Degradation of soil polluted with petroleum products using enzymatic accelerating reagent

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Abstract. Pollution with petroleum wastes is a serious environmental problem that must be treated considerably. Artificially polluted soil with oily contaminants mixtures was treated biologically using aerated bioreactor. The used oily mixtures used in this study are petroleum sludge, lubricating oil and crude oil. Crude oil and petroleum sludge were obtained from oil refinery of Al-Romayla at Al-Basrah governorate, tests were examined at the Ministry of Science and Technology. These wastes were mixed separately with soil in a ratio 7(pollutant):100 (soil) and treated by using the bioreactor. The degradation ability of microorganisms existed in the soil had been examined in low weather temperature with the aid of enzymatic reagent. The efficiency of removing the organic hydrocarbons after five weeks of treatment was 68.2 %, 74.4 % and 77.7 % for the petroleum sludge, lubricating and crude oil, respectively. The obtained results show the ability of the enzymatic reagent in the activation of microorganisms to degrade heavy hydrocarbons substances even in cold weather.

Key Word: Biodegradation, Petroleum, Soil, Crude, Accelerating Reagent.

1. Introduction

Among all environmental pollutants, oil sources are one of the most significant pollutants that can lead to serious and long-term damages to environment. Every time, enormous quantities of petroleum products contaminate environment in different ways. Due to oil extraction, transportation and refining, oil spills contaminate soil, ground water and surface water via leakages in tanks of pipelines. Oily liquids can move both directions, vertically and horizontally into the soil because of gravity and capillary forces. They can cause severe damages to soil as it can change its chemical, physical, and mechanical properties [1].

Among those items that consist of multi hydrocarbons, organic matters and some metallic substances, lubricating oil is a complex one that used to lubricate parts of vehicles engine [2]. Engine oil contains metals and heavy aromatic hydrocarbons that cause chronic risks like mutagen city and carcinogen [3]. The field of oil-soil contaminations treatment obtains huge interest all over world. Many authors suggested several methods for soil remediation. Not only soil contaminated with oil, but also contaminated with heavy metals can be remediated by soil extraction as it seemed significant and potential [4]. Taking into consideration that chemical soil remediation has the probability of generating by-compounds such as secondary remains, after the main crude oil pollutant removal that can be even
higher toxicity levels than the parent crude oil contaminates [5]. In another hand, bioremediation processes are better compared with soil extraction and remediation in terms of their enormous effectiveness and economic benefits [6].

One of the effective and main methods of eliminating petrol and petroleum products that contaminate environment is biodegradation by natural bacteria and fungi strain existed in nature [7]. Bacterial strains viz., Arthrobacter globiformis, Pseudomonas aeruginosa, Acinetobacter baumannii, Flavobacterium chungangense, Rhodococcus equi were isolated from soil and other environmental samples which are well-known organic petroleum eaters [8]. Yet, it had been recorded that functionalizing mixed bacterial–fungal consortium or bacterial culture can lead to better degradation results. Besides that, pure strain cannot simulate the biodegradation of contaminated soils that occur naturally [9].

Molecular methods are being applied to increase the ability for desulfurization. Biological treatments are being used for the reduction of H2S and sulfoxides from petroleum wastes. Nitric oxides can also be reduced from crude oil by using this type of treatment in a technique similar to that used in biodesulfurization [10].

The major physical properties of the oil that affected directly by temperature are density, viscosity and surface tension. Relation between temperature and physical properties affect the biodegradability. Different types of bacteria can be found over a wide range of temperature, which influence on the degradation of oil [11]. Hydrocarbon biodegradation can occur on various ranges of temperature, the rate and amount of bacterial degradation is increased by increasing temperature [11] and [12].

The reaction rate of enzymes for biodegradation increases with increasing temperature, the reaction rate is generally doubled with increasing temperature 10 °C [11].

In the current research study, bioremediation of manufactured soil contaminated with petroleum-based pollutants had been conducted via simply manufactured bioreactor. In order to challenge unusual ambient conditions of winter i.e., temperature was below the level of microorganism’s limits of biodegrading high toxic organic compounds. Accelerating enzymatic reagent that consists of nutrients and enzymes extracted from living microorganisms had been functionalized as described in the succeeding sections.

2. Material and Methods

The lubricating oil had been used as pollutant delivered from local oil changing station. While crude oil and petroleum sludge were obtained from oil refinery of Al-Romayla at Al-Basrah governorate. A Container made of plastic with dimensions of 58 cm length, 40.5 width and 10 cm depth had been used as bioreactor. Reagent which is complex of bio enzymes extracted from living bacteria and many nutrients had been supplied by OSEI. Ink company. The examined physical properties and compositions of petroleum sludge used in the current study are listed in Table 1, while Figure. 1 shows the petroleum sludge used in the current research.

| Properties       | Value |
|------------------|-------|
| Density (gm/cm³) | 0.935 |
| Viscosity (cP) @ 30 °C | 902.7 |
| pH               | 6.1   |
| Humidity %       | 23    |
| Total N (mg/dm³) | 170   |
| Total P (mg/dm³) | 320   |
| Total Fe (mg/dm³) | 510   |
Figure 1. The petroleum sludge used in the current study.

The physical and chemical content of crude oil used in the current study were tabulated in Table 2.

| Property                        | Value   | Method of measurement |
|---------------------------------|---------|-----------------------|
| Density @ 15 °C (gm/ml)         | 0.865   | ASTM D-4052           |
| Specific gravity 60/60 F        | 0.883   | Calculated            |
| Kinematic viscosity (cSt) @ 70F | 9.09    | ASTM D-445            |
| API gravity                     | 28.77   | Calculated            |
| Salt content (mg/L)             | 2.2-3.4 | IP 77                 |
| Sulphur content (wt %)          | 2.11    | ASTM D-4294           |

2.1. System set up

The lubricating oil, crude oil and petroleum sludge used were mixed separately with clean soil in a ratio of [7(pollutant):100 (soil)] as illustrated in Figure 2. The mixture then mixed thoroughly and spread homogenously in the bioreactor.
Figure 2. Soil to used lubricating oil mixture ready to be treated.

An accelerating reagent had been diluted with de-ionized water, where 29 ml of the reagent was mixed with 725 ml of water as presented in Figure 3. The diluted reagent had been sprayed on the surface of oil-soil mixture.

Figure 3. Adding water to accelerating reagent mixing step.
With constant period of three days, 2 litre of water had been sprayed on the oil-soil mixture in order to obtain humidity ratio of 60% in the bioreactor. The mixture had been flipped every week of treatment in order to allow fresh air to ventilate the bioreactor.

2.2. Sampling and measurements

In order to calculate the percentage of removal, the samples had been taken every week. The Zero sample had been supplied before starting treatment and the results had been used as blank to be compared with other results. Percentage of removal had been calculated as follows:

\[
\text{percentage of removal (POR)} = \frac{C_i - C_w}{C_i} \times 100\%
\]

Where:
\( C_i \) is the initial concentration of total hydrocarbons in the sample.
\( C_w \) is the weekly measured concentration of total hydrocarbons in the sample.

Used lubricating oil and samples of oil-soil mixture had been analysed via gas chromatography (GC-2010 Plus SHIMADZU) equipped with non-polarized column (30 m x 0.3 mm x 5 µm). At sampling, 5 random samples were taken from different locations of the bioreactor and mixed well to form the required sample sent to chromatography analyses [13] and [14].

2.3. Biological testing

For the contaminated soil, biological tests were conducted to reveal the already existing microorganisms in the soil. Morphological and biochemical tests were invested. The results revealed that three strains of bacteria were already available in the soil sample i.e.: Pseudomonas aeruginosa, Aeromonas hydrophila and Staphylococcus aureases.

3. Results and Discussion

As described above, percentage of removal (POR) had been calculated every week of treatment and the calculated values had been summarized in Figure 4.
As it can be noticed in the Figure 4 for the lubricating oil that used, percentage of removal (POR) increased as time was passing reaching to a plateau of 55% at the end of the forth week. While the increments were almost linear for crude oil and petroleum sludge. This can be ascribed to the rapid dissociation of pollutants via bacteria existed in soil combined with enzymatic dissociation by the pre-added reagent. It can be noticed that at the end of fourth week, degradation of crude oil was higher than that for used lubricating oil. This can be ascribed to the nature of crude oil that contains higher nitrates and sulphates that can be consumed by living microorganisms as nutrients leading to higher degradation of pollutant [10].

In another hand, increasing in POR had been noticed almost after 35 days (the end of the fifth week) to a value of 74.4% for used lubricating oil, 77.7% for crude oil and 68.2% for petroleum sludge. This gave a significant indication that the enzymatic reagent was sufficient in degrading heavy hydrocarbons of the pollutants that is used in spite of relatively low ambient temperature. Taking into consideration that recorded and well-known enzymatic activities required relatively high temperature (60°C and above). While in the current experiment, the bioreactor was installed outdoor with ambient temperature ranged between 26°C at mid noon and 4°C at night.

The reagent used consists of motivating complex organic enzymes with biological origin. These enzymes are simple protein molecules combined with some active chemical groups. The enzymes were extracted from types of bacteria that considered heavy hydrocarbons eaters and used to accelerate the biodegradation of the whole process. It is worth to say that the same experiment was already conducted depending on bacteria isolated from the same environment (soil mixed with used lubricating oil) without using the specified reagent. The results showed significant degradation at the end of three months of treatment with ambient temperature higher than that recorded for the current research. The soil with contaminated engine oil had been treated using bioremediation with three kinds of organic wastes by Abioye and Agamuthu[15]. Brewery spent grain (BSG), banana skin (BS) and spent mushroom compost (SMC) were used for the biological treatment of the contaminated soil for 84 successive days. The results revealed that total hydrocarbon loss was about 26.76% of the total weight.
The artificially contaminated soil with 5% by weight waste oil remediation was investigated by the work of Al Zubaidi & Al Tamimi [4]. Different soil particle diameters were contaminated with different dosage of waste oil. Batch procedure was used for solvent extraction technique. The contaminated soil was treated with many solvents, different solvents to oil ratios was also used. The reclaimed oil from contaminated soil showed better physical properties compared with original waste oil characteristics. This means that some of the heavy molecular weight hydrocarbons are not recovered from the pores of the soil and the resulting recovered oil has better physical properties comparing with the original waste lubricating oil.

Promising results were gained for the biodegradation by implementing 5% sawdust and aeration added to (soil + diesel oil) mixture by the work of Ibrahim et. al. [5]. The bacterial degradation rates were increased by adding sawdust up to 5%, these rates were reduced by increasing sawdust bulking materials proportion above this percent. The addition of sawdust without aeration increased the bacterial activity of the soil, but this did not make the diesel oil biodegradation more effective. Thus, it can be shown that the effective diesel oil bacterial degradation be more efficient in the samples exposed to aeration, and this considered to be the main factor for increasing oil biodegradation process [16], and [17].

4. Conclusions

Biodegradation of soil contaminated with used lubricating oil, crude oil, and petroleum sludge mixture was successfully conducted by using specified bioreactor and accelerated with the addition of enzymatic reagent. The findings of the current research revealed that the existing microorganisms combined with the reagent were capable in achieving promising results within five treatment weeks. Taking in the consideration that the process was conducted in winter season were low ambient temperatures were recorded. Yet the recoded results in terms of POR were significant indicating the validity and the active role of the used reagent.

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