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ADAPTATION OF IMMOBILIZED *Rhodococcus* CELLS TO INCREASING PETROLEUM CONCENTRATIONS IN A COLUMN BIOREACTOR

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Abstract

Biotreatment of petroleum contaminated wastewaters is an important aspect of environmental biotechnology based on microorganisms capable of degrading petroleum hydrocarbons, and it is regarded as an effective and potentially inexpensive cleanup technology increasingly applied today. Bioreactors with immobilized bacterial cells allow enhancing hydrocarbon bio-oxidation, adapting microorganisms to toxic compounds, and maintaining cell viability at extreme contaminant loads. *Rhodococcus* bacteria possessing broad catabolic and unique enzymatic capabilities are of ecological and biotechnological significance and play an important role in petroleum degradation in the environment. Since crude oil is a complex substrate composed of a wide range of hydrocarbons, we used the association of strains, *Rhodococcus ruber* IEGM 615 and *Rhodococcus opacus* IEGM 249 from the Regional Specialized Collection of Alkanotrophic Microorganisms (acronym IEGM, WFCC # 768). These strains differ by the range of substrates utilized suggesting complete degradation of petroleum hydrocarbons (www.iegm.ru/iegmcol/index.html). Bacteria were adapted to increasing petroleum concentrations in a column bioreactor. Oil-contaminated water (2%, v/v) was pumped through (flow rates, 0.6, 1.2 and 2.0 mL/min) the bioreactor packed with sawdust-immobilized rhodococcal cells at room temperature for two weeks. Maximal degradation of 88-99% was obtained for C_{11} to C_{19} aliphatic hydrocarbon fraction. Longer chain aliphatics and polycyclic aromatic hydrocarbons were biodegraded to a lower extent (30-55%). It was found that reuse of immobilized rhodococcal cells increased the efficiency of petroleum degradation in the reactor apparently due to *Rhodococcus* cell adaptation to toxic petroleum components. Moreover, the adapted bacterial cells isolated from the bioreactor after the operation cycle were more resistant to mono- (benzene, phenol) and polyaromatic (phenanthrene, anthracene) hydrocarbons and antibiotics. Currently, possible mechanisms of *Rhodococcus* cell adaptation to high concentrations of petroleum hydrocarbons are being investigated concerning to changes in cell-envelope lipid composition, plasmid profile and increase in the gene expression responsible for hydrocarbon biodegradation.

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