dihasilkan secara sintetik dengan menggunakan minyak sawit sebagai sumber karbon untuk mikroorganisma dalam mengkaji kesan perbezaan suhu dan perbezaan kepekatan minyak terhadap kadar penguraian minyak. Air buangan sintetik ini akan dirawat menggunakan inokulum daripada kultur campuran selama 20 hari pada suhu bermula dari 10^{o}C hingga 60^{o}C. Daripada eksperimen ini didapati kadar penguraian minyak adalah tinggi dalam keadaan suhu yang *mesophilic* iaitu pada suhu 30^{o}C dengan penguraian minyak sebanyak 4.722 g daripada 10 g minyak sepanjang tempoh rawatan. Keputusan turut menunjukkan kepekatan minyak yang tinggi akan menghadkan penguraian minyak. Kadar penguraian minyak adalah berkadar songsang terhadap kepekatan minyak. Daripada kajian ini, didapati bahawa kebolehan mengurai minyak oleh kultur campuran akan meningkat sekiranya suhu rawatan semakin tinggi dan keadaan yang optimum untuk penguraian minyak bagi kajian ini adalah pada suhu 30^{o}C dan pada kepekatan minyak yang rendah.

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LIST OF SYMBOLS

%	-	Percentage
BOD	-	Biochemical oxygen demand
cm	-	Centimeter
C_{oil}	-	Oil concentration
g	-	Gram
h	-	Hour
L	-	Liter
min	-	Minute
ml	-	Milliliter
N	-	Nitrogen
ºC	-	Degree celcius
P	-	Phosphorus
PAHs	-	Polyaromatic hydrocarbons
PCBs	-	Polychlorinated biphenyls
rpm	-	Revolution per minute
TCE	-	Trichloroethylene
V_{sample}	-	Sample volume

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Bioremediation is defined as any process that uses microorganisms or their enzymes to destroy or reduce the concentrations of hazardous wastes from contaminated sites without further disruption to the local environment. It is a relatively slow process, requiring weeks to months to effect cleanup. If done properly, it can be very cost-effective. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and the environment. This is an attractive process due to its cost effectiveness and the benefit of pollutant mineralization to CO_2 and H_2O (Mills *et al.*, 2004). The microorganisms may be endogenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes (Margesin and Schinner, 2001).

Biodegradation of a compound is often a result of the actions of multiple organisms. During biodegradation, oil is used as an organic carbon source by a microbial process, resulting in the breakdown of oil components to low molecular weight compounds. In another words, biodegradation of oil contaminants can be described as the conversion of chemical compounds by microorganisms into energy, cell mass and biological products. The key component in bioremediation is the microorganisms, which produce the enzymes involved in the degradative reactions leading to the elimination or detoxification of the chemical pollutant (Rahman *et al.*,

2002). The goal of bioremediation is to degrade organic pollutant which is oil to concentrations below the limits established as safe or acceptable by regulatory agencies. Effective bioremediation requires nutrients to remain in contact with the oiled material and the concentrations should be sufficient to support the maximal growth rate of the oil-degrading bacteria throughout the cleanup operation. The success of oil wastewater bioremediation depends on our ability to establish those conditions in the contaminated environment (Reynolds *et al*., 1989).

1.2 Problem statement

Oil contaminated wastewater has posed a great hazard for environment and marine ecosystems. Oil is major component in domestic wastewater that causes severe environment pollution. It can form oil films on water surfaces, preventing the diffusion of oxygen from air into water and leading to the death of many forms of aquatic life. The traditional treatment of oil contaminated wastewater such as use of straw or plant material as an absorbent of oil, biosurfactants to cleanup oiled surfaces (Banat *et al*., 1991), oil-water separation and other methods. But, all of these physical and chemical methods can not degrade and remove the oil thoroughly (Ollis, 1992). Biological methods can be most effective in the removal of thin oil films spread on the surface of water, where physical or chemical methods are not effective. So far, bioremediation suggests an effective method.

During bioremediation, oil is used as an organic carbon source by a microbial process, resulting in the breakdown of oil components to low molecular weight compounds. This technology accelerates the naturally occurring biodegradation under optimized conditions such as oxygen supply, temperature, pH, the presence or addition of suitable microbial population and nutrients, water content and mixing. Like other technologies, bioremediation has its limitations. Some contaminants, such as chlorinated organic or high aromatic hydrocarbons, are resistant to microbial attack. They are degraded either slowly or not at all, so it is not easy to predict the rates of clean up for a bioremediation exercise and there are no rules to predict if a contaminant can be degraded (Banat *et al*., 1991).

Bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. So, bioremediation methods have focused on the addition of microorganisms or nutrients concentration and the temperature dependant condition of the process environment. The main requirements for degradation of oil by microorganism are energy sources and carbon sources. Biostimulation is the addition of substrates, vitamins, oxygen and other compounds that stimulate microorganism activity, so that they can degrade the waste faster. The addition of materials to encourage microbiological biodegradation of oil which has received the most attention, notably after the "Exxon Valdez" incident (Swannel *et al*., 1996), however, such as low water temperature are not favorable for bioremediation.

The bioremediation treatment of oils contaminated wastewater under high temperature conditions is expected to be advantageous due to favorable changes in most physical properties of these hydrophobic compounds with increasing temperature (Thomas *et al*., 1987). The melting point of oil is often well above ambient temperatures. Above their melting temperature, these substances become more accessible to microorganisms and their enzymes. Both diffusion coefficients and the solubility of oil in aqueous media increase significantly with rising temperatures allowing for a better mass transfer (Thomas *et al*., 1987).

1.3 Objectives of study

The objectives of this study are as follows:

a) To study the effect different temperature on the rate oil degradation
b) To study rate of degradation based on different oil concentration in wastewater treatment

1.4 Scope of study

The scope of this study is to find out the different in the rate of oil degradation of oil contaminated wastewater using mixed culture origin from the sewage system located at Perak in different incubation temperature and different oil concentration.

CHAPTER 2

LITERATURE REVIEW

2.1 Bioremediation

Bioremediation means to use biological organisms to solve an environmental problem such as contaminated soil or contaminated water. In other words it is a technology for removing pollutants from the environment thus restoring the original natural surroundings and preventing further pollution. Bioremediation may be employed in order to attack specific contaminants, such as chlorinated pesticides that are degraded by bacteria, or a more general approach may be taken, such as oil contaminated wastewater that are broken down using multiple techniques including the addition of biostimulation to facilitate the decomposition of oil by bacteria (Jorgensen *et al*., 1999). Oil may contaminate water well below the surface of the water, injecting the right organisms, in conjunction with oxygen-forming compounds, may significantly reduce concentrations after a period of time. It will not always be suitable, however, as the range of contaminants on which it is effective is limited, the time scales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate (Ayotamuno *et al*., 2002).

Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result (Jorgensen *et al*., 1999). Generally, bioremediation technologies can be classified as in situ or ex situ. In situ bioremediation involves treating the contaminated material at the site

while ex situ involves the removal of the contaminated material to be treated elsewhere (Sasikumar and Papinazath, 2003). Different techniques are employed depending on the degree of saturation and aeration of an area. In situ techniques are defined as those that are applied to soil and groundwater at the site with minimal disturbance. Ex situ techniques are those that are applied to soil and groundwater at the site which has been removed from the site via excavation for soil or pumping for water (Vidali, 2001)

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2.2 In situ bioremediation

The bioremediation methods employed will depend on the area contaminated, the properties of the compounds involved, the concentration of the contaminants and the time required to complete the bioremediation. The in situ process includes bioventing, biosparging, biostimulation, bioaugmentation and phytoremediation (Vidali, 2001).

2.2.1 Bioventing

Bioventing is the most common in situ treatment and involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. Bioventing employs low air flow rates and provides only the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It works for simple hydrocarbons and can be used where the contamination is deep under the surface (Chipasa and Medrzycka, 2006)

2.2.2 Biosparging

Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological

degradation of contaminants by naturally occurring bacteria. Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system (Vidali, 2001)

2.2.3 Bioaugmentation

Bioaugmentation is the addition of microorganisms that specifically degrade the oil at the site of the oil spill. The oil-degradation organisms were collected from other sites and commercially cultivated them. They are selected to withstand harsh environmental conditions such as high salt and variable temperature combined with a superior ability to use the resources such as oxygen, nitrogen, phosphorus and others sources available. They also able to out compete indigenous microorganisms, so they can clean up the site rapidly (Campo *et al.*, 2007). It is proposed by proponents of bioaugmentation, once the oil which is the carbon source or substrate is used up, these organisms have no advantage over the native microorganisms present so eventually they decrease in numbers and disappear. The increase in the efficiency of the system was the result of an increased concentration of bacterial cells, which was accompanied by increased microbial activity, growth and maintenance of microbial populations that were associated with attached growth systems (Chipasa and Medrzycka, 2006)

2.2.4 Biostimulation

Biostimulation is the addition of substrates, vitamins, oxygen and other compounds that stimulate microorganism activity so that they can degrade the waste faster. Biostimulation of microorganisms by the addition of nutrients because the input of large quantities of carbon sources tends to result in a rapid depletion of the available pools of major inorganic nutrients such as N and P (Sang-Hwan *et al.*,

2007). An example of this is the addition of fertilizer to an oil wastewater. This works by supplying nutrients that are limiting the growth of the bacteria for the oil contaminated wastewater such as nitrogen and phosphorous. With this addition, the organisms can rapidly degrade the oil utilizing it as the carbon source and the fertilizer as the nitrogen and phosphorous source (Campo *et al.*, 2007).

2.2.5 Phytoremediation

Vegetation based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. In natural ecosystems, plants act as filters and metabolize substances generated by nature. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water. Its potential for encouraging the biodegradation of organic contaminants requires further research, although it may be a promising area for the future (Truu *et al*., 2003)

2.3 Ex situ bioremediation

If the contaminated material is excavated it can be treated on or off site which is often a more rapid method of decontaminating the area. The techniques that can be used are include land farming, composting, biopiles and bioreactors (Vidali, 2001)

2.3.1 Land farming

Land farming is a simple technique in which contaminated soil is excavated and spread over a prepared bed and periodically tilled until pollutants are degraded. The goal is to stimulate indigenous biodegradative microorganisms and facilitate their aerobic degradation of contaminants. In general, the practice is limited to the treatment of superficial 10–35 cm of soil. Since land farming has the potential to

reduce monitoring and maintenance costs as well as clean-up liabilities, it has received much attention as a disposal alternative (Vidali, 2001)

2.3.2 Composting

Composting is a technique that involves combining contaminated soil with nonhazardous organic amendants such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting (Vidali, 2001)

2.3.3 Biopiles

Biopiles are a hybrid of land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Biopiles provide a favorable environment for indigenous aerobic and anaerobic microorganisms (Sang-Hwan *et al.*, 2007).

2.3.4 Bioreactors

Slurry reactors or aqueous reactors are used for ex situ treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material (soil, sediment and sludge) or water through an engineered containment system (Vidali, 2001). A slurry bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing condition to increase the bioremediation rate of soil bound and water-soluble pollutants as a water slurry of the contaminated

soil and biomass (usually indigenous microorganisms) capable of degrading target contaminants.

In general, the rate and extent of biodegradation are greater in a bioreactor system than in situ or in solid-phase systems because the contained environment is more manageable and hence more controllable and predictable. Despite the advantages of reactor systems, there are some disadvantages. The contaminated soil requires pre treatment (excavation) or alternatively the contaminant can be stripped from the soil via soil washing or physical extraction (vacuum extraction) before being placed in a bioreactor (Vidali, 2001)

2.4 Microorganisms in bioremediation

Many different types of bacteria and fungi can be used for bioremediation. Microorganisms are nature's original recyclers. Their capability to transform natural and synthetic chemicals into sources of energy and raw materials for their own growth suggests that expensive chemical or physical remediation processes might be replaced with biological processes that are lower in cost and more environmentally friendly. Microorganisms therefore represent a promising, largely untapped resource for new environmental biotechnologies (Truu *et al*., 2003). Research continues to verify the bioremediation potential of microorganisms. Even dead microbial cells can be useful in bioremediation technologies. These discoveries suggest that further exploration of microbial diversity is likely to lead to the discovery of many more organisms with unique properties useful in bioremediation (Vidali, 2001). Microbes able to degrade the contaminant increase in numbers when the contaminant is present.

The use of microorganisms is not limited to one field of study of bioremediation, it has an extensive use. Oil slicks caused by oil tankers and petrol leakage into the marine environment and oil contaminated wastewater are now a constantly occurring phenomenon. A number of microorganisms can utilize oil as a

source of food and many of them produce potent surface active compounds that can emulsify oil in water and facilitate its removal. Unlike chemical surfactants, the microbial emulsifier is nontoxic and biodegradable (Truu *et al*., 2003). The microorganisms capable of degrading oil include *Pseudomonas*, various *Corynebacteria*, *Mycobacteria* and some yeast. These microorganisms can be subdivided into aerobic, anaerobic, ligninolytic fungi and methylotrophs:

2.4.1 Aerobic

An aerobic organism or aerobe is an organism that has an oxygen based metabolism. Aerobes, in a process known as cellular respiration, use oxygen to oxidize substrates like fatty acid from oil in order to obtain energy. Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus* and *Mycobacterium* (Giavasis *et al*., 2006). These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds (Vidali, 2001). Many of these bacteria use the contaminant as the sole source of carbon and energy.

2.4.2 Anaerobic

An anaerobic organism or anaerobe is an organism that does not need oxygen as based metabolism. Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform (Vidali, 2001)

2.4.3 Ligninolytic fungi

Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs (Adenipekun and Fasidi, 2005).

2.4.4 Methylotrophs

The aerobic bacteria that grow by utilize methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1,2-dichloroethane (Vidali, 2001).

2.5 Environmental factors on bioremediation

Environmental variables can also greatly influence the rate and extent of biodegradation. Variables such as oxygen and nutrient availability can often be manipulated at treatment sites to enhance natural biodegradation. Other variables, such as salinity, are not usually controllable. Lack of sufficient knowledge about the effect of various environmental factors on the rate and extent of biodegradation is another source of uncertainty (Harris *et al*., 1999).

2.5.1 Oxygen

Oxygen is one of the most important requirements for microbial degradation of oil (Giavasis *et al*., 2006). However, its availability is rarely a rate limiting factor in the biodegradation of oil contaminated wastewater. Microorganisms employ oxygen incorporating enzymes to initiate attack on oil. Anaerobic degradation of

certain oil which is the degradation in absence of oxygen also occurs, but usually at negligible rates. Such degradation follows different chemical paths and its ecological significance is generally considered minor. For example, studies of sediments impacted by the Amoco Cadiz spill found that, at best, anaerobic biodegradation is several orders of magnitude slower than aerobic biodegradation (Niblock, 1991).

Oxygen is generally necessary for the initial breakdown of oil, and subsequent reactions may also require direct incorporation of oxygen. Requirements can be substantial, 3 to 4 parts of dissolved oxygen are necessary to completely oxidize 1 part of oil into carbon dioxide and water. Oxygen is usually not a factor limiting the rate of biodegradation on or near the surface of the ocean, where it is plentiful and where oil can spread out to provide a large, exposed surface area. When oxygen is less available, the rates of biodegradation decrease (Niblock, 1991). Thus, oil that has sunk to the sea floor and been covered by sediment takes much longer to degrade. Oxygen availability there is determined by depth in the sediment, height of the water column and turbulence (Giavasis *et al*., 2006).

2.5.2 Nutrients

Nutrients such as nitrogen, phosphorus and iron play a much more critical role than oxygen in limiting the rate of biodegradation in marine waters. Nitrogen addition stimulated the biodegradation of alkane and polyaromatic hydrocarbons (PAHs), while phosphorus addition increased the biodegradation rate of alkane but not PAHs (Harris *et al*., 1999). Although oil is rich in the carbon required by microorganisms, it is deficient in the mineral nutrients necessary to support microbial growth. Wastewater ecosystems are often deficient in these substances because non-oil degrading microorganisms including phytoplankton consume them in competition with the oil degrading species.

Phosphorus precipitates as calcium phosphate at the high pH. Lack of nitrogen and phosphorus is most likely to limit biodegradation, but lack of iron or

other trace minerals may sometimes be important (Vidali, 2001). These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon. Carbon is the most basic element of living forms and is needed in greater quantities than other elements (Vidali, 2001). Table 2.1 showed the composition of a microbial cell.

Table 2.1: Composition of a microbial cell

Element	**Percentage**
Carbon	50
Nitrogen	14
Oxygen	20
Hydrogen	8
Phosphorus	3
Sulfur	1
Potassium	1
Sodium	1
Calcium	0.5
Magnesium	0.5
Chloride	0.5
Iron	0.2
All others	0.3

2.5.3 Temperature

At low temperature, the rate of oil metabolism by microorganisms decreases. So, lighter fractions of petroleum which is palm oil become less volatile, thereby leaving the oil constituents that are toxic to microbes in the water for a longer time and depressing microbial activity (Phillips *et al*., 1974). The rates of biodegradation are faster at higher temperature (Thomas, 1987). The diffusion coefficients and the solubility of lipids in aqueous media increase significantly with rising temperature. Under thermopile conditions, lipids become more accessible to microorganisms (Chipasa *et al.,* 2006). A temperature increase affects a decrease in viscosity, thereby affecting the degree of distribution and increasing diffusion rates of organic compounds. Therefore, higher reaction rates due to smaller boundary layers are

expected at elevated temperatures (Margesin and Schinner, 2001). The increased in volatilization and solubility of some oil at elevated temperature affects toxicity and allows biotransformation with high substrate concentrations (Müller *et al*., 1998).

2.5.3.1 Cold-adapted microorganisms

Cold-adapted, psychrophilic and psychrotrophic microorganisms are able to grow at temperatures around 0°C. They are widely distributed in nature since a large part of the Earth's biosphere is at temperatures below 5°C (Margesin and Schinner, 2001). Psychrophiles have an optimum growth temperature of lower than 15°C and do not grow above 20°C whereas psychrotrophs (cold-tolerant) have optimum and maximum growth temperatures above 15°C and 20°C (Morita, 1975). Cold-adapted indigenous microorganisms play a significant role in the in situ biodegradation of hydrocarbons in cold environments, where ambient temperatures often coincide with their growth temperature range (Margesin and Schinner, 1999)

2.5.3.2 Thermophilic Microorganism

Microorganisms that grow optimally above 40°C are designated as thermophiles. Most thermophiles known are moderate and show an upper temperature border of growth between 50 and 70°C. Optimal growth of extreme thermophiles and hyperthermophiles occurs at 70–80°C and above 80°C, respectively (Stetter, 1998). Thermophiles, predominantly bacilli, possess a substantial potential for the conversion of environmental pollutants, including all major classes (Müller *et al*., 1998)

2.5.4 pH

pH must remain within tolerance range for degradative microorganism to growth and survive (Reynolds *et al*., 1989). The optimal pH for oil biodegradation is between pH 6 and 9 (Atlas and Bartha, 1972). pH of water can be adjusted by addition of chemical reagents. For acidic wastewater, agriculture lime may be used to raise the pH. Then, aluminum sulfate, ferrous sulfate or sulfur that is a slow acting chemical that requires microbial activities to generate acid may be used to lower the pH of alkaline water (Vidali, 2001).

2.6 Wastewater

Wastewater is used contaminated water. It includes substances such as human waste, food scraps, oils, soaps and chemicals. In homes, this includes water from sinks, showers, bathtubs, toilets, washing machines and dishwashers. Businesses and industries also contribute their share of used water that must be cleaned and can encompass a wide range of potential contaminants and concentrations (Benitez *et al*., 1997). In most common usage, it refers to the municipal wastewater that contains a broad spectrum of contaminants resulting from the mixing of wastewaters from different sources.

2.6.1 Composition of oil contaminated wastewater

Wastewater is mostly water by weight, but other materials make up only a small portion of wastewater but can be present in large enough quantities to endanger public health and the environment because practically anything that can be flushed down a toilet, drain or sewer can be found in wastewater, even household sewage contains many potential pollutants. Major wastewater components are organisms, organic matter, oil and nutrients (Benitez *et al*., 1997).

2.6.1.1 Organism

Many different types of organisms live in wastewater and some are essential contributors to treatment. A variety of bacteria, protozoa and worms work to break down certain carbon-based that is organic pollutants in wastewater by consuming them. Through this process, organisms turn wastes into carbon dioxide, water or new cell growth. Bacteria and other microorganisms are particularly plentiful in wastewater and accomplish most of the treatment (*Husain et al.*, 2002). Most wastewater treatment systems are designed to rely in large part on biological processes.

2.6.1.2 Oil

Fatty organic materials from animals, vegetables and petroleum also are not easily degrade by bacteria and can cause pollution in receiving environments. When large amounts of oils are discharged to receiving waters from community systems, it will increase the water BOD and may float to the surface and harden, causing aesthetically unpleasing conditions (Fadil *et al.*, 2002). In some cases, too much oil causes septic conditions in ponds and lakes by preventing oxygen from the atmosphere from reaching the water. Onsite systems also can be harmed by too much oil which can clog onsite system drainfield pipes and soils, adding to the risk of system failure (Fadil *et al.*, 2002). Petroleum-based waste oils used for motors and industry are considered hazardous waste and should be collected and disposed of separately from wastewater.

2.6.1.3 Organic matter

Organic materials are found everywhere in the environment. They are composed of the carbon-based chemicals that are the building blocks of most living things. Organic compounds normally are some combination of carbon, hydrogen,

oxygen, nitrogen, and other elements. Many organics are proteins, carbohydrates or fats and are biodegradable, which means they can be consumed and degraded by organisms (Adenipekun and Fasidi, 2005). Large amounts of biodegradable materials are dangerous to lakes, streams and oceans, because organisms use dissolved oxygen in the water to break down the wastes. This can reduce or deplete the supply of oxygen in the water needed by aquatic life, resulting in death of aquatic living, odors and decreasing of water quality. The amount of oxygen organisms need to degrade wastes in wastewater is referred to as the biochemical oxygen demand (BOD) and is one of the measurements used to assess overall wastewater strength (Giavasis *et al*., 2006). Some organic compounds are more stable than others and cannot be quickly degraded by organisms, posing an additional challenge for treatment.

2.6.1.4 Nutrients

Wastewater often contains large amounts of nitrogen and phosphorus in the form of nitrate and phosphate which promote plant growth. Organisms only require small amounts of nutrients in biological treatment, so normally these nutrients were excess available in treated wastewater. In severe cases, excessive nutrients in receiving waters cause algae and other plants to grow quickly thus contribute to oxygen depletion in water (Chakchouk *et al*., 1994). Nutrients from wastewater are also linked to ocean "red tides" that poison fish and cause illness in humans. Nitrogen in drinking water may contribute to miscarriages and is the cause of a serious illness in infants called methemoglobinemia or "blue baby syndrome.

CHAPTER 3

METHODOLOGY

3.1 Mixed culture collection

Mixed culture used in this study was obtained from the sewage system located at Perak. Oil contaminated wastewater samples were enriching with palm oil as the sole of carbon source. Samples were collected in 1 L bottle and kept aseptically at 20 to 25^{o}C for about 4 weeks to encourage the development of the microbial species (Tano-Debrah *et al*., 1999).

3.2 Stock culture preparation

About 300 ml of oil contaminated wastewater sample were homogenized in a blender for 2 min and 50 ml of the homogenate is pipette into 450 ml of sterilized buffered NaCl-peptone solution in a 1000 ml Erlenmeyer flask. Buffered NaCl-Peptone solution was prepared. The composition of buffered solution in 1 L distilled water were 2 g of KH_2PO_4, 5 g of K_2HPO_4, 4.5 g of NaC1 and 1 g of peptone. pH was adjusted to 7.0. The flask contents were thoroughly mixed by shaking and incubated at 25°C for 5 days. After incubation, samples of the culture were appropriately diluted and plated on Agar medium at 25°C for 5 days. The colonies formed were aseptically scraped using a sterile plastic inoculating rod and inoculated into 20 ml of sterilized buffered peptone water and incubated at 28°C for 2 days to form the inoculum (Tano-Debrah *et al*., 1999).

3.3 Wastewater preparation

The wastewater for the study was synthetically made with known type, mass and volume of oil. Palm oil was used as the contaminant source of wastewater. The oil was added to the basal medium in 250 ml Erlenmeyer flasks based on controlled parameter, covered and then sterilized. The volume of wastewater was fixed for 100 ml. Composition of the basal medium in 1 L distilled water were 2 g of KH_2PO_4, 5 g of K_2HPO_4; 0.5 g of $MgSO_4$-$7H_20$, 0.02 g of $CaCI_2$, 0.001 g of $FeSO_4$-H_20 and 1 g of peptone (Tano-Debrah *et al*., 1999).

3.3.1 Effect of temperature on biodegradation

Study on the effect of temperatures to the rate of oil degradation is set to 0.10 g/ml for the concentration of oil in water with different temperatures, starting at 10, 20, 30, 45, and 60^{o}C. Others components were remain constant.

3.3.2 Effect of various concentrations of oil on biodegradation

The temperature for study on the effect of oil concentration to the rate of oil degradation is set to room temperature. The amount of oil concentration was varied with 0.05, 0.10, 0.15, 0.20 and 0.25 g/ml, whereas others components were kept constant. Oil concentration in water is calculated using the following equation:

$$C_{oil} = \frac{\text{mass of oil}}{V_{sample}} \tag{3.1}$$

Where C_{oil} is the concentration of oil (g/ml) and V_{sample} is volume of analyzed wastewater sample (100 ml). The mass of oil is weigh using an analytical balance.

3.4 Wastewater treatment

About 10 ml of the inoculum were added to the oil contaminated wastewater and incubated with continuous shaking at 120 rpm, for up to 20 days. The shaking was to provide aeration and mixing of the culture. Oil contaminated wastewater without inoculum was also included as controlled (Tano-Debrah *et al*., 1999).

3.5 Sampling

Samples were taken at 5 day interval during the incubation for microbial testing. Samples were then heated in a boiling water bath for about 10-15 min and the mass of oils were estimated by measured the weight of flask. The Extractive-gravimetric method is using to extract and estimate the residual oils at the end of wastewater treatment (Tano-Debrah *et al.,* 1999).

3.5.1 Analysis procedure

Extractive-gravimetric method was carried out with n-hexane as a solvent. Sample was adjusted to pH 2 with HCl or H_2SO_4. Then, the sample was transferred to a separating funnel. Rinsed the volumetric flask with 20 ml extracting solvent (n-hexane) and the solvent washing were poured in the separating funnel and vigorously shake for 2 min. The separating funnel was leaved stand undisturbed for at least 10 minutes to ensure separation of water and solvent layer. Then, the lower water layer slowly drained from the separating funnel into the volumetric flask. Next, the water layer is saved for the next step.

After that, using analytical balance, a dried and cleaned distillation flask containing 3-5 boiling chips are weighed. The weight of the flask is recorded. Next, the glass funnel was putted in the neck of the distillation flask and the folded 12.5 cm filter paper is placed at the filtering funnel. 10 g of anhydrous Na_2SO_4 were added to

the filter paper. After that, the Na_2SO_4 were rinsed with a small amount of the n-hexane. The n-hexane was discarded properly. Use the same filter funnel and Na_2SO_4 for the second and third extraction. Then, solvent layer drained through the filter paper which contained 10 g Na_2SO_4 into the distillation flask. The water layer that we saved is returned to the separating funnel. The steps were repeated for 2 more times. After the 3rd extraction, the water layer was discarded. Then, the separating funnel with 3 separate 5 ml aliquots of fresh n-hexane were rinsed to remove any oil film left on the funnel walls. Each aliquot was drain through the funnel containing the Na_2SO_4 into the distillation flask. Next, the tip of the glass funnel with 5 ml n-hexane is rinsed while removing it from the distillation flask. The distillation assembly shown in figure 3.1 was used, the n-hexane were distilled off.

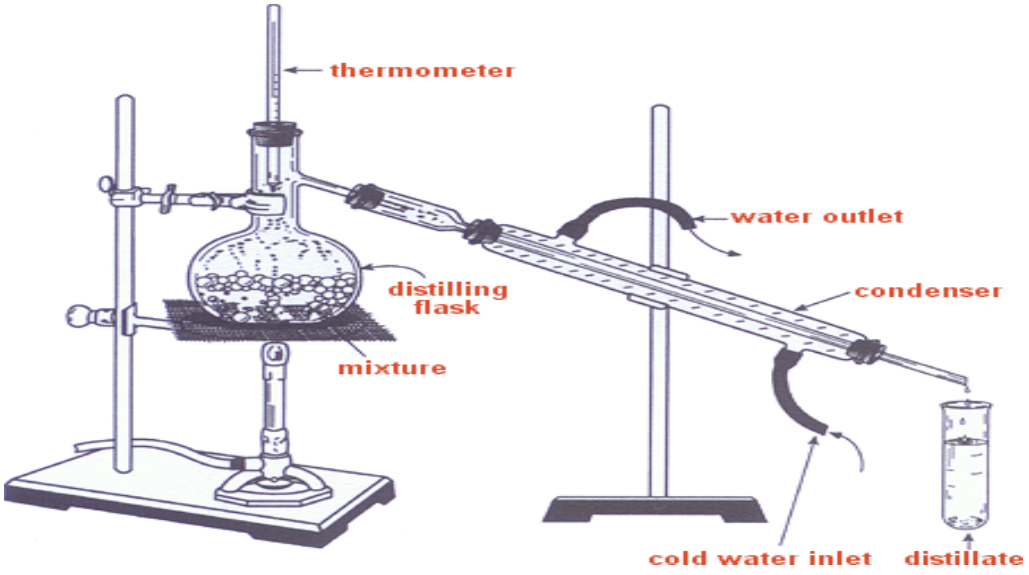

Figure 3.1: Distillation assembly

Distillation is complete when there are no boiling bubbles or the distillation flask appears dry. The remaining solvent vapors were removed from the distillation flask by attaching the vacuum connector/gas inlet adapter to the flask. Then, a vacuum is applied for 1-2 minutes or until all n-hexane solvent vapors have been removed. The flask was placed in desiccators for 30 minutes until it cools to room temperature. Finally, using an analytical balance, the distillation flask that contains oil was weighed. The weight is recorded. The oil volume is measured.

The mass of oil is calculated using the following equation:

$$\text{Mass of oil} = \text{Mass of flask with oil} - \text{Mass of flask without oil} \tag{3.2}$$

The rate of oil degradation is calculated using the following equation:

$$\text{Rate of oil degradation} = \frac{\text{Initial mass of oil} - \text{Final mass off oil}}{\text{Initial mass of oil}} \times 100\% \tag{3.3}$$

CHAPTER 4

RESULT AND DISCUSSION

4.0 Introduction

The mixed culture for this study was obtained from the domestic oil contaminated wastewater origin from the sewage system located at Perak (Appendix B.1). The wastewater for the study was synthetically made with known type, mass and volume of oil. Palm oil was used as the contaminant source of wastewater. About 10 ml of the inoculums were added to the oil contaminated wastewater and incubated with continuous shaking at 120 rpm, for up to 20 days (Appendix B.2). Samples were taken at 5 day interval during the incubation for microbial testing and the mass of oils were estimated by measured the weight of flask. The study are based on effect of temperature and effect of oil concentration on oil degradation

4.1 Effect of temperature

For the study effect of temperature on oil degradation, the concentration of oil in water is fixed to 0.10 g/ml and treated with different temperatures, starting at 10, 20, 30, 45, and 60^{o}C for 20 days of incubation time (Appendix A.1). From the Figure 4.1, the oil degradation is highest at temperature of 30^{o}C with 50% degradation followed by 10% degradation occurs at 20^{o}C. The lowest rate of oil degradation occurs at 10^{o}C with less than 1% degradation. For the temperature of 45^{o}C and 60^{o}C, the rate of oil degradation cannot be obtained because the wastewater has been vaporized after 14 days.

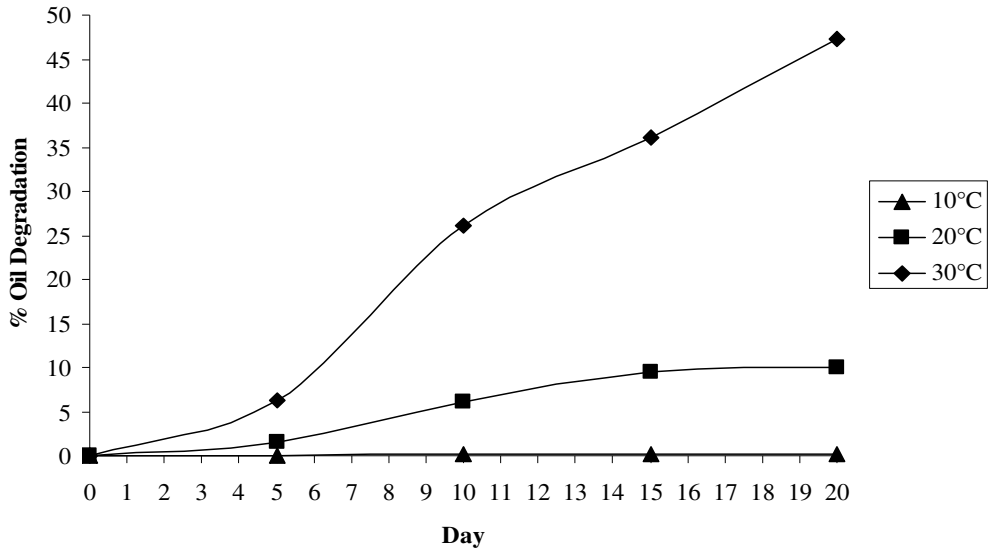

Figure 4.1: Effect of temperature on oil degradation

For the temperature of 30°C, the rate of oil degradation has been increased gradually during the treatment until it reaches 50% degradation after 20th day (Appendix A.4). The microorganisms were isolated from the environment with temperature around 30°C. So, at 30°C, the microorganism actively growth and degraded the oil as the carbon source. About 4.722 g of oil have been degraded during the incubation time of 20 days. Even though the diffusion coefficients and the solubility of oil in water increase significantly with rising temperature, but at the temperature as high as 45°C and 60°C, the result cannot be obtained because of the wastewater starting to vaporize.

The rate of oil degradation at 20°C increased slowly and only 10% degradation after 20 days of incubation time (Appendix A.3). At temperature of 20°C, the oil has started to cool and the phase of oils change from the liquid to solid form resulted the microorganisms cannot degrade the oil. After 2 weeks of treatment, the rate of oil degradation has been decreased from 4% to 1%. The reducing of temperature from 30°C to 20°C will decrease the growth of the microorganism and therefore lower the degradation of oil. About 1.030 g from 10 g of oil has been degraded during the incubation time of 20 days at 20°C compared to 4.722 g degraded at temperature of 30°C with same incubation time.

At incubation temperature of 10°C, the oil degradation was almost not happened which is less than 1% even after 20 days of incubation time (Appendix A.2). About 0.025 g of oil has been degraded after the 20th day. At low temperature of 10°C, the microorganisms were inactivated and only psychrophilic microorganisms are able to degrade the oil at low temperature. Mixed cultured used in this treatment was from mesophilic microorganisms which growth in temperature between 20-42°C. The oil mixture also has been cooled in solid form at temperature as low as 10°C which have resulted the microorganisms cannot degrade the oil (Appendix B.8). The rate of oil degradation will increase as temperature increase.

4.2 Effect of oil concentration

Various concentration of oil which is 0.05, 0.10, 0.15, 0.20 and 0.25 g/ml water were treated with mixed cultured for 20 days of incubation time at 30°C (Appendix A.5). From Figure 4.2, the oil degradation is the highest at concentration of 0.05 g/ml with 93% degradation followed by 0.10 g/ml with 30% degradation. For oil concentration of 0.15 g/ml, the oil degradation is 25% and 16% degradation for oil concentration of 0.20 g/ml. The lowest rate of oil degradation occurs at oil concentration of 0.25 g/ml which is the highest level of oil need to be treated.

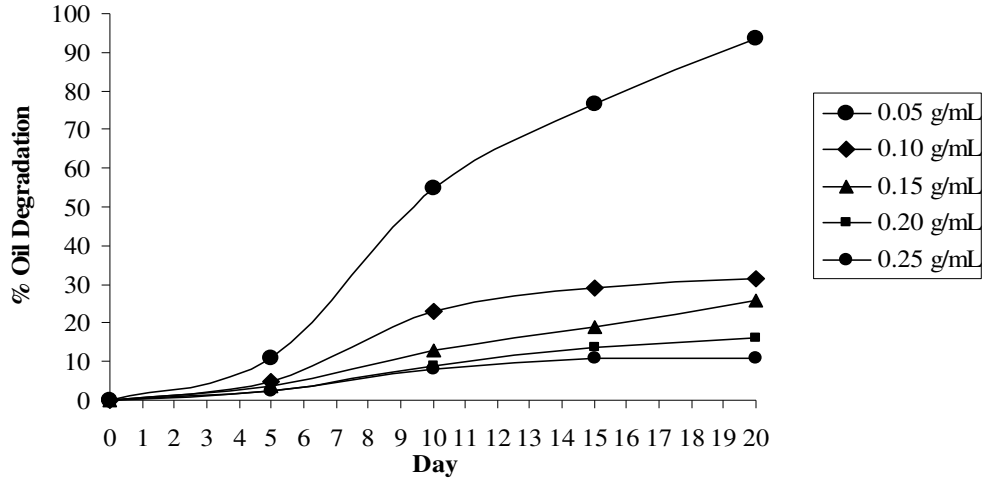

Figure 4.2: Effect of oil concentration on oil degradation

At oil concentration of 0.05 g/ml which is the lowest level of oil to be treated, the oil was highly degraded during the incubation time of 20 days (Appendix A.6). The rate of degradation gradually increased from 4th day and continuing increased until the end of treatment with 93% degradation. The nutrients such as nitrogen, phosphorus and carbon must be sufficient to support the microbial growth and increase oil degradation. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. At oil concentration of 0.05 g/ml, the composition of the nutrients is well balanced. So, about 4.682 g of oil have been degraded during the treatment (Appendix B.3). The difference of oil degradation for the concentration of 0.10 g/ml compare to 0.05 g/ml is 60% (Appendix A.7). The effect of oil concentration to the oil degradation is depending on carbon source which is from the oil. At this concentration, the rate of oil degradation gradually increased until the 9th day and starts to decrease at rate of 20% to just about 4% and maintained until the end of the treatment. This is because the large quantities of carbon sources which came from oils tends to result in a rapid depletion of the available pools of major inorganic nutrients such as nitrogen and phosphorus from the wastewater. About 3.161 g of oil have been degraded during the incubation time of 20 days at the oil concentration of 0.10 g/ml (Appendix B.4).

Even though the carbon sources can give an effect to the degradation of oils, large quantity of carbon can also inhibited the growth of the microorganisms. The oil concentration 0.15 g/ml has resulted in oil degradation of 25% after 20 days of incubation time (Appendix A.8). The rate of oil degradation increased about 7% after the 4th day and maintained until the end of treatment. The large volumes of oils limit the growth of the bacteria for the oil degradation from wastewater. For 0.15 g/ml of oil concentration, about 3.847 g of oil have been degraded (Appendix B.5). Even though the mass of oil for the concentration of 0.15 g/ml been degraded is higher than the oil concentration of 0.10 g/ml, the rate of oil degradation for the concentration of 0.15 g/ml is much lower than 0.10 g/ml. About 16% oil degradation occurs for the oil concentration of 0.20 g/ml after 20 days of treatment (Appendix A.9). The rate of oil degradation increased by 5% at the starting of treatment but after the 10th day, the rate of degradation decreased to 2% until the 20th day and resulted 3.246 g of oil degraded (Appendix B.6).

For the lowest oil degradation which the concentration of oil is 0.25 g/ml, the rate of degradation also increased by 4% at the starting of the treatment but after the 9^{th} day, the rate of degradation decreased to 2% until the end of treatment at the 20^{th} day (Appendix A.8). When oxygen is less available, the rate of biodegradation decreases because there are large volumes of oils at the surface of wastewater and limits the diffusivity of oxygen into water. This resulted that microorganism cannot degraded the oils because microorganisms employ oxygen incorporating enzymes to initiate attack on oil. Anaerobic degradation of oil which is the degradation in absence of oxygen also occurs, but usually at negligible rates. This was showed in the rate of oil degradation at 11% and 2.755 g of oil have been degraded during the treatment time (Appendix B.7). Duration of treatment would be proportional to the quantity of oil, suggesting lengthy treatment periods when the oil concentration is high can be done to treat the high level of oil.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The oil degradation is depending on many factors, such as temperature and oil concentration. From this study it is show that the optimum condition for the oil degradation using mixed culture must be at temperature of 30^{o}C and at low concentration of oil which at 0.05 g/ml. At certain temperature condition, the degradation of oil will increase. An increasing in temperature will decrease the viscosity of the oil in waste water, therefore affecting the degree of oil distribution and will increase the diffusion rates of organic compounds. The result showed that the rate of oil degradation is proportional to the temperature but at high temperature, for example 45^{o}C, will limit the degradation rate. The oil concentration was also found to give an effect to the oil degradation rate. It showed that rate of oil degradation was inversely proportional to the concentration of oil. In this study, the optimum concentration of oil and temperature for the optimum rate of oil degradation is depending on many factors such as pH, agitation speed, temperature and the concentration of inoculum used.

5.2 Recommendation

In order to improve this research, there are some recommendations like:

a) Duration of treatment would be proportional to the quantity of oil, suggesting lengthy treatment periods when the oil concentration is high can be done to treat the high level of oil.

b) More variation for the parameter of this study should be introduced such as agitation speed and pH to find the optimum condition

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APPENDICES A: TABLE

Appendix A.1: Result for the effect of temperature

Sampling Time (Day)	Temperature (°C)				
	10	**20**	**30**	**45**	**60**
Day 0	230.723	235.958	238.535	233.674	234.705
Day 5	230.717	235.803	237.905	222.981	209.855
Day 10	230.710	235.340	235.930	211.856	184.740
Day 15	230.708	235.012	234.929	Water Vaporize	
Day 20	230.698	234.955	233.813		

Appendix A.2: Result for temperature of 10^{o}C

Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	220.723	230.723	10.000	0.000
5	220.723	230.717	9.994	0.060
10	220.723	230.710	9.987	0.130
15	220.723	230.708	9.985	0.150
20	220.723	230.698	9.975	0.250

Appendix A.3: Result for temperature of 20^{o}C

Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	225.958	235.958	10.000	0.000
5	225.958	235.803	9.845	1.550
10	225.958	235.340	9.382	6.180
15	225.958	235.012	9.054	9.460
20	225.958	234.955	8.997	10.030

Appendix A.4: Result for temperature of 30^{o}C

Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	228.535	238.535	10.000	0.000
5	228.535	237.905	9.370	6.300
10	228.535	235.930	7.395	26.050
15	228.535	234.929	6.394	36.060
20	228.535	233.813	5.278	47.217

Appendix A.5: Result for the effect of oil concentration

Sampling Time (Day)	Concentration (g/mL)				
	0.05	**0.10**	**0.15**	**0.20**	**0.25**
Day 0	231.502	233.740	240.228	245.005	250.177
Day 5	230.959	233.248	239.703	244.510	249.612
Day 10	228.753	231.422	238.285	243.215	247.135
Day15	227.666	230.825	237.380	242.255	247.487
Day 20	226.820	230.579	236.381	241.759	247.422

Appendix A.6: Result for oil concentration of 0.05 g/ml

Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	226.502	231.502	5.000	0.000
5	226.502	230.959	4.457	10.867
10	226.502	228.753	2.251	54.987
15	226.502	227.666	1.164	76.713
20	226.502	226.820	0.318	93.640

Appendix A.7: Result for oil concentration of 0.10 g/ml

Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	223.740	233.740	10.000	0.000
5	223.740	233.248	9.508	4.923
10	223.740	231.422	7.682	23.183
15	223.740	230.825	7.085	29.150
20	223.740	230.579	6.839	31.613

Appendix A.8: Result for oil concentration of 0.15 g/ml

Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	225.228	240.228	15.000	0.000
5	225.228	239.703	14.475	3.502
10	225.228	238.285	13.057	12.953
15	225.228	237.380	12.152	18.989
20	225.228	236.381	11.153	25.644

Appendix A.9: Result for oil concentration of 0.20 g/ml

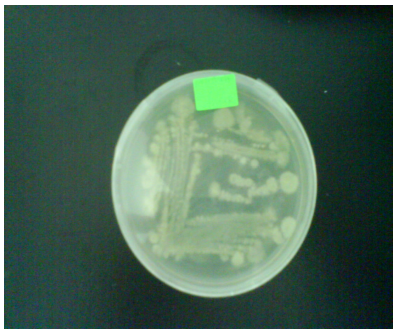
Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	225.005	245.005	20.000	0.000
5	225.005	244.510	19.505	2.473
10	225.005	243.215	18.210	8.950
15	225.005	242.255	17.250	13.748
20	225.005	241.759	16.754	16.228

Appendix A.10: Result for oil concentration of 0.25 g/ml

Sampling Time (Day)	Without Oil	With Oil	Mass of Oil	% Oil Degradation
0	225.177	250.177	25.000	0.000
5	225.177	249.612	24.435	2.259
10	225.177	248.135	22.958	8.168
15	225.177	247.487	22.310	10.760
20	225.177	247.422	22.245	11.021

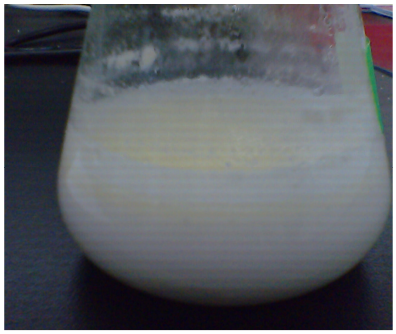
APPENDICES B: PICTURE

Appendix B.1: Oil contaminated wastewater



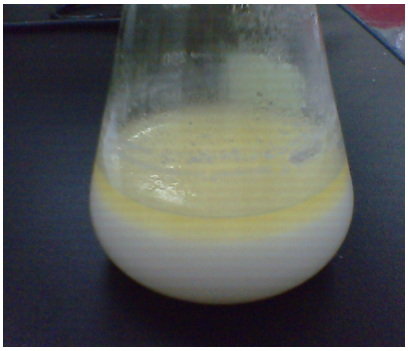
Appendix B.2: Mixed culture of oil degrading microorganism

Appendix B.3: Result for concentration of 0.05 g/ml

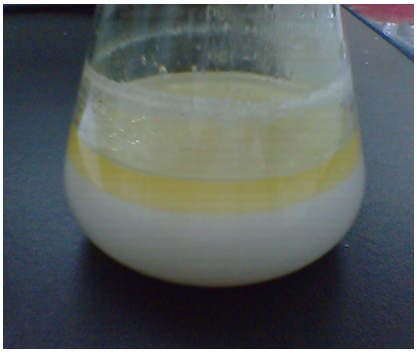


Appendix B.4: Result for concentration of 0.10 g/ml

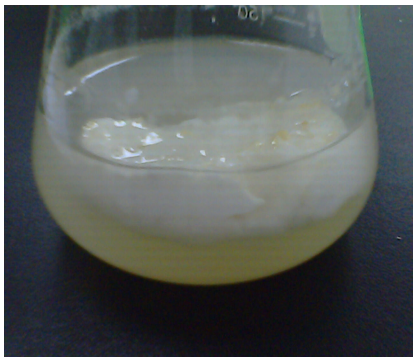
Appendix B.5: Result for concentration of 0.15 g/ml



Appendix B.6: Result for concentration of 0.20 g/ml



Appendix B.7: Result for concentration of 0.25 g/ml



Appendix B.8: Result for temperature of $10^{o}C$