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Biofilm mediated synergistic degradation of hexadecane by a naturally formed community comprising Aspergillus flavus complex and Bacillus cereus group

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Abstract

Background: The hydrophobic nature of hydrocarbons make them less bioavailable to microbes, generally leading to low efficiency in biodegradation. Current bioremediation strategies for hydrocarbon contamination, uses induced mixed microbial cultures. This in-vitro study demonstrates the utilization of naturally occurring communities in suitable habitats for achieving highly efficient, synergistic degradation of hydrocarbons in a simple community structure without additives.

Methods: Enrichment media supplemented with 1% (7652.53 mg/L) hexadecane (HXD) as the sole carbon source were inoculated with samples of soil with waste polythene, collected from a municipal landfill in order to isolate microbial communities. Gas Chromatography-Mass Spectrometry (GC-MS) analysis was performed on HXD grown co-cultures and individual counterparts after 14 days incubation and percentage degradation was calculated. Microbes were identified using 16S rRNA gene and Internal Transcribed Spacer region sequencing. Biofilm formation was confirmed through scanning electron microscopy, in the most efficient community.

Results: Three mixed communities (C1, C2 and C3) that demonstrated efficient visual disintegration of the HXD layer in the static liquid cultures were isolated. The C1 community showed the highest activity, degrading > 99% HXD within 14 days. C1 comprised of a single fungus and a bacterium and they were identified as a Bacillus sp. MM1 and an Apsergillus sp. MM1. The co-culture and individual counterparts of the C1 community were assayed for HXD degradation by GC-MS. Degradation by the fungal and bacterial monocultures were 52.92 ± 8.81% and 9.62 ± 0.71% respectively, compared to 99.42 ± 0.38% by the co-culture in 14 days. This proved the synergistic behavior of the community. Further, this community demonstrated the formation of a biofilm in oil-water interface in the liquid medium. This was evidenced from scanning electron microscopy (SEM) showing the Bacillus cells attached on to Aspergillus mycelia.

Conclusions: This study demonstrates the utilization of naturally formed fungal-bacterial communities for enhanced biodegradation of hydrocarbons such as hexadecane and reports for the first time, synergistic degradation of hexadecane through biofilm formation, by a community comprising of Bacillus cereus group and Aspergillus flavus complex.

Keywords: Aspergillus; Bacillus; Biodegradation; Biofilm; Hexadecane; Hydrocarbon; Microbial community; Synergism.