UNIVERSITI TEKNOLOGI MARA

REMOVAL OF PHENANTHRENE BY *RHODOCOCCUS ZOPFII* IN AN ATTACHED NATURAL BIOFILM

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Thesis submitted in fulfillment of the requirements for the degree of **Doctor of Philosophy** (Civil Engineering)

Faculty of Civil Engineering

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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

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds, which present as contaminants in wastewater treatment plants. The bacterial mitigation technique was employed in this study, which was *Rhodococcus* species to degrade one of the potential PAHs, phenanthrene. Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) were implemented for the identification of functional groups and the absorption characteristic on the natural biofilm surface. The polymerase chain reaction (PCR) method has successfully identified and verified the bacteria as Rhodococcus zopfii DSM 44. In this study, a complete biodegradation of 1mg/L phenanthrene by R. zopfii was achieved within 7 days, indicating the attachment of R. *zopfii* on the natural biofilm surface. The biodegradation rate of phenanthrene in the presence of natural biofilm was 0.26 mg/L/day (R²=0.95), which was 1.52 times higher than that without natural biofilm (0.17 mg/L/day) (R²=0.95). Approximately, 46%, 99%, and 100% biodegradation of phenanthrene by R. zopfii significantly occurred in the wastewater at initial bacteria populations of 10⁶, 10⁸, and 10¹⁰, respectively. The biodegradation rate of phenanthrene was accelerated by 54% in the presence of natural biofilm and high initial bacteria population (10^{10}) to 0.33 mg/L/day (R²=0.93), which was 1.08 times higher than the biodegradation rate at 10^8 bacteria population (0.30 mg/L/day) (R²=0.94). Meanwhile, the optimum pH condition required to enhance the biodegradation of phenanthrene by R. zopfii was at neutral pH (pH 7.0-7.2), with 0.44mg/L/day (R²=0.86) biodegradation rate was achieved at an initial pH of 7, which was 1.30 times higher than that at initial pH 7.2 (0.34mg/L/day) (R^2 =0.98). In this study, coumarin, catechol, and phthalic acids were identified in the phenanthrene degradation, and detailed phenanthrene pathway was constructed based on the metabolic results. The two (2) mathematical equations were successfully formulated to simulate the whole system of biodegradation of phenanthrene by R. zopfii with natural biofilm, namely, non-inhibition (below 50mg/L) and inhibition (above 50mg/L) equations. This study ultimately contributes to the remediation efficiency of municipal wastewater containing phenanthrene. Moreover, this study verified the non-toxic intermediate compound on the by-products study of degradation of phenanthrene.

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