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Research Article

Phytoremediation of Oil Sludge Using Bacterial Consortium (*Bacillus sphaericus* and *Pseudomonas aeruginosa*) and Mycorhized (*Paraserianthes falcataria* (*L.*) Nielsen) to Reduce Pb and Ni in Oil Sludge Contaminated Soil

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ABSTRACT

Bioremediation is considered as a method to manage petroleum waste and contaminated soil by decomposition process of biological waste by utilizing microorganisms (Indonesian Minister of Environment stipulation No.128 year of 2003). Oil sludge is categorized as hazardous waste that must be treated by Environmental friendly method such as Phytoremediation. Phytoremediation is utilization of microorganism (bacterial /fungal) combined with the plant for bioremediation. Phytoremediation of oil sludge by using bacterial consortium (*Bacillus sphaericus* and *Pseudomonas aeruginosa*) and mycorrhized (*Paraserianthes falcataria (L.) Nielsen*) against Pb and Ni value has been conducted in the media containing 35% oil sludge for 18 weeks. Oil sludge was composted for 6 weeks and then the media was planted with mycorrhized until 18 weeks. The main parameters measured were the heavy metals content Pb and Ni, the number of bacterial colonies before during the process of phytoremediation with 3-week intervals for 18 weeks and after composting. Heavy metals content Pb and Ni were analyzed using AAS (Atomic Absorption Spectrophotometer), while the number of bacterial colonies was counted by Total Plate Count (TPC). Data analysis was performed by regression. Secondary parameters were the percentage of mycorrhizal infection on the roots of albizia, acidity (pH), humidity (%) and temperature (°C) of medium. These parameters were analyzed descriptively. Our results revealed that phytoremediation decreased content of Pb and Ni as much as 57% and 89.2%, respectively. Regression analysis showed that the number of bacterial colonies strongly related to reduction of heavy metals content. The extensive time of remediation resulted a better reduction in heavy metals content with 18 weeks as the most favorable time.

Keywords: Bacteria consortium; Mycorrhized; Ni; Oil sludge; Pb

INTRODUCTION

Oil sludge is one of the wastes produced from the oil exploration and production activity. Governmental Regulation Republic of Indonesia No. 101 year 2014 states that oil sludge is categorized as toxic and hazardous. Hazardous waste is defined as a waste that either directly or indirectly can pollute, destruct or endanger the environment, health, survival of human beings and other organisms. Oil sludge contains oil (hydrocarbon), water, ash, rust tank, sand and heavy metals such as Pb and Ni. Analysis from the Central Laboratory of Geological Survey (2015) reveals that heavy metals content especially Pb and Ni in oil sludge is significantly higher compared to other heavy metals (51.41 ppm and Ni 52.00 ppm, respectively). In contrast, the quality standard based on the Ministry of Environment Decree states that quality standard for petroleum processing for Ni and

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Pb are 0.4 ppm and 5 ppm, respectively.

Oil sludge-polluted environment with heavy metal content can be treated physically, chemically and biologically. In accordance with the Decree of the Ministry of Environment No. 128 year 2003, petroleum waste and soil contaminated area can be treated biologically by bioremediation technology as clean production technology. Utilization of microorganisms both bacterial and the combination between fungal and plants for bioremediation can be used as an alternative option. Mechanism of plants in phytoremediation is facilitating microbial activity in soil by forming an association. Additionally, the plant roots are able to attract the microbes to approach them and associates with plants thus the roots secrete exudates that are generally required by microbes. According to [1], legume plants secrete flavonoids that stimulate the association between the plants and bacteria. Sengon (*Paraserianthes falcataria (L.)* Nielsen) is a legume plants that is considered to be a suitable candidate to restore oil sludge-contaminated soils. The aim of present study is to evaluate the utilization of sengon as bioremediator in oil-contaminated soils.

METHODOLOGY

In this study, experimental method with the addition of bacterial consortium (*Bacillus sphaericus, Pseudomonas aeruginosa*) on the oil sludge medium was used. Preparation of starter medium with a mixed culture into a solid waste composting (oily sludge) with a concentration of 2% mixed with sand, soil sterilized. Starter medium was stored in bottles at 300 g and was made by inserting isolate of bacteria), this inoculum allowed to stand for ± 1 week. Implanted medium starter in to treatment 30% and 35% oily sludge mixed with sand and soil 2:1 in the pot, composted for one month with inoculum and then planted with mycorrhizal as phytoremediation bacterial consortium was added into 35% oil sludge that previously composted during 6 weeks. Afterwards, medium was planted with mycorrhized sengon for 12 weeks.

The main parameters measured in this study was the number of bacterial colonies formed and heavy metals content Pb and Ni, which were analyzed before and after composting as well as during phytoremediation with 3 weeks interval during 18 weeks. Bacterial colony was counted by Total Plate Count method [2], whereas heavy metals content Pb and Ni were analyzed using AAS (Atomic Absorption Spechtrophotometer). The obtained data was analyzed by regression. On the other hand, supporting parameters measured were the percentage and mycorrhizal infection on sengon roots using the modified color method as described in [3]. Acidity (pH), humidity (%) and temperature (°C) were analyzed descriptively.

RESULTS AND DISCUSSION

The heavy metals content (ppm) and colony of bacterial consortium (CFU mL^{-1}) was analyzed before and after composting stage as well as phytoremediation stage with 3 weeks interval during 18 weeks of observation.

Mycorrhizal infection on sengon roots

The presence of mycorrhizal infection was observed prior to the treatment. Our results showed that the level of mycorrhizal infection in sengon roots prior to treatment was considerably high and internal hypha was clearly visible as well as vesicle in roots. Furthermore, observation on root's infection with modified Vierheilig's method is provided in Table 1.

Table1: Percentage of mycorrhizal infection in sengon' roots.

Before phytoremediation				
Replicates	**Number of infected roots**	**Percentage of infected roots (%)**	**Class of infected roots**	**Type of infection**
1	30	100	5	Vesicle, Internal hypha
2	30	100	5	Vesicle, Internal hypha
3	29	96	5	Vesicle, Internal hypha
4	30	100	5	Vesicle, Internal hypha
5	30	100	5	Vesicle, Internal hypha
6	30	100	5	Vesicle, Internal hypha
After phytoremediation				
Replicates	**Number of infected roots**	**Percentage of infected roots (%)**	**Class of infected roots**	**Type of infection**
1	30	100	5	Vesicle, Internal hypha
2	26	87	5	Vesicle, Internal hypha
3	25	83	5	Vesicle, Internal hypha
4	27	90	5	Internal hypha

5	27	90	5	Vesicle, Internal hypha
6	30	100	5	Vesicle, Internal hypha

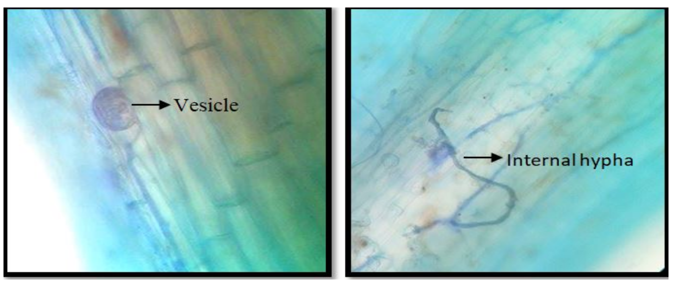

Figure 1: Mycorrhizal infection in sengon' roots with 100x magnification (Private documentation, 2015).

Percentage of infection on sengon roots was categorized in class 5 (Table 1 and Figure 1). This explains that mycorrhizal was able to infect the roots and able to adapt with the medium containing high content of oil sludge, thus able to oblige with bacteria. Interestingly the presence of hypha and vesicle in addition to the growth of external hypha demonstrated that mycorrhiza capable of filtering heavy metals such as Pb and Ni that infiltrate into the roots tissue. In addition, glomalin compound, as one of the glycoprotein produced by mycorrhiza, able to bind heavy metals in the soil [4] and has significant role in soil microbial-rhizosphere interaction. This process can therefore increase the plants survival [5].

Correlation between bacterial population and heavy metals content in oil sludge 35% medium

Linear regression analysis revealed that negative relationship was obtained between Pb content and bacterial population in oil sludge 35% medium, which explained by the equation Y=17.3-0.560X, meaning that there was a reduction in Pb content as much as 0.560 on each bacterial addition (CFU mL^{-1}). In other words, the increase in the number of bacterial consortium causing the decrease of Pb content in the oil sludge medium. Correlation coefficient between Pb content and the number of bacterial colony appeared to be strong (r=0.982). Moreover, the obtained determination coefficient (R) was 96.5%, which means Pb content was 96.5% affected by the number of bacterial colony where as 3.5% was affected by other factors. Obtained p-value showed that the confidence level on regression equation was 100%.

Negative relationship was observed on the linear regression analysis between Ni content and the number of bacterial colony in oil sludge 35% medium (Y=34.4-1.57X). This equation explains that Ni content decrease as much as 1.57 for each CFU mL^{-1} of bacterial colony. Thus, increase in the number of bacterial colony resulting the decrease in Ni content in oil sludge 35% medium. Correlation coefficient between Ni content and the number of bacterial colony was slightly lower than in Pb (0.827). However, this value was still showing a strong relationship between the two parameters. The obtained determination coefficient (R) was 68.4%, meaning that Pb content was 68.4% affected by the number of bacterial colony while the other 31.6% was affected by other factors. Furthermore, the p-value obtained showed that the confidence level in regression coefficient was 99.98%.

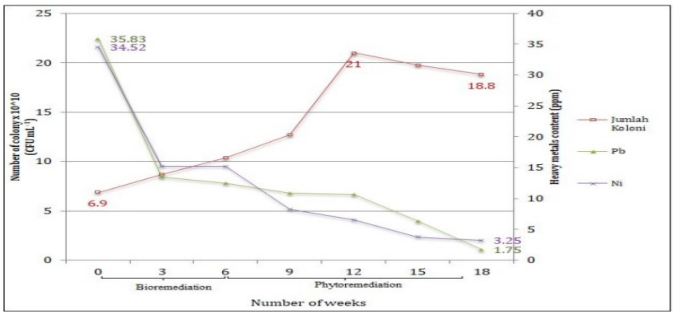

Figure 2: The number of bacterial colony and heavy metals content (Pb and Ni) during composting-phytoremediation. **Note:** (-◦-) Jumlah Koloni, (-▴-) Pb, (-✶-) Ni

Figure 2 showed the relationship between the number of bacterial population and the heavy metals content (Pb and Ni) during 18 weeks of experiment. The bacterial population increased during 12 weeks of experiment with populasi 21.00 x 1010CFU mL^{-1} at 26°C, 71.5% of humidity and pH 6.5 by the time of measurement on 12th week of experiment. Afterwards, this number decreased to18.80*1010 CFU mL^{-1} on 18th week of experiment.

Apart from the carbon contained in oil sludge, microorganisms also require additional nutrients from the vermin-compost. By the presence of this nutrition, organic decomposer microbes can be well developed and work faster in decomposing organic materials [6]. This process can be achieved due to the presence of biosorbent and bioremoval mechanism, which can be defined as the accumulation and bio concentration of pollutants in the environment by the biological materials that contribute to the reduction of heavy metals content [7]. Biologically, this bioremoval process involves 2 mechanisms, the active uptake and the passive uptake.

Passive uptake is the process when the heavy metal ion bound to the bacterial cell wall. Passive uptake can be achieved by two means first by the ions exchange on cell wall which are replaced by heavy metal ions. Second by the complex compounds formation between heavy metal ions and functional groups for instance carbonyl, amino, thiol, hydroxyl, phosphate and hydroxy-carboxyl [7,8]. Whereas the active uptake can be performed in various types of living cells. This mechanism is simultaneously occurred in accordance with metallic ions consumption for the microbial growth and intracellular accumulation of these ions. This process is strongly affected by the energy availability in the cell and its sensitivity to environmental parameters such as pH and temperature [9].

Bacteria have the metal-binding ability due to their functional groups with negative charge, such as phosphate group. Therefore, the bacteria can absorb heavy metals such as Pb and Ni because they are positively charge. This hypothesis confirmed by [10], which demonstrated that the bacteria are the heavy metals biosorbent agent. Heavy metals bio sorption mechanism is a complex mechanism that consists of metal transport across cell membrane, ion exchange and production of organic acids by microorganisms.

In fact, bacteria produce organic acids that chelate toxic metals. The metals are absorbed by the carboxyl group, which contained in microorganisms cell membrane polysaccharides [11]. Nevertheless, decreased in metal content could be also accompanied by the increase of heavy metal content in the medium. This increase can be probably caused by the microorganism that accumulate inactive metals, then the accumulated metals re-appear and re-bind with soil colloid thus enhance metal content in soil.

The mycorrhized sengon also contributes in heavy metals accumulation. Sengon is capable of removing pollutants by either absorbing or transporting pollutants into the plant tissue, which subsequently transformed or mineralized the pollutants [12]. This hypothese was in accordance with [13], which demonstrated that the absorption mechanism and heavy metals accumulation by the plants consist in 3 sustainable stages:

(1) Absorption by the roots through chelate substance formation (phytosiderophore), which able to bind metals and carry them into the roots cell by active transport. (2) Metals translocation from plant roots onto other part of the plants through transporter tissue such as xylem and floemand. (3) Metals localization in particular cell to prevent plant metabolic inhibition.

Endomycorrhize able to adapt in soil containing metals and able to bind heavy metals [14]. Endomycorrhize reduce the heavy metal content by: 1) resisting and unforward the metals into the plant tissue thus maintaining the metal concentration. 2) Explorating ability of external hypha that can explorate soil volume thus absorbing the heavy metals. 3) Reducing the heavy metals in plant tissue by the presence of glomalin, which produced in external hypha wall. Additionally [12], confirmed that

mycorrhiz has significant role inprotecting the roots from toxic s ubstance for instance heavy metals.

CONCLUSION

This study reveals that the population of B. *spaericus* and P. *aeruginosa* as well as mycorrhized sengon can reduce Pb and Ni content in oil sludge35% medium as much as 95 and 90.5%. The highest reduction of Pb and Ni content was achieved on week 18 where Pb concentration decreased from 35.83 into 1.75 ppm, while Ni content decreased from 34.52 into 3.25 ppm.

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