

REDUCTIVE DISSOLUTION OF PYRITE BY METHANOGENS. L. M. Bueter¹, C. M. Johnson², P. Chanda², B. L. Beard², E.E. Roden², and E.S. Boyd¹, ¹Montana State University, Department of Microbiology and Immunology, Bozeman, MT, ²University of Wisconsin, Department of Geosciences – Madison, WI.

Introduction: Pyrite (FeS₂) is the most abundant sulfide mineral in the earth's crust today and has been detected in peridotites of mantle origin. In the presence of oxygen (O₂), FeS₂ can be oxidized to form Fe³⁺ and SO₄²⁻, the latter of which can lead to acidification of natural waters. Microbial mediated aerobic oxidation of FeS₂ is a key process in contemporary global iron (Fe) and sulfur (S) cycles but is only thought to have become important after the rise of oxygen ~ 2.5 Ga [1,2]. In the absence of O₂ and in the presence of hydrogen (H₂), FeS₂ can undergo reductive dissolution to yield hydrogen sulfide (H₂S) and iron monosulfide (FeS) according to reaction (1):



However, this process has only been demonstrated at metamorphic conditions with temperatures (>200°C) that are not conducive to microbial life [3]. As such, the reductive dissolution of FeS₂ has never been demonstrated biologically, and there is no known pathway for the direct utilization of FeS₂ by microorganisms in anoxic conditions. This has led to the hypothesis that prior to the widespread oxygenation of the planet sulfide minerals, such as FeS₂, were biounavailable [2].

Methanogenic archaea are strict anaerobes which produce methane as a byproduct of energy metabolism. Isotopic evidence for methanogenesis in rocks dated to 3.4 Ga suggests that methanogens were present and active on early Earth [4]. Moreover, contemporary methanogens are thought to be the descendants of one of the earliest evolving lineages of life [5]. Recent phylogenomics suggests that ancestral methanogens emerged in a hydrothermal vent environment [6], where oxidation of FeS by H₂S to yield FeS₂ and H₂ (reverse of reaction 1) may support hydrogenotrophic activity [7]. Volcanic CO₂ in these hydrothermal settings could have served as an oxidant and a source of carbon for methanogen energy metabolism.

Like other forms of life methanogens require an external source of sulfur that must be supplied by the environment. Methanogens assimilate reduced sources of sulfur such as sulfide (H₂S) or cysteine for synthesis of iron-sulfur (Fe-S) clusters, in addition to other cellular components. Despite methanogens having elevated requirement for Fe-S clusters (e.g., 15-fold more clusters than *Escherichia coli*) key aspects of their S metabolism, including the pathway for S acquisition during formation of Fe-S clusters, remain un-

known [8]. Interestingly, abundant and active H₂-dependent methanogen assemblages have been identified in anoxic environments where FeS₂ is the primary reservoir of Fe and S and where H₂S and cysteine are not detectable [9]. These observations suggested that other forms of reduced sulfur may be capable of supporting methanogens, both in contemporary and past environments. Here we test the ability of three of the five primary lineages of methanogens to catalyze the reductive dissolution of FeS₂.

Results: Methanogens provided with H₂ were capable of reducing FeS₂ to H₂S and FeS, the latter of which is assimilated. In separate experiments we show that FeS can directly serve as an Fe and S source to meet biosynthetic demands. Both FeS₂ and FeS support growth efficiencies (cells/mol CH₄ produced) and growth rates equal to or greater than the canonical reduced S sources, H₂S and cysteine. These results indicate that FeS and FeS₂ can both be used to meet Fe-S cluster demands in methanogens and that FeS₂ can be mobilized in anoxic environments, such as the Archean when O₂ was scarce and methanogenesis was prevalent.

Conclusions: These observations challenge the paradigm that FeS₂ was biounavailable prior to the rise of O₂ and beg a reconsideration of past and contemporary Fe and S cycles. The importance of Fe-S clusters in methanogen metabolism and the apparent ability of methanogens to meet Fe and S biosynthetic demands using FeS₂ and other Fe-S minerals are consistent with Wächtershäuser's hypothesis for the origin of life which suggests biological catalysts originated on iron-sulfur minerals using the formation of FeS₂ as a driving force [5,6,10].

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