Estimation of Phenol Degradation by Selected Bacteria Isolated from Contaminated Site in Kolonnawa

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Abstract

Surface and ground water contamination by aromatic hydrocarbons like phenol and its derivatives significantly contribute to water pollution. As phenol is a highly toxic and one of the prime pollutants in the industrial effluent, removal of phenol is required. Bioremediation is a holistic approach that can be used to remove phenol in contaminated wastewater. Isolation and identification of phenol degrading bacteria will provide vital tools for the bioremediation process. Therefore, this study was conducted to examine the phenol biodegradation ability of isolated bacteria from a petroleum contaminated site in Sri Lanka.

Wastewater samples were collected from the petroleum contaminated water canal in Kolonnawa, Colombo. Initially bacteria were isolated in mineral salt media (MSM) containing 200 mg/L phenol as the sole carbon source. Optimized protocol was used to develop liquid cultures of bacterial isolates and then the bacterial isolates were grown in different phenol concentrations from 1000 to 1700 mg/L in liquid MSM. DNA was extracted from the selected bacterial isolates grown in the higher phenol concentrations. The extracted DNA was amplified using 16 rRNA gene specific primers. Amplified products were sequenced and the homology search of sequences was done by NCBI - Basic Local Alignment Search Tool (NCBI - BLAST) in order to identify the selected bacteria. The selected bacterial isolates, KOL 03 and KOL 12 were examined to determine their phenol degradation capacity by inoculating to MSM containing 1500 mg/L and 1600 mg/L phenol respectively. Samples were drawn aseptically from the cultures at regular intervals to test the growth of bacteria by measuring optical density (OD) at 600 nm, the level of residual phenol by 4-aminoantipyrine colorimetric method and the total cellular protein concentration using Bradford assay.

Two bacterial isolates, KOL 12 and KOL 03 were identified as *Klebsiella pneumonia* and *Enterobacter asburiae* respectively. *Klebsiella pneumonia* was able to grow at higher phenol level

of 1600 mg/l where as *Enterobacter asburiae* showed growth at 1500 mg/L (Figure 01). However, *Klebsiella pneumonia* was able to degrade 99.96% of 1600 mg/L phenol while *Enterobacter asburiae* degraded 99.97% of 1500 mg/L phenol within 9 days (Figure 01).

Klebsiella pneumonia expressed higher production of total cellular proteins under higher phenol concentration compare to the *Enterobacter asburiae* (Figure 02). Expression of cellular catabolic enzymes may be one of the deciding factors for the biodegradation of phenol.

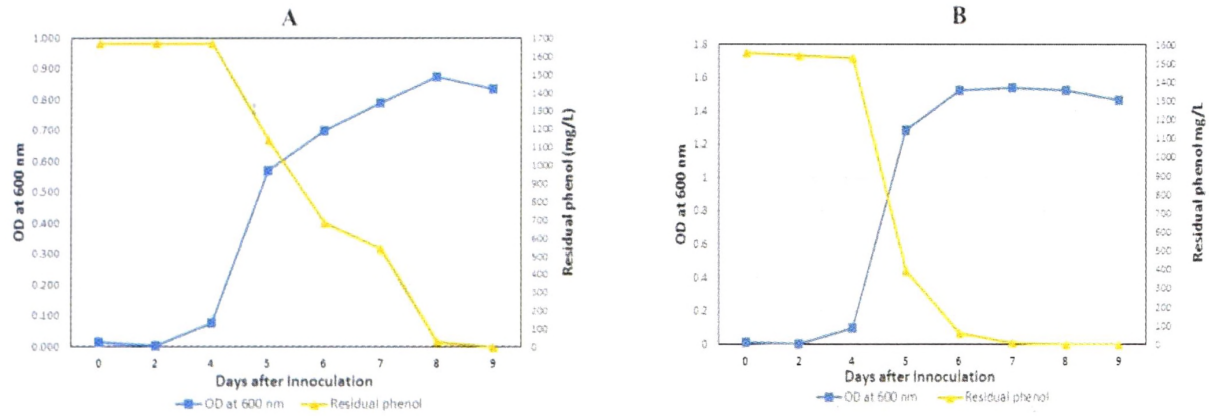

Figure 01: Bacterial growth and residual phenol in two bacterial cultures

A. *Klebsiella pneumonia* and B. *Enterobacter asburiae.*

In conclusion, both bacteria show nearly 100% degradation ability of higher concentrations of phenol. Therefore these two bacteria; *Klebsiella pneumonia* and *Enterobacter asburiae* can be identified as the potential biodegraders of phenol. However, further studies are needed to understand cellular biodegradation mechanism along with the detection of catabolic genes which will provide promising tools to treat phenol contaminated environments in future.

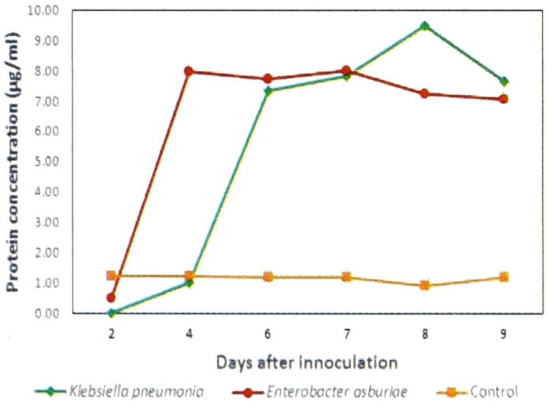

Figure 02: Total cellular protein concentration of bacteria

Keywords: Biodegradation; Enterobacter asburiae; Klebsiella pneumonia; Phenol; Waste water

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