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Characterization of phenols biodegradation by compound specific stable isotope analysis

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Biodegradation of phenol and alkylphenols has been described under both oxic and anoxic conditions. In the absence of molecular oxygen, the degradation of phenolic compounds is initiated by microorganisms through carboxylation, fumarate addition to the methyl moiety or anoxic hydroxylation of the methyl moiety. Comparatively, under aerobic condition, the initiation mechanisms are revealed to be monoxygenation or dihydroxylation for phenol and ring hydroxylation or methyl group oxidation for cresols. While several studies biochemically characterized the enzymes and reaction mechanisms in the relevant degradation pathways, isotope fractionation patterns were rarely reported possibly due to constraints in current analytical methods.
In this study, the carbon isotope fractionation patterns upon the degradation of phenol and cresols by several strains were analyzed by using isotope ratio mass spectrometry connected with liquid chromatography (LC-IRMS). The corresponding enrichment factors for carbon (C) have been obtained.
Cresols degradation by various strains showed generally moderate carbon isotope fractionation patterns with notable differences. For p-cresol degradation, five strains were examined. The aerobic strain Acinetobacter calcoaceticus NCIMB8250 exploits ring hydroxylation by molecular oxygen as initial reaction, and a C value of -1.4±0.2‰ was obtained. Pseudomonas pseudoalcaligenes NCIMB 9867, an aerobic strain initiating cresols degradation via oxygen-dependent side chain hydroxylation, yielded a C value of -2.3±0.2‰Under nitrate-reducing conditions, Geobacter metallireducens DSM 7210 and Azoarcus buckelii DSM 14744 attacks p-cresol at the side chain by monohydroxylation using water as oxygen source; the two strains produced C values of -3.6±0.4‰ and -2±0.1‰ accordingly.
The sulfate-reducing Desulfosarcina cetonica DSM 7267 activating cresols by fumarate addition to the methyl moiety yielded C values of -1.9±0.2‰ for p-cresol degradation and 2.2±0.3‰ for m-cresol degradation, respectively.
The carbon isotope fractionation patterns of phenol degradation differed more profoundly. Oxygen-dependent monooxygenation of phenol by A.calcoaceticus as the initial reaction yielded C values of -1.5±0.02‰In contrast, the anaerobic degradation initiated by ATP-dependent carboxylation performed by Thauera aromatia DSM 6984, produced no detectable fractionation (C 0±0.1‰. D. cetonica showed a slight inverse carbon isotope fractionation (C 0.4±0.1‰.
In conclusion, a validated method for compound specific stable isotope analysis was developed for phenolic compounds, and the first data set of carbon enrichment factors upon the biodegradation of phenol and cresols with different activation mechanisms has been obtained in the present study. Carbon isotope fractionation analysis is a potentially powerful tool to monitor phenolic compounds degradation in the environment.