

Microbially mediated dissolution of select carbonate minerals by a moderately alkaline, methanogenic enrichment community

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Carbonate minerals represent Earth's largest reservoir of carbon. Because carbonates are insoluble under alkaline conditions, they are often considered metastable. Existing research on microbial interaction with carbonates focuses heavily on mineral precipitation. However, microbially mediated dissolution could have deleterious effects on both natural and artificial carbon sequestration. Here, we demonstrate dissolution of solid-phase carbonates by a methanogenic microbial consortium at fixed pH, with a preference for calcium carbonate polymorphs. A moderately alkaline (pH 8.3), anaerobic microbial community containing a novel *Methanobacterium* sp. was enriched using synthetic calcium carbonate as a sole carbon source for methanogenesis. To assess the utilization of other common carbonate minerals as sources of inorganic carbon for methane production, a series of batch reactor experiments were initiated at a pH of 7.6, based on prior characterization of optimal culture conditions. The microbial consortium was cultivated in minimal, defined medium with hydrogen and 1 g/L of either calcite, aragonite, dolomite, siderite, magnesite, or synthetic calcium carbonate. Parallel reactors were initiated to serve as negative controls, including autoclave-killed cells for each mineral amendment as well as a live treatment in which no mineral was added other than residual calcium carbonate carried over from the inoculum. After three weeks of incubation, headspace methane increased considerably in cultures amended with natural or synthetic calcium carbonates (calcite, aragonite, and synthetic calcium carbonate accumulating 3407, 2800, and 3412 $\mu\text{mol CH}_4 \text{L}^{-1}$ respectively), with no significant difference between treatments. In cultures amended with dolomite, siderite, and magnesite, comparatively low levels of methane were measured (370, 143, and 15 $\mu\text{mol L}^{-1}$), underperforming the negative control containing only residual calcium carbonate (694 $\mu\text{mol L}^{-1}$). No methane was detected in autoclave-killed controls, and no change in pH was observed within or between treatments over the course of the incubation. Together these results demonstrate that calcium carbonate polymorphs are a preferential source of inorganic carbon for hydrogenotrophic methanogenesis by the novel *Methanobacterium* sp. in this microbial consortium. These results further indicate that calcium carbonate minerals are the most vulnerable to microbially mediated dissolution. As such, carbon sequestration strategies should consider long-term mineral stability if methanogenic dissolution is anticipated.