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Effect of rhamnolipid solubilization on hexadecane bioavailability: enhancement or reduction?

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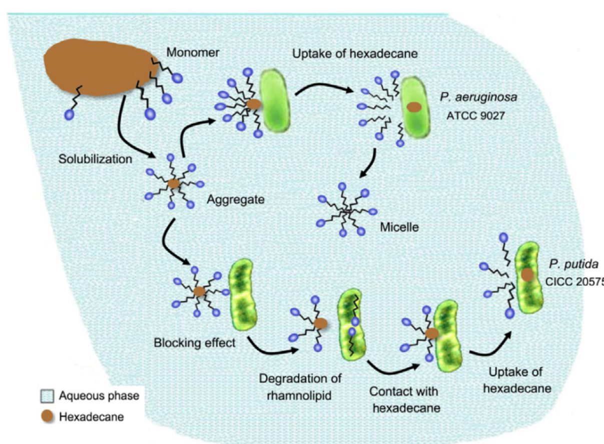
HIGHLIGHTS

- Rhamnolipid-solubilization increases availability of hexadecane for *P. aeruginosa*.
- Rhamnolipid-solubilization reduces bioavailability of hexadecane for *P. putida*.
- Blocking effect of surfactants accounts for reduction in hexadecane bioavailability.
- Bacterial compatibility is important to overcome the blocking effect.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, liquid culture systems containing rhamnolipid-solubilized, separate-phase, and multi-state hexadecane as the carbon source were employed for examining the effect of rhamnolipid solubilization on the bioavailability of hexadecane. Experimental results showed that the uptake of rhamnolipid-solubilized hexadecane by *Pseudomonas aeruginosa* ATCC 9027, a rhamnolipid producing strain, was enhanced compared to the uptake of mass hexadecane as a separate phase, indicating rhamnolipid solubilization increased the bioavailability of hexadecane for this bacterium. For *Pseudomonas putida* CICC 20575 which does not produce but degrade rhamnolipid, the uptake of either rhamnolipid-solubilized hexadecane or multi-state hexadecane was inhibited. The reduction of bioavailability was assumed to be the consequence of the blocking effect caused by the partition of rhamnolipid molecules at the hexadecane-water interface. The results show that how rhamnolipid solubilization changes the bioavailability of hexadecane depends on the bacterial compatibility to rhamnolipid. The study adds insight into the knowledge of biosurfactant-associated bioavailability of hydrophobic organic compounds (HOCs), and is of importance for application of biosurfactants in bioremediation of HOCs.

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1. Introduction

Water and soil contamination by hydrophobic organic compounds (HOCs) due to anthropogenic activities, e.g., oil exploitation and transportation, downstream petroleum product manufacturing and processing, and improper discharge of industrial wastes, has attracted increasing attention in recent decades. Bioremediation technology, which has high ecological significances, is widely used for cleaning of these contaminations. However, the hydrophobic nature (low water solubility) of HOCs is a major obstacle for their uptake by microbes since most of these compounds either resided in non-aqueous phase liquids (NAPLs) or adsorbed on soil, which greatly limit their bioavailability [1–4]. Unlike bioaccessibility, bioavailability does not only refer to whether the organism has access to the chemical in the environment. Bioavailability is defined as "the compound is freely available to cross the cellular membrane of an organism from the medium the organism inhabits at a given time" [5,6].

Surfactants may play a role in degradation of HOCs by enhancing the apparent solubility and hence greater partitioning of these compounds in aqueous phase [7–9]. Such surfactant-aided solubilization of HOCs is usually based on aggregate-formation mechanism [10,11]. Biosurfactants are promising alternatives to synthesized surfactants due to their advantages such as environmental compatibility and high efficiency [12–14]. For example, biosurfactants generally show stronger hydrocarbon solubilization capacity than synthetic surfactants [15,16], and significant solubilization of alkanes at concentrations below critical micelle concentration (CMC) was observed for rhamnolipid [17,18], which is the most extensively studied and commonly used biosurfactant [19,20].

Petroleum hydrocarbons are some of the most intensively studied HOCs. For hydrocarbon degradation, both positive and negative effects of biosurfactant-solubilization were observed in prior studies. Zhang and Miller [21] observed in a batch liquid degradation experiment that 20% of octadecane as the carbon source was mineralized in 84 h by *Pseudomonas aeruginosa* ATCC 9027 in the presence of rhamnolipid, compared to 5% of mineralization without rhamnolipid. The enhancement of degradation was attributed to rhamnolipid-facilitated octadecane dispersion by forming octadecane/rhamnolipid aggregates (octadecane solubilized in rhamnolipid micelles). These aggregates, as tiny hydrocarbon reservoirs, are likely to enhance mass transfer of hydrocarbon to microbial cells [22,23]. Guha and Jaffé [24] formulated a mathematical model to describe the interaction of the biomass-contaminant-water surfactant system, and the results showed that a fraction of the micellar-phase phenanthrene is directly bioavailable. Further researches showed that surfactant sorption and the formation of hemi-micelles on the bacterial cell surface have a strong influence on the surfactant-enhanced bioavailability of HOCs [10,25,26].

A major concern regarding this aggregate-based solubilization mechanism is that the biosurfactant layer on the surface of aggregates could be a barrier, which blocks microbial cells from capturing the inside hydrophobic compounds. In our prior study it was observed that a *Pseudomonas aeruginosa* strain failed to grow on the hexadecane in thoroughly solubilized form in the presence of rhamnolipid at a concentration higher than CMC [27]. Similar observation that microbial cells were not able to directly ingest compounds closed in surfactant micelles was also reported by Pęziak et al. [28]. Variation of the results of these studies indicates uncertainty for biosurfactant solubilization to enhance hydrocarbon bioavailability. An insight into such uncertainty is required for better application of biosurfactants in bioremediation.

This study is focused on examining whether biosurfactant solubilization of hydrocarbons enhances the bioavailability of hydrocarbons, and why if it does. Hexadecane in various forms was used as the carbon source for degradation by *Pseudomonas aeruginosa* ATCC 9027 and *Pseudomonas putida* CICC 20575. These forms included: a) rhamnolipid-solubilized hexadecane, b) mass hexadecane, and c) multi-state hexadecane (including rhamnolipid-solubilized, emulsified and mass hexadecane). Dur-

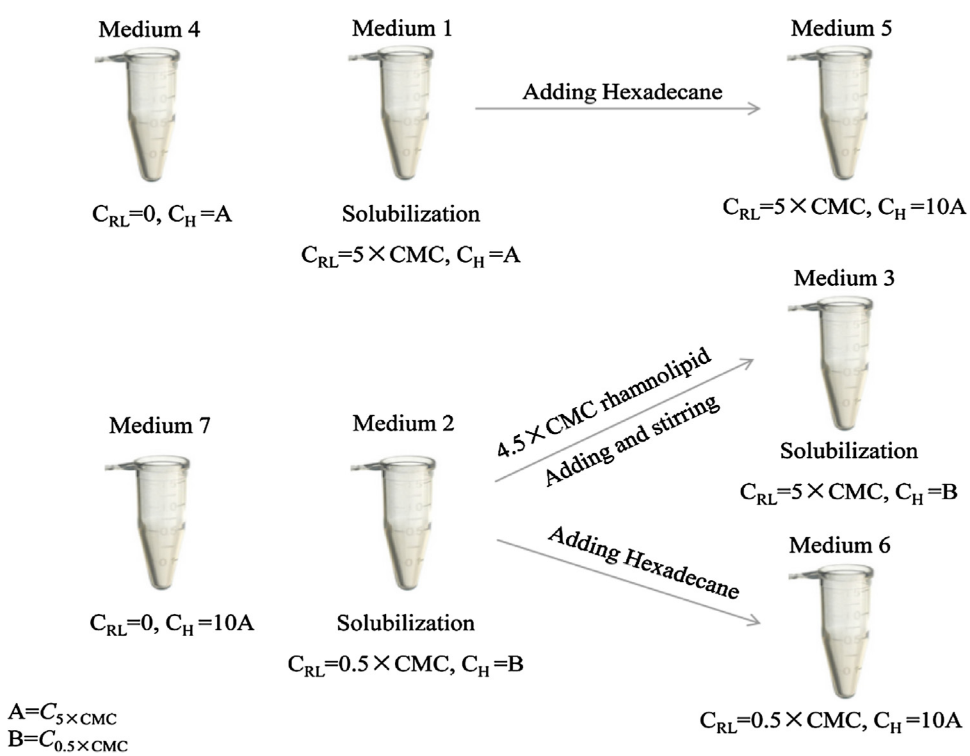

Fig 1. Preparation of seven sets of MSM-based culture medium.