

Enhanced aquifer bioremediation with bioactive microbubble dispersions

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Introduction

Subsurface soil contamination by both organic waste and heavy metals is a problem widespread around the world due to intensive industrial activities. The release of these chemicals may be caused by accidental spillage, leakage from pipes and storage tanks, and improper disposal. Fuel hydrocarbons, for example, are one of the mostly widespread pollutants in soils (Scullion, 2006). With time, the contamination will spread and migrate into the aquifer, and will threaten the viability of the groundwater system. In many parts of the world, such as South Australia, China and Southwest North U.S., groundwater is the primary source of water for daily consumption (Scanlon et al., 2006). Contaminated groundwater can damage the health of humans and animals. The clean-up of contaminated subsurface and aquifer is important not just to safeguard our water supply, but also to maintain a healthy ecosystem.

The traditional approach in contaminated soil remediation emphasizes containment of the soils. The polluted soils are either excavated for disposal to landfills or kept in situ by building various types of barriers to prevent pollutants from moving off-site. In the past few decades, due to increasing land cost and regulatory changes, various treatment options have been developed to clean up the polluted soils.

Bioremediation is a rapidly developing biological treatment technology for the clean-up of a range of ubiquitous pollutants present in our environment (Atlas and Cerniglia, 1995). It is a process whereby living organisms, particularly microorganisms are used to degrade or transform hazardous contaminants to benign products. This technology, especially when it can be carried out *in-situ* in the aquifer environment, offers benefits of being effective, economical and eco-friendly when compared to chemical and physical methods. Nevertheless, a commonly cited drawback of in-situ bioremediation is that relatively long treatment time is needed, especially when the contaminants are recalcitrant to biological degradation. Many factors have been identified to hinder the rate of degradation, including: availability of molecular oxygen, absence of contaminant degrading bacteria, low availability of contaminant to the bacteria due to mass transfer limitation, heterogeneous aquifer environment, and lack of trace nutrients (Atlas and Cerniglia, 1995; Romantschuk et al., 2000; Makkar and Rockne, 2003; Abalos et al., 2004).

More recently, it has been proposed that microbubble dispersions – a suspension of minute fine gas bubbles generated from surfactant solution – could help to remedy the primary limitations in in-situ bioremediation.

Bioactive Microbubble Dispersions

Microbubble dispersion, which is also termed colloidal gas aphron (Sebba, 1987), is essentially a suspension of a large number of minute spherical gas bubbles encapsulated in a soapy liquid film in an aqueous surfactant solution. It is claimed that the structure of microbubbles is different from conventional bubbles that are simply surrounded by surfactant monolayer. Each microbubble, as per the structure proposed by Sebba (1987), is encapsulated in a soapy shell by three surfactant layers (see Figure 1). The presence of the ordered layers of surfactant film retards coalescence of the microbubbles, thereby increasing their stability (Sebba, 1987).

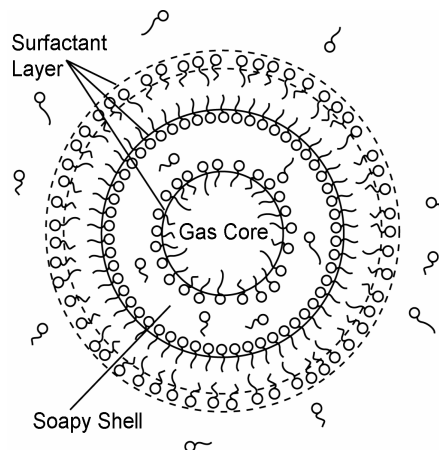


Figure 1. Microbubble structure proposed by Sebba (1987).

Microbubble dispersion is generated by intensive stirring (> 4,500 rpm) of surfactant solution with a spinning disk (Sebba, 1985). Synthetic chemical surfactants have been used in the past studies to make the microbubbles. Many of these chemical surfactants are produced by synthesis of petroleum products and are non-biodegradable, resulting in secondary contamination once they were injected into the subsurface, while some inhibit bacteria growth (Tiehm, 1994; Rothmel et al., 1998). Biosurfactants are secreted by microorganisms and are more biocompatible with the natural environment than the synthetic surfactants. In this study, rhamnolipid biosurfactant is used to make the microbubble dispersions. Rhamnolipid is primarily produced by the *Pseudomonas* bacteria species (Desai and Banat, 1997) and is one of the most known biosurfactant.

Experimental Methodology

Pseudomonas putida (*P. putida*) 852 a laboratory bacteria strain was grown overnight in 3.27g/l Bushnell-Hass broth (Sigma) with 2g/l D-glucose (BDH) as sole carbon source under constant shaking in a 30°C incubator. Bacteria cells were harvested by centrifugation and washed with 0.85% saline solution to remove soluble extracellular substances before being resuspended in surfactant solution to achieve a final cell concentration of approximately 1×10^9 cfu ml⁻¹. To enable enhanced visualisation of the bacteria by fluorescent microscopy, the cells were labelled with the fluorescent stain acridine orange (AO; Sigma). The stained cell suspension was added to 1,000 mg l⁻¹ rhamnolipid solution. Bioactive microbubble dispersion was generated from intensive mixing of the rhamnolipid mixture at 8,000 rpm for 3 minutes.

To investigate the interaction between bacteria and organic contaminant in the microbubble dispersion environment, n-Hexadecane (Sigma), an example petroleum hydrocarbon was stained with fluorescent dye Nile Red (Sigma). The hydrocarbon was then mixed with the bioactive microbubble dispersion in a microfuge tube. Subsequent microscopic observation of the microbubble-bacteria and microbubble-bacteria-hydrocarbon samples was carried out in cavity slides protected with cover slip.

Results and Discussions

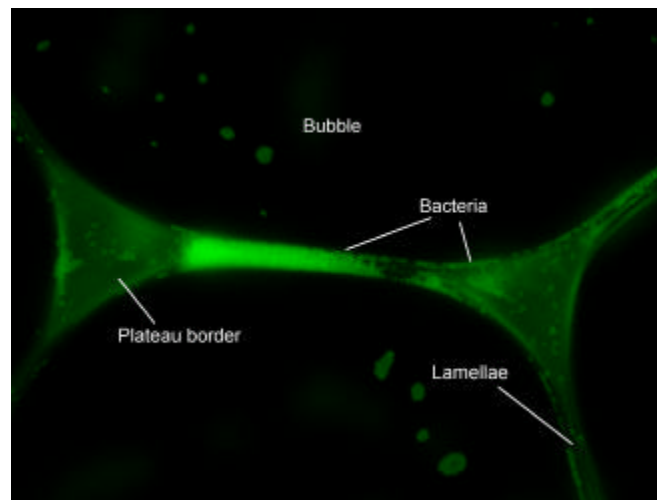


Figure 2. Fluorescence photomicrograph (400x) illustrating sorption of *P. putida* at the gas-liquid interface of the microbubbles.

The fluorescent image in Figure 2 reveals preferential sorption of *P. putida* cells at the gas-liquid interface of the microbubbles. It is consistent with observations by other researchers (Wan et al., 1994; Ripley et al., 2002). The sorption is irreversible because of capillary forces (Wan et al., 1994). The result suggests that microbubble dispersion could be an effective carrier of contaminant-degrading bacteria.

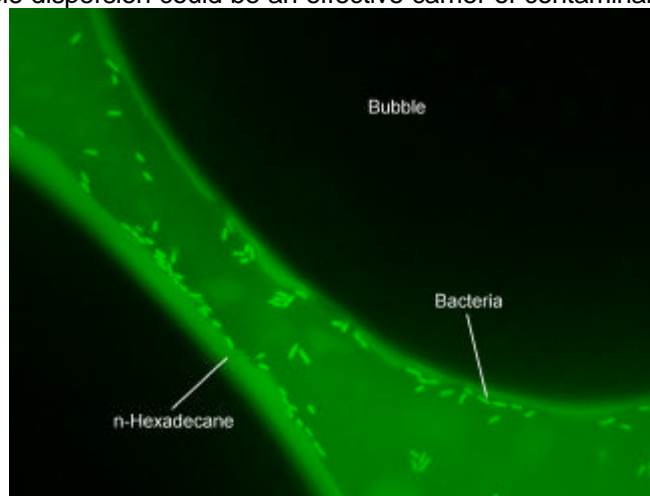


Figure 3. Fluorescence photomicrograph (1000x) illustrating association of *P. putida* with n-hexadecane at the gas-liquid interface of the microbubbles.

Figure 3 shows that n-Hexadecane adheres to the microbubble surface, with the microbial cell sorbed to the hydrocarbon layer. The arrangement suggests that microbubbles promote contact between the bacteria and hydrocarbon. It is likely that the aggregation would lead to enhanced degradation of the contaminant.

Conclusions

Our study revealed that microbubble dispersions could be an effective carrier of contaminant-degrading bacteria due to preferential sorption of the bacteria at the bubble surface. The dispersion also facilitates aggregation of the bacteria and contaminant, which could lead to enhanced contaminant degradation.

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