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Development of a cell immobilization technique with polyvinyl alcohol for diesel remediation in seawater

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ABSTRACT

The objective is to develop and evaluate a PVA cell immobilization technology in degrading diesel in seawater. The inclusion of activated carbons improved the TPHd degradation efficiency up to 47%. The optimal combination of the immobilized cells, Design H (dominant *Rhodococcus pyridinivorans* CC-HCCH11), achieved relatively high TPHd degradation efficiency of 92%, and reduced TPHd from 2168.65 ± 74.52 to 164.42 ± 3.26 mg L^{-1}. Design J (dominant *Gordonia alkanivorans* CC-JG39) achieved the highest degradation rate *k* (0.0041 hr^{-1}) with the final degradation efficiency of 88%. The chromatograph explained the superiority of Design H and J. The plate counts revealed a consistent degradation pattern with the genomic DNA's, with correlation coefficient ranging from 0.774 to 0.915. The water quality analysis indicated adequate consumptions and evidence of microbial activity. The developed PVA immobilization technique is helpful in solving the potential environmental problem by using it as resources for environmental remediation. Design H is a recommended bacterial cell combination.

1. Introduction

For removal or degradation of petroleum hydrocarbons from contaminated seawater, bioremediation has frequently been proposed as a useful strategy due to their advantages of being effective, economic, and environmentally friendly. Either carried out by bioaugmentation or biostimulation, bioremediation for oil spill cleanup in seawater and soils has been attempted with varying degrees of success (Hou et al., 2013; Das and Chandran, 2011; Wu et al., 2016). For a successful biostimulation, ideal concentrations of nutrients are required for maximum growth of the hydrocarbon degraders. To resolve the problem of diesel contamination in seawater, Liu et al. (2015) has identified the optimal nutrient composition as Bushnell Haas medium (Bushnell and Haas, 1941).

However, micro-species may show high degradation rates in a laboratory, but when introducing to the real world, they may fail due to harsh environmental conditions including competition with indigenous microorganisms and limited nutrients. The problems of suspended bacterial cells could be dilutions caused by wave and tidal movements. According to existing studies (Banerjee and Ghoshal, 2011; Duarte et al., 2013) , immobilized cells are superior to suspended free cells in different ways, including relatively high cell density, continuous operational, reduction of lag phase, improved cell viability, and higher tolerance to toxicants.

Various immobilization techniques are available for keeping microbes in an appropriate environmental that is neither nutrient nor oxygen limited. Polymeric gel beads of calcium-alginate was one of the most useful supporting materials for entrapping microbial cells due to their advantages of good biocompatibility, easy preparation, less costly, and high availability (Banerjee and Ghoshal, 2011; Duarte et al., 2013). Polyurethane-polyurea copolymer (PPC) has shown the successful examples in entrapping bacteria cells and performed successful degradability (Chou, 2005; Fang, 2007; Chen, 2012; Sun et al., 2014; Liu et al., 2015). Chen's (2012) application using PPC as entrapment material to the indigenous microbes in seawater samples around Taiwan, a degrading efficiency of 47.25% was given at virtual marine condition with nutrient as a limiting factor. The four consortia: *Gordonia alkanivorans* CC-JG39, *Rhodococcus pyridinivorans* CC-HCCH11, *Alcaligenes piechaudii* CC-ESB2, and *Comamonas testosterone* CC-CF3 have been employed with the same PPC immobilization technique as

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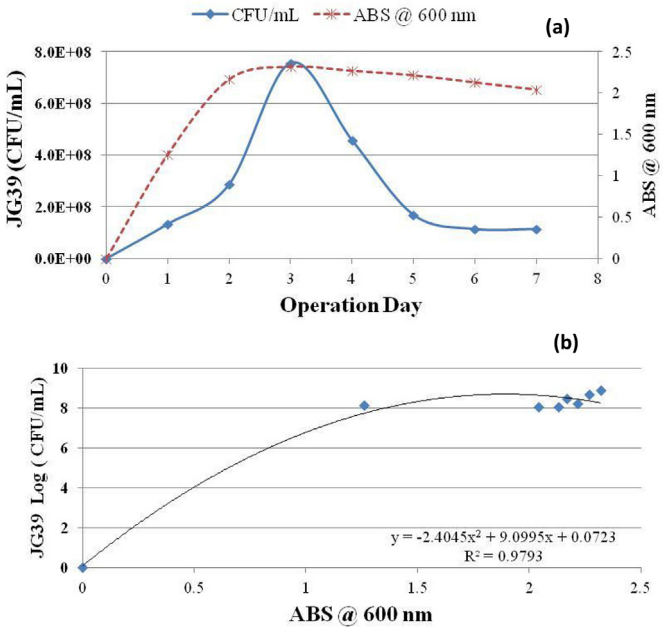

Fig. 1. Bacterial concentration to be entrapped: (a) optimal concentration at ABS = 2.32 at the 3rd inoculation day; (b) non-linear relationship between the log JG39 and the ABS at 600 nm.

single immobilized cell beads. The highest degradability achieved by the CC-HCCH11 single strains was 53% (Sun et al., 2014). Polyvinyl alcohol (PVA) cryogelation as an entrapment technique was applied to indigenous microbes for degradation of diesel-contaminated soil. A successful removal efficiency was achieved by using constructed the laboratory biopiles, and the superiority of the immobilized cells to the suspended liquid culture was observed.

The PVA approach proved the success over the suspended liquid one after 32 day-operation of diesel remediation (Cunningham et al., 2004). Activated carbons have been deployed in certain soil and sediment for remediation purposes (Qin et al., 2013) and they were also observed in improving bacteria releasing from entrapped gel beads (Fang, 2007). Another approaches including using biofilms of two different bacterial consortia to immobilize the cells also improved the diesel oil degradation efficiency (Ławniczak e al., 2011). Chitosan and celite were used and successfully flocculated and immobilized *Pseudomonas delafieldii* R-8 cells into the culture broth, so that diesel oil biodesulfurization with one bacterial strain was applicable (Li et al., 2010).

An optimal bacterial consortia combination was found by using PPC (Liu et al., 2015). The cell beads containing single bacterial strain individually: CC-JG39, CC-ESB2, and CC-HCCH11, and the optimal degradability of 78% was achieved with the combination of CC-HCCH11: CC-JG39 equals to 5:1. A question was raised about the applicability of the same optimal combination, by using another kind of material. Whether or not changing the entrapment material would cause a different result? Therefore, we are proposing an alternative material, PVA (Cunningham et al., 2004), and to test the inclusion of activated carbons. PVA has been widely used as warp sizes for cotton-synthetic blends in the textile industry (Huang et al., 2014a,b; Pang, 2012) , and for polarizing film light in liquid crystal displays. The molecular structure of $[-CH_2-CH(OH)]_n$ makes it to be well water soluble (Chang et al., 2011). Wastewater containing PVA also cause environmental problems (Huang et al., 2014a,b; Giroto et al., 2006), since the PVA-degrading capacity of most microorganisms is extremely restricted and specific (Lim and Park, 2001).

The objective of the study is to develop and evaluate PVA cell immobilization technology to effectively degrade diesel in seawater, with existing bacterial consortium combinations (Liu et al., 2015). The development of the PVA immobilization technique could be helpful in solving the mentioned environmental problems by using it as resources for environmental remediation. The optimal combination of the entrapped diesel-degrading strains was decided based on their TPHd degradability. Prior to testing the degradability of different combined bacterial beads, a parallel degradation test was carried out as a screen test for inclusion of activated carbons in the formation of PVA.

Table 1
Experimental design.

	CC-HCCH11	CC-JG39	Total bead number
Design H	50	10	60
Design HJ	30	30	60
Design J	10	50	60
BK	0	0	60

Note: numbers stand for the gel beads containing diesel-degrading bacteria.

2. Materials and methods

2.1. Experimental design for testing activated carbon inclusion

This experiment design goal is to test two types of composition, with and without inclusion of activated carbons in the entrapped cell beads (namely JC and J, respectively), on total petroleum hydrocarbon-diesel degradability (namely TPHd). CC-JG39 was chosen for the test. Sixty gel beads containing CC-JG39 with and without the activate carbons were tested. The concentration entrapped in each of the cell beads was about 10^8 CFU mL^{-1} based on the non-leaner relationship between the absorbance under 600 nm and the corresponding concentration (Fig. 1). CC-JG39 particularly showed floating activity and can utilize aromatic compounds which were frequently found in petroleum hydrocarbon oils. CC-JG39 was isolated from sludge drainer of a gas station located in central Taiwan (Young et al., 2005).

Serum bottles of 500 mL were used and the space gas was analyzed. In each of the bottles, the cell beads were added to the 100 mL medium and shaken at about 100 rpm at room temperature for 336 h. The initial TPHd concentration was about 1638 ± 12 mg L^{-1}. The diesel oil in this study was Super Low Sulfur Premium Diesel (Vehicle), which were purchased from Formosa Petrochemical Corporation for our study. The diesel is produced for vehicle. The Cetane Index is 48, and the density ranged from 820 to 845 kg m^{-3}.

Entire sample from one bottle was analyzed for the TPHd analysis. Mixed liquid volatile suspended solid (MLVSS), pH, normal phosphate, nitrite, nitrate, and gases of CO_2 and O_2 were analyzed periodically. Also, the pH was adjusted to around 8.10 and 8.15 periodically, with 0.1 N of sodium hydroxide. Oxygen was provided adequately to avoid becoming a limiting factor. Phosphate buffer solution (PBS) was used to clean the cell beads before the remediation tests were initiated. The key ingredients appeared in PBS were 0.4 M of $NaH_2PO_4{\cdot}2H_2O$, 0.1 M of $Na_2HPO_4{\cdot}12H_2O$, and saturated H_3BO_3. Dichloromethane (DCM) was used to dissolve the attached diesel on the gel beads.

2.2. Experimental design for optimal bacterial combination entrapped by PVA

In order to test and verify the superiority of the PVA entrapment approach on seawater diesel degradability, comparable