

**COMBINED CARBON AND HYDROGEN ISOTOPE FRACTIONATION INVESTIGATIONS FOR  
ELUCIDATING BENZENE AND TOLUENE BIODEGRADATION PATHWAYS**

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The two-dimensional isotope fractionation approach (TDIFA) was applied in order to differentiate benzene and toluene biodegradation pathways under oxic and anoxic conditions in laboratory experiments. Using the slope derived from hydrogen vs. carbon isotope discriminations or the ratio of hydrogen to carbon enrichment factors, degradation mechanisms could be distinguished.

Although experimentally determined slopes for hydrogen and carbon discrimination partially overlapped, specific ranges could be determined for different benzene biodegradation pathways. Slopes were  $< 2$  for dihydroxylation, between 7 and 9 for monohydroxylation and  $> 17$  for anaerobic degradation. Moreover, variations in slopes suggest that more than one reaction mechanism exists for benzene monohydroxylation as well as for anaerobic benzene degradation.

For toluene biodegradation, the highest slope was estimated for methyl monohydroxylation ( $> 53$ ). The lowest slope was observed for dihydroxylation ( $< 2$ ). Slopes for organisms attacking toluene under anoxic conditions by benzylsuccinate synthase were significantly different and ranged from 4 for a phototrophic strain, 11 - 14 for nitrate reducers to 28 - 33 for sulfate reducers.

Our results show that TDIFA has great potential to elucidate biodegradation pathways of pollutants in field and microcosm studies.