ABSTRACT

Biodegradation of phthalate esters (PAEs), namely dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP) and their mixture by a bacterial strain (BS1) is reported. This bacterium was isolated from soil that was contaminated with plastic wastes and shown in laboratory assays to have the potential to utilize PAEs as the sole source of carbon and energy. The isolate was identified as *Variovorax* sp. based on 16S r-DNA sequence analysis. The extent of biodegradation of DMP, DEP, DBP and their mixture (300 mg L^{-1}) by *Variovorax* sp. BS1 were: >97%, >99%, >92% and >99%, respectively after 24 h of incubation at 30°C. The *Variovorax* sp. BS1 was able to biodegrade 200 to 600 mg L^{-1} of individual PAEs and their mixture within 30 h of incubation. The specific growth rate of *Variovorax* sp. BS1 increased with increasing concentrations of PAEs up to 200 mg L^{-1} and further increase in concentrations did not change the specific growth rate. The transient intermediates and persistent end product formed due to biodegradation of DMP, DEP, and DBP by *Variovorax* sp. BS1 were not detectable by either HPLC or LC-MS analyses in liquid culture medium. However, using GC-MS, trace amounts of phthalic acid and its derivatives were observed. Total organic carbon (TOC) analysis suggested complete mineralization of DMP, DEP, DBP and their mixture by *Variovorax* sp. BS1.

In order identify the enzymes involved in the biodegradation of PAEs, cell free crude extracts were prepared and assayed for the presence of enzymes using PAEs and *para*-nitophenyl acetate (*p*NPA) as the substrates. The extent of biodegradation of 1 mM of DMP using cell free extracts derived from *Variovorax* sp. BS1 grown on DMP was observed to be >80% at the end of 60 min of incubation at 30°C. Results suggested that the cell-free extracts contained the mixture of enzymes responsible for the biodegradation of DMP. Further it was observed that the cell free enzymes were more efficient in biodegrading various concentrations of DMP (25 to 1000 μM). The enzyme(s) responsible for the biodegradation of DMP exhibited highest activity in freshly prepared cell-free crude extracts while the activity declined rapidly upon storage. Zinc ions (Zn^{+2}) inhibited the phthalate dioxygenase activity in the cell free extracts which resulted in the generation and accumulation of phthalic acid. Cell free crude extracts were also prepared from *Variovorax* sp. BS1 grown on a mixture of DMP, DEP and DBP and used for biodegradation of individual PAEs as well as mixture. The extent

of removal of 1 mM of DMP or DEP or DBP and mixture of PAEs (0.33 mM of each) were: 59%, 67%, 65% and 53% after 60 min of incubation using cell free enzyme preparations.

Variovorax sp. BS1 cells were immobilized in calcium alginate beads and used for the biodegradation of PAEs in batch experiments. Degradation efficiency of immobilized cells in mineral salts medium (MSM) and growth non supportive medium, de-ionized water was compared. Results showed higher degradation rate in MSM as compared to de-ionized water. Higher rate of degradation in MSM was due to the growth of cells in/on beads as well as in spent medium. Therefore, MSM was replaced with de-ionized water and used for repeat batch experiments. The results suggested that the immobilized cells of *Variovorax* sp. BS1 may be re-used upto ten cycles with diminutive loss of DMP degradation activity. The results presented suggest the bacterial isolate, *Variovorax* sp. BS1 has the potential for the bioremediation of water contaminated with PAEs.

Keywords: Biodegradation, Dimethyl phthalate, Diethyl phthalate, Dibutyl phthalate, Phthalate esters, *Variovorax*, Enzyme, Immobilization