

Biodegradation of Benzene Under Microaerobic Conditions: a Groundwater Microcosm Experiment Combined with a Multiomics Approach and DNA Stable Isotope Probing

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Abstract

Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX) are the most common contaminants of the groundwater and can easily contaminate drinking water sources due to their relatively high water solubility. Among these compounds, benzene is known to have carcinogenic properties and is considered to be persistent under anoxic conditions. Although deep knowledge was acquired both on the aerobic and anaerobic degradation of benzene in the past three decades, the diversity of those bacteria which are able to degrade it under microaerobic conditions, is still unknown. To come over this limitation aerobic and microaerobic benzene-degrading microcosms were established using groundwater sediment of a BTEX-contaminated site and the evolved bacterial communities were investigated through a polyphasic approach including multiomics analysis and DNA stable isotope probing. The obtained results shed light on the fact that the aerobic and microaerobic benzene-degrading bacterial communities were distinctly different. In the aerobic microcosms members of the genus *Pseudomonas* overwhelmingly dominated the bacterial communities by showing even 40-50% abundance. In contrast, under microaerobic conditions members of the genera *Azovibrio* and *Malikia* dominated the communities, while the abundance of *Pseudomonas* drastically decreased compared to that of was observable in the aerobic microcosms. Results obtained by the analysis of microcosms spiked with ¹³C₆-benzene confirmed these observations. In heavy DNA fractions obtained from the aerobic microcosms the enrichment of *Pseudomonas* and Rhizobiaceae-related 16S rRNA gene fragments was observable. At the same time, labelled DNA from the microaerobic microcosms contained mostly *Azovibrio* and *Malikia*-related sequences, while the

abundance of *Pseudomonas* 16S rRNA gene sequences was below 2%. Besides, by using metagenomic dataset of a previous microaerobic experiment we managed to reconstruct an *Azovibrio* genome, in which a complete *meta*-cleavage pathway for the aerobic degradation of aromatic hydrocarbons was identified. Overall, it can be concluded that under microaerobic conditions members of Comamonadaceae and Rhodocyclaceae can be the key benzene degraders in contaminated subsurface environments.

Keywords

Azovibrio, Malikia, aromatic hydrocarbons, microaerobic conditions

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