The Effectiveness of Bioremediation Treatment for Diesel-Soil Contamination

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Abstract. Soils are increasingly threatened by spillage of petroleum products such as petrol, diesel fuel, gasoline at oil refineries, underground storage tanks and pump stations pipelines. This study aims to investigate the effectiveness of *Pseudomonas putida* as oil-biodegradable agent in soil contaminated with diesel (D100). The effectiveness on bioremediation have been conducted by examining several physico-chemical tests on diesel-contaminated soil before and after seeding with *P. putida*. The spillage stimulation of D100 was conducted at laboratory scale for 24 days of incubation time. The results show that the bioremediation treatment was able to remove up to 82%, 55%, 48%, and 34% of nitrogen, total organic carbon, phosphate, and sulfate, respectively. The pH of soil sample was changed from pH 7.8 (Day 0) to 6.78 (Day 24) after the treatment. Meanwhile, the moisture content in the sample has increased from 39% (Day 0) to 59% (Day 24). These results show the good indication of quality improvement on polluted soil after treated with *P. putida*. It is apparent from the acquired results that the application of *P. putida* is suitable as effective microorganism and is a potential diesel-soil biodegradable agent in polluted soil.

Introduction

The fuel products penetrate and disperse in the soil when it spills on the surface. The hazardous constituents of fuel are currently on the Environmental Protection Agency (EPA)'s priority pollutants list [1]. Some of hazardous substances tend to persist in the environment for a long time, where they pose a risk humans and also aquatic life. Their harmful effects from lower doses include immune system-dysfunction, organ failure and physiological impairment [2].

The soil contamination can be clean-up by a bioremediation method that involving transformation of hazardous organic chemical to another less harmful substances by using microbial activities [3]. Many aerobic bacteria found in environment biologically used substances such as sugar, proteins, fats and hydrocarbons of plant and animal wastes and can also metabolize petroleum hydrocarbons [4]. Natural soil bacteria can present in dormant or slow-growing state, but when stimulated by optimum environmental conditions, they multiply rapidly and then adapt to new environment. Some of the more common genera involved in the biodegradation of oil products include *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Micrococcus*, *Arthrobacter* and *Corynebacterium* [5].

There are several factor must be considered in order to achieve successful bioremediation. Physical contact between bacteria and pollutants is among of these factors. Following inoculation, mixing of soil particles is required in order to bring microorganisms in close contact with pollutants [6]. Nutrient availability is vital for soil bioremediation as it can enhance bacteria activities and their potential co- metabolism. Previous research proved that bioremediation occurred rapidly when the nutrients such as nitrogen (N), phosphorus (P), sulfur (S) and carbon added equivalently into soil [6]. Another important factor is suitable temperature throughout the soil bioremediation. The temperature can significantly alter the biodegradation rate. The suitable temperature for hydrocarbons biodegradation is between 20 and 35 °C, although it occurs less at 0 °C and at as high as 70 °C [7]. Factors such as environmental pH, redox conditions, pollutants concentration and presence of predator organisms are also vital for successful biodegradation of pollutants.

Previously, many studies have described the capabilities of microorganisms especially *Pseudomonas spp.* to biodegrade petroleum hydrocarbons. Wongsa [8] proved the degradation of gasoline, kerosene, diesel oil and lubricating oil by *Pseudomonas aeruginosa* and *Serratia marcescens* species. The results from their study revealed that about 90-95% of excess amount of total diesel oil and kerosene could be degraded by *Pseudomonas aeruginosa* within 2 and 3 week, respectively. However, *Serratia marcescens* species was able to degrade kerosene, diesel and lubricating oil with a capacity of 50-60%. Thus, these bacteria especially *Pseudomonas aeruginosa* (*Pseudomonas* genus) are useful biological agent and also have potential to be useful for bioremediation of sites that highly contaminated with petroleum hydrocarbons. According to Bento [9] studies, the results found that light (C_{12}-C_{23}) fractions and heavy (C_{23}-C_{40}) fraction of total petroleum hydrocarbons (TPH) in Long Beach, California were significantly degraded by bacteria consortia (*Bacillus cereus, Bacillus sphaericus, Bacillus fusiformis, Bacillus pumilus, Acinetobacter junii* and *Pseudomonas* spp.). The acquired results from the study indicated that the consortia can degrade a 72.7% of light fractions and 75.2% of heavy fractions of the TPH. This indicated that these consortia of bacteria are good as an oil-biodegradable agent due to their ability to remove hydrocarbons from environment contaminated with diesel oil. Most of reported researches were focused on bacteria consortia not on specific sole species. Therefore, this study aims to investigate the effectiveness of bioremediation treatment for diesel-soil contamination by *Pseudomonas putida*. The effectiveness of this species for soil bioremediation was observed, evaluated and analyzed.

Materials and Methods

Biodiesel and diesel. The petroleum diesel oil (EN 590:2004) was purchased from commercial diesel pump station at Parit Raja, Johor. The collected diesel was filled in clean dark bottles made of plastic with a volume of 1500mL for further experimentations.

Soil. The soil samples were taken in campus area. The soil samples were collected in the range of approximately 7-8 kg from the surface of approximately 5-10 cm deep layer of soil. The collected soil samples were then put into the clean dark plastic containers with a volume of 5000mL.

P. putida broth culture. Pure *P. putida* ATCC 49128 was purchased from United States of America (USA) in dry culti-loop form. This *P. putida* samples were stored in chiller at 4°C prior to cultivation procedure. Approximately 16g of medium (nutrient broth) was filled into the 2000 mL Erlenmeyer flask containing 2000 mL of distilled water. The mixtures were stirred gently and then dissolved by heating on hot plate at 100 °C for approximately 2 hours until complete dissolved. After that, the medium was sterilized in autoclave at 121 °C for about 15 minutes. It was then allowed to cool down for a few minutes until its temperature drop in between 35 °C to 37°C. One loop shaft was removed from the handle straight into the medium according to the manufacturer's instruction. The mixtures were then stirred until homogeneous and keep in the chiller at 4°C for 48 hours.

Soil-biodiesel/diesel and P. putida mixture. Spill simulations with diesel (D100) in soil were carried out in accordance with Taylor [10], with modification. The soil-diesel mixture samples were then inoculated with approximately 200mL of 1.0×10^6 cfu/mL *P. putida* broth culture and placed in dark condition.

Physico-chemical test. The moisture content of the soil samples were determined by oven-drying method (BS 1377: Part 2: 1990: 3.2), which wet soil samples were weighted and dried in an oven at 105 °C for 24 hours. The dried samples were weighted again once more and final weight was recorded. The moisture content of soil samples was then calculated by percentage of dry soil mass formula. The pH of the soil samples were determined by electrometric method (BS 1377: Part 3: 1990), which gives a direct reading of the direct reading of the pH value of soil suspension in water. Both TOC (total organic count) and TN (total nitrogen) in soil samples were determined by TOC analyzer. The phosphate and sulfate for soil samples were determined by using ion chromatography (IC) analyzer. Preparation of soil samples for testing was conducted according to Jackson [11] prior to analysis.

Results and discussion

The Fig. 1 shows the moisture content of sample D100 after treatment with *P. putida* for 24 days. The moisture content of sample D100 was lowest detected on Day 0 at 40 % while the highest was on Day 12 at 75.74%. This is due to the inoculation of *P. putida* with sample of D100. The degradation of contaminants in diesel by *P. putida* produced carbon dioxide (CO_2), water (H_2O) and biomass as a by-product of bioremediation. The moisture content of sample D100 was in the range of soil standard guidelines (40-60%) after treatment with *P. putida* for 24 days (shown in Table 1).
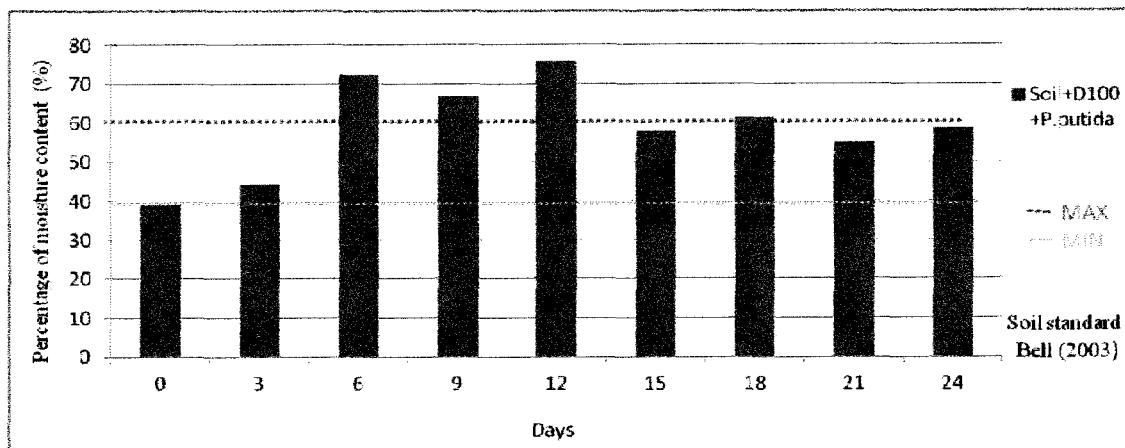

Fig. 1 Result of moisture content in 24 days

The Fig. 2 shows the pH of sample D100 after treatment with *P. putida* for 24 days. The pH of soil sample was at pH 7.5 (Day 0). The pH of sample D100 was decreased from Day 0 to Day 12 at pH 7.8 and pH7.0, respectively. However, the pH of sample D100 was increased on Day 15 (pH 7.87) and then decreased from Day 21 (pH 7.44) to Day 24 (pH 6.77). According to Demirbas [12], commercial diesel fuel contains free fatty acid of 45.62-46.48 mg KOH/g oil. The addition of pure diesel as well as *P.putida* into soil sample might be contributed to the decrement of pH for sample D100. Majority of sample D100 have pH in the range of soil standard guidelines (pH 6-7.5) during 24 days of treatment with *P. putida* except on Day 0 (pH 7.8) and Day 15 (pH 7.87).
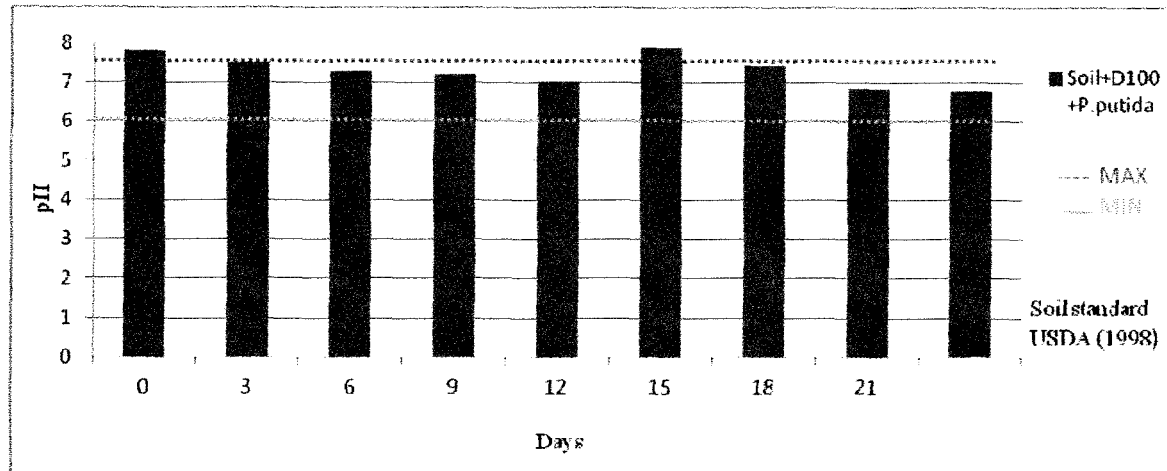
Fig. 2 Result of pH in 24 days

The Fig. 3 shows the TOC of sample D100 after treatment with *P. putida* for 24 days. The bioremediation process was able to reduce TOC content in D100 sample (Table 1). This might be due to the *P. putida* has used carbon as an energy source using their metabolic process throughout the microbiological process in order to degrade, break down or transform hazardous contaminants in diesel to less toxic or nontoxic forms, thereby remedying or removing and eliminating contaminants from diesel-contaminated soil [13]. Nevertheless, all sample of D100 has TOC in the range of soil standard guidelines (0-0.05%) as shown in Fig. 3 since Day 0.
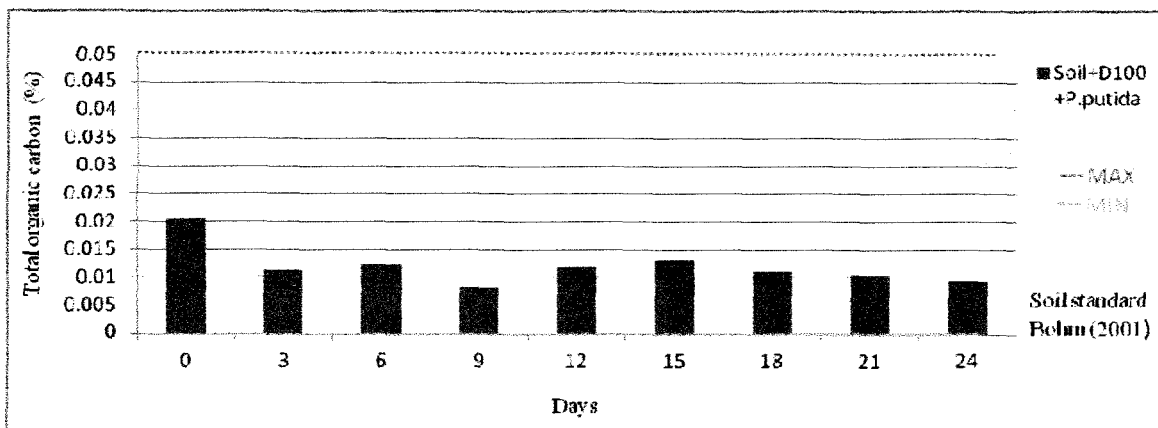

Fig. 3 Result of TOC in 24 days

The Fig. 4 shows the TN of sample D100 after treatment with *P. putida* for 24 days. The highest amount of TN for sample D100 was detected on Day 0 while the lowest one on Day 24 at 31.86 ppm and 5.823 ppm, respectively. The removal of TN from Day 0 to Day 24 is up to 82%. The nutrient like nitrogen is vital in soil bioremediation as it can enhance bacteria activities and their potential co-metabolism [14]. Throughout the experiment, the total nitrogen was used by *P. putida* to perform its microbiological process and result in reduction of TN in the sample of D100. The TN of sample D100 was observed in the range of soil standard guidelines (5-15 ppm) along 24 days of treatment except on Day 0 (31.86 ppm), Day 3 (23.76 ppm), Day 6 (20.95 ppm) and Day 9 (15.81 ppm).
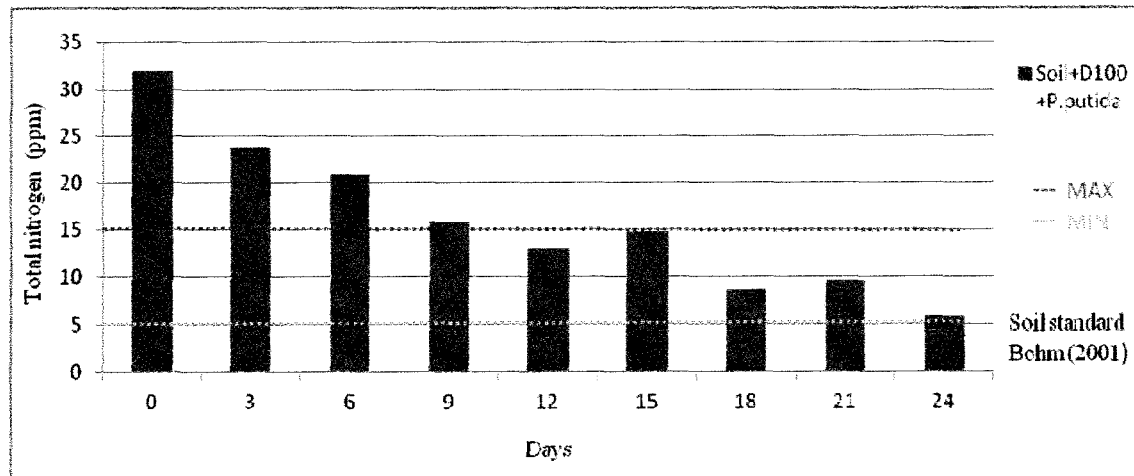
Fig. 4 Result of TN in 24 days

The Fig. 5 shows the amount of sulfate for sample D100. The sulphate in sample D100 was decreased from Day 0 to Day 24. There is up to 34% removal of sulfate from Day 0 (15.44 ppm) to Day 24 (10.14 ppm) as shown in Table 1. Sulfate is also vital in soil bioremediation as it can enhance bacteria activities and their potential co-metabolism [14]. Throughout the experiment, there is indication that sulphate was used by *P. putida* to perform its microbiological process. This indicated by the reduction of sulphate in the sample of D100. The sample of D100 was in the range of soil standard guidelines (5-20 ppm) after treatment with *P. putida*.
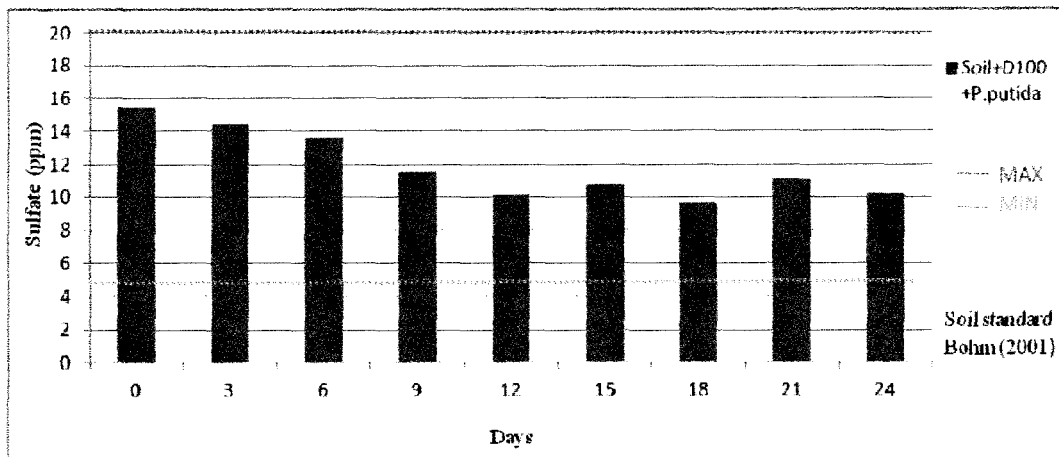

Fig. 5 Result of sulfate in 24 days

The Fig. 6 shows the amount of phosphate for sample. The phosphate of sample D100 was decreased from Day 0 to Day 24. The highest amount of phosphate for sample D100 was detected on Day 0 (98.76 ppm). Phosphate is also vital in soil bioremediation as it can enhance bacteria activities and their potential co metabolism [14]. Throughout the experiment, the phosphate was possibly used by *P. putida* to perform its microbiological process and it result in reduction of phosphate in the sample of D100.
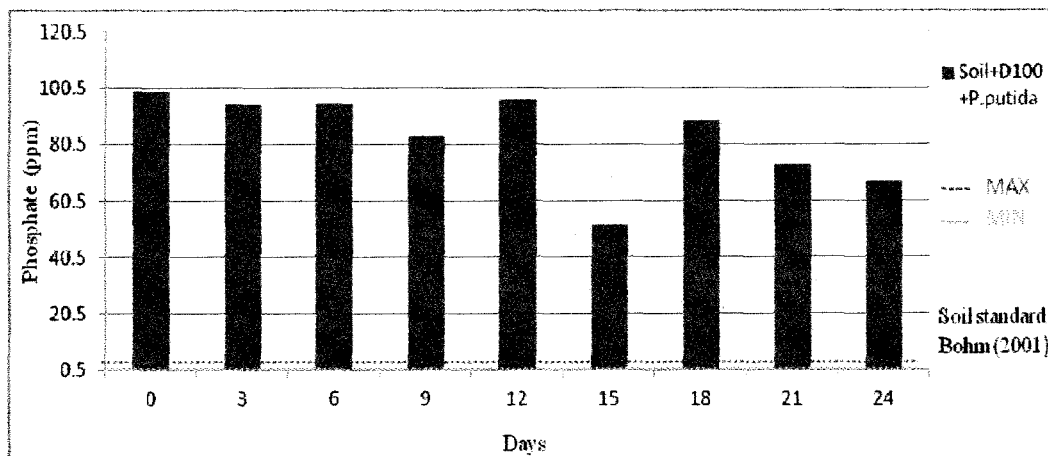
Fig. 6 Result of phosphate in 24 days

Conclusion

This study can be concluded that *P. putida* is an effective microorganism and suitable as diesel-soil biodegradable agent. Physico-chemical tests proved that there is indication of nutrient consumed in diesel-soil mixture after 24 days of bioremediation. The *P. putida* was suitable for the bioremediation of diesel-soil contamination based on these results.

Table 1: Summary of the physico-chemical results.

Parameter	Sample		Standard	Reference
	Soil + D100 + P. *putida* (Day 0)	Soil + D100 + P. *putida* (Day 24)		
pH	7.45	7.25	6-7.5	[14]
Total organic carbon (%)	0.0332	0.030	0-0.05%	
Total nitrogen (ppm)	34.17	6.05	5-15 ppm	[15]
Sulfate (ppm)	21.52	18.00	5-20 ppm	
Phosphate (ppm)	2.29	0.75	0.5-0.9 ppm	
Moisture content, %	38.88%	58.73	40-60%	[16]

Acknowledgment

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