

## Dark O<sub>2</sub> production in subsurface environments

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Recent evidence has revealed potentially widespread molecular oxygen (O<sub>2</sub>) production in permanently dark environments, challenging the idea that non-photosynthetic O<sub>2</sub> production was negligible in Earth's system. This process, termed dark oxygen production (DOP), may occur through abiotic reactions, such as water radiolysis, or through biotic processes, such as microbial nitric oxide or chlorite dismutation. We hypothesize that DOP may be a globally relevant process in today's subsurface ecosystems and that aquifers and fracture fluids represent model systems to study O<sub>2</sub> production and consumption in the deep geologic past.

Our multi-disciplinary approach combines triple- (and clumped-) oxygen isotope analyses, nanomolar-scale measurements of microbial DOP rates and fluxes, and high-throughput genomic and proteomic analyses of O<sub>2</sub>-producing enzymes and pathways. This integrated methodology aims to provide comprehensive insights into the geochemistry, microbiology, and ecology of DOP in diverse groundwater ecosystems.

As a first step, we have collected water samples from shallow groundwater wells in Thuringia (Germany), multiple aquifers in Alberta (Canada), and fluid inclusions from 1.2 and 2.9 km depths in a South African gold mine. Preliminary oxygen isotopic analyses of these samples will reveal whether the measured O<sub>2</sub> has atmospheric or DOP sources. To complement these findings, we aim to characterize the isotopic fractionation factors and triple-oxygen isotope signatures of select biotic and abiotic DOP processes. This approach will enhance our ability to identify and differentiate the various O<sub>2</sub> producing and consuming processes in subsurface environments, ultimately improving our understanding of O<sub>2</sub> cycling in the deep biosphere and its implications for early Earth environments.