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Microbubble suspension as a carrier of oxygen and acclimated bacteria for phenanthrene biodegradation

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ABSTRACT

The applicability of microbubble suspension made of saponin as a biodegradation-enhancing carrier where oxygen and pollutant-degraders are limited was studied. The saponin-microbubble suspension was used to deliver phenanthrene-degrading bacteria, inorganic nutrients, and oxygen. Bench-scale study was carried out to determine the physical properties of the microbubble suspension and to verify whether the delivered bacteria and oxygen were effectively used to degrade phenanthrene. A concentration of 2 g saponin/L H_2O generated stable microbubble suspension with a long half-drainage time and a high gas hold-up, and the addition of phenanthrene-degraders and inorganic salts to the saponin solution did not affect such properties. The flow of the microbubble suspension through a heterogeneous sand/clay-packed column occurred in two phases, with the liquid front advancing faster and the retarded gas front. The retarded gas front provided oxygen with bacteria, which enables phenanthrene biodegradation. Approximately 30% of the spiked phenanthrene was degraded in 21 days when one pore volume of 2.0 g/L saponin-microbubble suspension was applied whereas no phenanthrene decrease was observed following the application of the same saponin solution without microbubble generation. The decrease mainly occurred at the lower part of the column where the supply of oxygen by the microbubble was concentrated.

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1. Introduction

Organic contaminants in the subsurface have a well known tendency for natural attenuation, which is mostly achieved by microbial degradation [1]. However, the efficiency and duration of the microbial degradation cannot be assured due to the wide variety of environmental factors involved in the process that do not work independently [2,3]. In particular, organic contaminants suitable for aerobic biodegradation are less degradable when located in the subsurface due to the shortage of electron acceptors and micronutrients [4]. Therefore, providing limiting factors such as oxygen and inorganic nutrients might enhance the natural biodegradation potential to change organic contaminants into harmless water and carbon dioxide.

Microbubbles, which can be generated by simply agitating a surfactant solution with a high-speed stirrer [5], have many advantages in material transport, subsurface remediation and soil flushing. These merits can be usefully adapted for more successful introduction of microbubbles to bioremediation. First, microbubbles have the potential to enhance bacterial transport [6]. Second, it shows plug-flow characteristics, which can overcome the matrix heterogeneity [7]. Therefore, it can provide the materials essential for aerobic biodegradation uniformly to the subsurface. Furthermore, it is easy to control microbubble flow because they can be stably pumped due to its enhanced stability and water-like viscosity [8]. Third, it can be used in a soil flushing process [9,10], because surfactant shells have layers of surfactant molecules in a tail-to-tail manner that can act as a partitioning medium for hydrophobic compounds [6,11].

Microbubble suspensions, same as other foam systems, are a collection of gas bubbles dispersed in an aqueous surfactant solution. When generated from a surfactant solution, a gas–liquid dispersion is formed with a high gas content of around 50% [12], which can be enhanced successfully up to 70% using combination of surfactants [13]. This enables the microbubble suspensions to act as a carrier for materials in gaseous form (e.g., gas phase oxygen) and dissolved form (e.g., micronutrients). Moreover, microbubble suspensions can carry microorganisms suspended in bulk liquid and/or attached to the bubbles. Therefore, oxygenation, bioaugmentation, and biostimulation effects can be achieved simultaneously through the application of the microbubble suspensions when generated from a solution containing target contaminants' degraders and micronutrients.

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A comprehensive bench-scale study was carried out to test the feasibility of a microbubble suspension as an *in situ* bioremediation technology. The physical parameters such as gas hold-up and half-drainage time of the microbubble suspension were measured and its flow properties were visually observed by visualization cell test. In addition, the biological degradation of a target contaminant (i.e., phenanthrene) by the introduced microbubble suspension containing phenanthrene-degraders and micronutrients was determined. The patterns of the materials distribution (i.e., bacteria and gas) after the introduction of the microbubble suspension into a test column were also investigated.

2. Experimental

2.1. Components for microbubble generation

The solution used to generate the microbubble suspension consisted of a surfactant, a phenanthrene-degrading bacterial species, and inorganic nutrients. A heterogeneous saponin mixture extracted from *Quillaja* bark (S7900, Sigma–Aldrich, St. Louis, MO, USA) was used as a base surfactant. Saponin is a plant-origin biosurfactant that is commonly found in soil [14]. The critical micelle concentration (CMC) of saponin was determined experimentally to be 1.0 g/L by measuring the changes in surface tension of a saponin solution as a function of concentration.

The phenanthrene degrader was isolated from an oil-contaminated soil and identified by 16S rDNA sequencing as *Burkholderia cepacia* and named *B. cepacia* RPH1. The bacterium was maintained in an inorganic salt medium containing 1.0 g/L $(NH_4)_2SO_4$, 0.8 g/L K_2HPO_4, 0.2 g/L KH_2PO_4, 0.2 g/L $MgSO_4{\cdot}7H_2O$, 0.1 g/L $CaCl_2{\cdot}7H_2O$, 0.005 g/L $FeCl_3$, and 0.001 g/L $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ with phenanthrene at the concentration of 100 mg/L as the sole energy and carbon source. After allowing the bacteria to grow for 9 days at 30 °C, the bacterial cells were harvested by centrifugation, washed twice with a phosphate buffer (pH 7.0), and resuspended in an appropriate solution prior to use. The same concentrations of inorganic salts were added to a 2.0 g/L saponin solution containing phenanthrene degraders for microbubble generation below. The supplement of inorganics maintained the pH of the saponin solution near neutral, otherwise the pH was about 4.5.

2.2. Microbubble generation

A generating device was developed as suggested by Sebba [15]. The generator consisted of a mixer (Silverson SL2T Model, Silverson Machines, Chesham Bucks, England) and a generating vessel (15 cm diameter × 23 cm height) with two baffles. One liter of each microbubble-generating solution (i.e., 0.5, 1.0, or 2.0 g/L saponin solution) was agitated thoroughly at 7000 rpm for 5 min [6,16]. The size of the microbubbles generated, which was determined using a particle counter (Coulter Multisizer II 9900266-F, Beckman Coulter, Miami, FL, USA), ranged from 20 to 250 μm with an average value of 49 μm.

2.3. Determination of microbubble suspension characteristics

Half-drainage time and gas hold-up were chosen as physical parameters to represent the stability and quality of the generated microbubble. A drainage curve of the changes in the drainage ratio as a function of time was constructed. Two hundred milliliters of the microbubble suspension were transferred to a graduated cylinder and the volume of the drained liquid was read with time. Half-drainage time was determined to be the time taken for half the total volume of liquid in microbubble suspension to drain away [8]. At the same time, gas hold-up (ε) was calculated using the initial microbubble suspension volume (V_{a0}) and the total liquid volume (V_{l0}) as follows [12]:

$$\varepsilon = \frac{V_g}{V_{a0}} = \frac{V_{a0} - V_{l0}}{V_{a0}} \quad (V_g, \text{gas volume})$$

The effect of the saponin concentration (0.5, 1.0, and 2.0 g/L) on the properties of the microbubble suspension was investigated by observing the above mentioned parameters.

2.4. Visualization cell test for microbubble suspension flow properties

A transparent visualization cell (15 cm × 1.5 cm × 25 cm) was designed to observe the flow properties of the microbubble suspension. The cell test was conducted in two ways; (1) a homogeneous medium packed with 920 g of Ottawa sand (20–30 mesh; Fisher Scientific, Fairlawn, NJ, USA) to give a final porosity of 0.334 and (2) a heterogeneous medium with a low permeability patch consisting of Ottawa sand mixed with 10% kaolinite at one side. The homogeneous medium was used to examine the effect of saponin concentration on microbubble suspension flow, and the heterogeneous medium was used to determine the plug-flow characteristic of microbubble suspension in comparison with a saponin solution (i.e., direct injection of saponin solution into the test cell without microbubble generation). After flushing the cell with carbon dioxide and saturating it with distilled water, a saponin-microbubble suspension was injected into the cell from the bottom using a peristaltic pump at a flow rate of 10 mL/min. A blue dye, bromophenol blue was added (0.25 g/L) to the saponin solution before microbubble generation to improve microbubble observation.

2.5. Biodegradation enhancement by microbubble suspension

The enhancement in biodegradation by saponin-microbubble suspension was determined using a semi-batch type column (4 cm diameter × 15 cm height) made of Pyrex® glass with Teflon seals. The influent and effluent ports were connected to Viton® tubing in order to prevent phenanthrene and bacterial adsorption. The inlet part of the column (i.e., bottom section) was packed with glass beads (4 mm diameter) to generate uniform influent flow.

Phenanthrene was dissolved in acetone (10^4 mg/L) and was spiked onto 120 g of Ottawa sand to yield a phenanthrene concentration of 100 mg/kg. The acetone to sand ratio was set to 1:100 (v:w) in order to prevent a cosolvent effect [17]. The spiked sand sample was then mixed vigorously by spatula and left to stand in a fume hood for 3 h to evaporate the acetone. A 20-g portion of the spiked sand was taken for phenanthrene analysis, the remaining 100 g was packed at the bottom of the column and another 200 g of clean Ottawa sand was then overlaid on top of the spiked sand to give a final porosity of 0.346.

After the column was saturated with distilled water, one pore volume (PV) of microbubble suspension was injected into the phenanthrene-spiked sand column at a flow rate of 10 mL/min in an upflow manner. The microbubble suspension was generated from a 2.0 g/L saponin solution containing *B. cepacia* RPH 1 (10^8 CFU/mL) and inorganic salts at the same concentrations as described earlier. The column was incubated at 25 °C for 21 days to allow biological degradation. One pore volume of 2.0 g/L saponin solution containing the same bacterial species and inorganic salts, but without microbubble generation, was also injected in the same manner for comparison. An additional column was also prepared to determine the initial distribution of phenanthrene and mass recovery. All experiments were duplicated to confirm the results of each column set.

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