NANO-SCALE IMAGING OF CULTURED AND UNCULTURED ANAEROBIC MICROBIAL CONSORTIA BY SYNCHROTRON X-RAY FLUORESCENCE MAPPING. J. B. Glass[1*], S. E. McGlynn[2], S. Chen[3], K. S. Dawson[2], G. Chadwick[2], J. Deng[4], S. Vogt[3], E. D. Ingall[1], B. S. Twining[5], and V. J. Orphan[2]

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This study evaluated the potential of synchrotron x-ray fluorescence (SXRF) microscopy for imaging elemental distributions in anaerobic microbial co-cultures and enrichment cultures. Anaerobic co-cultures of the methanogenic archaeon *Methanosarcina acetivorans* C2A and the sulfate-reducing bacterium (SRB) *Desulfococcus multivorans* DSM 2059 were grown on methanol and lactate, respectively. Otherwise, media composition (DSMZ Medium 141) for the two cultures was identical. Cells were digested in trace metal clean nitric acid for analysis by HR-ICP-MS. SRB had the highest metal:P ratios, followed by the co-culture. Lowest metal:P ratios were measured for the methanogen. No major differences in relative abundances was observed between the methanogen, SRB and co-culture measured by ICP-MS; the trend was consistently Zn >> Fe > Co >> Ni > Cu > Mn > Mo > V, with concentrations ranging from >200 to <1 mmol metal mol P^{-1}. With the exception of Mn, the biomass metal content generally reflected the dissolved metal composition of the growth media as measured by HR-ICP-MS (21 μM Mn, 7 μM Zn, 4 μM Fe, 2 μM Co, 1 μM Ni, 0.4 μM Mo, 0.3 μM Cu and 0.008 μM V).

For SXRF imaging, co-cultures were either directly deposited onto silicon nitride membranes or fixed in trace-metal clean paraformaldehyde and glutaraldehyde with or without subsequent embedding and thin sectioning in Technovit resin. Thin sections (5 μm) or whole cells were imaged on the Bionanoprobe at sector 21 at the Advanced Photon Source at 100 nm resolution with 10 keV hard x-rays [1]. SRB from co-cultures analyzed by SXRF contained similar Zn:P, Fe:P, Ni:P and Mn:P ratios as SRB measured in pure culture by HR-ICP-MS, but significantly higher Co:P and Cu:P. Methanogens from co-cultures analyzed by SXRF contained similar Co:P, Cu:P, Ni:P and Mn:P ratios as pure cultures measured by HR-ICP-MS, but significantly higher Zn:P and Fe:P. Thin-sectioned cell images were much better resolved than whole cells.

Enzymes involved in methanogenesis require Fe, Zn, Co and Ni cofactors [2]. The ~5-10 mmol Ni mol P^{-1} in methanogen cells measured by both methods was similar to Ni:P ratios measured by ICP-OES for *Methanosarcina* spp. grown on methanol [3]. There was significantly more Zn in methanol-grown *Methanosarcina* cells in this study than in the previous study, likely reflecting the higher Zn in the DSM 141 growth medium.

The elevated Co:P ratios (up to 1000 mmol Co mol P^{-1}) in SRB cells grown in co-culture and measured by SXRF was unexpected based on SRB metabolism [4]. Methanogens would be expected to utilize cobalt for methyltransferases to convert the methanol in the media to methane, while SRBs would be predicted to have fewer uses for cobalt-containing enzymes when grown on lactate. The cobalt accumulation in SRB suggests that these cells are either highly expressing cobalt-utilizing enzymes, or possibly sequestering this metal for another purpose.

Methane seep enrichment cultures were dominated by microbial consortia (~10-500 μm^2) comprised of ANME-2c (anaerobic methanotrophic Euryarchaeota) and Desulfobacteraceae SRB that were phylogenetically related to co-culture species. Enrichment cultures were analyzed by SXRF after fixation in trace-metal clean paraformaldehyde and glutaraldehyde, followed by embedding in Technovit resin and thin sectioning to 5 μm. Interspecies differences in elemental content were more difficult to discern in uncultured microbial consortia. Some aggregates were surrounded by a Si-K-Fe-Ti-rich ring, likely comprised of an aluminosilicate clay, as previously observed [5]. Fluorescent *in situ* hybridization (FISH) was attempted unsuccessfully after SXRF, likely due to radiation damage of ribosomes. Further tests will be needed to determine if FISH can be performed prior to SXRF analysis or if it results in trace metal contamination.

References: [1] Chen S. et al. (2014) *J. Synchroton Rad.,* 21, 66-75. [2] Glass J. B. and V. J. Orphan (2012) *Front. Microbiol.*, 3, 1-20. [3] Scherer P. et al. (1983) *Bio. Trace Element Res.*, 5, 149-163. [4] Barton L. L. et al. (2007) *Biometals*, 20, 291-302. [5] Chen Y. et al. (2014) *Sci. Rep.*, 4, 5696.