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O_2-Uncoupling Modulates the Observable Substrate Isotope Fractionation Associated with Oxidative Biodegradation of Organic Soil and Water Contaminants

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Oxygenations of aromatic and aliphatic moieties with molecular O_2 are frequent initial steps of biodegradation of emerging organic contaminants in soil and aquatic environment as well as in engineered systems like wastewater treatment plants. Because oxygenated products are more polar that the parent contaminants and often prone to further transformation in standard metabolic pathways, the assessment of oxidative contaminant biodegradation is particularly challenging. As has been shown successfully for many legacy contaminants, compound-specific isotope analysis (CSIA) offers well-established procedures for evaluating the extent of biological transformation through quantification of changes of stable isotope ratios in the residual substrate. Extending the CSIA-based approach to emerging contaminants, however, silently implies similar substrate-specificities, kinetic mechanisms, and kinetic isotope effects for contaminant degrading oxygenases.

Here, we examined the validity of this assumption and, thus, the applicability of CSIA to assess emerging contaminant oxygenations, by studying the aromatic hydroxylations catalyzed by Rieske non-heme ferrous iron oxygenases (ROs), the class of enzymes responsible for initial steps of aromatic contaminant metabolism. To that end, we quantified hydroxylation efficiencies of a suite of structurally similar nitroaromatic substrates and derived the corresponding substrate isotope effects of the organic compounds and molecular O_2 from the observable C and O stable isotope fractionation. Our characterization of the catalytic mechanism of ROs revealed that substrate hydroxylations occur after the rate-limiting activation of molecular O_2. Substrate C isotope fractionation is nevertheless observable because of the phenomenon of unproductive O2 activation. This so-called O_2 uncoupling leads to formation of reactive oxygen species with concomitant release of unreacted substrate from the active site [1,2]. Despite originating from the identical sequence of elementary reaction steps, substate ^{13}C isotope effects, however, are highly variable. The substrate- and enzyme-dependent occurrence of O_2 uncoupling discovered in this work modulates the extent of observable substrate isotope fractionation in an unpredictable way and therefore could preclude the quantitative interpretation of isotope fractionation associated with the (di)oxygenation processes of emerging organic contaminants.

[1] Bopp et al, ACS Environ. Au 2022, 2, 428-440
[2] Pati et al., ACS Catal. 2022, 12, 6444−6456