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Research Article

The effectiveness of oil spill dispersant addition for phytoremediation of petroleum-contaminated soil using *Ricinus communis* L.

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Abstract: Phytoremediation is one of the most effective and environmental-friendly methods in retrieving oil-contaminated soil. The decline of Total Petroleum Hydrocarbon (TPH) oil in the phytoremediation process will be accelerated by Oil Spill Dispersant (OSD) as a surfactant and the use of *Ricinus communis* L. plant. Commercial OSD products used in the remediation process are S200 OSD which contains LAS surfactant, which is a US EPA recommendation and is known to be less environmental-friendly, so SBRC-IPB develops OSD containing palm oil surfactants and is environmental-friendly. The purpose of this study was to determine the performance of OSD and *R. communis* to reduce TPH. This study used a completely randomized design with two factors, namely OSD and plant use. The results of this study indicated that OSD from SBRC-IPB improved plant growth, increased dry plant biomass, accelerated the decrease in TPH and increased the bacterial population compared to the S200 OSD and control. The combination of OSD from SBRC and *R. communis* was able to degrade TPH better than S200 OSD, with and without *R. communis,* with TPH concentrations of 30,000 ppm (3%) to 2,333 ppm (0.2%) for 20 weeks. This showed that the use of OSD was able to provide a positive response in the phytoremediation process of land contaminated with petroleum.

Keywords: *OSD, phytoremediation, petroleum-contaminated soil, TPH*

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Introduction

The increase in the use of petroleum is directly proportional to the increase in oil waste produced causing pollution to the environment. Environmental pollution can be caused by oil exploration and exploitation activities which include drilling, refining and transportation. The amount of oil spill in these activities to the surface of the land has the potential to pollute the environment, especially soil and water. One of the contaminants originating from petroleum or petroleum waste is a hydrocarbon compound. Toxic concentrations of petroleum contaminated soil it is usually known to have high Total Petroleum Hydrocarbon (TPH) of more than 3% (Charlena et al., 2009). Many plants die at the seedling level in the soil with TPH greater than 3% (Setiadi, 2014). Hu et al. (2013) reported that generally, petroleum waste of soil could reach 5-86.2%. Petroleum pollution is carcinogenic and causes changes in the metabolism of microorganisms in the environment, even destroying primary species from the food chain (Vaziri et al., 2013). To avoid this, biological processing efforts that are known as phytoremediation can be employed (Sekoohiyan et al., 2016; Brzeszcz et al., 2016). Phytoremediation is a remediation method utilizing plants and microorganisms to clean environment contaminated with heavy metals, pesticides, and toxic organic compounds in the soil, water or sediments, especially from petroleum contamination (Zhang et al., 2016; Seckin, 2016). One of the plants that can be used for phytoremediation is *Ricinus communis* L. The potential of *R. communis* for phytoremediation of mine tailings and oil production has been reported by Olivares et al. (2013). The plant has an excellent ability to extract many toxic metals such as Cd, Pb, Ni, As, Cu, and it is tolerant to salinity (Bauddh and Singh, 2012). Some phytoremediation strategies in petroleum-contaminated soils can be successful if aided by Oil Spill Dispersant (OSD). OSD is a mixture of surfactants and solvents designed to decompose the oil layer into small granules so that it is naturally dispersed and it can reduce surface and interface voltage (EMSA, 2006). Some research results showed that the addition of OSD affected the performance of biodegradation of petroleum and bacteria. Currently, the OSD that is widely used by the petroleum industry is OSD S200 type commercial product. The Surfactant and Bioenergy Research Center (SBRC-IPB) has developed surfactant materials derived from palm oil to be used as dispersant materials. The materials used are not toxic and that are biodegradable. The purpose of this study was to determine the performance of OSD and *R. communis* against the reduction of Total Petroleum Hydrocarbon (TPH) in the phytoremediation process.

Materials and Methods

This study was conducted from August 2018 to January 2019 in the greenhouse of the experimental field in Cikabayan, Soil Biotechnology Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, Bogor Agricultural University, Surfactant and Bioenergy Research Center (SBRC) Laboratory, Bogor. Materials used in this study were the SBRC-IPB OSD (Oil Spill Dispersant), Commercial S200 OSD, petroleum-contaminated soil, *R. communis* seeds (from the Sweetener and Fiber Crops Research Institute in Malang), oil palm empty fruit bunch, hexane and chemical reagents for analysis. The material used for making the SBRC-IPB OSD was surfactant product from SBRC-IPB. This surfactant comes from palm oil consisting of 1.5% Diethanolamine (DEA) and 0.9% Methyl Ester Sulfonate (MES) with 7:3 formulation ratio (Adlina et al., 2016).

Preparation of planting media

Preparation of planting media was carried out by formulating a pot containing media contaminated with petroleum in the amount of a minimum TPH of 3% (30,000 ppm) and mixed with sand and compost with a ratio of 4:2:1; so that it was used in the amount of 7 kg/pot of soil, 3.5 kg/pot of sand, and 1.75 kg/pot of compost. The size of the pot used in this study was 30 x 35 cm. This proportion referred to a previous study conducted by Arifuddin et al. (2016).

Seed preparation and seeding

Seed preparation began with a viability test aiming to select good seeds. The seeds were soaked in water (room temperature) for 12 hours, and submerged seeds in water were selected for seeding. The seeds were sterilized by using a fungicide for 24 hours for protecting the seeds from the attack of fungi. Seeding was done by planting seeds into polybags containing soil media. Seeding was done for 2 weeks, up to 30 cm in height.

Trial design and OSD application

This study used a completely randomized design with two factors. The first factor was the type of Oil Spill Dispersant (OSD) consisting of two levels, namely O1 = OSD from SBRC-IPB with DOR 0.5: 1, and O2 = Commercial S200OSD with Dispersant to Oil Ratio (DOR), 0.5: 1. The second factor was the use of vegetation, consisting of two levels, namely V0 = without *R. communis*, and V1 = without *R. communis*. This study consisted of four treatments and two controls with three replications, so the total experimental units were 18. The OSD was applied by spraying with the same DOR, which was 0.5:1, using a sprayer bottle according to the treatment carried out to the prepared soil media. Spraying was done the day before planting.

Planting and maintenance

Two weeks old plants that grew well and had a uniform height were selected, transferred to the prepared planting media. Watering was made every day so that the planting media remained moist. Physical weed and bug controls were done regularly. At ten weeks after planting, oil palm empty bunch (as an organic fertilizer) was incorporated into the rhizosphere of the plant.

Phytoremediation performance test

Plant height and number of leaves if the plant were measured every two weeks. Other parameters measured during this study were pH, water content, TPC, and TPH. Soil samples were collected using a stainless-steel hollow tube with a diameter of 1.27 cm and a height of 50 cm.

Data analysis

The data obtained were subjected to analysis of variance. In the treatment that had a significant effect, further analysis was carried out with the Duncan Multiple Range Test (DMRT) with the SAS 9.4 program.

Results and Discussion

The growth of R. communis

The response of plant growth was one of the important indicators in phytoremediation. The average height of *R. communis* for each treatment started from 2 to 20 WAP is presented in (Table 1). The application of OSD from SBRC-IPB (O1V1) resulted in higher growth with the height increase of 203 cm at 20 WAP compared to that of the control treatment (KO2) of 195 cm and that of the commercial S200 OSD (O2V1) of 194 cm. This indicated that the application of surfactants originating from SBRC-IPB resulted in fairly good response to plant growth compared to the commercial S200 OSD. Provision of OSD can break down the oil layer into small granules so that it is naturally dispersed, then it has the ability to reduce surface tension and interface stress. Therefore, nutrients contained in the soil can be absorbed by plant roots well. Generally, the condition of petroleum-contaminated soil gains low porosity which can inhibit the roots of plants penetrating the soil in obtaining water and nutrients (Lolomari, 1979). However, in this study, the statement was not applied to the treatment of O2V1 (commercial S200 OSD) as the plant height was lower than the treatment with no addition of OSD (KO2). This illustrates that the main ingredient of the surfactants used by S200 OSD is LAS. This material contains benzene-bonds in it so that it is less able to support the growth of the plant. The number of leaves formed for each treatment did not show any significant effect, but the application of the SBRC-IPB OSD gave more responses to the number of leaves than that of the S200 OSD and control (Table 1). The average number of leaves increased along with the O1V1 treatment from the beginning of planting until the fourth week reaching 50%, 48.5% for O2V1 and41.5%for control. However, entering the sixth week until the 20th week, the average number of leaves increased by only around 9%. The number of leaves declined along with the changes in TPH concentration in the soil, while the amount of nutrients found in the soil had also declined.

Table 1. Observation results of plant height and number of leaves for 20 weeks of phytoremediation process

Observation Time (WAP)	Plant Height (cm)			Number of Leaves (sheet)		
	KO2 (Control)	O1V1 (SBRC OSD)	O2V1 (S200 OSD)	KO2 (Control)	O1V1 (SBRC OSD)	O2V1 (S200 OSD)
2	34.2(±3.33)^{b}	40.2(±2.08)^{a}	31.3(±2.06)^{b}	7(±0.58)^{a}	8(±0.00)^{a}	7(±0.58)^{a}
4	62.1(±3.49)^{ab}	68.4(±4.37)^{a}	59.2(±3.01)^{b}	10(±1.53)^{a}	11(±1.73)^{a}	11(±0.58)^{a}
6	82(±4.37)^{ab}	86(±5.19)^{a}	77(±1.53)^{b}	11(±1.53)^{a}	12(±1.53)^{a}	12(±1.00)^{a}
8	98(±5.84)^{a}	101(±5.10)^{a}	92(±3.83)^{a}	11(±0.58)^{b}	13(±1.00)^{a}	12(±0.58)^{ab}
10	108(±6.56)^{a}	110(±6.54)^{a}	99(±6.66)^{a}	12(±0.58)^{a}	14(±1.15)^{a}	13(±0.58)^{a}
12	129(±6.24)^{a}	131(±4.16)^{a}	125(±7.70)^{a}	13(±0.00)^{a}	14(±1.00)^{a}	13(±0.00)^{a}
14	146(±6.08)^{a}	149(±6.03)^{a}	143(±8.41)^{a}	14(±0.00)^{b}	15(±0.58)^{a}	15(±0.58)^{ab}
16	162(±5.97)a	167(±5.01)^{a}	163(±7.79)^{a}	15(±1.00)^{a}	16(±0.58)^{a}	16(±0.58)^{a}
18	182(5.10)^{a}	185(±7.00)^{a}	180(±3.40)^{a}	16(±0.58)^{b}	18(±0.58)^{a}	17(±0.58)^{ab}
20	195(±4.16)^{b}	203(±3.21)^{a}	194(±2.65)^{b}	16(±0.58)^{b}	18(±0.58)^{a}	17(±0.58)^{ab}

Remarks: Mean values within a column followed by the same letters are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test. WAP = week after planting

Root performance and dry weight of R. communis

Results of observation of plant root length showed 25% difference in mean root length of the SBRC-IPB OSD (O1V1) treatment from that of the S200 OSD treatment, while the difference in mean root length of the control (KO2) to that of the S200 OSD treatment was around 6% (Table 2). This demonstrates that the SBRC-IPB OSD has a better sensitivity response to the performance of the *R. communis* roots than the application of the S200 OSD and without OSD application. However, the S200 OSD treatment showed a poor root length performance compared to the control. A good root system will show the level of effectiveness of plant in absorbing nutrients and contaminants in the form of hydrocarbons. The wider the root system, the higher the efficiency of the reduction of hydrocarbon compounds in petroleum-contaminated soil (Figures 1 and 3). In addition to plant height, leaf number and root performance, the response of plant growth can be seen from the amount of dry weight of the plant that is closely related to nutrient absorption for growth and development of vegetative parts.

Table 2. Root length and dry weight of *R. communis* at 20 weeks after planting

Treatment	Plant Root Length (cm)	Plant Dry Weight (g)
KO2	48.7(±5.13)	106.67(±11.01)
O1V1	51.7(±4.93)	128.00(±15.50)
O2V1	44.7(±5.69)	113.73(±15.21)

The average dry weight of the plant of the SBRC-IPB OSD (O1V1) treatment was higher than those of the S200 OSD (O2V1), and control (KO2) treatments (Table 2). This indicates that the SBRC-IPB surfactant material derived from oil palm did not cause toxicity in plants so that it could boost the biomass of *R. communis* for 20 weeks phytoremediation process.

Figure 1. Root performance of *R. communis*

The response of oil spill dispersant (OSD) to total petroleum hydrocarbon (TPH) concentration

The oil-contaminated soil used in this study had an initial amount of TPH of 30,000 ppm (3%), and *R. communis* as one of the plants that had been able to grow well on oil-contaminated soil with that concentration. The results indicated that the TPH concentration decreased in line with increasing time of phytoremediation in each treatment, with or without *R. communis* (Figure 2). The largest decrease in TPH concentration at 20 weeks of phytoremediation was shown in the O1V1 treatment with the use of *R. communis* and the addition of the SBRC-IPB OSD. The decrease of TPH from 30,000 ppm (3%) to 2,333 ppm (0.23%) indicating the average degradation efficiency of 92% (Figure 3). This proves that the use of the SBRC-IPB OCD combined with *R. communis* can reduce the hydrocarbon concentration in the phytoremediation process of oil-polluted soil, and can help to accelerate the process of hydrocarbon degradation.

The treatments of SOD from SBRC-IPB and commercial S200 OSD, with or without. *R. communis*, had a significant difference in the trend of TPH decrease. The treatment of SBRC-IPB OSD mostly resulted in a better trend of TPH decrease compared to that of the commercial S200 OSD and control treatments. Chanif et al. (2017) have proven that the use of OSD from SBRC-IPB reduced greater the number of hydrocarbon compounds than the commercial S200 OSD in the bioremediation process with degradation efficiency in the amount of 89.62%.

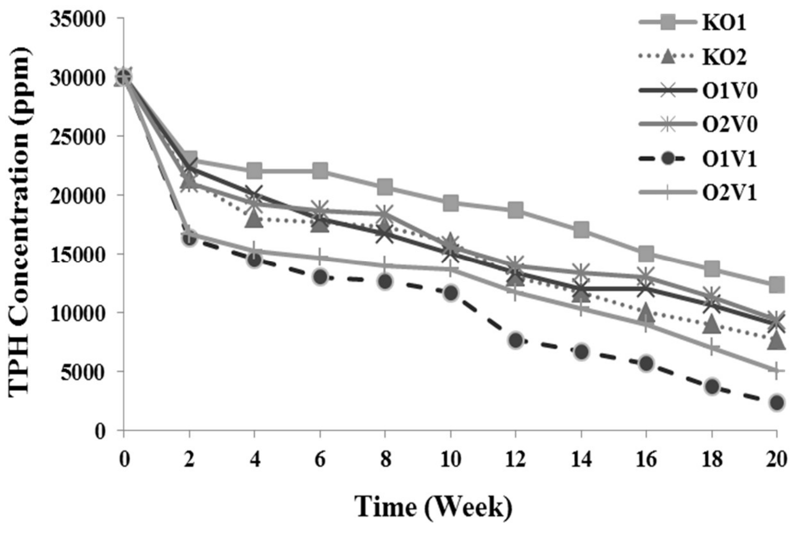

Figure 2. The decreasing trend in TPH concentration during 20 weeks of the phytoremediation process

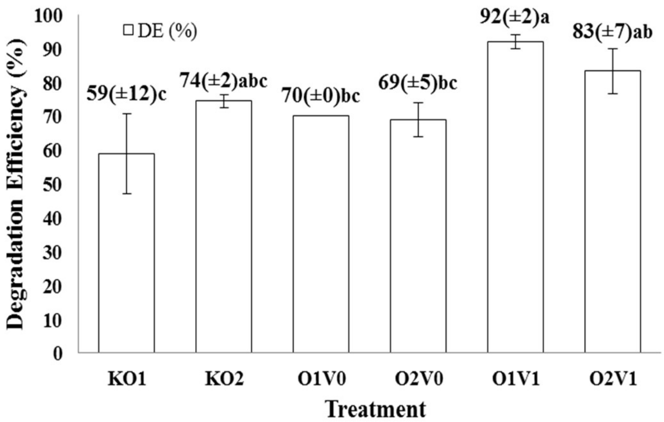

Figure 3. TPH degradation efficiency value

The choice of a plant has an important role in remediating polluted soil, which is adjusted to plant that is tolerant and thriving on extreme location (Hernandez-Ortega et al., 2012). The new finding of this study can be seen from the results of decreasing trend of hydrocarbon compounds during the phytoremediation process. This was evidenced by the difference in treatment without the addition of surfactant with or without the plant as in the KO1 and KO2 treatments. The KO2 treatment with *R. communis* was able to reduce hydrocarbon compounds with degradation efficiency of 74%, while that of the KO1 treatment without *R. communis* was only 59% during the 20 weeks phytoremediation process (Figure 3).

Significant changes occurred not only in decreasing the value of hydrocarbon compounds but also in the change in colour of oil found in the soil during the study. Figure 4 shows that at 20 WAP, the oil colour changed due to the treatments applied compared to the initial colour of the oil (before treatment). The use of the SBRC-IPB OSD at 20 WAP yielded a brighter colour change compared to the use of OSD S200 (both with and without plant). The discolouration of hydrocarbons occurred also indicated shorter C chain changes.

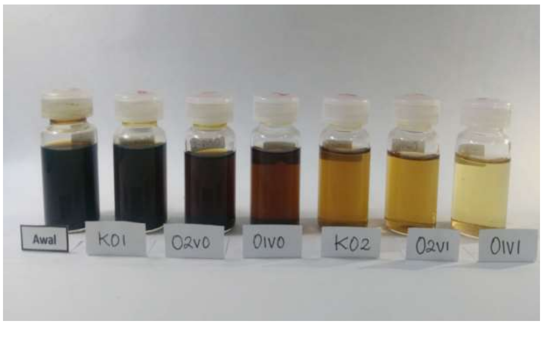

Figure 4. Oil colour change at every treatment for 20 weeks of the phytoremediation process

This may be influenced not only by organic matter but also by the role of the plant directly (adsorption to plant's roots through enzymatic activity) and indirectly (through enzymes release and exudates released by plant roots) (Cunningham et al., 1996). Therefore, the number of indigenous microorganisms that could degrade hydrocarbon rises. According to Liao et al. (2016), there are 3 mechanisms of phytoremediation of petroleum hydrocarbons include: (1) degradation by microorganisms in the soil; (2) containment, the accumulation of contaminants occurs either directly or indirectly by plant roots, and (3) phytovolatilization, the reduction of contaminant number through washing or evaporation.

Changes in pH and water content during the phytoremediation process

Changes in the degree of soil acidity (pH) and water content are important environmental conditions to be considered in the phytoremediation process. Changes in pH and water content will affect the metabolic processes of plants and soil microorganisms in degrading hydrocarbons. The initial soil pH ranged from 5.0 to 5.36 increased after the application of organic matter (oil palm empty bunch) in the second-week observation that ranged from 6.22 to 6.38. Figure 5 shows that during 20 weeks of the phytoremediation process, there were fluctuating changes in pH values although the changes were not large in range. In this study, each treatment with *R. communis* (KO2, O1V1, and O2V1) had a relatively lower pH value at each observation time than that of each treatment without *R. communis* (KO1, O1V0, O2V0). The low pH value was probably due to the presence of organic acids produced from the activity of soil microorganisms in remodelling hydrocarbon compounds. The development of pH value was likely caused by the nitrification process which released H^+ then replaced by alkaline cations (Sari et al., 2015) during the phytoremediation process. Fluctuations in pH values could naturally occur due to the condition of the water content found in the soil during the treatments. According to Soepardi (1983), soil solutions will experience fluctuations in hydrogen ion levels over time, in the summer (at dry condition due to evaporation); pH value often falls as a result of microbial activity, whereas in the rainy season (humid conditions) pH value often rises. Soil with pH ranging from 6 to 7 is in good condition. The pH conditions in this study until the end of the observation ranged from 6.22 to 6.41. These conditions were considered suitable for plant growth and soil microorganisms in reducing hydrocarbon compounds. Beside pH condition, the availability of sufficient water will also support the growth of plants and microorganisms in the maximum phytoremediation process. In this study, the water content obtained by each treatment was fluctuating (up and down) until the last week of observation (Figure 6).

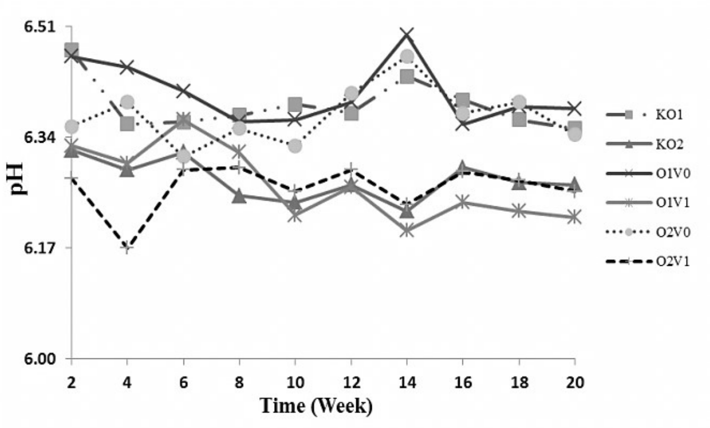

Figure 5. Changes in pH values for 20 weeks of the phytoremediation process

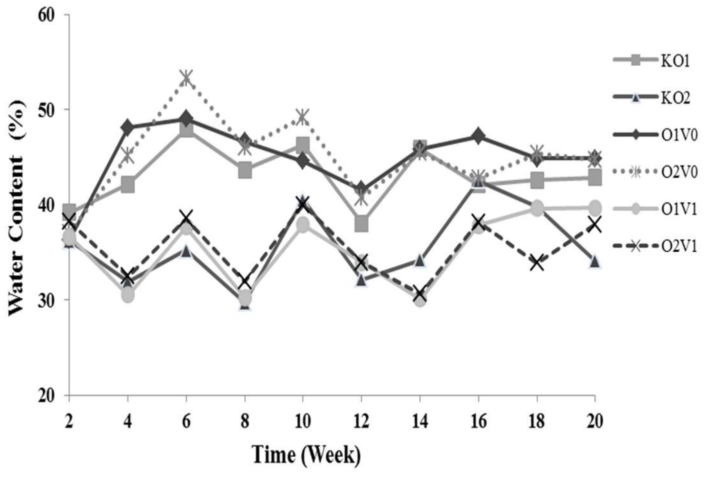

Figure 6. Changes in water content values for 20 weeks of the phytoremediation process

The average water content of the plant in each treatment with *R. communis* until the end of the experiment decreased by 36% (KO2), 35% (O1V1), 36% (O2V1), whereas in treatments without *R. communis* the water content were 43% (KO1), 45% (O1V0), and 45% (O2V0). This happened because of the water absorption by plant roots in the process of metabolic growth so that the amount of water content decreased along with the rise of the phytoremediation process. Soil water content during this study was maintained at least at 20% because high water content in the soil would cause the decline of oxygen level so that the aerobic bioremediation process would not be optimum. According to Thapa et al. (2013), optimal humidity to degrade hydrocarbons is in the interval of 30-

90%. Oxygen needed by bacteria can be obtained from the air through the process of stirring and watering water. Too high water content affects the difficulty of oxygen to penetrate into the soil and the decreasing water content caused by evaporation.

Microbial population during the phytoremediation process

The microbial population has a role in the degradation process of hydrocarbons in petroleum-contaminated soil. In this study, there was no addition of consortium of hydrocarbon-degrading bacteria and only rely on indigenous microbial groups as well as those from compost added. The results of this study showed that the addition of OSD gave a better response to the bacterial population than without the addition of OSD. The treatment using *R. communis* contained greater bacterial population than without using *R. communis*. Figure 7 shows that the longer the duration of the phytoremediation process, the lower the number of bacterial populations. The highest bacterial populations until the end of the study (6.92 Log CFU/g soil) was obtained from the O1V1 treatment (with OSD from SBRC-IPB and with *R. communis*). The bacterial populations from the KO2 treatment (without OSD and with *R. communis*) and O2V1 (with OSD S200 and with *R. communis*) were in the amount of 6.73 and 6.72 Log CFU/g soil, respectively.

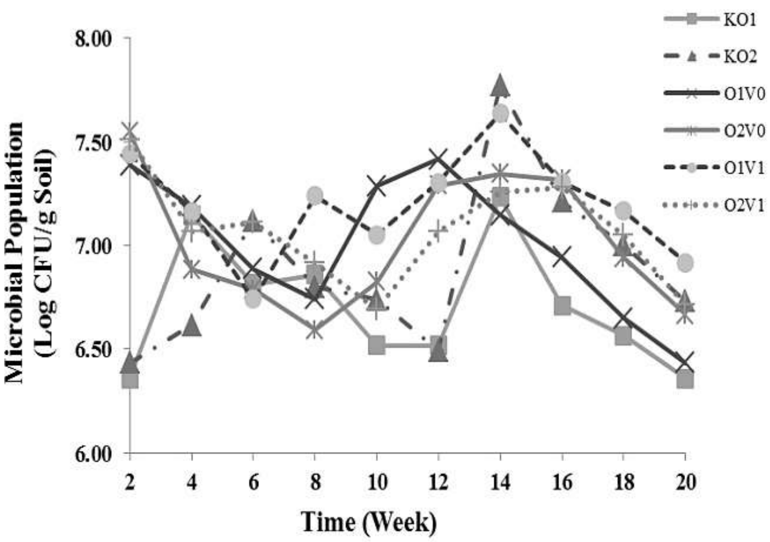

Figure 7. Condition of microbial populations

In the treatments without *R. communis*, the use of commercial S200 OSD (O2V0) had larger bacterial population than those of OSD from SBRC-IPB (O1V0) and control (KO1) treatments. The number of bacterial populations in each treatment ofO2V0, O1V0, and control KO1at 20 WAP were 6.67, 6.43, and 6.36 Log CFU/g soil, respectively. This shows that the use of *R. communis* affected the increasing number of bacterial populations. Plant roots produce exudates that can give better response in boosting carbon source and energy for bacteria so that these mechanisms boost bacterial growth. However, there was no significant difference in the number of bacterial populations for each treatment statistically. The addition of OSD affected the ability of bacteria to degrade hydrocarbons in the phytoremediation process. However, the high number of bacteria could not ascertain the high process of degradation of hydrocarbons. Data presented in Figure 7 show that the number of bacterial populations in the O2V0 treatment was higher than in the O1V0 treatment. Inversely, the decrease of TPH concentration in the O1V0 treatment was better than in the O2V0 treatment (Figure 2). According to Trinidade et al. (2002), the number of cells that is possible to degrade hydrocarbons ranges from 1 x 10^6 CFU/g to 1 x 10^8 CFU/g soil. Although the number of bacteria contained in soil samples met the criteria for degrading hydrocarbon compounds, the total of active bacteria would determine the ability of bacteria to degrade pollutants. The disadvantage of observing bacteria using the TPC method is that it is not known whether the bacteria are still alive and active in degrading hydrocarbons so that the high number of bacteria in the soil does not determine the efficiency of the bacteria in degrading hydrocarbons.

Conclusion

The use of *R. communis* in phytoremediation of petroleum-contaminated soil increased the efficiency of hydrocarbon degradation. Application of OSD from SBRC-IPB in the phytoremediation process increased the compound reduction from 30,000 ppm to 2,333 ppm with degradation efficiency of 92% compared to those of the commercial OSD S200 treatment of 83% and the control of 74%. The amount of bacterial population in a particular time unit did not determine the amount of TPH degraded, but the presence of microbes is the key to the success of hydrocarbon degradation in the soil.

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