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A Feasibility Study on the Formation of the Permeable Reactive Biobarrier treated with *Beijerinckia indica*

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ABSTRACT

Authors evaluated the feasibility of a permeable reactive biobarrier (PRB) treated with biofilm formed by *Beijerinckia indica (B. indica).* This study focused on evaluating two potentials of *B. indica* for the requirements of PRB: reducing the hydraulic conductivity and degrading the polycyclic aromatic hydrocarbons (PAHs). The hydraulic conductivity was estimated by rigid wall column test and finally the values were converted to the values of intrinsic permeability. The nutrient solution was passed through the biobarrier column to activate the bacterium and then leachate was in turn carried into the column to evaluate the durability of the biobarrier. Phenanthrene was selected as a representative substance of PAHs. The ability of degrading phenanthrene by *B. indica* was evaluated by two-phase partitioning bioreactor after estimating the possibility with two pretests: observing the colony formation and the optical density on glucose-free medium containing phenanthrene.

With the results, *B. indica* produced large amount of strongly adhesive exopolysaccharides (EPS) and reduced several orders of magnitude of the hydraulic conductivity after 2 weeks of cultivation. Furthermore, about 1000mg/L of phenanthrene could be degraded by *B. indica* in the two-phase partitioning bioreactor. In conclusion, the application of the bacterium, *B. indica,* was found to have a potential role of a PRB to retain and remove contaminants in porous media.

Key word : Permeable reactive biobarrier (PRB), Exopolysaccharides, Hydraulic conductivity, PAHs, Two-phase partitioning bioreactor

1. INTRODUCTION

As increasing the contaminated sites, many types of treatment technologies have been developed for containment and remediation of the pollutants. Besides the physical and chemical soil treatments, biological treatments using the indigenous microorganisms in the soils or injecting some useful microorganisms into the subsurface have been focused on many sorts of advantages.

Among the biological treatments, clogging effect by the exopolysaccharides (EPS) produced by some bacteria has been applied to reduce the hydraulic conductivity under the subsurface. The biofilm composed of biomass and EPS in the porous media retains the flow of contaminants and degrades the pollutants, the key roles of the PRB.

In this study, we evaluated the feasibility of a PRB treated with biofilm developed by *B. indica* in view of reducing the hydraulic conductivity and degrading the polycyclic aromatic hydrocarbons (PAHs).

2. MATERIALS AND METHODS

1) Bacterial Strains and Nutrient Solution

The bacterial strain, *Beijerinckia indica* (DSM 1715), used in this study was selected as it can produce lots of tough, strongly adhesive EPS and degrade PAHs. *B. indica* is a free-living and non-pathogenic bacterium that is capable of adapting to the soil environment and surviving under low oxygen conditions. The durability of the bacterium against the limited pH condition, is relatively stronger than that of other microorganisms.

The nutrient solution used for activating the bacterium was Azotobacter (AB) #13 supplement that added the following constituents to the tap water to make 1000mL volume: 20g glucose, 1g NaCl, 1g yeast extract (Difco), 5mL 10% $MgSO_4$, 8mL 10% K_2HPO_4, 2mL 10% KH_2PO_4 and 1mL 0.75% $FeCl_2$. The pH of the solution was adjusted to 6.5 with 1N HCl and the solution was autoclaved at 121℃ for 15min, except for the carbon source, glucose, that was separately autoclaved.

2) Soil Medium

The soil used for this study was classified as SW-SC by Unified Soil Classification System (USCS). The test soil was sterilized at 121℃ for 90min to ensure the effect of *B. indica* on the clogging phenomenon in porous media. The bacteria were precultured in 200mL nutrient solution for about 40 hours and then 5% of the precultured solution was seeded to fresh 200mL nutrient solution. The bacteria were cultured for about 30 hours. After that, soil specimens were inoculated with the culture solution corresponding to the optimum moisture content (12%).

3) Flow System and Hydraulic conductivity

Four stainless columns (length, 12cm: inside diameter 5cm) were connected to the acrylic reservoir in a parallel line. The apparatuses of flow system were autoclaved at 121℃ for 90min. Low pressure (below 5psi) was used for saturating the column with nutrient solution at the first stage. The measurement of the initial hydraulic conductivity was conducted by using the equation of falling head test and then the change of the hydraulic conductivity was recorded by the effluent flows. The values were finally converted to the values of intrinsic permeability due to the difference of density of each permeant liquid.

4) Potential for Phenanthrene Degradation

Two pretests were performed to ensure the potential of phenanthrene degradation by *B. indica*: observing the colony formation in agar plates and the change of optical density in the glucose-free medium. Phenanthrene was added to the glucose-free medium for a carbon and energy source.

Finally, two-phase partitioning bioreactor was used to evaluate the ability of degradation. The

1400mL of glucose-free medium and the 700mL of Oleyl alcohol containing 5g phenanthrene were mixed in the bioreactor. The 2.5mL of Oleyl alcohol for phenanthrene concentration and the 5mL of the medium for optical density were sampled every day for two weeks. All the experiments were conducted in duplicates.

3. RESULTS

1) Initial Intrinsic Permeability without Biofilm

Three columns were compacted in three equal layers by a hammer (25mm in diameter and 340g in weight) that delivered 3, 7 and 12 blows to each layer, respectively. The initial intrinsic permeability of the soil specimens are shown in Table 1.

Table 1 The Initial Intrinsic Permeability

Specimen	Dry Unit Weight	Pore Volume	Intrinsic Permeability
3 Blows	1.53 t/m3	94.0mL	1.02×10^{-8} cm^2
7 Blows	1.64 t/m3	85.3mL	9.15×10^{-10} cm^2
12 Blows	1.85 t/m3	67.5mL	2.02×10^{-10} cm^2

2) Reduction of the Intrinsic Permeability

As shown in Figure 1(a), significant reduction of the hydraulic conductivity was occurred in the first week. The values of hydraulic conductivity were converted into those of intrinsic permeability. In the 3-blow-specimen, two orders of magnitude of the reduction of the hydraulic conductivity were observed after two weeks of cultivation. In Figure 1(b), part A is the region of permeation of nutrient solution, part B is permeation of sterilized tap water and part C is permeation of leachate.

Leachate was passed through the biofilm columns to evaluate the durability of the biobarrier. After that, one order of magnitude in the intrinsic permeability was approximately increased; however, re-permeation of nutrient solution could restore the former low permeability.

3) Potential of phenanthrene degradation

The change of optical density in the 200mL of glucose-free medium containing 0.1g and 0.05g phenanthrene was observed. In Figure 2, part P indicates the possibility of phenanthrene degradation. The conditions of each medium are shown in Table 2. Two-phase partitioning bioreactor was used to evaluate the degradation of phenanthrene. Figure 3 shows that *B. indica* could degrade about 1g of phenanthrene in two weeks.

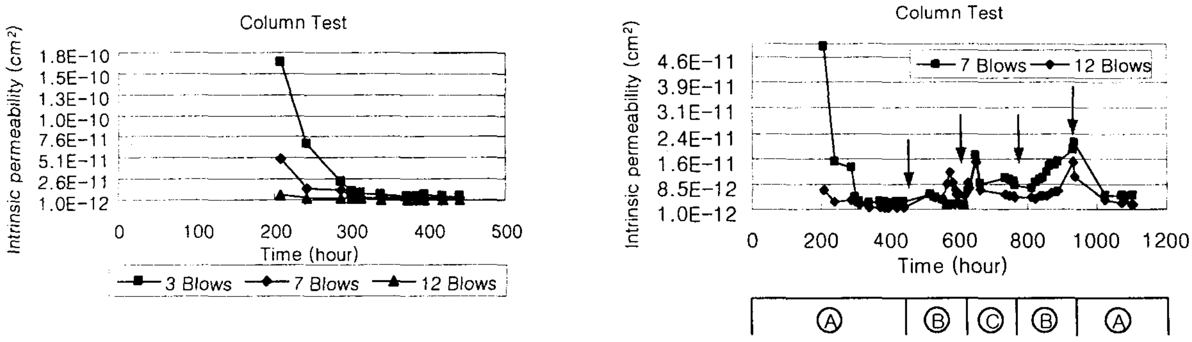
Table 2 The medium conditions

	Inorganic media		Inorganic media + Yeast extract
A	No phenanthrene	D	No phenanthrene
B	0.1g phenanthrene	E	0.1g phenanthrene
C	0.05g phenanthrene	F	0.05g phenanthrene

4. CONCLUSION

The authors concluded that *B. indica* produced large amount of strongly adhesive EPS and thus reduced several orders of magnitude of the hydraulic conductivity during 2 weeks. As the results of the durability test of biofilm, the biofilm formed by *B. indica* has an ability to resist increasing hydraulic conductivity and re-permeation of nutrient solution to the damaged biofilm could restore the former low permeability. The facts indicate indirectly that the biofilm formed by *B. indica* can be applied to PRB system. In addition, the bacterium could degrade phenanthrene and thus contaminated sites with PAHs can be controlled by *B. indica*.

In conclusion, the application of the bacterium, *B. indica*, was found to have a potential role of a PRB to retain and remove contaminants in porous media.



(a) Reduction of intrinsic permeability (b) Permeation of leachate into the column

Figure 1 Reduction of intrinsic permeability and the change of the permeability due to permeation of leachate

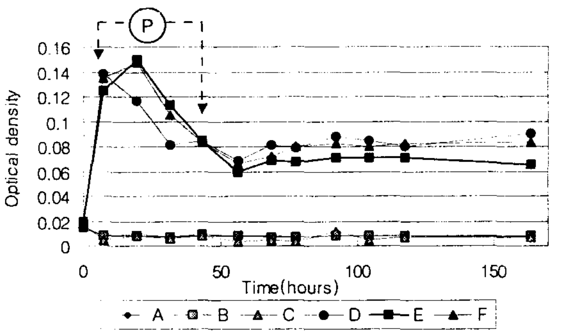


Figure 2 The growth of *B. indica* on the glucose-free medium

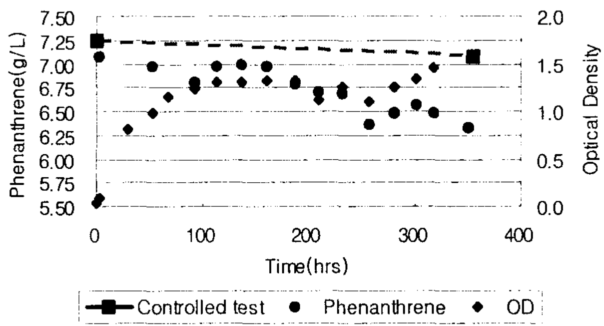


Figure 3 The growth of *B. indica* and the degradation of phenanthrene

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