UNIVERSITI TEKNOLOGI MARA

APPLICATION OF BIOSURFACTANT IN ENHANCING BIOREMEDIATION OF ANTHRACENE AND BENZO(A)PYRENE IN CONTAMINATED POROUS MEDIA

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Biodegradation of PAHs in contaminated sand is a slow process due to the low bioavailability of these persistent pollutants, owing to their low aqueous solubility and high sorption tendency to soil. Biosurfactant could enhance the bioavailability of PAHs and accelerate the bioremediation process. Therefore, the aspirations of the present study were to investigate the feasibility of using biosurfactant produced by isolated bacteria from waste streams to enhance the bioremediation of PAH contaminated sand, as well as to develop a more effective bioremediation technology. In this study, bacteria from three different waste streams were tested through qualitative and quantitative screening test. From the tests, three bacteria isolated from palm oil mill effluent were found to have biosurfactant production and identified using molecular technique. These biosurfactant-producing bacteria namely *Bacillus subtilis*, *Bacillus tequilensis* and *Bacillus* sp. were capable of reducing surface tension of water from 72 mN/m to 27 mN/m which indicate their effectiveness in producing biosurfactant. The individual treatment of PAH biodegradation was conducted using uncultured *Pseudomonas* sp. and *Pseudomonas stutzeri* as PAH-degrading bacteria. The results showed that uncultured *Pseudomonas* sp. and *P.stutzeri* were able to degrade 9 - 20% anthracene and contributes higher degradation percentage of benzo(a)pyrene where these bacteria successfully degraded 22 – 37% of benzo(a)pyrene. In the sequential treatment, supplementing Triton X-100 on the third day resulted in an increase degradation of 12% anthracene for uncultured *Pseudomonas* sp. and 21% anthracene for *P.stutzeri*. The addition of Triton X-100 also exhibited an increased on the biodegradation of benzo(a)pyrene. *P.stutzeri* was able to degrade 44% benzo(a)pyrene. In the presence of surfactin, an increased degradation of 11 - 17% anthracene by uncultured *Pseudomonas* sp. and *P.stutzeri*. Uncultured *Pseudomonas* sp. and *P.stutzeri* successfully increased the percentage removal of benzo(a)pyrene to 31% and 51% respectively with the addition of surfactin. The synergistic effects of PAH-degrading and biosurfactant-producing bacteria showed that the biodegradation PAHs was significantly increased by the inoculation of *B.subtilis* and *B.tequilensis*. The synergistic treatment between *P.stutzeri* and *B.subtilis* was considered the most effective as the combination effectively removed 80% of benzo(a)pyrene. The performance of PAH-degrading bacteria in degrading anthracene and benzo(a)pyrene significantly improved with the co-presence of biosurfactant-producing bacteria. Therefore, biosurfactant released by *Bacillus subtilis* and *Bacillus tequilensis* in soil effectively enhanced the rate and extend of PAHs biodegradation by the PAH-degrading bacteria. From this study, a method was developed to enumerate PAH-degrading bacteria and biosurfactant-producing bacteria in the biodegradation experiment using Mannitol salt and MacConkey agar. Apparently, this is the only study to date that accentuate the interaction of biosurfactant-producing bacteria instead which focuses on its ability to improve the bioavailability of PAH in sand. A mathematical model for the biodegradation of PAH in contaminated sand was also developed converging on the synergistic effect of PAH-degrading and biosurfactant-producing bacteria. The proposed models were able to provide prediction of benzo(a)pyrene removal for planning and designing the remediation strategy in order to minimize impact of PAH contamination.

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