

## **Anaerobic oxidation of methane coupled to iron reduction via cycling of sulfur**

CAROLINE R. BUCKNER<sup>1\*</sup>, TIMOTHY G. FERDELMAN<sup>1</sup>,  
MARCEL M. M. KUYPERS<sup>1</sup> AND JANA MILUCKA<sup>1</sup>

<sup>1</sup>Max Planck Institute for Marine Microbiology, Department of Biogeochemistry, Bremen, Germany

(\*Correspondence: cbuckner@mpi-bremen.de)

Recently, it has been proposed that organisms capable of sulfate-dependent anaerobic oxidation of methane (AOM) are able to replace their standard electron acceptor with iron and manganese oxides when grown in the absence of sulfate. This metabolic flexibility appears problematic, as dissolved sulfate is reduced inside the cell, while iron and manganese oxides are insoluble and would require extracellular transfer of electrons obtained from methane oxidation. We hypothesize that, in the apparent absence of sulfate, sulfate-dependent AOM can occur and be indirectly linked to iron reduction via a hidden sulfur cycle.

In this scenario, iron oxides are chemically reduced by sulfide producing iron (II) sulfides and zero-valent sulfur. As shown previously, zero-valent sulfur can subsequently be disproportionated, thus producing sulfate, which can be then used to fuel classical AOM.

To test this hypothesis, we used a highly enriched AOM culture, which has been continuously grown on sulfate for over 10 years. Experiments were conducted in sulfate-free medium to which iron oxide (ferrihydrite) was added as a sole electron acceptor. Methane oxidation was instantaneous, reaching rates ca. 40x lower than those with sulfate. Iron was reduced stoichiometrically to the amount of methane oxidized. After all iron was reduced, methane oxidation ceased as well. In agreement with our initial assumption, we also observed concomitant sulfide production in our experiment and thus conclude that iron reduction can be coupled to methane oxidation indirectly, via cycling of reduced sulfur compounds. Such a mechanism might be responsible for apparent iron reduction in many settings where AOM occurs under non-detectable sulfate concentrations.