Advances in Environmental Research, *Vol. 9, No. 3 (2020) 215-232*
DOI: https://doi.org/10.12989/aer.2020.9.3.215

In situ isolation and characterization of the biosurfactants of *B. Subtilis*

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(Received November 26, 2019, Revised September 11, 2020, Accepted September 24, 2020)

Abstract. Crude oils are essential source of energy. It is majorly found in geographical locations beneath the earth's surface and crude oil is the main factor for the economic developments in the world. Natural crude oil contains unrefined petroleum composed of hydrocarbons of various molecular weights and it contains other organic materials like aromatic compounds, sulphur compounds, and many other organic compounds. These hydrocarbons are rapidly getting degraded by biosurfactant producing microorganisms. The present study deals with the isolation, purification, and characterization of biosurfactant producing microorganism from oil-contaminated soil. The ability of the microorganism producing biosurfactant was investigated by well diffusion method, drop collapse test, emulsification test, oil displacement activity, and blue agar plate method. The isolate obtained from the oil contaminated soil was identified as *Bacillus subtilis*. The identification was done by microscopic examinations and further characterization was done by Biochemical tests and 16SrRNA gene sequencing. Purification of the biosurfactant was performed by simple liquid-liquid extraction, and characterization of extracted biosurfactants was done using Fourier transform infrared spectroscopy (FTIR). The degradation of crude oil upon treatment with the partially purified biosurfactant was analyzed by FTIR spectroscopy and Gas-chromatography mass spectroscopy (GC-MS).

Keywords: biosurfactant; crude oil; well diffusion method; drop collapse test; emulsification test; oil displacement activity; blue agar plate method; 16SrRNA gene sequencing; FTIR; GC-MS

1. Introduction

Petroleum products are often released either accidentally or intentionally into the environment through the spill, leakage, transport, or other incidents that affect residential, agricultural or recreational land use and it also affects the water areas. It damages the ecosystems and it negatively affects the health of plants, animals, and human beings. Therefore, remediation of oil-polluted sites by various chemical and biological methods has become crucial to control oil pollution. Most of the hydrocarbons that are present in oils are insoluble in water and therefore, the process of remediation becomes complex and needs several chemicals and reagents, which otherwise causes deleterious effects to the environment. Bioremediation by using microorganisms

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http://www.techno-press.org/?journal=aer&subpage=7 ISSN: 2234-1722 (Print), 2234-1730 (Online)

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