AEM Accepted Manuscript Posted Online 17 July 2020
Appl. Environ. Microbiol. doi:10.1128/AEM.00967-20






# Biomineralization of $Cu_2S$ nanoparticles by *Geobacter sulfurreducens*

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Running Head: Biomineralization of $Cu_2S$ nanoparticles

Keywords: *Geobacter sulfurreducens*; copper, nanoparticles, $Cu_2S$, bioreduction

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## Abstract

Biomineralization of Cu has been shown to control contaminant dynamics and transport in soils. However, very little is known about the role that subsurface microorganisms may play in the biogeochemical cycling of Cu. In this study, we investigate the bioreduction of Cu(II) by the subsurface metal-reducing bacterium, *Geobacter sulfurreducens*. Rapid removal of Cu from solution was observed in cell suspensions of *G. sulfurreducens* when supplied with Cu(II), while transmission electron microscopy (TEM) analyses showed the formation of electron dense nanoparticles associated with the cell surface. Energy-dispersive X-ray spectroscopy (EDX) point analysis and EDX spectrum image maps revealed the nanoparticles are rich in both Cu and S. This was confirmed by x-ray absorption near edge structure (XANES) and extended X-Ray absorption fine structure (EXAFS) analyses which identified the nanoparticles as $Cu_2S$. Biomineralization of $Cu_xS$ nanoparticles in soils has been reported to enhance the colloidal transport of a number of contaminants including Pb, Cd, and Hg. However, formation of these $Cu_xS$ nanoparticles has only been observed under sulfate-reducing conditions and could not be repeated using isolates of implicated organisms. As *G. sulfurreducens* is unable to respire sulfate, and no reducible sulfur was supplied to the cells, these data suggest a novel mechanism for the biomineralization of $Cu_2S$ under anoxic conditions. The implications of these findings for the biogeochemical cycling of Cu and other metals as well as the green production of Cu catalysts are discussed.


## Importance

Dissimilatory metal-reducing bacteria are ubiquitous in soils and aquifers and are known to utilize a wide range of metals as terminal electron acceptors. These transformations play an

important role in the biogeochemical cycling of metals in pristine and contaminated environments and can be harnessed for bioremediation and metal bioprocessing purposes. However, relatively little is known about their interactions with Cu. As a trace element that becomes toxic in excess, Cu can adversely affect soil biota and fertility. In addition, biomineralization of Cu nanoparticles has been reported to enhance mobilization of other toxic metals. Here, we demonstrate that when supplied with acetate under anoxic conditions, the model metal-reducing bacterium, *Geobacter sulfurreducens*, can transform soluble Cu(II) to $Cu_2S$ nanoparticles. This study provides new insights into Cu biomineralization by microorganisms and suggests that contaminant mobilization enhanced by Cu biomineralization could be facilitated by *Geobacter* species and related organisms.

## Introduction

Dissimilatory metal-reducing bacteria are ubiquitous in soils and aquifers and are able to respire a wide range of metals coupled to the oxidation of organic or inorganic compounds (1, 2). These processes play an important role in the biogeochemical cycling of metals in soils and subsurface environments (3, 4). In addition, microbial metal reduction can be harnessed for catalytic applications (5, 6) and bioremediation purposes (7, 8). Species of *Geobacter* and *Shewanella* are among the most intensively studied metal reducers due to their presence in many soil and freshwater environments, respiratory diversity and availability of genomic data (9-11). These organisms are able to utilise a range of metals as terminal electron acceptors including Fe(III), Mn(IV), U(VI), Pu(VI), Cr(VI), Ag(I), Pd(II), and V(V) (3, 12-18). These processes typically involve c-type cytochromes, which facilitate electron transfer from the inner membrane to the periplasm and outer membrane, where

62 many of these metals are reduced (19-21). Although the versatility of these metal-reducers
63 is well established, relatively little is known about their interactions with Cu.

64 Cu is a widely encountered trace element with its distribution in soil affected by climate,
65 geology and soil properties (22). In addition, anthropogenic sources can lead to elevated Cu
66 concentrations (23-26). Cu is an essential trace metal found in almost all forms of life,
67 however, it can also be highly toxic by binding to proteins and inactivating enzyme function
68 or by catalysing Fenton chemistry to produce reactive oxygen species. Thus at elevated
69 concentrations, the inherent toxicity of Cu can limit plant growth, lowering crop yield and
70 quality (27-29), and decrease microbial community diversity and activity (30). Microbial
71 processes play an important role in controlling the environmental fate of metals, potentially
72 immobilizing them via redox changes including bioreduction or through sulfidation reactions
73 (31). However, biomineralization has also been reported to enhance the mobilization of Cu
74 and other co-contaminants through the formation of colloidal Cu nanoparticles (25, 32). For
75 example, Hofacker *et al*. suggested *Clostridium* sp. were responsible for the Cu nanoparticle
76 formation under soil reducing conditions, however, they were unable to directly observe
77 Cu(II) reduction in cell suspensions of the *Clostridium* isolates (33). Therefore further work is
78 required to identify subsurface microorganisms which can potentially influence the
79 biogeochemical behaviour of this important micronutrient.

80

81 We demonstrated recently that *S. oneidensis* is able to reductively precipitate Cu(0)
82 nanoparticles from a Cu(II)-containing solution (6). Interestingly, the use of deletion mutants
83 revealed that *c*-type cytochromes in the Mtr-pathway, commonly used to reduce metals in
84 *Shewanella* species, did not play a role in Cu(II) reduction. The as-prepared Cu-

nanoparticles were shown to be catalytically active towards a range of 'click-chemistry' cycloaddition reactions, which have applications in the pharmaceutical industry (34).

Here we investigate the fate of Cu(II) when supplied to cultures of another well characterised model metal-reducing bacterium, the obligate anaerobe *G. sulfurreducens*, in order to better understand its potential role in the biogeochemical cycling and fate of Cu in contaminated soils and sediments, including the ability to produce Cu nanoparticles. Cu toxicity towards *G. sulfurreducens* was examined and its ability to bioreduce Cu(II) and remove the metal from solution monitored using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Any biomineralization products were analysed using transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM), energy-dispersive X-ray spectroscopy (EDX), X-ray absorption near edge structure (XANES), and extended X-Ray absorption fine structure (EXAFS).

## Results

### Cu(II) toxicity towards *G. sulfurreducens*

The effect of a range of concentrations of Cu on the anaerobic growth of *G. sulfurreducens* in minimal medium with lactate and fumarate supplied as the electron donor and acceptor respectively, is shown in Figure 1. Supplementation of the medium with 100 nM and 1 µM Cu(II) had little or no effect on the growth of *G. sulfurreducens* relative to a control with no added Cu(II). However, supplementation with 10 µM Cu(II), caused an extended lag phase with little to no growth seen over the initial 24 hours and only 45 % growth seen after 48 hours relative to the no added Cu(II) control. With 100 µM Cu(II) supplementation, no

growth was observed after 48 hours. Hence, under anoxic conditions growth of *G. sulfurreducens* is strongly inhibited by Cu at 10 µM and above.

Removal of Cu(II) and formation of Cu nanoparticles

Based on data from the toxicity assay, and guided by Cu pore-water concentrations in contaminated soils (25, 35), Cu(II) concentrations of 5 and 50 µM were chosen to be used in resting cell experiments to investigate potential bioreduction of Cu(II) and possible formation of lower oxidation state Cu nanoparticles, noted recently in cultures of other metal-reducing bacteria (6). Initial Cu(II) concentrations were confirmed via sampling of the solution prior to the addition of cells (t0). A decrease in the soluble Cu solution concentrations of 30.3 % and 12.0 % was observed in control experiments performed with dead (autoclaved) cells with initial concentrations of 5 µM and 50 µM Cu(II), respectively (Figure 2). This decrease was observed at the first time point, taken immediately after inoculating the Cu(II)-containing medium with cells (t1), and then remained constant, consistent with initial Cu removal via rapid passive sorption to the biomass. In the presence of live cells and electron donor (acetate), a substantial further increase in Cu removal from solution compared to the dead (autoclaved) cell controls was observed, suggesting that the majority of Cu removal in these treatments was facilitated by metabolic processes, rather than by passive biosorption to the cell biomass (Figure 2). When supplied with 5 µM Cu(II), 50 % Cu removal was observed immediately after adding the cells (t1) which then increased to 80% removal at 1 hour. Cu removal remained stable at ~80% at 24 hours, with a small decrease to 73% seen at the final 72 hour time point. When supplied with an initial concentration of 50 µM Cu(II), only 36% of the Cu was removed by the live cells at the first

time point (t1). Removal of Cu continued slowly for up to 24 hours, reaching a maximum of 63% removal at 24 hours before decreasing again slightly to 58% removal at 72 hours. Oxygenated cell controls (with acetate) showed decreased Cu removal at all time points compared to the anoxic cells when challenged with either 5 µM or 50 µM Cu(II). When no electron donor was supplied, initial Cu removal was slightly enhanced relative to the acetate-amended system when challenged with 5 µM Cu(II). However, after 3 hours, Cu solution concentrations remained relatively stable in the acetate-amended experiment but a significant increase in Cu solution concentrations was observed in both the electron donor free and oxygenated cell controls. When the final time point was taken at 72 hours, Cu removal was greatest in the anoxic cells supplied with acetate for both the 5 µM and 50 µM Cu(II) challenged systems.

TEM images of samples taken at 24 hours demonstrated that when supplied with acetate as an electron donor under anoxic conditions, removal of Cu by *G. sulfurreducens* resulted in the biomineralisation of Cu nanoparticles (Figure 3). These Cu nanoparticles were typically spherical and predominately associated with the cells, ranging in size from 10 to 90 nm.

EDX point analysis indicated that the particles were both Cu and S rich (Figure 3). This was further supported by EDX spectrum imaging performed during STEM that revealed a close association between Cu and S in the nanoparticles (Figure 4). A small number of particles were also seen in the dead (autoclaved) control, however these were typically larger (100 - 200 nm) agglomerates and were far fewer than with live cells supplied with acetate. EDX analysis indicated these particles were also Cu and S rich (Figure S1, Supporting

Information). No nanoparticles were observed in the oxygenated cells control or no electron donor control (Figure S2, Supporting Information).

X-ray absorption spectroscopy (XAS) characterization

To identify the speciation of Cu and the local valence structure of the metallic nanoparticles, XAS characterisation was performed on B18 at the Diamond Light Source. The Cu K-edge X-ray absorption near edge structure (XANES) spectrum collected on the Cu nanoparticles precipitated by *G. sulfurreducens* had an absorption edge energy ($E_0$) of 8980.4 eV. This aligned well with the $E_0$ energy of a $Cu_2S$ (chalcocite) standard (8980.1 eV). The excellent match between the XANES spectra for the Cu nanoparticles and the $Cu_2S$ standard clearly demonstrates that washed cell suspensions of the metal-reducing bacterium *G. sulfurreducens* catalysed the formation of $Cu_2S$ particles (Figure 5a). The oxidation state of Cu in $Cu_2S$ is thought to be dominated by Cu(I), however, the presence of significant Cu(II) and Cu(0) has also been suggested (36, 37). XANES data were identical whether the Cu nanoparticles were formed when the cells were supplied with Cu(II) as $CuSO_4$ or $CuCl_2$, ruling out any impact from the salt (data not shown).

Given the similarity between the Cu nanoparticles and $Cu_2S$ XANES spectra, the Cu nanoparticle EXAFS data were fitted assuming a similar coordination environment as the mineral chalcocite ($Cu_2S$) (38). The best fit (Figure 5b) was obtained with one shell of 3 S atoms at 2.30 ± 0.01 Å with a Debye-Waller factor of 0.009 ± 0.001. This is consistent with a $Cu_2S$-like structure where Cu is coordinated by 3 S atoms at approximately 2.3 Å. $Cu_2S$ has 6 Cu atoms in the second shell, however, the Cu-S interatomic distance is poorly constrained (2.60 - 3.30 Å), and which is likely to reduce the contribution from Cu scatterers to the

175 EXAFS spectrum. Given this, the EXAFS data do not preclude the formation of a $Cu_2S$ phase.
176 See Table S1 (Supporting Information) for complete description of the EXAFS fitting.
177 Collectively, the XANES and EXAFS data support the biomineralization of the Cu(II) as poorly
178 ordered $Cu_2S$.

179 Cu $L_{2,3}$-edge XAS was also performed on selected samples at the Advanced Light Source,
180 Berkeley. When live cells were supplied with an electron donor, peaks at 930.7 and 933.4 eV
181 were observed (Figure S3, Supporting Information). The peak at 930.7 eV is indicative of
182 Cu(II) whereas the peak at 933.4 eV reflects the presence of Cu(I) (39, 40). Although the
183 peak at 930.7 eV is qualitatively larger than the peak at 933.4 eV, the Cu(II) is known to be
184 approximately 25 times more intense than the Cu(I) peak (39), suggesting a significant
185 presence of Cu(I) in the sample, confirming bioreduction took place. Cu $L_{2,3}$-edge XAS data
186 from the autoclaved and no electron donor controls also display peaks indicative of Cu(II)
187 and Cu(I), although with a noticeable shift of +0.3 eV in peak positions. The intensity of the
188 Cu(I) peaks are qualitatively smaller in both controls compared to the live cells supplied with
189 electron donor, suggesting less Cu bioreduction occurred in the controls.

190

191 **Discussion**

192 Cu toxicity towards *G. sulfurreducens*

193 Our results demonstrate that under anoxic conditions, concentrations as low as 10 µM Cu(II)
194 strongly inhibit the growth of *G. sulfurreducens*. This is over an order of magnitude lower
195 than the Cu(II) concentrations (>100 µM) that are reported to be required to inhibit the
196 growth of the enteric model organism *Escherichia coli* under similar conditions (anoxic

chemically defined media) (39). In a recent study, the model Fe(III)-reducing bacterium *Shewanella oneidensis*, also appears to have greater resistance to Cu toxicity than *G. sulfurreducens* with some growth of *S. oneidensis* still observed at 100 µM Cu in a similar chemically defined medium (6, 40), whereas growth of *G. sulfurreducens* is inhibited completely (Figure 1). Hence, *G. sulfurreducens* appears to be particularly vulnerable to Cu toxicity.

Biomineralization of Cu nanoparticles

The relatively low tolerance of *G. sulfurreducens* towards Cu(II) compared to *S .oneidensis* is also reflected in their respective ability to reduce Cu(II). In our previous study, complete reduction of 50 µM and partial reduction of up to 200 µM Cu(II) to Cu(0) was observed by *S. oneidenis* (6). In the present study, *G. sulfurreducens* was only capable of removing up to 80 % and 63 % of 5 µM and 50 µM Cu(II) from solution, respectively. No removal at 200 µM Cu(II) was observed (data not shown). Our data suggest that Cu removal from solution is greatest in the presence of live cells, suggesting that microbial metabolism increases removal of Cu from solution compared to biosorption by dead biomass. The highest removal was seen when live cells were supplied with an electron donor under anoxic conditions, with TEM and XAS data indicating that formation of Cu nanoparticles was significant only under these conditions. This suggests that the presence of an electron donor and anoxic conditions are required for significant biosynthesis of Cu nanoparticles. Characterisation of these nanoparticles as $Cu_2S$ is supported by EDX point analysis, EDX mapping, XANES and EXAFS data. As no nanoparticles were seen in the oxygenated or electron donor free controls and XAS data support limited reduction to Cu(I) species, we attribute the observed removal of Cu from solution under these conditions to biosorption of Cu(II) to the cell surface and/or

intracellular accumulation of the metal. Interestingly, the Cu solution concentrations in the oxygenated and electron donor free controls were found to increase after 3 hours, but remained relatively stable under anoxic conditions with an electron donor (acetate). We suggest that the rerelease of Cu into solution observed in the oxygenated and electron donor free controls may be due to export of intracellular Cu and/or desorption of cell-bound Cu, which was limited in the anoxic cells supplied with acetate due to immobilization of Cu via bioreduction and $Cu_2S$ precipitation.

The formation of $Cu_2S$ nanoparticles here is surprising as previous work on Cu biomineralization by anaerobic bacteria found that, in the absence of sulfate-reducing conditions, nanoparticles formed were metallic Cu or Cu oxides (6, 25, 41, 42). A number of mechanisms have been proposed for the formation of Cu(0) nanoparticles among different bacteria. Ramanathan *et al.* suggested that intracellular reduction and cellular efflux systems may play a role in the synthesis of extracellular Cu(0) nanoparticles by *M. morganii* (42). *Clostridium* species were implicated in the formation of Cu(0) nanoparticles in a flooded Cu contaminated soil via cellular efflux of Cu(I) followed by disproportionation to Cu(II) and Cu(0) (25, 33). Conversion of the Cu(0) nanoparticles to $Cu_xS$ in the flooded soil was observed following the onset of sulfate reduction. As *G. sulfurreducens* is unable to carry out dissimilatory sulfate reduction, this cannot account for the of $Cu_2S$ nanoparticles seen here. Identical XANES spectra from Cu nanoparticles formed when *G. sulfurreducens* were supplied with Cu(II) as $CuSO_4$ and $CuCl_2$ further supports a mechanism which does not directly involve sulfate reduction. Clearly, a different mechanism is responsible for the biomineralization of $Cu_2S$ nanoparticles in *G. sulfurreducens* compared to the Cu(0) nanoparticles typically produced by other bacteria studied to date.

243 Cell bound thiol sites have been shown to dominate metal(oid) complexation in a range of
244 microorganisms, including *G. sulfurreducens* (43, 44). In addition, intracellular Cu(I) is known
245 to target Fe-S clusters in *E. coli* under anoxic conditions with Cu(I) displacing the Fe (45-47).
246 Ligation of Cu with these sulfur groups could potentially play a role in the formation of $Cu_xS$
247 nanoparticles in organisms which are unable to reduce Cu(II) to Cu(0), such as *G.*
248 *sulfurreducens*. However, elucidating the mechanisms of Cu nanoparticle formation in
249 metal-reducing bacteria requires further work and will be the target of future studies.

250 This study demonstrates that *G. sulfurreducens*, a metal-reducing bacteria, is able to
251 produce $Cu_2S$ nanoparticles from aqueous Cu(II). This provides direct evidence of Cu
252 biomineralization by a ubiquitous subsurface microorganism which may suggest a role for
253 this organism in the biogeochemical cycling of Cu and potential mobilization of co-
254 contaminants in soil systems (25). $Cu_xS$ nanoparticles have previously been reported to form
255 only following the onset of sulfate reduction. *G. sulfurreducens* is unable to respire sulfate,
256 suggesting that biomineralisation of $Cu_xS$ nanoparticles could also occur in the absence of
257 sulfate-reducing conditions. In addition to the biogeochemical implications discussed above,
258 biomineralization of Cu nanoparticles offers a promising green method for the production of
259 Cu catalysts. We have previously demonstrated that Cu(0) nanoparticles produced by *S.*
260 *oneidensis* are active click chemistry catalysts (6). $Cu_2S$ nanoparticles also have a number of
261 catalytic applications, including as electrocatalysts for oxygen evolution and $CO_2$ reduction
262 (48, 49). Therefore, tailored Cu nanoparticle catalysts could potentially be produced using
263 specific microorganisms.

264

265 **Materials and Methods**

*Geobacter sulfurreducens*

All cultures of *G. sulfurreducens* (ATCC 51573) were grown anaerobically in a fully defined, pre-sterilized, liquid minimal medium (NBAF) (50) at pH 7.1. Serum bottles (100 ml) containing NBAF were inoculated with a late log/early stationary phase culture to give an optical density of 0.02 at 600 nm. The cultures were grown for 24 hours at 30 $^{o}$C. Late log cultures were transferred under anoxic conditions to centrifuge tubes, and the cells pelleted by centrifugation at 4,960 rpm for 20 minutes at 4 $^{o}$C. The cells were washed two times with fresh anoxic, sterilised MOPS buffer (50 mM) and then were resuspended in the same buffer at pH 7.1.

Toxicity assay

Cells were grown in minimal media as described above. Late-log phase aliquots were used to inoculate 50 ml of anoxic minimal media to give a starting optical density of 0.015. Cu ($CuSO_4$) was added from a stock solution to give a final concentration of 0.1, 1, 10, 100 or µM Cu(II). A Cu free control was also used. Incubation was carried out at 30°C. Samples were taken under anoxic, aseptic conditions at 0, 4, 8, 24, and 48 hours and the optical density measured to determine growth. All conditions were performed in triplicate with a standard deviation of less than 0.012 between $OD_{600}$ readings.

Cu removal experiments

The removal of Cu(II) by *G. sulfurreducens* was determined using pre-grown and washed "resting cell" cultures supplied with excess electron donor. The cultures contained either 5 µM or 50 µM Cu(II) as $CuSO_4$ (unless stated otherwise) and 30 mM sodium acetate as the electron donor in 50 mM MOPS adjusted to pH 7.1. The medium was purged with a gas mix

of $N_2$:$CO_2$ (80:20) for 20 minutes to remove $O_2$, sealed with thick butyl rubber stoppers and autoclaved. Washed late log/early stationary phase cells were then added aseptically to achieve a final optical density of 0.2 and incubation was carried out at 30 $^{o}$C. Soluble Cu was determined by taking aliquots centrifuging at 14,900 g for 10 minutes to pellet the cells and insoluble Cu. Samples were taken from the supernatant and Cu in solution measured using ICP-AES. All experiments were performed in triplicate.

TEM imaging

All sample preparations were performed under anoxic conditions in an anaerobic cabinet. 1 ml of cell suspension from the Cu reduction assay (5 and 50 µM) was taken after 24 hours and centrifuged at 14900 g for 10 minutes, the supernatant discarded and the pellet resuspended in 1 ml deionised water. 1.5 µl of the cell suspension was pipetted onto a gold TEM grid with a carbon-coated formvar or holey-carbon support film and air dried in an anaerobic chamber. Samples were kept anoxic until they were transferred into the TEM chamber. TEM imaging and EDX analysis were performed in an FEI TF 30 FEG Analytical TEM operated at 300kV and equipped with an Oxford X-max80 Windowless SSD EDS system. EDX analysis was performed with the sample tilted at the optimum angle towards the detector to increase collection efficiency.

STEM

STEM imaging and EDX analysis were performed in a FEI Talos F200A AEM with anX-FEG electron source operated at 200 kV. High angle annular dark field (HAADF) STEM imaging was performed using a probe current of approximately 250 pA. EDX analysis was performed

using a Super-X four silicon drift EDX detector system with a total collection solid angle of 0.7 srad, all four detectors were turned on and the sample was not tilted.

X-ray absorption spectroscopy (XAS) characterization

For XAS characterisation at the Cu K-edge, 1 ml aliquots of the *G. sulfurreducens* Cu reduction (5 and 50 µM of $CuSO_4$ and $CuCl_2$) assays were taken and centrifuged at 14900 g for 10 minutes. The supernatant was discarded and the pellet resuspended in 1 ml anoxic deionised water. The sample was centrifuged again, resuspended in 1 ml anoxic deionised water before 200 µl of the suspension was pipetted onto a plastic weighing boat and air dried. Samples were mounted onto a layer of kapton tape which in turn was mounted onto an aluminium sample holder. A further layer of kapton tape was applied over the samples to maintain anaerobicity. X-ray absorption fine structure (XAFS) spectra were collected at the Cu K-edge (~8980 eV) at room temperature on beamline B18 at the Diamond Light Source. A 36-element solid-state Ge detector with digital signal processing for fluorescence XAFS, high energy resolution, and high count rate was used to measure with the beam at 45° incidence with respect to the sample holder plane. All spectra were acquired in quick-EXAFS mode, using the Pt-coated branch of collimating and focusing mirrors, a Si(111) double-crystal monochromator and a pair of harmonic rejection mirrors.

XAS processing and background subtraction was carried out using Athena whereas EXAFS data were modelled using Artemis (Demeter(51); 0.9.24). Fitting was calculated using multiple k-weights (k, $k^2$, and $k^3$) and the best fit was calculated in R space by minimisation of the reduced $X^2$. At no point did the parameterisation utilise more than $^2/_3$ of the independent points available.

Cu $L_{2,3}$-edge spectroscopy was performed at the Advanced Light Source, Berkeley, USA on beamline 6.3.1.1. For sample preparation 1 ml aliquots were taken and centrifuged at 14900 g for 10 minutes. The supernatant was discarded and the pellet resuspended in 1 ml anoxic deionised water. The sample was centrifuged again before final resuspension in 1 ml anoxic deionised water. 200 µl of the suspension was dried as a powder onto a carbon sticky pad before being placed on an aluminium sample holder and stored under anoxic conditions prior to analysis. All manipulations were performed in an anaerobic chamber. X-ray absorption spectra were collected in total-electron yield (TEY) mode and normalised to the incident beam intensity.

## Acknowledgments

The authors would like to thank NERC for funding under the Resource Recovery from Waste program (NE/L014203/1), and also acknowledges support from the BBSRC (grants BB/L013711/1 and BB/R010412/1). The authors would also like to P. Lythgoe (University of Manchester) for ICP-AES analysis. K.S. would like to acknowledge EnvRadNet and ST/K001787/1 for funding. The authors acknowledge beamtime awarded at the Diamond Light Source for XANES and EXAFS on beamline B18 under proposals SP-13705 and SP-16136. We would like to thank Giannantonio Cibin for his assistance on B18. This research used resources of the Advanced Light Source, a DOE Office of Science User Facility under contract no. DE-AC02-05CH11231

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501 **Figure 1** Anaerobic growth of *G. sulfurreducens* in minimal medium (NBAF) supplemented with different Cu(II)
502 concentrations. Each concentration was performed in triplicate with error bars representing the standard
503 deviation of the replicates.

504

505 **Figure 2** Concentration of Cu in solution in the presence of *G. sulfurreducens* when supplied with an initial
506 Cu(II) concentration of (a) 5 µM and (b) 50 µM. In both cases, Cu(II) was added to the media prior to cell
507 addition. The initial concentration of Cu was confirmed via ICP-AES. The first time point (t1) was then taken
508 immediately after cell addition. Cu in solution was calculated as the concentration of Cu at a given time point
509 (C) divided by the initial concentration prior to cell addition ($C_0$) as determined by ICP-AES. Experiments were
510 performed under anoxic conditions except where indicated with the addition of O2 (purple diamonds). The
511 addition or omission of acetate as electron donor is indicated by $+e^-$ or $-e^-$, respectively. Each experiment was
512 performed in triplicate with errors representing the standard deviation of these replicates.

513

514 **Figure 3** TEM images of *G. sulfurreducens* with associated Cu nanoparticles after being supplied with (a-c) 5 µM
515 Cu(II) and (d-f) 50 µM Cu(II). The bottom row shows the corresponding EDX spectra of particles from panel a, c,
516 and e (left to right). The x axis displays energy (keV) with the y axis displaying total counts. Samples for TEM
517 imaging were taken at 24 hours.

518

519 **Figure 4** (a) TEM image of cells with Cu nanoparticles (b) High-angle annular dark field (HAADF) image of the
520 red dashed square from (a). EDX spectrum imaging of (b) taken under STEM showing (c) Cu and (d) S.

521

522 **Figure 5** (a) XANES for the Cu K-edge of Cu nanoparticles produced by *G. sulfurreducens* (black line) and Cu
523 standards. b) $K^3$ weighted EXAFS data and c) corresponding Fourier transform for the Cu K-edge of the Cu
524 nanoparticles (Cu-NPs). Data are shown by the black (solid) line and the fit is shown by the red (dotted) line.