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Biodegradation of *Tapis* blended crude oil in marine sediment by a consortium of symbiotic bacteria

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ABSTRACT

Biodegradation rate and the high molecular weight hydrocarbons are among the important concerns for bioremediation of crude oil. Inoculation of a non-oil-degrading bacterium as supplementary bacteria increased oil biodegradation from 57.1% to 63.0% after 10 days of incubation. Both the oil-degrading bacteria and the non-oil-degrading bacteria were isolated from Malaysian marine environment. Based on the 16S rDNA sequences, the oil-degrading bacteria was identified as *Pseudomonas pseudoalcaligenes* (99% similarity) while the non-oil-degrading bacterium was *Erythrobacter citreus* (99% similarity). *E. citreus* does not grow on crude oil enriched medium under present experimental condition but it withstands 5000 mg kg^{-1} *Tapis* blended crude oil in sediment. Under optimal condition, the oil-degrading bacterium; *P. pseudoalcaligenes*, alone utilized 583.3 ± 3.8 mg kg^{-1} (57.1%) at the rate of 3.97×10^{-10} mg kg^{-1} $cell^{-1}$ day^{-1} *Tapis* blended crude oil from 1000 mg kg^{-1} oil-contaminated sediment. Inoculation of *E. citreus* as the supplementary bacteria to *P. pseudoalcaligenes* enhanced biodegradation. The bacterial consortium degraded 675.8 ± 18.5 mg kg^{-1} (63.0%) *Tapis* blended crude oil from the 1000 mg kg^{-1} oil-contaminated sediment. Biodegradation rate of the bacterial consortium increased significantly to 4.59×10^{-10} mg kg^{-1} $cell^{-1}$ day^{-1} ($p = 0.02$). Improvement of the oil degradation by the bacterial consortium was due to the synergetic reaction among the bacterial inoculants. There are two implications: (1) *E. citreus* may have a role in removing self-growth-inhibiting compounds of *P. pseudoalcaligens*. (2) *P. pseudoalcaligenes* degraded *Tapis* blended crude oil while *E. citreus* competes for the partially degraded hydrocarbons by *P. pseudoalcaligenes*. *P. pseudoalcaligenes* forced to breakdown more hydrocarbons to sustain its metabolic requirement. The bacterial consortium degraded 78.7% of (C_{12}–C_{34}) total aliphatic hydrocarbons (TAHs) and 74.1% of the 16 USEPA prioritized polycyclic aromatic hydrocarbons.

1. Introduction

Spillage of 10,810 tons of crude oil from Heibei Spirit in the West Coast of South Korea in December 2007 is drawing attention of the world on the hazards of oil pollution. The surging global oil demand, has drive the transportation of large volume of oil through the busy sea lanes including the Straits of Malacca. The heavy maritime traffic exposed the marine environment to the risk of oil spills. When the oil spills into the marine environment, sediment is the ultimate fate of the spilled oil (Samanta et al., 2002). However, less attention was given to oil-degradation in the sediment. Sediment is an important matrix for the quality and productivity of an ecosystem (Wezel and Vegter, 2001). Oil has longer residential time in sediment as compared to water. Oil residues in sediment posed long-term hazards to the marine organisms (Wezel and Vegter, 2001; Albrechtsen et al., 1997). The level of hydrocarbons in the sediment is usually used to classify the status of oil-contamination in the marine environment. There are many reports on oil toxicity in the sediment, though there is little unequivocal evidence to suggest a linkage to higher order biological effects (Lee and Anderson, 2005). To date, there are many techniques to remove oil contamination from the sediment, such as, solvent extraction, bioremediation and chemical dispersant (Eweis et al., 1998). Biodegradation is one of the promising treatments for combating oil pollution. It costs less and contributed less destruction to the environment as compared to other treatments. However, biological remediation is the least practice techniques to remediate the oil-contaminated sediment (Wood, 1997). Oil biodegradation is normally slower as compared to other treatments (Oh et al., 2001; Yuan et al., 2000).

Biodegradation of crude oil is not a new technology for combating oil pollution. Isolation of active oil-degrading bacteria from the marine environment was conducted as early as 1960s

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