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# Coal induced production of a rhamnolipid biosurfactant by *Pseudomonas stutzeri*, isolated from the formation water of Jharia coalbed

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## HIGHLIGHTS

- *Pseudomonas stutzeri* was isolated from the formation water of an Indian coalbed.
- The *P. stutzeri* isolate produced biosurfactant in response to coal supplementation.
- The coal induced biosurfactant produced by *P. stutzeri* was a rhamnolipid.
- *P. stutzeri* produced more biosurfactant with lignite than bituminous or anthracite.

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## ABSTRACT

A strain of *Pseudomonas stutzeri* was isolated form an enrichment of perchlorate reducing bacteria from the formation water collected from an Indian coalbed which solubilized coal and produced copious amount of biosurfactant when coal was added to the medium. It produced maximum biosurfactant with lignite coal followed by olive oil and soybean oil which was able to emulsify several aromatic hydrocarbons including kerosene oil, diesel oil, hexane, toluene etc. Haemolytic test, growth inhibition of *Bacillus subtilis* and FTIR analysis showed rhamnolipid nature of the biosurfactant. The stability of the coal induced biosurfactant in pH range of 4–8 and up to 25% NaCl concentration and 100 °C temperature suggests that due to its ability to produce biosurfactant and solubilize coal *P. stutzeri* may be useful in the coalbed for *in situ* biotransformation of coal into methane and in the bioremediation of PAHs from oil contaminated sites including marine environments.



## 1. Introduction

India is the 5th largest proven reservoir of coal in the world utilization of which as energy source results into atmospheric pollution including emission of green house gases (Hayward, 2010). *In situ* microbial conversion of coal into methane gas is considered an environment friendly alternative to produce the clean energy source. Thus, microbial biotransformation of coal into simpler, low molecular weight product is considered an economic and effective way of improving the utility of coal. A variety of microorganisms including fungi (Hatakka, 1994), bacteria (Standberg and Lewis, 1988) and actinomycetes (Quigley et al., 1989a) are capable of coal solubilization/degradation. Studies suggested the mechanism involved in coal biotransformation mainly comprises of (1) microbial production of alkaline substances (Quigley et al., 1989b), (2) biocatalysts especially produced by fungi (Hatakka, 1994), (3) metal ion chelators and surface active agents (Fakoussa, 1988; Polman et al., 1994). Surfactant decreases the coal surface tension and increases the solubility of coal in aqueous solutions (Fakoussa, 1988; Yuan et al., 2006). It is quite possible that both enzymatic and non enzymatic mechanisms may be involved in coal biotransformation process in the same microorganism. In a study it was shown that chemically synthesized surfactants and surfactant from *Bacillus licheniformis* and *Candida bombicoal* were able to solubilize different portion of coal (Breckenridge and Polman, 1994; Polman et al., 1994).

Biosurfactants can be used in coal dust control, enhancement of gas permeability of coal, removal of coal ash and bioremediation of soil and groundwater contaminated with hydrocarbon. Microorganisms producing biosurfactant can participate in oil degradation. Alternatively, they can function in a bacterial consortium, supplying the emulsifier for other bacteria that carry out the degradation of hydrocarbons (Ron and Rosenberg, 2002). In addition biosurfactant can be used in petroleum industry for transportation of crude oil, enhanced oil recovery by increasing the apparent solubility of petroleum components and effectively reducing the interfacial

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