

# The High-Temperature Fate of the Hydrothermal Deep Biosphere: An Experimental Investigation at 250–360°C

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The hydrothermal ‘deep biosphere’ - a reservoir of microbially produced organic carbon, extends below the heated axial oceanic crust. It has been suggested that thermal breakdown of this highly functionalized carbon could release small organic molecules into circulating fluids [1], potentially providing additional sources of metabolic energy. Only a single experimental study, however, implies that biomass may be reactive in hydrothermal solutions, but low molecular weight (LMW) organic-inorganic products were not characterized [2]. To address this, we used a Au-TiO<sub>2</sub> flexible cell hydrothermal apparatus to simulate thermal decomposition of *Bacteria* and *Archaea* under realistic, single-phase hydrothermal conditions. *Methanocaldococcus villosus* and *Sulfurovum riftiae* cells, representing vent archaeal and bacterial domains, were grown autotrophically (minimizing background organics), and aqueous suspensions of ~0.1g of each biomass were heated in artificial hydrothermal fluid (Mg, SO<sub>4</sub>-free seawater) to 250–360°C (20–35MPa) over several months without added minerals.

Time-series sampling of both experiments showed formation of abundant thermogenic dissolved organic carbon (DOC), light hydrocarbons (C<sub>1</sub>–C<sub>5</sub> alkanes, alkenes), H<sub>2</sub>, CO<sub>2</sub>, H<sub>2</sub>S, CH<sub>3</sub>SH and LMW carboxylic acids (OAs) over several months. LMW species production and destruction trends were broadly similar between both biomass types. H<sub>2</sub> - the dominant LMW product besides CO<sub>2</sub>, was continuously produced, increasing from low μmolar (250°C) to mmolar concentrations (330–360°C). Initial heating produced higher concentrations of alkenes than alkanes, a trend which reversed with increasing temperature. CH<sub>3</sub>SH, Σpyruvate and H<sub>2</sub>S were also rapidly produced at the initial time points at 250°C but disappeared at higher temperatures. ΣC<sub>1</sub>–C<sub>4</sub> OAs were continuously produced at 250–330°C, reaching high μmolar levels, but these decreased at 360°C, possibly due to decarboxylation reactions. Additional ongoing analysis of CH<sub>3</sub>OH, amino acids, ΣNH<sub>4</sub>, DOC and lipid biomarker residues

will also be presented. Our results imply that microbial biomass is highly reactive at moderate hydrothermal conditions, even in the absence of minerals, on timescales of hours to weeks. Such reactivity and product diversity have implications for hydrothermal deep biosphere carbon recycling and potential microbial heterotrophy [3] in heated oceanic crust.

[1] McCollom (2008) *AGU Monograph*, 178

[2] Konn *et al.* (2011) *Geobiology*, 9

[3] Li *et al.* (2020) *Nature*, 579