Utilization of kapok fiber as a natural sorbent in petroleum hydrocarbon biodegradation by Pestalotiopsis sp.

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Abstract

Utilization of sorbents is considered have a beneficial effect on crude oil cleanup. In this study, a natural sorbent was used in association with Pestalotiopsis sp. NG007 to enhance the biodegradation of petroleum hydrocarbons (PHCs) in soil. Among six natural sorbents, namely, pulp sheet from softwood (PS), pulp sheet from hardwood (PH), pulp sheet manila (PM), cellulose sponge (CS), polypropylene (PP), and kapok fiber (KF), KF showed high sorption capacity toward asphalt (34.76 g asphalt/g kapok). Addition of 7.5% KF-associated NG007 to crude oil-contaminated soil increased 25.6% degradation compare with no addition of kapok. The effective adsorption of all fractions in PHCs to kapok which induce the high accessibility of fungus and the increasing enzymatic activities by the fungus may responsible to the higher degradation of PHCs. This study suggested that kapok may has the potential to use in bioremediation of crude oil by microorganism.

Keywords: Adsorption, Biodegradation, Crude oil, Fiber, Kapok

Introduction

Crude oil can cause environmental pollution during various stages of their production, storage, transportation, refining, and utilization. In such cases, PHCs affect aquatic life, human life, local economies, tourism, and leisure activities because of the coating properties of PHCs. The basic methods for oil spill collection and cleanup are chemical, mechanical, and biological treatments. The mechanical methods involve the transfer of oil from spill site to temporary storage using oil sorbents or skimmers.

Oil sorbents can be classified into inorganic mineral, organic synthetic, and natural products (Adebajo et al., 2003). Perlite as a mineral oil sorbent has been
used by Bastani et al. (2006) but showed lower capacity than that of natural fibers. Most of inorganic oil sorbents include vermiculites, organoclay, zeolite, and diatomite have poor buoyancy, oil sorption capacity, reusability, and oil recovery. In addition, they are difficult to handle on site due to their granular or powder forms. Due to inadequate hydrophobicity, they may also experience collapse of their microstructure due to sorption of water (Adebajo et al., 2003). As an organic synthetic products, polypropylene and polyurethane are recently the most commercially available oil sorbents. However, they have a limited-biodegradable and can be difficult to deal with after use due to their xenobiotic nature. Natural oil sorbents such as rice straw, corn cob, peat moss, cotton, cotton grass, barks, milkweed, kenaf, wool, and kapok have been known to have higher sorption capacities for oil than that of commercially available synthetic (Johnson, Manjreker and Halligan., 1973; Adebajo et al., 2003; Saito et al., 2003; Radetic et al., 2003; Suni et al., 2004). These agricultural products and residues are inexpensive and available locally giving rise an advantages to be used as a low-cost alternative for the removal of oil spilled on water surface.

The use of oil sorbent during incubation of Pseudomonas sp. has showed effective biodegradation of crude oil (Setti, Mazzieri and Pifferi., 1999). Naturally, fungi or bacteria capable of using petroleum hydrocarbons can form a colonization in natural oil sorbents. Ali et al. (2011) reported that fungi that associated with sawdust could effectively consume crude oil. The utilization of sorbent that associated with PHC-degrading fungi is projected to not only remove oil pollutants by sorption, but also to simultaneously mineralize this pollutants microbiologically. Therefore, this study aims to investigate the effect of kapok absorbent in enhancing the biodegradation of crude oil in soil.

Materials and Methods

Adsorption study of sorbents

A-100 mL sample of crude oil was placed in a beaker glass before any sorbent was immersed in the ice bath. One piece of sorbent (with initial weight was measured) was place in the system, which was shaken for 3 hours. The wetted sorbent material was weighed after being drained for 1 min in the sustainer. The amounts of oil sorbed by the sorbent were determined by a gravimetric method (Zhu et al. 2011).

Pre-incubation of fungal culture in kapok adsorbent

Three gram of 2-cm length-air dried kapok was added with 50 mL of malt extract (ME) liquid medium (20 g/L malt extract, 20 g/L glucose, 1 g/L polypeptone in distilled water) and 1% of Tween 80 in an autoclaved plastic box. After all surfaces was wetted, the mixture of kapok was autoclaved at 121°C for 20 min. The fungal culture Pestalotiopsis sp. NG007 was added to kapok adsorbent, then were grown until fully cover the surface of kapok.

Effect of kapok adsorbent on biodegradation and enzymatic activities of Pestalotiopsis sp. NG007

Experimental design of addition kapok adsorbent and Pestalotiopsis sp. to asphalt contaminated soil is shown in Fig. 1. Approximately after 1 month pre-incubation in kapok, the kapok containing NG007 with final composition 0, 1, 2.5, 5, 7.5, and 10% was added to soil containing crude oil and incubated for 15 and 30 days in dark condition at 25°C. Non-inoculated NG007 was used as a negative control.

![Figure 1](image-url)
Chemical analysis of crude oil degradation

The detailed biodegradation analysis of crude oil and their fractions (aliphatic, aromatic, resin, and asphaltene) were described in our previous study (Yanto and Tachibana, 2013). The biodegradation of total petroleum hydrocarbons (TPHs), resin, and asphaltene fractions were analyzed using the gravimetric method. The biodegradation of aliphatic and aromatic fractions were analyzed using gas chromatography (GC-FID Shimadzu 2014), with a TC-5 capillary column (30 m, id x 0.25 mm x 0.25 μm).

Aliphatic compounds were identified by gas chromatography-mass spectrometry (GC-MS) using a Shimadzu QP-2010 with a TC-1 capillary column (30 m, id x 0.25 mm x 0.25 μm) (Yanto and Tachibana, 2014). The carrier gas, helium, was delivered at a constant rate of 1.5 mL min⁻¹, with a column pressure of 100 kPa and interface temperature of 280°C. The column temperature was started at 60°C, and increased at 10°C min⁻¹ intervals to 280°C, at which it was maintained for 10 min. The injection volume was 1 μL with a split ratio of 100. The conditions for GC-MS were adjusted to a scan interval of 1.3 eV s⁻¹, a threshold of 100, and a mass range of 40–900. Each peak was compared with the NIST08 library and results were confirmed with authentic standards.

Enzymatic assay

Enzymatic activities were assayed using the UV-Vis spectrophotometer (Shimadzu UV-1600). Catechol 1,2-dioxygenase (C12O) activity was assayed using catechol 0.01 M as the substrate by measuring the formation of cis,cis-muconic acid at 260 nm (Ɛ260nm = 16000 M⁻¹ cm⁻¹) (Nakazawa and Atsushi, 1970). Catechol 2,3-dioxygenase (C23O) activity was assayed using the same protocols as those for C12O based on changes in absorbance at 375 nm due to the production of 2-hydroxymuconate semialdehyde (Ɛ375 nm = 44000 M⁻¹ cm⁻¹) (Nozaki, 1970). Laccase activity was measured by monitoring the oxidation of syringaldazine at 525 nm (Ɛ525 nm = 6500 M⁻¹ cm⁻¹) (Leonowicz and Grzywnowicz, 1981). Manganese peroxidase (MnP) activity was determined based on the oxidation of 2,6-dimethoxyphenol (2,6-DMP) at 470 nm (Ɛ470 nm = 49600 M⁻¹ cm⁻¹) (Warishiri, Vali and Gold., 1992). Lignin peroxidase (LiP) activity was determined by the oxidation of veratryl alcohol to form veratryl aldehyde at 310 nm (Ɛ310 nm = 9300 M⁻¹ cm⁻¹) (Tien and Kirk, 1984). All experiments were performed at 25°C. Activities were expressed as units per gram wet soil matter (U g⁻¹), in which one enzyme unit (U) was defined as that forming 1.0 μmol of the product per minute under the assay conditions.

Results and Discussion

Adsorption study of the natural fibers

The adsorption study of the natural fibers is performed in an ice-container box using crude oil as a model PHCs. The results in Fig. 2 showed that among the fibers tested, kapok has the highest capacity of sorption. Maximum sorption capacity of kapok in crude oil is 34.76 g crude oil/g kapok. The lowest capacity has showed for pulp sheet prepared from softwood (1.00 g crude oil/g sorbent). Polypropylene sheet fibers that widely used commercial sorbent for oil spill cleanup showed lower capacity than kapok in this study. The hollow structure with large lumen in kapok fiber may attribute to the high capacity of kapok to adsorb the oil (Lim and Huang, 2007). The high adsorption capacities of kapok have also been reported by Hori et al. (2000) with 40 g oil/g sorption capacity and by Lim and Huang (2007) with 36–45 g oil/g sorption capacity, at different oils. Other synthetic adsorbent like polyvinyl chloride/polystyrene prepared by electrospinning method has showed a high capacity in oil sorption compare to polypropylene sorbent (Zhu et al., 2011). The mechanism of oil sorption by sorbents can be adsorption, absorption, capillary action, or a combination of these (Radetic et al., 2008).

Figure 2. Asphalt sorption of different sorbents. PS: pulp sheet from softwood, PH: pulp sheet from hardwood, PM: pulp sheet manila, CS: cellulose sponges, PP: polypropylene sheet, KF: kapok fiber.
Biodegradation of PHCs by Pestalotiopsis sp. NG007 in the presence of kapok

Effect of kapok composition on biodegradation of COC and enzyme activities of Pestalotiopsis sp. NG007 is shown in Fig. 3. The results showed that addition of kapok enhanced the biodegradation of COC by Pestalotiopsis sp. NG007 in soil. Optimum biodegradation was obtained when 7.5% of dry weight of kapok was used. About 25.6% degradation of COC could be enhanced in kapok-assisted biodegradation by Pestalotiopsis sp. NG007. Comparing with control no addition of kapok, the biodegradation of all fractions in three PHCs, COA, COC, and asphalt were increased in kapok treatment by Pestalotiopsis sp. NG007. The effect of kapok was more significant in degradation of aliphatic, aromatic, and resin fractions in all PHCs than the asphaltene fractions (Fig. 4). However, for overall, the addition of kapok promotes enhancing biodegradation of all fractions in all PHCs. This may indicate that the effective adsorption of all fractions in PHCs to kapok can induce the high accessibility of fungi to degrade and led to higher degradation. Setti, Mazzieri and Pifferi. (1999) reported that utilization of oil sorbents can increase enormously n-alkane biodegradation rate when a Pseudomonas sp. was used in crude oil fermentation as compared with that observed in the absence of the sorbents due to an improved oil and microorganism interaction at the water/cell/oil/sorbent interphase.

Addition of kapok also showed an increasing enzymatic activities produced by Pestalotiopsis sp. (Fig. 3). All the enzymatic activities such as catechol 1,2-dioxygenase (C12O), catechol 2,3-dioxygenase (C23O), laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP) showed enhanced 1.84-, 2.02-, 6.13-, 4.98-, and 6.25-fold than without kapok addition.

![Figure 3. Biodegradation of Crude oil C (COC) and enzyme activities of Pestalotiopsis sp. NG007 in the presence of kapok in soil.](image)

![Figure 4. Biodegradation of fractions in COA (Crude oil A), COC (Crude oil C), and asphalt by Pestalotiopsis sp. NG007 in the absence and the presence of kapok in soil.](image)
The study proposes that in addition to improve the oil adsorbed and microorganism interaction, kapok adsorbent can also enhance the production of ligninolytic and non-ligninolytic enzymes of Pestalotiopsis sp. NG007 in the presence of COC. Ligninolytic and non-ligninolytic enzymes have showed a play important role in degradation of PHC fractions. It was reported that dioxygenases or monooxygenases can initiate degradation of PHCs by degrading first the aliphatic fraction via mono-, di-, or sub-terminal oxidation (Yanto and Tachibana, 2013). In addition, both non-ligninolytic and ligninolytic enzymes were needed to improve simultaneously the degradation of PHCs in soil (Yanto and Tachibana, 2014).

**Conclusion**

Addition of 7.5% KF-associated NG007 to crude oil-contaminated soil increased 25.6% degradation compare with no addition of kapok. Associating Pestalotiopsis sp. NG007 with kapok adsorbent enhanced the biodegradation rate of PHCs in soil due to both facilitating interaction of the fungal culture with PHCs or enhancing enzymatic activities in the presence of PHCs. This technology can be projected to bioremediation of PHCs in contaminated environments.

**References**


