

APPLICATION OF EZ-ENZYME IN BIOREMEDIATION OF OILY SLUDGE

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Abstract. Sludge farming is commonly used in petroleum refinery as a method of treatment for oily waste containing degradable constituents into materials that are not hazardous to human health or the environment. The sludge is left on a designated plot of land to be biodegraded naturally, but the process could take a considerable length of time. In this study, an enzyme known as EZ-Enzyme was tested to bioremediate the oily sludge sample in a laboratory scale experiment. Results shown that there was a consistent reduction in the oil and grease (O&G) content of the tested sludge (~ 60% in average). The O&G content decreased with increase in the concentration of enzyme. The O&G content in a sludge sample decreased the most with the application of 100 pm EZ-Enzyme, which decreased from 8% to 2% within a period of nine weeks. In general, the temperature and pH of the system were maintained at optimum condition, which were 35°C and pH 7 respectively. The quality of the leachate was also improved by the end of the study, where chemical oxygen demand (COD) was 25 mg/l and pH was 6.45 respectively.

Keywords: Bioremediation, enzyme, oil and grease content, sludge farming

Abstrak. *Sludge farming* adalah cara biasa yang digunakan dalam loji penapisan petroleum untuk merawat sisa minyak yang mengandungi bahan berupaya terdegradasi kepada bahan yang tidak toksid terhadap kesihatan manusia dan alam sekitar. Sisa tersebut akan dibiarkan di atas tanah supaya terdegradasi secara semula jadi tetapi proses ini akan mengambil masa yang lama. Dalam kajian ini, sejenis enzim bernama Enzim EZ telah diuji untuk bioremediasi sampel sisa minyak dalam uji kaji yang berskala makmal. Keputusan menunjukkan bahawa terdapat pengurangan secara konsisten untuk kandungan minyak dan gris dalam sampel yang diuji (secara rata ~ 60%). Kandungan tersebut berkurangan dengan meningkatnya kepekatan Enzim EZ. Kandungan minyak dan gris terdegradasi dengan paling banyak dalam sampel mengandungi 100 ppm Enzim EZ iaitu dari 8% ke 2% dalam eksperimen selama sembilan minggu. Secara umum, suhu dan pH dikekalkan pada keadaan optimum, iaitu 35°C dan pH 7. Kualiti air serapan yang dihasilkan juga telah menunjukkan peningkatan kualiti di akhir eksperimen, iaitu keperluan oksigen kimia (COD) ialah 25 mg/l dan pH ialah 6.45.

Kata kunci: Bioremediasi, enzim, kandungan minyak dan gris, *sludge farming*

1.0 INTRODUCTION

Petroleum refinery activity generates oily type sludge that is regulated under the 1989 Environmental Quality Act (Schedule Waste) Regulation. The Acts classify

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the sludge as a scheduled waste, which restricts over its storage, transportation and disposal.

Land farming, which is also referred to as land spreading, land application, sludge farming, land disposal, soil cultivation or land treatment, is a widely used method in the refinery to treat the oil sludge before its final disposal [1, 2]. This method has gained popularity over other techniques due to its relatively low operating cost. The objective of land farming is to convert or treat substantial quantities of waste containing degradable constituents into materials that are not hazard to human health or the environment [3, 4]. This technique is based on spreading the bio-sludge in a thin layer on the soil surface and stimulating aerobic microbial activity within the soil through aeration and the addition of minerals, nutrients and moisture. The repeated application of the bio-sludge to a given soil, and controlled promotion of naturally occurring microbial assimilation converts the hydrocarbons to the end products of CO_2 and H_2O , and increases the humus content of the soil [5].

The sludge farming utilizes the physical, chemical and biological capabilities found in the soil to serve as an ultimate receiver of the wastes by converting it into harmless by-products. It bio-remediates the soil contaminants based on the biological oxidation of hydrocarbons by natural soil microflora [3]. Most of the biodegradation process takes place at the surface soil layer (0.1–0.5 m) known as ‘zone of incorporation’. Additional treatment and immobilization of the waste could also occur to a depth of 1.5 m from the surface known as the ‘treatment zone’. Finally, soil conditions below 1.5 m are not favourable because oxidation process will not take place beyond this depth [2].

Hejazi [6] studied on the degradation of petroleum hydrocarbons under natural and enhanced conditions using land farming and bioreactor techniques in the Juaymah area in the Eastern Province of Saudi Arabia. The author found that weathering (evaporation) and not biodegradation was the dominant degradation mechanism under arid conditions. Of the three operating parameters studied, i.e. tilling, addition of water and/or addition of nutrients, tilling was the main parameter caused for the highest reduction in O&G concentrations. The study found that the addition of nutrients and water influenced the soil properties and hence minimized the weathering effect. The author concludes that such land farming treatment is a slow process, which must be accompanied with an enhanced strategies to fasten the biological activity.

Thus, microbes are usually applied in land farming plots. However, the present knowledge of the complex assemblages of microbes in both natural and polluted environments is limited due to inability to culture so many of the organisms by conventional microbiological methods. In addition, many questions arise about what impact a pollutant or a treatment regime may have on the microbial community still remained to be answered.

Alternatively, enzyme, known as a biocatalyst, is used to stimulate and accelerate natural biological reactions by reducing the energy of activation [7, 8]. EZ-Enzyme is an exclusive, proprietary formula that effectively removes oils, grease, waxes and other organic contaminants without the use of environmentally dangerous chemical. The application and effectiveness of the EZ-Enzyme on environmental samples have been reported [9, 10]. Enzyme can easily be applied by power sprayers or through dosing pumps and it is effective in water or soil medium. It is a liquid concentrate containing diverse oxidative preformed enzymes that oxidise organic molecules at an extremely fast rate.

Several studies have established that many factors such as temperature, moisture content and pH, have a strong influence on the bioremediation of oily sludge in sludge farming [1, 4, 11]. However, application and performance of enzyme in the bioremediation of oily sludge have not been studied in detail. This paper reports on the application of EZ-Enzyme in biodegradation of petroleum refinery oily sludge. The oil and grease contents of sludge have been monitored for a period of time and the characteristic of leachate has also being collected and analysed. This paper can guide future studies in application of EZ-Enzyme for industrial scale of sludge farming.

2.0 MATERIALS AND METHODS

2.1 Sludge Farming Plot

The oil sludge sample taken from a petroleum refinery was equally allocated into five sets of already prepared experimental sludge farming plots outside the Environmental Pollution Control Laboratory, Universiti Teknologi Malaysia. Figure 1 illustrates the schematic diagram of an example of the experimental sludge farming plot equipped with a leachate collection bottle.

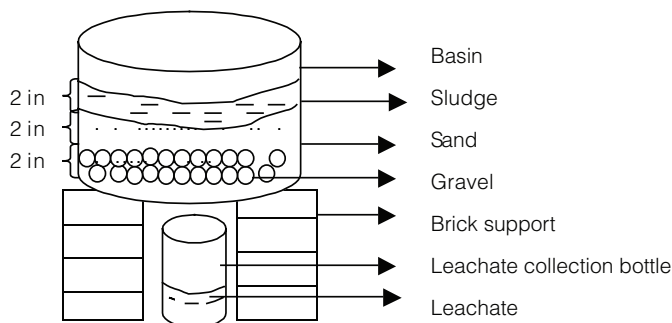


Figure 1 The experimental sludge farming plot

The sludge was layered (*ca* 2 inches height) on top of sand and gravel in each of the plot container, which was drilled at its bottom as drainage for the collection of leachate. One of the plots was sprayed with 500 ml of distilled water (as a control plot) while the other four plots were sprayed with a similar quantity water containing different concentrations (i.e. 10, 40, 60 and 100 ppm) of EZ-Enzyme solution. The plot was sprayed every morning while the oil and grease contents were analysed on a weekly basis. Other parameters such as sludge moisture content, pH and temperature were also monitored during the experiment.

2.2 Analysis of Sludge

The oil and grease (O&G) content in the sludge was analysed on a weekly basis. The loss of O&G content in sludge represents the degree of bioremediation of the sludge sample in each of the plot. The O&G was determined gravimetrically where 20 gram of sludge was extracted with 100 ml n-Hexane [10]. The mixture was mixed thoroughly and left to stand for 30 minutes. Then, it was filtered into a pre-weighted conical flask and immersed in a warm water bath at approximately 80°C for 1 hour to evaporate the hexane. The final weight of flask and O&G remains in it was noted and the difference between the final and the tare weight represents the mass of O&G recovered in the sludge sample.

However, the percentage of the O&G was calculated based on the dry basis of the sludge sample, whereby a same quantity of sludge was taken and placed in an oven at 105°C for a period of 24 hours. The dried sample was weighed, and the difference determined the moisture content in the sludge taken in from the designated plot.

The pH of the sludge sample was determined with a sludge/water ratio of 1/10 (weight/volume). A 20-gram sludge sample was mixed with 200 ml of distilled water and stirred for 5 minutes before the pH was measured. The optimal pH required in a land farm sludge, as reported in the literature, should be between 6 and 8 [11]. The pH of the raw sludge was 6.9, hence, pH adjustment was not necessary. The temperature of sludge farming sample was performed *in-situ* by using a digital thermometer.

2.3 Analysis of Leachate

The quality of the collected leachate was performed on a regular basis for the chemical oxygen demand (COD) and pH using a standard HACH method.

3.0 RESULTS AND DISCUSSION

3.1 Effect of EZ-Enzyme on O&G Content

Figure 2 shows the percentage of O&G remaining under different concentrations of enzyme, which showed that there was a consistent reduction of O&G with increased concentration of enzyme applied to the sludge sample. Three distinctive phases representing changes in the O&G content were observed in all samples. There was a significant reduction ($\sim 60\%$ in average) of O&G during the first phase of the experiment, which took place between week zero (initial) and week three, compared to the second and third phase of the study. This is mainly because the higher concentration of reactant at the beginning, the higher reaction rate for enzyme [12-14].

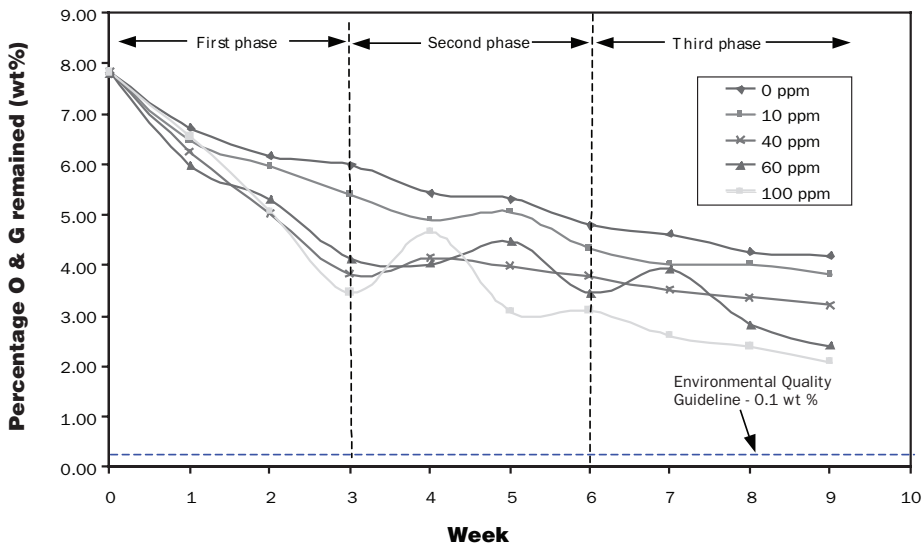


Figure 2 Effect of different concentrations of EZ-Enzyme on the O&G content in sludge

In the second phase (between week three and six), the decrease in O&G content was apparent, however, it was not as drastic as during the first phase of the study. In this phase, the data seemed to fluctuate but steadily remained in the downward trend consistently with the concentration of enzyme introduced in the sample. The downward trend was observed to continue in the third phase of the study period i.e. between week six and nine, where the O&G content continuously decreased with time in less drastically manner.

Envas and Furlong [4] stated that the biodegradation rate will slow down as the sludge farm practice proceed due to less O&G remains in the sludge compared with initial conditions. A similar finding was observed in this study. Initially, the average O&G content in the sludge sample was 7.83 ± 0.27 wt%, but decreased to as low as 2.10 wt% in sample that was dosed with 100 ppm enzyme (Note: The Environmental Quality Guideline for O&G content is 0.1 wt%). This represents a degradation of O&G content by 73.2%. This is followed by the 69.1% for 60 ppm enzyme, 59.0% (40 ppm), 51.1% (10 ppm), and 46.3% (0 ppm) being introduced into the sludge sample. The percentage loss in O&G content was found to be consistent with the concentration of enzyme dosed in the sludge sample. These findings showed that the presence of enzyme considerably influenced the final O&G content of the oily sludge sample.

Table 1 lists the calculated rate of O&G degradation in all sludge samples. It indicates that the highest rate of O&G degradation was the application of 100 ppm enzyme, which is 0.0122 O&G degraded per day. The finding demonstrates that the concentration of enzyme influenced the degradation rate of the O&G in the oily sludge sample, and that the higher the enzyme concentration, the higher is the degradation rate of O&G content in a given sample. The degradation rate of O&G in the sample was calculated by using the following Equation (1).

$$C_i dt_o = \frac{C_i a_o - (C_i r_o - C_i s)}{C_i a_o} \quad (1)$$

where $C_i dt_o$ = Fraction of component i degraded in sludge over time, $C_i a_o$ = The amount of component i in the sludge during application, $C_i r_o$ = The amount of residual component i in degraded sludge, and $C_i s$ = Component i in soil (component i background level in soil).

Table 1 The O&G degradation rate for sludge sample with different concentrations of EZ-Enzyme

Sample	Degradation rate (Fraction O&G degraded / day)
0 ppm	0.0077
10 ppm	0.0085
40 ppm	0.0098
60 ppm	0.0115
100 ppm	0.0122

It seems that the biodegradation rate of introducing 100 ppm enzyme solution could speed up the rate by as high as 1.6 times compared to the one without any enzyme introduced. Sludge sample that was left to degrade naturally (i.e. without any enzyme introduced which acts as control) showed the least effects of biodegradation and took a longer time to achieve the targeted treatment goals as compared to enhanced treatment processes using enzyme solution. The enzyme can speedily remove oils, grease, waxes and other organic contaminants by oxidising organic molecules at a faster rate as illustrated in this study.

3.2 Evaluating Parameters Influencing the Degradation Process

3.2.1 Moisture Content

Figure 3 shows the moisture content of the sludge sample, which fluctuated throughout the study period due to weather conditions. The sludge sample was very watery (88% moisture) and odorous at the beginning of the study. Then, its moisture content decreased, maintaining above 50% within the first three weeks of the study period. It is mainly because the first three weeks of the field study were rainy days. The moisture content was quite consistent after the fourth week of the study, which was maintaining below 50%. At the end of the study, the sludge sample became dry and appeared like normal soil. This moisture content was at its minimum in week seven as there was no rainfall for the whole week.

The moisture content has a great impact on the biodegradation rate, and indirectly affects the O&G contents in the sludge sample. Too much water will hinder the supply of oxygen and as a result will decrease the rate of biodegradation. On the contrary, too little water will inhibit microbial activities. The moisture content in the sludge sample plot may be due to three sources i.e. water in the original sludge sample, the rain that occurred throughout the study period, and water introduced along the spraying of enzyme solution. Rainwater that falls directly onto, or runs onto the land farm area certainly increase the moisture content of the soils that may temporarily in excess of the required amount of moisture for effective bacterial activity. Decomposition is typically not restricted by moisture if the soil moisture content is maintained above a certain minimum (usually between 30% and 90% of the water-holding capacity of the soil). However, excess water reduces the available oxygen levels and can retard microbial decomposition of the waste applied. When anaerobic reaction of bacteria dominates, this would cause the rotten egg smell in the sludge itself [15, 16]. As depicted in Figure 3, the moisture contents in all of the sludge samples in the plots were always more than the required range (1.6% and 4.9%) [3] throughout the study period, which means that the sludge samples had enough water to support microbial activities.

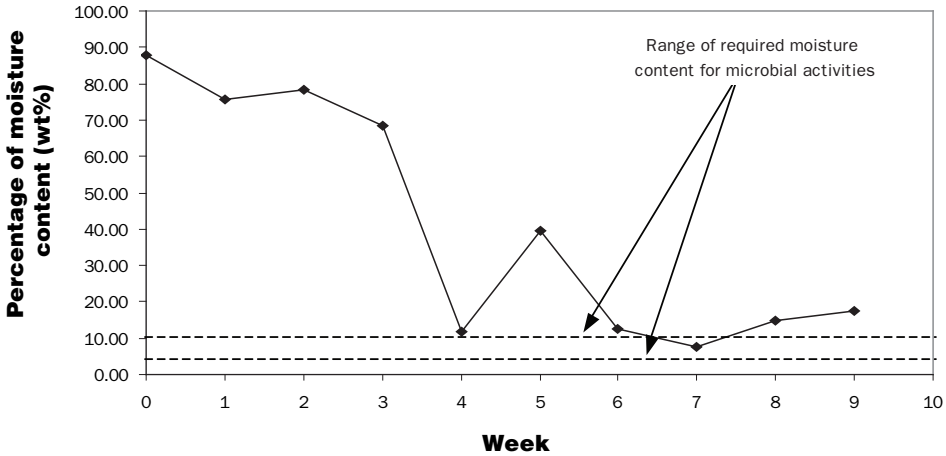


Figure 3 Moisture content in sludge throughout the study

3.2.2 Temperature

Figure 4 presents the overall average temperature as well as the pH of the sludge plots obtained during the study, which showed that the sludge temperature ranged from 35.2°C to 36.7°C during daytime. However, the temperature and pH were only monitored after the fifth week of the study period. The optimal growth of microbial populations was responsible for the biodegradation of petroleum products.

Temperature exerts a major control on the metabolic activity of micro-organisms because the entire microbiological organic breakdown occurs through the activity of enzymes. Generally, as temperature increases, the rate of metabolic activity increases due to the presence of more energy in the system. However, microbial activities increase until approximately 45°C and that beyond this temperature, microbiological activities decrease and eventually cease. Soil microbial activity has been shown to decrease greatly at 10°C and essential cease at 5°C [3]. But this should not be a problem to tropical countries like Malaysia with ambient temperature of around 24-35°C, where EZ-Enzyme can bio-remediate effectively up to 50°C. Atlas *et al.* [1] stated that the optimal degradation rate occurs at a temperature between 20°C and 35°C. This coincides with the sludge temperature obtained in this study where the temperature fluctuated above 20°C but maintained within the range of optimum values. In general, the temperature should not be seen as one of the inhibiting factors of biodegradation process in this part of the world.

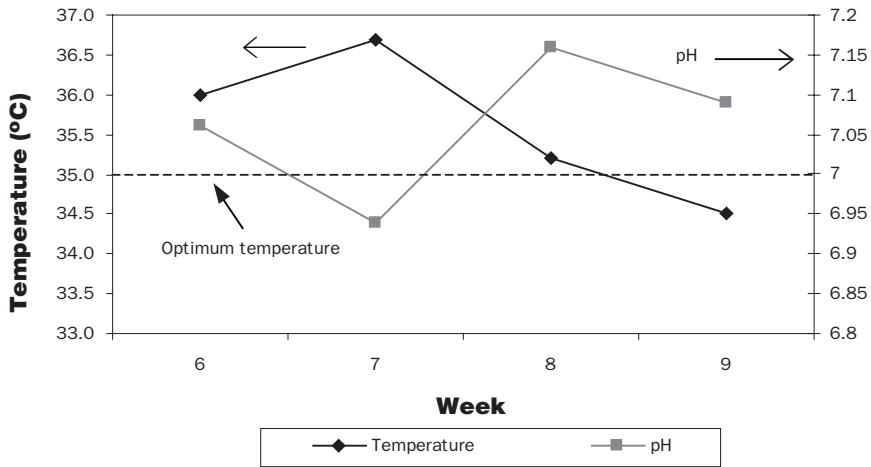


Figure 4 Sludge temperature and pH monitored throughout the study

3.2.3 pH

pH is another important parameter in sludge farming. The pH must be maintained between 6 and 8 to immobilize the leaching of heavy metals in the waste and maximizes the rate of biodegradation [11]. As depicted in Figure 3, the monitoring of pH only started at week six and data was not available before this. It seemed that the pH value in each of the plot was maintained within 6.94 - 7.16, which was nearly neutral that certainly would support or facilitate bacterial activity for bioremediation of the sludge sample. This is evidence by the fact that the O&G content was found in the decreasing trend with respect to time. To support bacterial growth, the soil pH should be within these ranges, with a value of about 7 (neutral) being optimal. If the pH is higher or lower than the above limits, microbial growth will be affected and the soil chemistry will be modified. Soils with pH values outside this range prior to landfarming will need to be adjusted prior to and during landfarming operations.

3.3 Characteristics of Leachate

In sludge farming facilities, it is always required to adequately control the leachate from contaminating the surface and ground water. Soluble degraded and non-degraded waste contaminant in soil could penetrate deep into the soil through leaching process. Thus, it is necessary to place impermeable layer as leachate collection system at a few feet below the treatment zone to prevent waste constituents from penetrating the groundwater [6]. Where the groundwater contamination occurs, remedial actions

can be very costly. Therefore, early detection is necessary to detect any possible migration of contaminants. With this in mind, the leachate generated as a result of the bioremediation of the sludge was collected and analysed. Figure 5 presents the overall characteristics of leachate collected from samples dosed with enzyme throughout the study period. The leachate were collected and mixed and divided into three samples before analysing the parameters of interest in triplicates.

In general (as depicted in Figure 5), the overall Chemical Oxygen Demand (COD) concentration showed a decreasing trend although there were some fluctuations of these parameters during the study due to experimental errors and different sampling point in the same sludge sample. The fluctuation of COD is not uncommon in previous studies [16,17]. Initially, a high concentration of COD was observed at the beginning of the study and improved significantly by the end of the study. The COD recorded the highest in the early week with 153 mg/l and continuously improved in the third and the following week as the COD level achieved as low as 25 mg/l by the end of the sixth week. At the end of the study, the COD was less than the Department of Environment limits for Standard A COD level (50 mg/l) in the Environmental Quality (Sewage and Industrial Effluents) Regulations 1979 [18]. This finding illustrates that the leachate collected which directly relates to degree of remediation of the sludge were becoming more stabilized and less hazardous with time.

As shown in Figure 5, the pH of the leachate was quite acidic at the beginning of this study. The pH level was initially 4.78 and continuously increased to neutral i.e. 6.45, by the end of the study. The biodegradation of sludge results in some acidic products, which will finally turn to neutral as it has stabilized [17]. On the whole, the characteristics of the leachate improved considerably as found in this study, which indicates the effectiveness of EZ-Enzyme in improving the degradation of sludge.

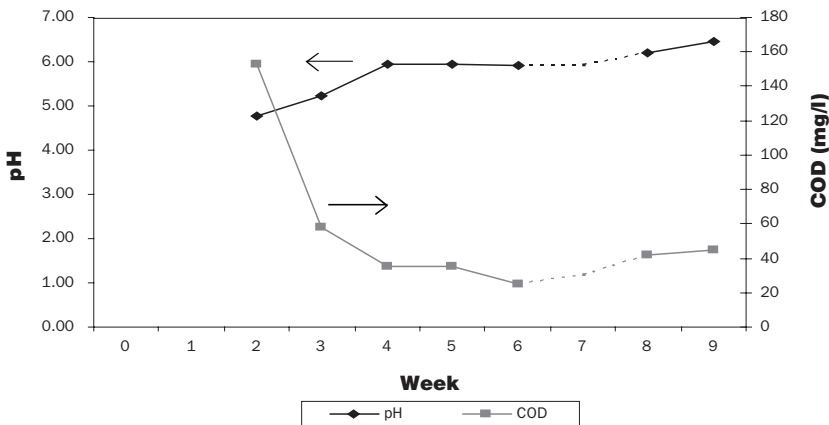


Figure 5 Characteristics (COD and pH) of leachate collected during the sludge farm experiment

3.4 Physical Observation

Figure 6 illustrates the pictorial observation of the sludge plot, which showed that the physical conditions of the sludge samples were significantly improved by the end of the experiment. In the early stage, the fresh dewatered sludge was in the form of a sticky paste, oily and blackish in colour, with mixed odour of hydrocarbon and rotten egg. However, during the second phase (between week three and week six), the sludge became less sticky and slightly dried. The sludge was much easier to handle with resemblance to soil. However, the sludge still contained some hydrocarbon odour and slightly oily. Nevertheless, the presence of grasses was noticeable in several sludge plots. As shown in Figure 6, the physical appearance of the sludge improved greatly during the third phase of field study (between week six and week nine). At this stage, the sludge was odourless and appeared like normal soil with a lot of grasses growing in most of the sludge samples. It indicates that the application of EZ-Enzyme in sludge farming could help improve the physical characteristics of sludge and make it suitable for plant growth.

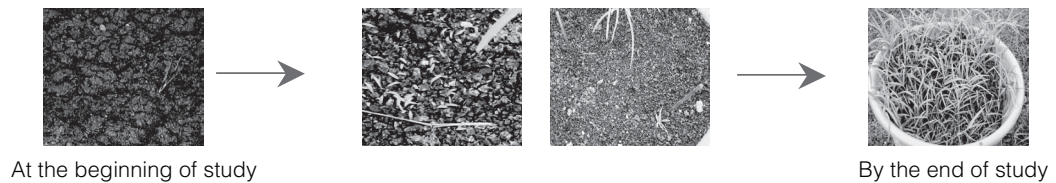


Figure 6 Physical appearance of sludge plot before and after treatment

4.0 CONCLUSION

Different concentrations of EZ-Enzyme had been tested to bioremediate oily sludge samples taken from a sludge farming facility of a petroleum refinery plant. Results showed that the bioremediation rate of the sludge increased with the increase in the concentration of EZ-Enzyme being added in the sample. The degradation was the highest with 100 ppm enzyme where nearly 75% of the O&G content was reduced within nine weeks of the study period. The bioremediation rate of sludge with the application of enzyme was higher than that without the use of enzyme and it was observed that the higher the concentration of EZ-enzyme, the higher the rate of biodegradation rate of the sludge sample.

In addition, the analysis of the leachate collected from sludge farming experiment seems to indicate that there was a considerable improvement on the quality of the leachate generated through the process. The enzyme helps to accelerate the stabilization of the sludge, which makes it non-hazardous, and reduce groundwater contamination. It was observed in the study that the appearance of the sludge

also improved significantly from very oily and watery with strong oily smell at the beginning of the experiment into a dry normal texture of soil like condition with no obnoxious smell by the end of the study.

As a conclusion, the application of EZ-Enzyme in bioremediation of oily sludge is promising where it helps to accelerate the stabilization of sludge within a shorter period of time as compared to solely relying on a natural and slow process.

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