Transformation of Benzene Derivatives in Acidic Conditions by the Fungus *Hormoconis Resinae* – Reductive, Oxidative, or Both?

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Abstract

Hormoconis resinae (or *Cladosporium resinae*), colloquially known as the kerosene fungus, is predominantly found in fuel tanks (Rafin and Veignie 2018). Its occurrence in fuel tanks was first reported in early 1960s. Since then, it has been considered as a serious threat by the petroleum industry for bio-deteriorating fuel quality, corroding storage tanks, and clogging pumps and filters (Sheridan et al. 1971). This fungus flourishes well in the presence of water and can thrive at a wider pH range (2-10), than most commonly studied bacteria, with optimum towards the acidic end (Rafin and Veignie 2018). As a biosafety level 1 organism (ATCC 2021) with wide natural prevalence, *H. resinae* is both safe to study and apply in the field. Thus, it can be utilized for developing bioremediation processes suitable for petroleum-contaminated sites.

Contamination of groundwater sources by fuel pollutants has been an important public health concern for decades (Mitra and Roy 2011). Several components of fuel are known to be toxic even at low concentrations with deleterious health effects including teratogenicity and carcinogenicity (ATSDR 1995). Past research has mainly focussed on the degradation of n-alkanes, a major component of fuel, by *H. resinae* which used the n-alkanes as sole carbon and energy sources (Rafin and Veignie 2018). Benzene derivatives like toluene, benzaldehyde, benzoic acid are also often found as fuel pollutants. Though some studies have investigated the effects of benzene derivatives on the survival and growth of *H. resinae* (Cofone et al. 1973, Oh et al. 2001, Qi et al. 2002), not much work has been done on their biodegradation (Kato et al. 1990).

Previous study showed a reductive transformation of benzoate to benzaldehyde, benzyl alcohol, and 1-phenyl-l,2-propanediol (Kato et al. 1990). More work was needed to study the further transformation of these products. Thus, the current study focussed on the

transformation of benzaldehyde and benzyl alcohol in acidic conditions by *H. resinae* ATCC 34066. The main objectives were to study the effects of:

1. culture media,
2. glucose, and
3. oxygen enrichment on the fungal growth in the presence of these benzene derivatives and their biodegradation kinetics and pathways.

Some experiments were also conducted with toluene as the contaminant.

H. resinae was not able to transform toluene (1-200 ppm) at all, though it was able to grow on it in the presence of 1% glucose. The fungus was able to transform benzaldehyde (≤550 ppm) to benzyl alcohol (reductive) and benzoic acid (oxidative). Many monoaromatics such as catechol, resorcinol, hydroxybenzoic acids and aliphatic compounds such as fumaric acid, levulinic acid were also detected as the oxidation products of benzaldehyde by high-resolution liquid chromatography-mass spectrometry. The presence of glucose slowed down benzaldehyde transformation but increased the benzyl alcohol formation relative to benzoic acid, probably due to the further slower transformation of benzyl alcohol. Oxygen enrichment enhanced the benzaldehyde transformation. Glucose was a preferred culturing media as fungus grown on potato dextrose agar (PDA) showed a 5-week lag phase for benzaldehyde transformation. However, this PDA-cultured fungus, after growing on benzaldehyde, did not exhibit a lag phase and started benzaldehyde transformation immediately. Transformation of benzyl alcohol, as target contaminant, was slower and incomplete in the presence of glucose. Benzyl alcohol was transformed mainly to benzoic acid via an oxidative pathway.

In summary, this study has shown that *H. resinae* can transform the benzene derivatives via both oxidative and reductive pathways. Moreover, *H. resinae* can use these compounds as sole carbon and energy sources.

Keywords

Biodegradation, toluene, benzaldehyde, benzoic acid, benzyl alcohol, ATCC 34066

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Conflicts of interest

The authors have declared that no competing interests exist.

References

- ATCC (2021) Hormoconis resinae (Lindau) von Arx et de Vries (34066). American Type Culture Collection, USA. URL: https://www.atcc.org/products/34066
- ATSDR (1995) Toxicological profile for fuel oils. Agency for Toxic Substances and Disease Registry, USA. URL: https://www.atsdr.cdc.gov/toxprofiles/tp75.pdf
- Cofone L, Walker JD, Cooney JJ (1973) Utilization of hydrocarbons by Cladosporium resinae. Microbiol 76.
- Kato N, Konishi H, Masuda M, Joung E, Shimao M, Sakazawa C (1990) Reductive transformation of benzoate by Nocardia asteroides and Hormoconis resinae. J Ferment Bioeng 69.
- Mitra S, Roy P (2011) BTEX: A serious ground-water contaminant. Res J Environ Sci 5.
- Oh K, Mar W, Chang I (2001) Biodegradation of hydrocarbons by an organic solvent-tolerant fungus, *Cladosporium resinae* NK-1. J Microbiol Biotechnol 11.
- Qi B, Moe WM, Kinney KA (2002) Biodegradation of volatile organic compounds by five fungal species. Appl Microbiol Biotechnol. 58.
- Rafin C, Veignie E (2018) Hormoconis resinae, The Kerosene Fungus. In: McGenity T (Ed.) Taxonomy, Genomics and Ecophysiology of Hydrocarbon-Degrading Microbes. Handbook of Hydrocarbon and Lipid Microbiology. Springer, Cham. https://doi.org/10.1007/978-3-319-60053-6_3-1
- Sheridan JE, Nelson J, Tan YL (1971) Studies on the 'kerosene fungus' Cladosporium resinae (Lindau) de Vries: Part I. The problem of microbial contamination of aviation fuels. Tuatara 19.