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Cobalt enrichment in anaerobic microbial cocultures revealed by synchrotron X-ray fluorescence imaging — Source link

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Trace metal imaging of sulfate-reducing bacteria and methanogenic archaea at single-cell resolution by synchrotron X-ray fluorescence imaging

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Abstract

Metal cofactors are required for many enzymes in anaerobic microbial respiration. This study examined iron, cobalt, nickel, copper, and zinc in cellular and abiotic phases at the single-cell scale for a sulfate-reducing bacterium (*Desulfococcus multivorans*) and a methanogenic archaeon (*Methanosarcina acetivorans*) using synchrotron x-ray fluorescence microscopy. Relative abundances of cellular metals were also measured by inductively coupled plasma mass spectrometry. For both species, zinc and iron were consistently the most abundant cellular metals. *M. acetivorans* contained higher nickel and cobalt content than *D. multivorans*, likely due to elevated metal requirements for methylotrophic methanogenesis. Cocultures contained spheroid zinc sulfides and cobalt/copper-sulfides.

Introduction

In anoxic natural and engineered environments, sulfate-reducing bacteria and methanogenic archaea perform the last two steps of organic carbon respiration, releasing sulfide and methane. Sulfate-reducing bacteria and methanogenic archaea can exhibit cooperative or competitive interactions depending on sulfate and electron donor availability (Brileya et al. 2014; Bryant et al. 1977; Ozuolmez et al. 2015; Stams and Plugge 2009). Methanol (CH_3OH), the simplest alcohol, is an important substrate for industrial applications (Bertau et al. 2014) and microbial metabolisms. In the presence of methanol, sulfate reduction and methanogenesis occur simultaneously in cocultures (Dawson et al. 2015; Phelps et al. 1985), anoxic sediments (Finke et al. 2007; Oremland and Polcin 1982), and anaerobic digesters (Spanjers et al. 2002; Weijma and Stams 2001). Methanol has also been studied as a substrate for stimulating organochlorine degradation in sediment reactors containing sulfate-reducing bacteria and methanogenic archaea (Drzyzga et al. 2002).

Metalloenzymes are essential for both sulfate reduction and methylotrophic methanogenesis (Barton et al. 2007; Ferry 2010; Glass and Orphan 2012; Thauer et al. 2010). Iron is needed for cytochromes and iron-sulfur proteins in both types of organisms (Fauque and Barton 2012; Pereira et al. 2011; Thauer et al. 2008). Cobalt and zinc are present in the first enzymes in sulfate reduction (ATP sulfurylase, Sat; Gavel et al. 1998; Gavel et al. 2008), and methylotrophic methanogenesis (methanol:coenzyme M methyltransferase; Hagemeier et al. 2006). Nickel is found in the final enzyme in methanogenesis (methyl coenzyme M reductase; Ermler et al. 1997), and zinc is present in the heterodisulfide reductase that recycles cofactors for the methyl coenzyme M reductase enzyme (Hamann et al. 2007). Nickel and cobalt are required by methanogenic archaea and sulfate-reducing bacteria that are capable of complete

48 organic carbon oxidization for carbon monoxide dehydrogenase/acetyl Co-A synthase in the
49 Wood-Ljungdahl CO_2 fixation pathway (Berg 2011; Ragsdale and Kumar 1996). Hydrogenases
50 containing Ni and Fe are functional in many, but not all, sulfate-reducing bacteria (Osburn et al.
51 2016; Pereira et al. 2011) and methylotrophic methanogens (Guss et al. 2009; Thauer et al.
52 2010). Evidence for high metabolic metal demands is provided by limited growth of
53 methanogenic archaea without Co and Ni supplementation in methanol-fed monocultures
54 (Scherer and Sahm 1981) and anaerobic bioreactors (Florencio et al. 1994; Gonzalez-Gil et al.
55 1999; Paulo et al. 2004; Zandvoort et al. 2003; Zandvoort et al. 2006).

56 Sulfate-reducing bacteria produce sulfide, which can remove toxic metals from
57 contaminated ecosystems due to precipitation of metal sulfides with low solubility (Paulo et al.
58 2015). Metal sulfides may also limit the availability of essential trace metals for microbial
59 metabolism (Glass and Orphan 2012; Glass et al. 2014). In sulfidic environments such as marine
60 sediments and anaerobic digesters, dissolved Co and Ni are present in nanomolar concentrations
61 (Glass et al. 2014; Jansen et al. 2005). These metals are predominantly present as solid metal
62 sulfide precipitates (Drzyzga et al. 2002; Luther III and Rickard 2005; Moreau et al. 2013)
63 and/or sorbed to anaerobic sludge (van Hullebusch et al. 2006; van Hullebusch et al. 2005; van
64 Hullebusch et al. 2004). The bioavailability of metals in these solid phases to anaerobic microbes
65 remains relatively unknown. Previous studies suggest that methanogenic archaea can leach Ni
66 from silicate minerals (Hausrath et al. 2007) and metal sulfides (Gonzalez-Gil et al. 1999; Jansen
67 et al. 2007). Sulfidic/methanogenic bioreactors (Jansen et al. 2005) and *D. multivorans*
68 monocultures (Bridge et al. 1999) contain high-affinity Co-/Ni- and Cu-/Zn-binding ligands,
69 respectively, which may aid in liberating metal micronutrients from solid phases when they
70 become growth-limiting.

Due to the importance of trace metals for anaerobic microbial metabolisms in bioremediation and wastewater treatment, extensive efforts have focused on optimizing metal concentrations to promote microbial organic degradation in anaerobic digesters (for review, see Demirel and Scherer (2011)). Numerous studies have investigated the effect of heavy metals on anaerobic metabolisms at millimolar concentrations in heavy-metal contaminated industrial wastewaters, whereas few studies have investigated interactions between anaerobic microbes and transition metals at the low micro- to nanomolar metal concentrations present in most natural ecosystems and municipal wastewaters (see Paulo et al. 2015 for review). Studies of the metal content of anaerobic microbes have primarily measured monocultures using non-spatially resolved techniques such as ICP-MS (Barton et al. 2007; Cvetkovic et al. 2010; Scherer et al. 1983). Little is known about the effect of coculturing on cellular elemental composition and mineralogy due to changes in geochemistry (e.g. via sulfide production) of the medium and/or microbial metabolisms (e.g. via competition for growth-limiting substrates).

In this study, we measured cellular elemental contents and imaged extracellular metallic minerals for sulfate-reducing bacteria and methanogenic archaea grown in mono- and co-culture. For the model sulfate-reducing bacterium, we chose the metabolically versatile species *Desulfococcus multivorans*, which is capable of complete organic carbon oxidation. *Methanosarcina acetivorans* C2A, a well-studied strain capable of growing via aceticlastic and methylotrophic methanogenesis, but not on H_2/CO_2, was selected as the model methanogenic archaeon. These species were chosen because they are the most phylogenetically similar to pure culture isolates available to syntrophic consortia of anaerobic methanotrophic euryarchaeota (ANME-2) and sulfate-reducing bacteria (*Desulfosarcina/Desulfococcus*) partner that catalyze the anaerobic oxidation of methane in marine sediments (see Dawson et al. (2015) for more on

coculture design). Individual cells of mono- and cocultures of these two species were imaged for elemental content on the Bionanoprobe (Chen et al. 2013) at the Advanced Photon Source (Argonne National Laboratory) and measured for relative abundance of bulk cellular metals by ICP-MS.

Materials and Methods

Culture growth conditions

The growth medium contained (in g L^{-1}): NaCl, 23.4; $MgSO_4 \cdot 7H_2O$, 9.44; $NaHCO_3$, 5.0; KCl, 0.8; NH_4Cl, 1.0; Na_2HPO_4, 0.6; $CaCl_2 \cdot 2H_2O$, 0.14; cysteine-HCl, 0.25; resazurin, 0.001, 3 x 10^{-6} Na_2SeO_3 supplemented with DSM-141 vitamin (including 1 µg L^{-1} vitamin B_{12}) and trace element solutions containing metal concentrations (provided below as measured by ICP-MS) and 1.5 mg L^{-1} nitrilotriacetic acid (Atlas 2010). The medium (pH 7.6) was filter sterilized in an anoxic chamber (97% N_2 and 3% H_2 headspace) and reduced with 1 mM Na_2S.

Monocultures of *Desulfococcus multivorans* (DSM 2059) and *Methanosarcina acetivorans* strain C2A (DSM 2834) were inoculated into 20 mL culture tubes containing 10 mL of media with N_2:CO_2 (80:20) headspace, and sealed with butyl rubber stoppers and aluminum crimp seals. *D. multivorans* monocultures were amended with filter-sterilized lactate (20 mM). *M. acetivorans* monocultures were amended with filter-sterilized methanol (66 mM). Equal proportions of dense monocultures in early stationary stage (as assessed by OD_{600} measurements; Fig. S1) were inoculated into sterile media and amended with filter-sterilized lactate (20 mM) and methanol (66 mM) to form the coculture. Cultures were grown at 30°C without shaking. After 12 days of growth (Fig. S1), mono- and cocultures were pelleted and frozen for ICP-MS analysis, or prepared for SXRF imaging.

Fluorescence in situ hybridization

In order to confirm that monocultures were free of contamination, and to determine the relative abundance of *D. multivorans* and *M. acetivorans* in coculture, fluorescence *in situ* hybridization (FISH) was performed on separate aliquots from the same time point of the cell culture used for SXRF analyses. One mL of cell culture was preserved in 3% paraformaldehyde for 1-3 hours, then washed and resuspended in 200 µL of 3x PBS:ethanol as described in Dawson et al. (2012). Four microliters of fixed cells were spotted onto a glass slide and hybridized with an oligonucleotide probe targeting *Methanosarcina acetivorans* MSMX860 (Raskin et al. 1994) and the deltaproteobacterial probe Delta495a (Loy et al. 2002) and cDelta495a (Macalady et al. 2006). The FISH hybridization buffer contained 45% formamide, and the hybridization was carried out at 46°C for 2 hours followed by a 15 minute wash in 48°C washing buffer (Daims et al. 2005). The slides were rinsed briefly in distilled water, and mounted in a solution of DAPI (5 µg/mL) in Citifluor AF-1 (Electron Microscopy Services). Imaging was performed with a 100x oil immersion objective (Olympus PlanApo). Cell counts were performed by hand. Multiple fields of view from replicate wells were compiled and counted on the basis of fluorescence in DAPI (all cells), Cy3 (bacteria), and FITC (archaea).

ICP-MS

Frozen cell pellets were dried (yielding ~4 mg dry weight per sample) in acid-washed Savillex Teflon vials in a laminar flow hood connected to ductwork for exhausting acid fumes. Cells were digested overnight at 150°C in 2 mL of trace metal grade nitric acid and 200 µL hydrogen peroxide, dried again, and dissolved in 5 mL 5% nitric acid. The medium was diluted 1:50 in nitric acid. The elemental content of microbial cells and media was analyzed by ICP-MS (Element-2, University of Maine Climate Change Institute). Sterile medium contained the

following concentrations (in μM): P, 800; Zn, 7; Fe, 4; Co, 2; Ni, 0.9; Cu, 0.3. Digestion acid blanks contained (in nM): P, 127; Zn, 12; Fe, 5; Co, 0.007; Ni, 0.9; Mo, 0.02; Cu, 0.1; V, 0.03.

SXRF sample preparation

Monocultures were prepared for SXRF analysis without chemical fixation by spotting onto silicon nitride (SiN) wafers (Silson Ltd., cat. 11107126) followed by rinsing with 10 mM HEPES buffer (pH 7.8). To enable FISH microscopy after SXRF analysis, cocultures were chemically preserved prior to analysis by incubation on ice for 1 hour in 50 mM HEPES and 0.6 M NaCl (pH 7.2) containing 3.8% paraformaldehyde and 0.1% glutaraldehyde that had been cleaned of potential trace-metal contaminants with cation exchange resin (Dowex 50-W X8) using established protocols (Price et al. 1988; Twining et al. 2003). Cells were then centrifuged, re-suspended in 10 mM HEPES buffer (pH 7.8) and either embedded in resin and thin sectioned following the methods described in McGlynn et al. (2015) or spotted directly onto SiN wafers.

SXRF analyses

Whereas ICP-MS measurements cannot delineate the elemental contributions of co-occurring cell types, SXRF imaging enables elemental quantification of the specific cell of interest (Fahrni 2007; Ingall et al. 2013; Kemner et al. 2004; Nuester et al. 2012; Twining et al. 2003; Twining et al. 2008). SXRF analyses were performed at the Bionanoprobe (beamline 21-ID-D, Advanced Photon Source, Argonne National Laboratory). Silicon nitride wafers were mounted perpendicular to the beam as described in Chen et al. (2013). SXRF mapping was performed with monochromatic 10 keV hard X-rays focused to a spot size of ~100 nm using Fresnel zone plates. Concentrations and distributions of all elements from P to Zn were analyzed in fine scans using a step size of 100 nm and a dwell time of 150 ms. An X-ray fluorescence thin film (AXO DRESDEN, RF8-200-S2453) was measured with the same beamline setup as a reference. MAPS

software was used for per-pixel spectrum fitting and elemental content quantification (Vogt 2003). Sample elemental contents were computed by comparing fluorescence measurements with a calibration curve derived from measurements of a reference thin film.

Regions of interest (ROIs) were selected with MAPS software by highlighting each microbial cell (identified based on elevated P content with care taken to avoid regions of elevated non-cellular metals) or particle (identified based on elevated metal content). Each ROI (n=14 and n=17 for *D. multivorans* (radius: 0.60 ± 0.01 μm) and *M. acetivorans* (radius: 0.48 ± 0.01 μm), respectively, and n=13 for the coculture (radius: 0.96 ± 0.01 μm)) was background corrected to remove elements originating from each section of the SiN grid on which cells were spotted. To do so, the mean of triplicate measurements of area-normalized elemental content for blank areas bordering the analyzed cells was subtracted from cellular ROIs. The background-corrected area-normalized molar elemental content was then multiplied by cellular ROI area to obtain molar elemental content per cell, which was then divided by the cell volume ($4/3\pi r^3$, assuming spherical cells) to yield total metal content per cell volume, in units of mmol L^{-1}. Visualization of elemental co-localization was performed with MAPS software. Statistical analysis was performed with JMP Pro (v. 12.1.0) using the Tukey-Kramer HSD test.

Results

Cellular elemental content of monocultures

Cellular metal contents of *M. acetivorans* and *D. multivorans* monocultures followed the trend Zn ≈ Fe > Cu > Co > Ni when measured by SXRF, and Zn ≈ Fe > Co > Ni > Cu when measured by ICP-MS (Fig. 1). When normalized to cell volume, cellular S measured by SXRF was 50x higher in methanol-grown *M. acetivorans* (n=14) than lactate-grown *D. multivorans* (n=17).

185 Cellular P, Fe, Co, Ni and Cu were 4-7x higher in *M. acetivorans* than *D. multivorans,* and
186 cellular Zn was not significantly different between the two microbes (Table 1).

187 ***Relative abundance of species in coculture***

188 Coculturing of both species for 12 days in media containing methanol and lactate resulted in
189 dominance of *M. acetivorans* (77%, or 1,753 cells hybridized with the MSMX860 FISH probe)
190 over *D. multivorans* (23%, or 522 cells hybridized with the Delta495a FISH probe) for 2,275
191 total cells counted in ten 100x (125 x 125 μm) fields of view. Cells were ~1 μm^2 cocci. No other
192 cells exhibited DAPI staining other than those that hybridized with MSMX860 and Delta495a
193 oligonucleotide probes. Attempts at FISH microscopy after SXRF analysis were unsuccessful
194 due to x-ray radiation damage of the cells.

195 ***Cellular elemental content of cocultures***

196 ICP-MS measurements showed that the relative abundance of cellular metals remained relatively
197 constant between mono- and cocultures, whereas SXRF data indicated that the coculture
198 contained a relatively higher proportion of Co than the monocultures (Fig. 1). SXRF imaging
199 showed no visual difference in elemental distribution between cells in the coculture (Fig. 2),
200 although the relatively small size of the cells relative to the focused x-ray spot may have limited
201 our ability to discern subtle differences. Cocultures, which were fixed with paraformaldehyde
202 and glutaraldehyde for subsequent fluorescence microscopy, were larger (radius: 0.96 ± 0.01
203 μm) than monocultures (*D. multivorans* radius: 0.60 ± 0.01 μm; *M. acetivorans* radius: 0.48 ±
204 0.01 μm), which were not fixed prior to analysis. When normalized on a per cell basis,
205 cocultures contained 5-20x higher P, Co and Ni than monocultures; however, when normalized
206 to cellular volume, the larger cell volumes of the cocultures resulted in significantly less Fe, Cu
207 and Zn per cellular volume than either of the monocultures (Table 1).

208 ***Non-cellular metals in cocultures***

209 In whole cell SXRF images, ~30 "hot spots" (discrete semi-circular areas with low-P and
210 elevated metals, indicative of nano-sized minerals) of Zn (max: 0.7 μg cm^{-2}), Co (max: 0.4 μg
211 cm^{-2}) and S (max: 2.7 μg cm^{-2}) were present in the center of a cluster of ~30 cocultured cells
212 identified as P-containing cocci (Fig. 2). In thin sections, semi-circular non-cellular small Zn hot
213 spots (0.6 ± 0.1 μm^2) containing ~1:1 molar ratios of Zn:S (17 ± 2 μg Zn cm^{-2}: 7.6 ± 0.7 μg S
214 cm^{-2}) were interspersed amongst cell clusters (n=8; Fig. 3a-e) along with more numerous
215 spheroid non-cellular Co hot spots of the same size (0.6 ± 0.1 μm^2) containing 2.1 ± 0.1 μg Co
216 cm^{-2}, 3.4 ± 0.2 μg S cm^{-2}, and 1.3 ± 0.1 μg Cu cm^{-2} (n=45; Fig. 3a-e). Discrete semi-circular hot
217 spots of elevated Ni (max: 2.9 μg cm^{-2}) with low S were observed in two imaging fields (n=8;
218 Fig. 3b,c).

219

220 **Discussion**

221 In this study, SXRF imaging and quantification of trace metals in cellular and abiotic phases was
222 performed at the single-cell scale. Our observation that Zn and Fe were the two most abundant
223 cellular trace metals in monocultures is consistent with previous studies of diverse prokaryotes
224 (Barton et al. 2007; Cvetkovic et al. 2010; Outten and O'Halloran 2001; Rouf 1964), including
225 diverse mesophilic and hyperthermophilic methanogens grown on a range of substrates, for
226 which, generally: Fe > Zn > Ni > Co > Cu (Cameron et al. 2012; Scherer et al. 1983). To our
227 knowledge, there are no previous reports of the trace metal content of sulfate-reducing bacteria,
228 but the abundance of Fe and Zn-containing proteins encoded by their genomes (Barton and
229 Fauque 2009; Barton et al. 2007; Fauque and Barton 2012) is consistent with the cellular
230 enrichment we observed in these trace metals.

231 Both normalizations for SXRF data (per cell and per cellular volume) showed that the
232 methanogenic archaeon contained more P, S, Co, Ni and Cu than the sulfate-reducing bacterium.
233 The higher cellular Co content of *M. acetivorans* vs. *D. multivorans* is likely due to due to
234 numerous methyltransferases involved in methylotrophic methanogenesis (Zhang and Gladyshev
235 2010; Zhang et al. 2009) that contain cobalt as a metal center in their corrinoid (vitamin B_{12})
236 cofactor, in addition to the corrinoid-containing Fe-S methyltransferase protein in the Wood
237 Ljungdahl pathway in both species (Ekstrom and Morel 2008; Fig. 4). Similarly, the higher Ni
238 content of *M. acetivorans* vs. *D. multivorans* is likely due to the presence of Ni-containing
239 cofactor F_{430} in methyl coenzyme M reductase, the final enzyme in the methanogenesis pathway.
240 Cofactor F_{430} is found only in methane-metabolizing archaea, in which it comprises 50-80% of
241 total cellular Ni (Diekert et al. 1981; Mayr et al. 2008). Additional Ni requirements in both *M.*
242 *acetivorans* and *D. multivorans* are used for Ni-Fe hydrogenases and carbon monoxide
243 dehydrogenase in the Wood-Ljungdahl pathway (Fig. 4).
244 Metabolic Cu requirements for methanogenesis are not well known, although high
245 accumulations have also been reported for other methanogens (Scherer et al. 1983). However, it
246 should be noted that our early trials analyzing S-rich cells on Au grids revealed artifacts resulting
247 from interactions of S and Cu underlying the grid's surface Au coating (data not shown); use of
248 SiN grids in this study appeared to eliminate such Cu artifacts, but potential reactions between
249 trace Cu in SiN grids and abundant S in the archaeal cells cannot be completely discounted.
250 Faster growth rates of methylotrophic methanogens than sulfate-reducing bacteria at
251 moderate temperatures have been reported in previous studies (Dawson et al. 2015; Weijma and
252 Stams 2001), and likely account for *M. acetivorans* outcompeting *D. multivorans* in our
253 cocultures. We consider it unlikely that differences in cellular trace metal contents in

monocultures were a result of harvesting *D. multivorans* earlier in their stationary phase than *M. acetivorans* (Fig. S1) because cellular metal reserves generally decline or remain constant in stationary phase (Bellenger et al. 2011). Our SXRF measurements of cocultures are more difficult to interpret due to apparent swelling of aldehyde-fixed cocultured cells (~1 μm radius) to ~2x the size of monocultures (0.5-0.6 μm radius). When normalized per cell, fixed cocultures showed significantly higher P, Co and Ni than unfixed monocultures, but the apparent swelling of cocultured cells erased this trend when normalized to cellular volume.

When grown at millimolar metal concentrations, sulfate-reducing bacteria efficiently remove metals from solution (Krumholz et al. 2003) and precipitate covellite (CuS; Gramp et al. 2006; Karnachuk et al. 2008), sphalerite/wurtzite (ZnS/(Zn,Fe)S; Gramp et al. 2007; Xu et al. 2016), and pentlandite (Co_9S_8) (Sitte et al. 2013). Based on its ~1:1 Zn:S ratio, the semi-circular nanoparticulate zinc sulfide phase(s) observed in thin sections imaged by SXRF in this study were likely sphalerite spheroids, also found in sulfate-reducing bacteria biofilms due to aggregation of ZnS nanocrystals (0.1-10 μm) and extracellular proteins (Moreau et al. 2004; Moreau et al. 2007). The abiotic phase with the approximate stoichiometry $(CoCu)S_2$ may be mineralogically distinct from those in previous studies.

Conclusions and Challenges

This study used two independent methods for assessing trace metal inventories in anaerobic microbial cultures. We found that SXRF is a promising method for imaging and quantifying first-row transition metals in anaerobic microbial cultures at single-cell resolution. This method's single-cell resolution enables more precise measurements of cellular metal content than ICP-MS analysis of bulk cells, which can include metals bound to extracellular aggregations such as

277 cation-binding exopolymeric substances produced by sulfate-reducing bacteria (Beech and
278 Cheung 1995; Beech et al. 1999; Braissant et al. 2007). We did not observe evidence of metal
279 contamination from aldehyde fixation in SXRF data, likely because we pre-cleaned fixatives
280 with metal-chelating resin prior to use, as previously described by Twining et al. (2003).

281 Challenges remain with accurate elemental quantification of microbial cocultures
282 preserved in a manner that would also allow assignment of identity for similar cell types. It was
283 not possible to distinguish methanogenic archaea from sulfate-reducing bacteria in coculture on
284 the basis of cell morphology or elemental content, and attempts to image cells with fluorescent
285 oligonucleotide probes after SXRF analysis were unsuccessful due to x-ray radiation damage.
286 We recommend method development for simultaneous taxonomic identification and elemental
287 imaging (e.g. gold-FISH (Schmidt et al. 2012)) for samples containing multiple microbial
288 species as a high priority for future work.

289 **Author Contributions**

290 J.B.G., V.J.O., S.C., and K.S.D. conceived and designed the experiments; K.S.D. performed the
291 microbial culturing, S.C., J.B.G., and S.V. performed the SXRF analyses; B.S.T. performed the
292 ICP-MS analysis, J.B.G., S.C., D.R.H., S.V., E.D.I., and B.S.T. analyzed the data; and J.B.G.
293 wrote the manuscript with input from all authors. All authors have given approval to the final
294 version of the manuscript.

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310

Table 1. Mean and standard error (in parentheses) of elemental contents normalized per cellular volume and per cell as measured by SXRF. Monocultures were prepared without chemical fixation, and cocultures were prepared with paraformaldehyde and glutaraldehyde fixation, followed by spotting onto silicon nitride wafers as described in the text. A, B and C superscripts indicate statistically different elemental contents ($p < 0.05$ based on Tukey-Kramer HSD test).

Culture	Substrate (mM)	P	S	Fe	Co	Ni	Cu	Zn
		Element per cellular volume (mmol L^{-1})						
100% *Methanosarcina acetivorans* DSM 2834 (n = 14)	Methanol (66 mM)	382^{A} (47)	4553^{A} (458)	38^{A} (4)	3.1^{A} (0.3)	0.44^{A} (0.04)	11^{A} (1)	38^{A} (3)
100% *Desulfococcus multivorans* DSM 2059 (n = 17)	Lactate (20 mM)	55^{B} (7)	96^{B} (11)	22^{B} (4)	0.5^{B} (0.1)	0.11^{B} (0.02)	3^{B} (1)	36^{A} (10)
77% *Methanosarcina acetivorans* DSM 2834, 23% *Desulfococcus multivorans* DSM 2059 (n = 13)	Methanol (66 mM), lactate (20 mM)	353^{A} (32)	234^{B} (19)	3.6^{C} (0.3)	2.0^{C} (0.2)	0.15^{B} (0.01)	0.13^{C} (0.04)	2.4^{B} (0.2)
		Element per cell (mol x 10^{-18} $cell^{-1}$)						
100% *Methanosarcina acetivorans* DSM 2834 (n = 14)	Methanol (66 mM)	178^{A} (28)	2167^{A} (318)	19^{A} (4)	1.5^{A} (0.2)	0.20^{A} (0.03)	5^{A} (1)	17^{AB} (2)
100% *Desulfococcus multivorans* DSM 2059 (n = 17)	Lactate (20 mM)	60^{A} (11)	107^{B} (19)	24^{A} (6)	0.5^{A} (0.1)	0.12^{A} (0.02)	3^{A} (1)	39^{A} (12)
77% *Methanosarcina acetivorans* DSM 2834, 23% *Desulfococcus multivorans* DSM 2059 (n = 13)	Methanol (66 mM), lactate (20 mM)	1252^{B} (69)	855^{C} (69)	13^{A} (1)	7^{B} (1)	2^{B} (1)	0.5^{B} (0.2)	9^{B} (1)

Figure Captions

Figure 1. Proportions of each cellular metal (Fe, Co, Ni, Cu and Zn) for monocultures of *Methanosarcina acetivorans* (n=14), monocultures of *Desulfococcus multivorans* (n=18), and cocultures of 77% *M. acetivorans* and 23% *D. multivorans* (n=12) measured by ICP-MS (bulk measurement) and SXRF (single cell average).

Figure 2. SXRF co-localization of P (red), Co (green), and Zn (blue; left panel), and S (red), Ni (green), and Cu (blue; right panel) for whole cells of 77% *Methanosarcina acetivorans* and 23% *Desulfococcus multivorans* in coculture. Values in parentheses are maxima in μg cm^{-2} for each element.

Figure 3. SXRF co-localization of P (red), Co (green), and Zn (blue) in left panels, and S (red), Ni (green), and Cu (blue) in right panels for five imaged fields of 5 μm thin sections of 77% *Methanosarcina acetivorans* and 23% *Desulfococcus multivorans* cocultures. Values in parentheses are maxima in μg cm^{-2} for each element.

Figure 4. Schematic of metalloenzyme-containing metabolic pathways in the complete carbon-oxidizing sulfate-reducing bacterium *Desulfococcus mutitvorans* and the methylotrophic methanogenic archaeon *Methanosarcina acetivorans* as confirmed by genomic analyses. Nickel (Acs, Cdh, Mcr) and cobalt (CFeSP, Mts, Mtr, and Sat) containing enzymes are labeled in bold. Enzyme abbreviations: Acs/CFeSP: acetyl-CoA synthase/corrinoid-FeS protein; Cdh: carbon monoxide dehydrogenase; Mts: methanol:coenzyme M methyltransferase; Mcr: methyl coenzyme M reductase; Mtr: methyl-tetrahydromethanopterin:coenzyme M methyltransferase; Sat: ATP sulfurylase.

338 **Figure S1.** Growth curves based on OD600 for the three cultures described in this study:
339 *Methanosarcina acetivorans* (white), *Desulfococcus multivorans* (light grey), and 77%
340 *Methanosarcina acetivorans* and 23% *Desulfococcus multivorans* cocultures (dark grey).
341

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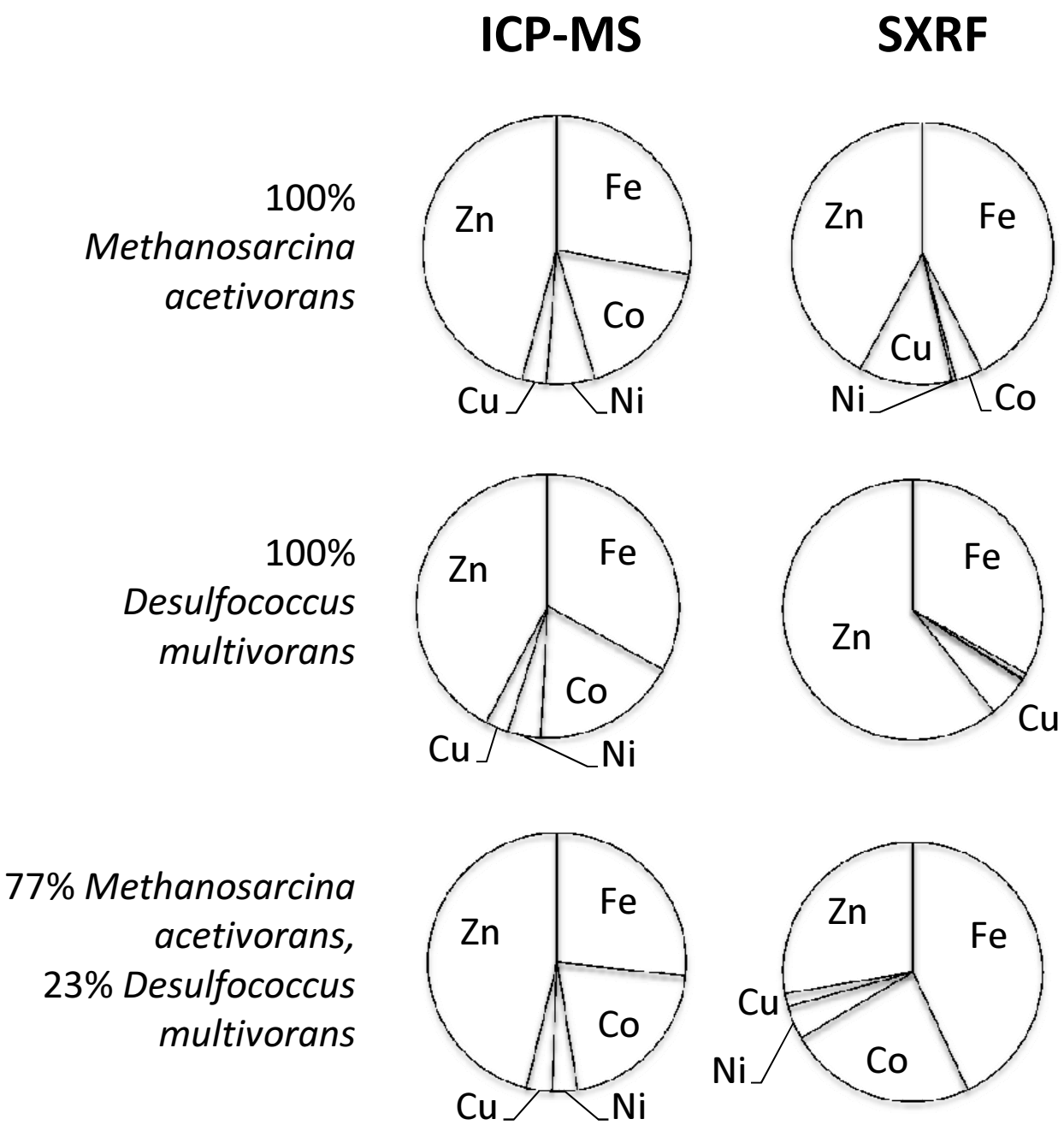

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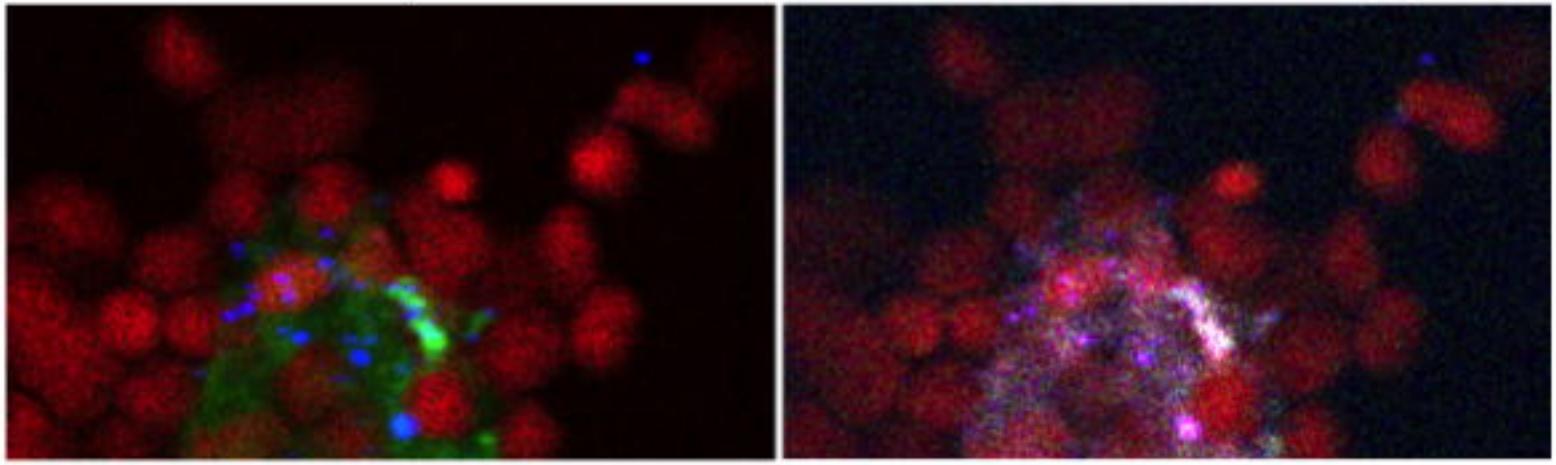
Figure 2



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S (2.7) Ni (0.03) Cu (0.04)

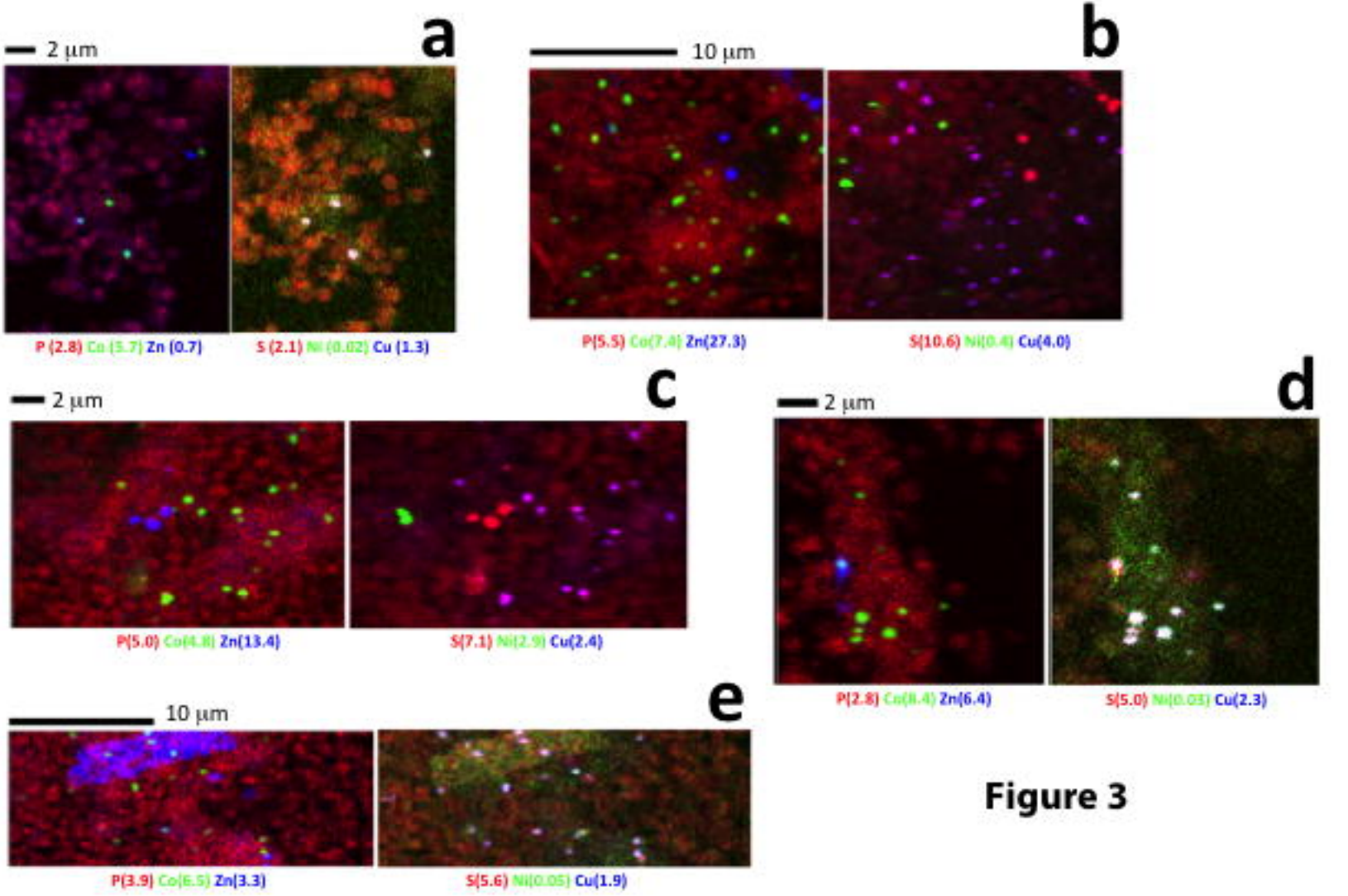
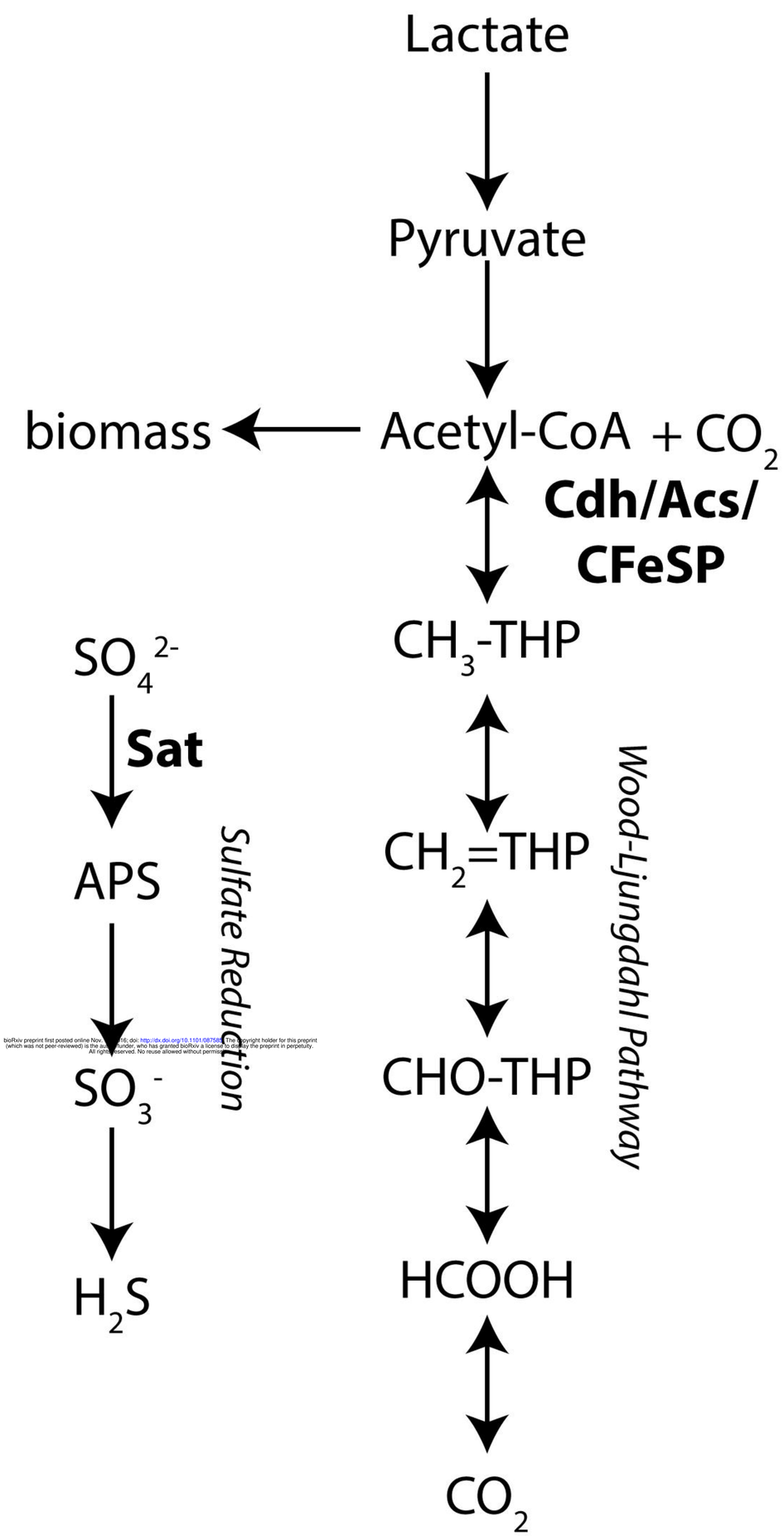

a
2 μm
P (2.8) Co (5.7) Zn (0.7)
S (2.1) Ni (0.02) Cu (1.3)
b
10 μm
P(5.5) Co(7.4) Zn(27.3)
S(10.6) Ni(0.4) Cu(4.0)
c
2 μm
P(5.0) Co(4.8) Zn(13.4)
S(7.1) Ni(2.9) Cu(2.4)
d
2 μm
P(2.8) Co(8.4) Zn(6.4)
S(5.0) Ni(0.03) Cu(2.3)
e
10 μm
P(3.9) Co(6.5) Zn(3.3)
S(5.6) Ni(0.05) Cu(1.9)

Figure 3

Desulfococcus multivorans



Methanosarcina acetivorans C2A