



## Potential application of a biosurfactant in phytoremediation technology for treatment of gasoline-contaminated soil



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### ABSTRACT

Biosurfactants are amphiphilic compounds excreted extracellularly that contain hydrophobic and hydrophilic moieties, allowing them to accumulate between the fluid phases on an organism and thus reduce the surface and interfacial tension. The objectives of this study were to evaluate the potential application of a biosurfactant to the phytoremediation of gasoline-contaminated soil and compare it to other additives such as hydrocarbon degrading bacteria, sodium dodecyl sulphate (SDS) and bacterial culture supernatant. The results showed that the biosurfactant removed a significant amount (up to 93.5% of the total petroleum hydrocarbons (TPH) compared with the other additives that removed only 85.4% (bacteria), 70.3% (culture supernatant) and 86.3% (SDS). Kinetic analysis showed that the phytoremediation of gasoline-contaminated soils by the biosurfactant fitted pseudo-second-order kinetics with a coefficient of determination ( $R^2$ ) of 0.9318 and a second-order rate constant ( $k_2$ ) of 0.0032 (g TPH/kg plant d). Thus, biosurfactants have strong potential as supporting biocatalysts to increase the performance of phytoremediation technology for soil treatment.

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### 1. Introduction

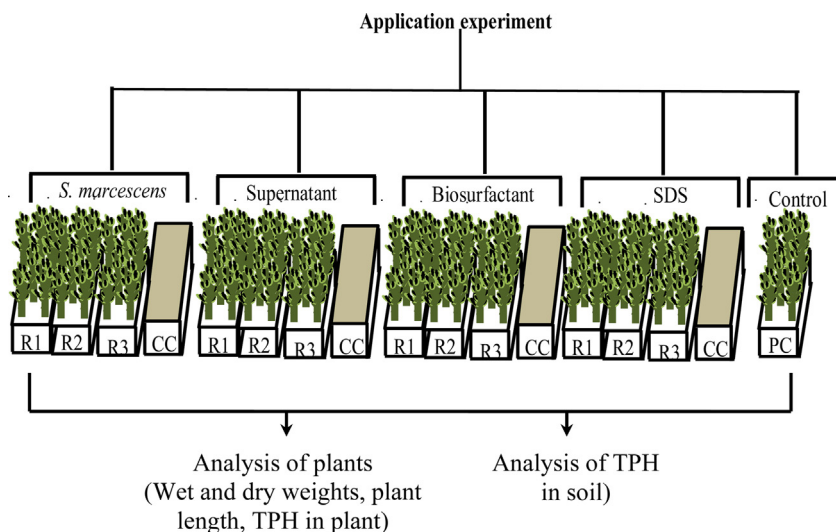
Biosurfactants are considered environmentally friendly because they are relatively nontoxic and biodegradable. Biosurfactants have unique structures that are only recently beginning to be appreciated for their potential application to many different facets of industry, ranging from biotechnology to environmental clean-up (Pornsunthorntawee et al., 2008). Some important selection criteria for potential biosurfactant applications in pollution are their environmental physicochemical behaviour, adsorption behaviour, ability to reduce surface tension and increase solubility, detergent power, wetting capability, and foaming capacity. The petroleum industry has traditionally been the major user of biosurfactants in

applications such as the enhancement of oil removal by increasing the solubility of petroleum components in contaminated soil and in the aquatic environment (Mulligan, 2005; Sanket and Yagnik, 2013). Biosurfactants can be used (i) to increase the mass transfer of petroleum hydrocarbons from activated sludge liquid to bacterial cells and (ii) to change the hydrophobicity of the cell, thereby enhancing direct cell attachment to petroleum hydrocarbons (Sponza and Gok, 2011).

Most biosurfactants produced by modern microbial technology can be used in various applications, such as reducing the bio-corrosion and fouling-related degradation of hydrocarbons within oil reservoirs and as enzymatic and bio-catalysis of hydrocarbons (Perfumo et al., 2010). Biosurfactants are amphiphilic molecules, which have distinguishable hydrophilic head and hydrophobic tail moieties that decrease surface and interfacial tension by accumulating at the interface between immiscible fluids. These properties confer varying degrees of polarity and hydrogen bonding at different interfaces, such as water–oil or air–water interfaces, resulting in detergency, foaming, emulsifying, and disparity traits (Cameotra and Makkar, 2010; Singh et al., 2007). Biosurfactants and

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**Fig. 1.** Experimental setup of gasoline-contaminated soil treatments for the phytoremediation process by *S. marcescens*, culture supernatant, biosurfactant and SDS.

bioemulsifiers are not only used for bioremediation in the petrochemical industry but are also potentially useful for cleaning oil storage tanks, reducing the viscosity of heavy oil, increasing flow through pipelines, and stabilizing fuel–water–oil emulsions (Jain et al., 2012). The capability of biosurfactants for biodegradation is probably due to an increase in cell surface hydrophobicity, which allows direct contact between the cell and the hydrocarbon droplets (Luis et al., 2000). Surfactants reduce the surface/interfacial tension at air–water and water–oil interfaces by increasing the aqueous solubility of non-aqueous phase liquids (NAPLs), increasing the aqueous surfactant concentration and decreasing the interfacial tension; the monomers aggregate or create a micro-emulsion, leading to micelle formation (Abdolhamid et al., 2009).

The main goal of this research was to investigate the potential of biosurfactants as catalysts in phytoremediation technology for the treatment of gasoline-contaminated soil. The biosurfactant used in this study was extracted from the bacterium *Serratia marcescens*. The performance of the biosurfactant in the phytoremediation of gasoline-contaminated soil was compared to that of other additives such as hydrocarbon-degrading bacteria (HDB), culture supernatant, and a commercial surfactant (sodium dodecyl sulphate, SDS).

## 2. Materials and methods

### 2.1. Experimental setup

The experiment was conducted under greenhouse conditions with dark-light cycle at the Universiti Kebangsaan in Malaysia. Seventeen glass aquariums were used throughout this study which includes three replicates for each additive (four additives) and one plant control (without gasoline-spiked soil). Each aquarium had dimensions of 60 × 30 × 30 cm ( $L \times W \times D$ ) and was filled with 30 kg of a mixture of 50:50 (w/w) garden soil:sand and planted with *Ludwigia octovalvis*. Gasoline-contaminated soil was simulated by spiking 2 g gasoline/kg of *L. octovalvis* to the soil mixture. The same plants were also planted in a control without gasoline. Fig. 1 shows the experimental design with four treatments (different additives) and one control. Three replicates of treatment (R1, R2 and R3) of each additive type were set up. An unplanted aquarium was used as a contamination control (CC), and another aquarium without gasoline-spiked soil was used as a plant control (PC).

Acetone was used as a solvent and combined with gasoline at a ratio of 1:1 (v/v). This mixture was then sprayed onto the soil,

which was stirred until homogeneous. The soil was left to stand for 5 to 7 days prior to planting in order to ensure that the gasoline well permeate into the surface, middle and bottom of soil level in glass aquarium. After this time, 18 healthy, three-month-old *L. octovalvis* plants were transplanted into each aquarium that contained gasoline-contaminated soil. All experimental plants were frequently watered per week with distilled water at a fixed calculated volume of 7.8 L per aquarium where the bulk density of the soil was 0.26 mL/g soil mixture. Sampling was conducted on days 0, 7, 14, 28, 42, and 72.

### 2.2. Biosurfactant as a biocatalyst in phytoremediation of gasoline-contaminated soil

A biosurfactant was produced by inoculating *S. marcescens* in 100 mL of MSM containing 5% glycerol as the carbon source, 4 g/L peptone and 5 g/L ammonium sulphate as the nitrogen source. The pH of the medium was adjusted to 8.0 using 1 N NaOH and HCl, and then the medium was autoclaved at 121 °C for 15 min. After autoclaved, the culture was incubated for five days at 37 °C with shaking at 150 rpm. After incubation, the *S. marcescens* cells were removed by centrifugation at 4 °C at a speed of 8000 rpm for 15 min. The supernatant was saved and adjusted to pH 2 with 1 N HCl. The biosurfactant was collected via centrifugation at 4 °C at a speed of 12,000 rpm for 15 min. The dry pellet was lyophilized, producing the biosurfactant in the form of a white sediment. This method was a modification of a method from a previous study by Anandaraj and Thivakaran (2010).

Approximately 1.5 g of the extracted biosurfactant was dissolved in 1 L of distilled water to prepare the biosurfactant solution that was then mixed into the soil at a bulk density of 10%. The weight of the soil mixture in the glass basin was 30 kg. The total water volume of the aquarium was 7800 mL, so to achieve a 10% bulk density, 780 mL (Eq. (1)) of the biosurfactant solution was poured into the aquarium for the phytoremediation of the gasoline-contaminated soil.

$$10\% \text{ bulk density} = 10\% \times 7800 = 780 \text{ mL} \quad (1)$$

### 2.3. Addition of the hydrocarbon-degrading bacteria (HDB) *S. marcescens*, SDS and culture supernatant for phytoremediation

This test examined the effects of adding the HDB *S. marcescens*, SDS and culture supernatant to gasoline-contaminated soil. The

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