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Bioremediation of Biodiesel / Diesel Blend (B5) in Soil Contamination by using *Pseudomonas putida*

Norshuhaila Mohamed Sunar[1*], Quin Anak Emparan[2], Nuramidah Hamidon[1], Nor Hazren Abdul Hamid[1], Mimi Suliza Muhamad[1], Najeeha Mohd Apandi[1], Hasnida Harun[1], Roslinda Ali[1], Paran Gani[3] and Nor Maizzaty Abdullah[1]

[1]*Department of Civil Engineering Technology, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia, Pagoh Higher Education Hub, KM 1, Jalan Panchor, 84600 Pagoh, Johor, Malaysia*

[2]*Department of Chemical & Environmental Engineering, Faculty Engineering, Universiti Putra Malaysia, 43400 Serdang, Selangor*

[3]*School of Applied Sciences, Faculty of Integrated Life Sciences, Quest International University, No. 227, Plaza Teng Seng (Ground Floor), Jalan Raja Permaisuri Bainun, Ipoh Perak, Malaysia*

The introduction of biodiesel or diesel mixes on various countries' markets may cause damage to the environment owing to spillage. This study investigates the efficacy *of Pseudomonasputida (P. putida)* as an oil-biodegradable agent in soil cotaminated with biodiesel or diesel blends (B5). Different physicochemical studies were conducted on biodiesel or diesel-contaminated soils before and after sowing of *P. putida* to investigate the significance of bioremediation. Spillage stimulation of B5 was carried out on a laboratory scale for a period of 24 days. The laboratory results reveal that the bioremediation treatment can eliminate up to 82 % of nitrogen, 59 % of organic carbon, 53 % of phosphate, and 51 % of sulfate, respectively. After the treatment, the pH of the soil sample increased from 7.43 (Day 0) to 7.17 (Day 24). Meanwhile, the moisture content in the sample was increased from 29.73% (Day 0) to 52.67% (Day 24). All of the data indicate that the quality of contaminated soil improved after being treated with *P. putida*. The obtained results show that the application of *P. putida* is appropriate as an effective microorganism and has significant advantages as a beneficial oil-soil biodegradable agent in polluted soil.

Keywords: Physico-chemical removal; *Pseudomonas putida*

I. INTRODUCTION

The biodiesel which is the palm-based biodiesel has been induced by using the transesterification method with the methanol acquired from Biodiesel Pilot Plant, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia. As there is a merchandising diesel pump station at Parit Raja, Johor, the petroleum diesel oil (EN 590: 2004) was purchased there. Collected soil sample at the campus area ranges from 7 to 8 kg, and it is also from surface of a 5-10 cm deep layer of soil. Pure *Pseudomonas putida* ATCC 49128 were purchased in dry culti-loop form and prepared based to the industrialist's instruction. Spill simulation on soil using biodiesel or diesel blends were performed in compliance with (Taylor & Jones, 2001), with modifications. The soil-biodiesel or diesel mixture samples were added with 1 × 106 cfu/mL of *P. putida* broth culture and placed in dark conditions. Another soil sample has been generated and then added with P. putida without the use of biodiesel or diesel mixes as a control sample. The soil samples' measured moisture content and pH were according to BS 1377: Part 2: 1990: 3.2 and BS 1377: Part 3: 1990. The TOC analyser has determined both Total Organic Carbon (TOC) as well as (Total Nitrogen) TN in soil samples. Meanwhile, an ion chromatography (IC) analyser determines both phosphate

*Corresponding author's e-mail: shuhaila@uthm.edu.my

and sulphur for soil samples. As per Jackson (2006), the performed test for the processing of soil samples testing was before analysis by these analysers.

II. MATERIALS AND METHOD

The biodiesel which is the palm-based biodiesel has been induced by using the transesterification method with the methanol acquired from Biodiesel Pilot Plant, Faculty of Engineering Technology, Universiti Tun Hussein Onn Malaysia. As there is a merchandising diesel pump station at Parit Raja, Johor, the petroleum diesel oil (EN 590: 2004) was purchased there. Collected soil sample at the campus area ranges from 7 to 8 kg, and it is also from surface of a 5-10 cm deep layer of soil. Pure *Pseudomonas putida* ATCC 49128 were purchased in dry culti-loop form and prepared based to the industrialist's instruction. Spill simulation on soil using biodiesel or diesel blends were performed in compliance with (Taylor & Jones, 2001), with modifications. The soil-biodiesel or diesel mixture samples were added with 1 × 106 cfu/mL of *P. putida* broth culture and placed in dark conditions. Another soil sample has been generated and then added with P. putida without the use of biodiesel or diesel mixes as a control sample. The soil samples' measured moisture content and pH were according to BS 1377: Part 2: 1990: 3.2 and BS 1377: Part 3: 1990. The TOC analyser has determined both Total Organic Carbon (TOC) as well as (Total Nitrogen) TN in soil samples. Meanwhile, an ion chromatography (IC) analyser determines both phosphate and sulphur for soil samples. As per Jackson (2006), the performed test for the processing of soil samples testing was before analysis by these analysers.

III. RESULT AND DISCUSSION

Figure 1 depicts the amount of moisture of specimen B5 after 4 weeks of *P. putida* treatment. During the treatments, the moisture content B5 varied. From the day 0 to the 24th day, the moisture content of sample B5 rose, probably because to the breakdown of pollutants in biodiesel or diesel blends by *P. putida*, which produced water (H2O), carbon dioxide (CO2) and biomass as a by- product of bioremediation (Mariano *et al.,* 2008). After treatment with *P. putida*, the sample of moisture content, B5 was in the range of soil standard limit (40-60 percent) but on Day 3, which was 29.73 percent. The pH of sample B5 on day 0 was (Figure 2) 7.43, and on Day 24, it was 7.17. Commercial diesel fuel includes 45.62 to 46.48 mg potassium hydroxide (KOH)/g oil free fatty acids, according to Demirbas (2009). The pH deacrese in sample B5 may have been caused by the addition of pure diesel and *P. putida* to the soil sample. Furthermore, palm-based biodiesel is constituted of methyl or ethyl esters of long-chain fatty acids (Pinto *et al.,* 2005) and the use of an acid catalyst in the transesterification process to improve reactivity and produce (Ma & Hanna, 1999); this results in biodiesel being in acidic circumstances. As a result, when the soil sample got mixed with B5, the pH of the soil sample fell. Furthermore, the pH decrease implies that *P. putida* could reduce pH from sample B5. Overall, the pH of both the control and the sample B5 were within the margin of soil minimum specifications (pH 6-7.5). The TOC results of sample B5 after 24th days of bioremediation are shown in Figure 3. The results suggest that TOC can be removed up to 59 percent from the day 0 to the day 24. This is because *P. Putida* has used carbon as a form of energy throughout the micro-biological cycle to decay, decompose, or alter harmful pollutants in biodiesel or diesel blends into less toxic or harmless forms (Walworth *et al.,* 2005). Hence, it is cleaning and removing pollutants from biodiesel or diesel polluted soil. After being treated with *P. putida*, both the control and the B5 samples had TOC levels that were within the range of soil standard requirements.

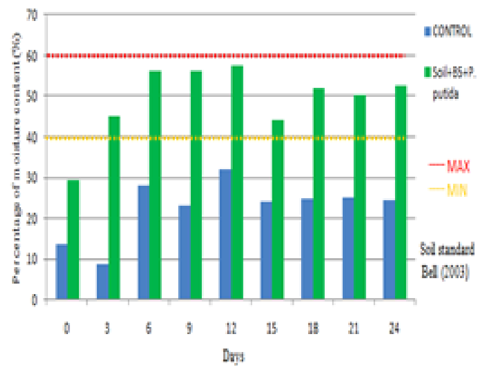

Figure 1. Percentage moisture content in 24 days

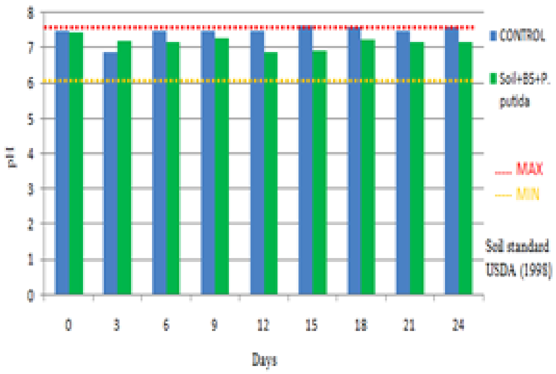

Figure 2. pH result in 24 days

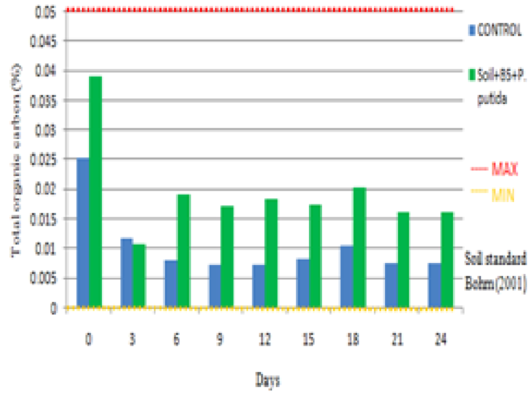

Figure 3. TOC result in 24 days

The TN (Figure 4) indicates a declining pattern after *P. putida* treatment. From the day 0 to the day 24, TN was removed at a rate of up to 82 percent. In soil bioremediation, nutrients like TN are crucial because they can boost bacteria activity and possibly co-metabolism. (Kucerova, 2006). In the experiment, *P. putida* has used total nitrogen as a provider of energy to thrive and complete its microbiological activity. As a result, the nitrogen in the B5 sample is reduced. Figure 5 depicts the sulfate quantity throughout the bio-remediation process. The findings demonstrate that sulfate was removed up to 51% from the day 0 to the day 24th. Sulfate is indeed important in soil bioremediation since that can boost bacterial activity and probable co-metabolism (Kucerova, 2006). Throughout the experiment, *P. putida* used sulfate to execute its microbiological process and minimise sulfate in the B5 sample. Some detected B5 sulfate was a bit beyond the range of soil standart limit which is 5 to 20 ppm. Nevertheless, from the day 12 to the day 24, the level of sulfate was within the permissible range. During the procedure, the phosphate (Figure 6) in sample B5 dropped. From the day 0 to the day 24, the experiment diminished the phosphate content of sample B5. The data demonstrate that sulfate was removed up to 53% from Day 0 to Day 24. The phosphate content of sample B5 was within soil standard limits (0.5-0.9 ppm) (0.95 ppm), except on the day 0. Phosphate is also important in soil bioremediation since it can boost bacterial activity and potential co-metabolism (Kucerova, 2006; USDA, 1998; Bohn *et. al.,* 2001; Bell *et. al.,* 2003; Gani *et. al.,* 2015a; Gani *et. al.,* 2015b; Gani *et. al.,* 2016a; Gani *et al.,* 2016b). Excess phosphorus in the soil, on the other hand, inhibits microbiological growth. The analysis indicate that *P. putida* utilised phosphate to execute its microbiological activity by lowering phosphate in the B5 sample.

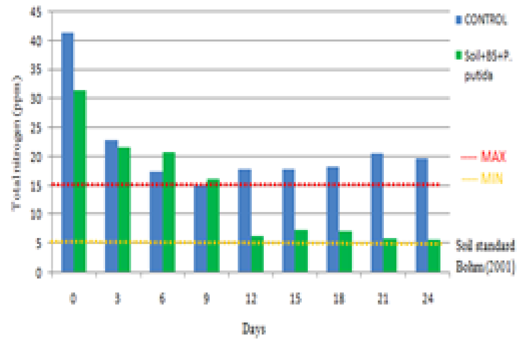

Figure 4. TN result in 24 days

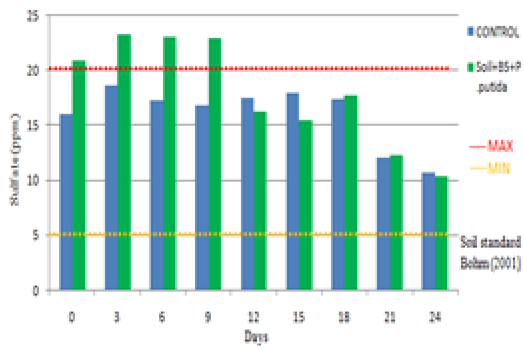

Figure 5. Sulfate result in 24 days

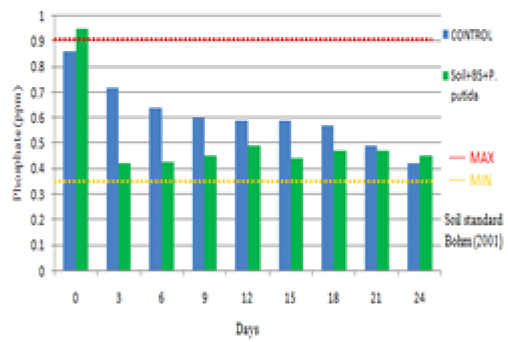

Figure 6. Phosphate result in 24 day

IV. CONCLUSION

This finding revealed that *P. putida* is a useful bacterium that can be used as an oil-soil compostable agent. Physicochemical test showed an indication of nutrient consumption in the soil-biodiesel or diesel combination after 24 days of bioremediation. As a result of the findings, *P. putida* is appropriate for biodiesel or diesel-soil pollution bioremediation.

V. ACKNOWLEDGEMENT

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