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## **Evaluating the aerobic xylene-degrading potential of the intrinsic microbial community of a legacy BTEX-contaminated aquifer by enrichment culturing coupled with multi-omics analysis: uncovering the role of *Hydrogenophaga* strains in xylene degradation**

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### **Abstract:**

To develop effective bioremediation strategies, it is always important to explore autochthonous microbial community diversity using substrate-specific enrichment. The primary objective of this present study was to reveal the diversity of aerobic xylene-degrading bacteria at a legacy BTEX-contaminated site where xylene is the predominant contaminant, as well as to identify potential indigenous strains that could effectively degrade xylenes, in order to better understand the underlying facts about xylene degradation using a multi-omics approach. Henceforward, parallel aerobic microcosms were set up using different xylene isomers as the sole carbon source to investigate evolved bacterial communities using both culture-dependent and independent methods. Research outcome showed that the autochthonous community of this legacy BTEX-contaminated site has the capability to remove all of the xylene isomers from the environment aerobically employing different bacterial groups for different xylene isomers. Interestingly, polyphasic analysis of the enrichments disclose that the community composition of the *o*-xylene-degrading enrichment community was utterly distinct from that of the *m*- and *p*-xylene-degrading enrichments. Although in each of the enrichments *Pseudomonas* and *Acidovorax* were the dominant genera, in the case of *o*-xylene-degrading enrichment *Rhodococcus* was the main player. Among the isolates, two *Hydrogenophaga* strains, belonging to the same genomic species, were obtained from *p*-xylene-degrading enrichment, substantially able to degrade aromatic hydrocarbons including xylene isomers aerobically. Comparative whole-genome analysis of the strains revealed different genomic adaptations to aromatic hydrocarbon degradation, providing an explanation on their different xylene isomer-degrading abilities.

**Keywords:** xylene, *Hydrogenophaga*, groundwater, aromatic hydrocarbons, biodegradation, catechol 2,3-dioxygenase