Role of Biosurfactants in Enhancing Bioremediation of Soils Contaminated by NAPLs

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CONTAMINATION of soils by non-aqueous phase liquids (NAPLs) has created serious problems in the past few years in many developed and developing nations. NAPL contaminated sites contain pollutants such as halogenated solvents, polychlorinated biphenyls (PCBs), pesticides, chlorinated benzenes, phenols and polycyclic aromatic hydrocarbon (PAHs). While current bioremediation technologies are seen as an affordable and effective tool for treating sites contaminated with NAPLs, these technologies are often not preferred due to the slow degradation process of NAPLs.

The effectiveness of the bioremediation process is typically governed by three important factors, namely, the presence of the degraders, the availability of the contaminants to the degraders and a favourable environment for the degradation to occur. Therefore, the intensity of biodegradation is highly dependent on microbial activity to degrade these contaminants and their rate limiting kinetics. Although very few microbes have been recognised as hydrocarbon degraders, the persistent characteristics possessed by NAPLs such as low aqueous solubility and recalcitrant response towards degradation makes it unavailable for microorganisms.

These conditions can substantially affect the microbial growth and biodegradation capabilities. This phenomenon is referred to as limited bioavailability. Limited bioavailability is often defined when the uptake rate by organisms is limited by physicochemical barrier between pollutant and the organism (Volkering *et al.*, 1997). Hence, the performance of the bioremediation can be improved by increasing the bioavailability of NAPLs.

Several technologies such as biostimulation and bioaugmentation are utilised in addressing problems on bioavailability to speed up the degradation rate of NAPLs. Biostimulation refers to the additional nutrients to stimulate the population of indigenous population microorganisms. Another approach is bioaugmentation which involves the addition of a specific strain that is capable of degrading NAPLs by increasing the bioavailability. However, these bioremediation enhancement strategies can incur high maintenance costs.

For that reason, the application of surface-active agents or surfactants has been considered as an alternative to improve the bioavailability of NAPLs. Yet, the knowledge on the interactions of surfactants and its capability in enhancing the bioremediation of NAPLs is relatively recent particularly on green surfactants or biosurfactants. Even though biosurfactants' potential in promoting the bioremediation process has been widely considered in many parts of the world, it is imperative that the mechanism of these emerging green surfactants should be grasped conceptually as measures to facilitate the remediation of contaminated lands.

SURFACTANTS AND BIOSURFACTANTS

Surfactants, of both biological and chemical origin, are amphipathic molecules that accumulate at interfaces, decrease interfacial tensions, and form aggregate structures that allow hydrocarbon solubilisation. Due to these properties, surfactants modify interfacial behaviour and impact the way other molecules behave at interfaces and in solutions.

In general, surfactants can be classified into two types, namely, chemically synthesised surfactants and biologically derived surfactants. Most common chemically synthesised types of surfactants are ethoxylates, ethylene, sorbitan esters, ester sulfonates, fatty acids and quaternary ammonium salts.

Chemical surfactants have been discovered to possess properties suitable for a vast variety of industrial applications. Their abilities in lowering the surface tension and increasing solubility have been applied to an important product in the oil industry to enhance oil removal and recovery. Nevertheless, the level of surfactant toxicity that could harm the environment and costly production are among the major drawbacks in their application.

Another group of surfactant is from biosurfactant-producing microorganisms of the genus *Pseudomonas*, *Candida* and *Bacillus* that is known to produce rhamnolipids, glycolipids and lipopeptide surfactants (Rahman and Gakpe, 2008). From the current perspective of moving towards green chemistry and the environment, biological surfactants or biosurfactants have exhibited in several studies similar emulsification properties and high surface activity characteristics compared to chemically synthesised surfactants (Banat, 1995). Table 1 illustrates some of the common biosurfactants with their producing microorganisms.

Table 1: Common biosurfactants with their producing microorganisms

Biosurfactants	Organisms	Sources	References
Rhamnolipids	*Pseudomonas aeruginosa*	Petrochemical wastewater, soap stock	(Whang *et al.*, 2009) (Wei *et al.*, 2005)
Lipopeptide	*Brevibacilisbrevis*	Oil field	(Haddad *et al.*, 2008)
Lipopeptide	*Azobacterchroococcum*	Water sample	(Thavasi *et al.*, 2009)
Lipopeptide	*Nocardiopsis alba*	Marine actinomycetes	(Gandhimathi *et al.*, 2009)
Rhamnolipids	*Pseudoxanthomonas sp.* PNK-04	Coal field	(Nayak *et al.*, 2009)
Surfactin	*Bacillus subtilis ATCC*	Petroleum sludge, waste soybean oil	(Lee *et al.*, 2008; Pornsunthorntawee *et al.*, 2008)
Mannosylerythritol lipids	*Candida Antarctica*	Vegetable oil	(Kim *et al.*, 2002)
Bioemulsan	*Gordoniaspp* BS29	Diesel contaminated soil	(Franzetti *et al.*, 2009)

Compared to synthetic surfactants, biosurfactants have the advantages of being less toxic, biodegradable and inexpensive as it can be produced using cheaper substrates. These advantages have triggered interests in the possible usage of biosurfactant technology to enhance the biodegradation performance in NAPL contaminated soil. Thus, its interactions with microorganisms and NAPLs would provide a better insight into the biological response on the addition of surfactant to enhance the degradation of NAPLs.

SURFACTANTS MECHANISM

In order to facilitate the bioavailability of NAPLs, the use of surfactants is commonly applied through three main mechanisms, namely, emulsification, solubilisation and mobilisation. The emulsification of NAPLs can reduce the surface tension and interfacial tension between an aqueous and non-aqueous phase. The dissolution of NAPLs is initiated when a surfactant monomer increases the contact between the soil and NAPLs, which promotes the separation of NAPLs entrapped in the soil media. The concentration of surfactants, surface tension and its relation to critical micelle concentration (CMC) is elucidated in Figure 1.

CMC is described as a measurement for the efficiency of surfactant. In reducing the surface and interfacial tension, surfactant monomer exists below the CMC. Low CMC indicates that less surfactant is needed to saturate interfaces and form micelles. The solubilisation of NAPLs will commence once the formation of micelles takes place at higher CMC. The presence of surfactant micelles will promote the partitioning of NAPLs from soil into the micelle hydrophobic core. Consequently, solubilisation NAPLs leads in mobilising the pollutant, which in turn improves their bioavailability.

The interaction between microorganisms, pollutant, soil and surfactants can be further described in Figure 2. NAPLs entrapped in the soil matrix can be dispersed by the sorption of surfactant molecules onto soil (1). Surfactant monomers are capable of mobilising the NAPLs into the water phase leading towards a micelle formation where NAPLs are solubilised into the hydrophobic core (2,3). As for the microorganisms, once the NAPLs are available, it can be utilised directly by the sorption of micelle onto microorganism (4), partitioning of NAPLs between the water phase and micelles (5), directly from the water phase (6) or directly from the solid phase (7).

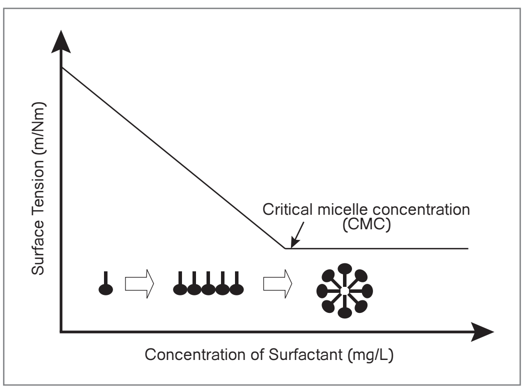

Figure 1: Effect of surface tension and concentration of surfactant on the formation of surfactant micelle

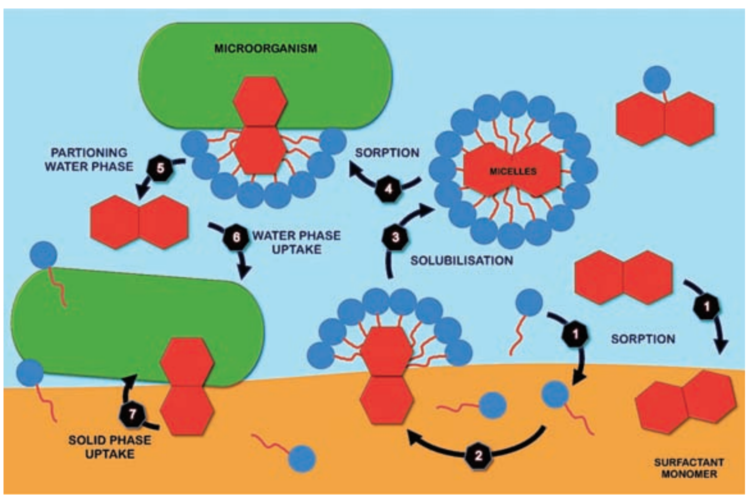

Figure 2: Mechanism of biosurfactants interaction between microorganism, soil and pollutant (Adapted from Volkering, 1997)

On the other hand, these relative interactions are strongly influenced by the physical state of the pollutant; i.e. whether it is adsorbed, absorbed or trapped in the soil. Although surfactants play an important role in promoting biodegradation, it is necessary to understand that the effectiveness of surfactant aided bioremediation depends on the selection of the surfactant and its most conducive working environment.

BIOSURFACTANT POTENTIAL IN BIOREMEDIATION APPLICATION

The emerging technology in applying biosurfactant to enhance the bioremediation of hydrocarbon contaminated soil has shown much success. Biosurfactants have considerable potential for remediation application because biosurfactants have better biocompatibility, lower toxicity and higher degradability compared to synthetic surfactants (Ron and Rosenberg, 2002; Volkering *et al.*, 1997). The ability of biosurfactant in solubilising NAPLs has been widely reported.

Biosurfactant (lipopeptides, rhamnolipids and surfactin) producing bacteria such as those belonging to the genus Pseudomonas, *Brevibacillus* and *Bacillus* have been reported to enhance phenanthrene and anthracene availability (Das *et al.*, 2008; Reddy *et al.*, 2010; Tecon and Van der Meer, 2010). In addition, some synthetic surfactants such as Triton X-100 and Tween 80 have been reported to accelerate the initial degradation of phenanthrene and fluoranthene (Avramova *et al.*, 2008; Hickey *et al.*, 2007).

On the other hand, there have been claims whereby the presence of rhamnolipid and Brij 80 resulted in slight phenanthrene degradation and exhibited an inhibitory effect (Lee *et al.*, 2007; Shin *et al.*, 2004; Volkering *et al.*, 1997). Findings by various researchers indicated that most of the surfactant helps in degrading insoluble pollutants while others reported that the presence of surfactant has inhibited the degradation process. Nevertheless, the potential of biosurfactants in many cases proved to be more effective than chemical surfactants and have the added benefit of being biodegradable.

CONCLUSION AND FUTURE CHALLENGES

Bioremediation is not widely practiced in most developing countries although there has been some significant success where bioremediation is employed. As technology is gradually being applied, there are many uncertainties in predicting the field performance. With a better understanding of the theoretical mechanism of biosurfactant, its use as a practical solution for the bioremediation of hydrophobic contaminants can be further explored for implementation. Future challenges in improving and developing biosurfactant technology to enhance the degradation of NAPLs must be extensively investigated and will require interdisciplinary efforts before it can be adopted as a viable green surfactant in bioremediation. ■

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