View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by LJMU Research Online

For Peer Review Only

A novel aerobic mechanism for reductive palladium biomineralization and recovery by *Escherichia coli*

Journal:	*Geomicrobiology Journal*
Manuscript ID:	UGMB-2015-0116.R1
Manuscript Type:	Special Issue
Date Submitted by the Author:	n/a
Complete List of Authors:	Foulkes, Joanne; Liverpool John Moores University, Pharmacy and Biomolecular Science Deplanche, Kevin; University of Birmingham, School of Biosciences Sargent, Frank; University of Dundee, College of Life Sciences Macaskie, Lynne; University of Birmingham, School of Biosciences Lloyd, Jon; University of Manchester, School of Earth, Atmospheric and Environmental Sciences
Keywords:	biomineralization, metal reduction, bioremediation

SCHOLARONE™
Manuscripts

A novel aerobic mechanism for reductive palladium biomineralization and recovery by *Escherichia coli*

JOANNE M. FOULKES[a], KEVIN DEPLANCHE[b], FRANK SARGENT[c], LYNNE E. MACASKIE[b], JONATHAN R. LLOYD[a*]

a. Williamson Research Centre for Molecular Environmental Science and School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, UK

b. Unit of Functional Bionanomaterials, School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

c. Division of Molecular Microbiology, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK

*Corresponding author, Jon.Lloyd@manchester.ac.uk

Tel: (0161) 275 7155

Fax: (0161) 306 9361

Aerobically-grown *E. coli* cells reduced Pd(II) via a novel mechanism using formate as the electron donor. This reduction was monitored in real-time using extended X-ray absorption fine structure. Transmission electron microscopy analysis showed that Pd(0) nanoparticles, confirmed by X-ray diffraction, were precipitated outside the cells. The rate of Pd(II) reduction by *E. coli* mutants deficient in a range of oxidoreductases was measured, suggesting a molybdoprotein-mediated mechanism, distinct from the hydrogenase-mediated Pd(II) reduction previously described for

For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

26 anaerobically-grown *E. coli* cultures. The potential implications for Pd(II) recovery
27 and bioPd catalyst fabrication are discussed.
28
29 **Keywords:** palladium nanoparticles, *Escherichia coli*, biomineralization.
30
31 **Introduction**
32 The microbial reduction of metals and radionuclides has attracted much interest, as it
33 can be potentially harnessed for bioremediation, metal recovery, the fabrication of
34 novel nanobiominerals and even energy generation in biobatteries (Lloyd 2003; Lloyd
35 et al. 2008; Lovley 2006;). For example, the sulfate-reducing bacterium (SRB)
36 *Desulfovibrio desulfuricans* has been shown to use a periplasmic hydrogenase
37 supplied with hydrogen to reduce soluble Pd(II), resulting in the precipitation of Pd(0)
38 nanoparticles in the periplasm of the cell ('bioPd'). However SRB produce H_2S, a
39 potent catalyst poison that must be removed before making the bioPd. Other
40 organisms capable of this metal bioreduction include the Gram-negative bacteria
41 *Shewanella oneidensis* (De Windt et al. 2005), *Escherichia coli* (Deplanche et al.
42 2010, 2014; Mabbett et al. 2006), *Pseudomonas putida*, *Cupriavidus necator* (Søbjerg
43 et al. 2009), *Cupriavidus metallidurans* (Gauthier et al. 2010), *Paracoccus*
44 *denitrificans* (Bunge et al. 2010), *Rhodobacter sphaeroides* (Redwood et al. 2008),
45 *Rhodobacter capsulatus* (Wood et al. 2010), and the Gram-positive bacteria *Bacillus*
46 *sphaericus* (Creamer et al. 2007), *Arthrobacter oxyidans* (Deplanche et al. 2014;
47 Wood et al. 2010), *Micrococcus luteus* (Deplanche et al. 2014), *Staphylococcus sciuri*
48 (Søbjerg et al. 2009) and *Clostridium pasteurianum* (Chidambaram et al. 2010). This
49 property has allowed the use of 'palladised' whole cells or processed biomineral
50 directly in industrially important reactions, often showing superior activity compared

For Peer Review Only

with a commercially available carbon-supported palladium catalyst. A number of studies have investigated the catalytic activity of bioPd, demonstrating its use in remediative reactions such as the reduction of Cr(VI) to Cr(III) (Beauregard et al. 2010; Mabbett et al. 2006), the dehalogenation of chlorophenol, polychlorinated biphenyls, polybrominated diphenyl ethers (Baxter-Plant et al. 2003; De Windt et al. 2005; Harrad et al. 2007), trichloroethylene (Hennebel et al. 2009a, 2009b), and the pesticide γ- hexachlorocyclohexane (Mertens et al. 2007), in 'greener' chemical synthesis such as the hydrogenation of itaconic acid (Creamer et al. 2007) and 2-pentyne (Bennett et al. 2010), in Heck and Suzuki reactions (Bennett et al. 2013; Deplanche et al. 2014), and also in the application of bioPd as a fuel cell electrocatalyst to produce electricity from hydrogen (Orozco et al. 2010; Yong et al. 2007). In each case where the bioPd was compared with an abiotically-produced palladium catalyst (finely-divided or supported on a carbon matrix), the bioPd was more active than or at least as active as the commercially available alternative.

Production of catalytically active bioPd also was reported by an aerobically-grown *Serratia* sp. (Beauregard et al. 2010; Deplanche et al. 2014) under which condition hydrogenases are not expressed. Also, cells of *E. coli* deficient in the three major hydrogenases reduced Pd(II) (albeit slowly: Deplanche et al. 2010), and showed larger Pd-nanoparticles located on the outer surface of the cells. This suggested an alternative mechanism of Pd(II) reduction which has not been investigated.

E. coli produces bioPd which is comparably active to that produced by *D. desulfuricans* (Deplanche et al. 2014). This also provides a very useful model organism since it is facultatively anaerobic and has well-defined molecular tools to

For Peer Review Only

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

76 elucidate reaction mechanisms under aerobic and anaerobic conditions. The enzymes
77 potentially involved in the bioreduction of palladium by *E. coli* under the latter
78 conditions are the nickel-dependent hydrogenase enzymes Hyd-1, Hyd-2, and Hyd-3,
79 and the formate dehydrogenase molybdoenzymes FDH-N, and FDH-H. Another
80 molybdoenzyme, FDH-O, is expressed under both aerobic and anaerobic conditions.
81 A possible role for FDH-O is to allow bacteria to adapt rapidly to a sudden shift from
82 aerobic respiration to anaerobiosis, before FDH-N has been produced in sufficient
83 amounts to continue formate metabolism (Abaibou et al. 1995). Hyd-1, Hyd-2, FDH-
84 O, and FDH-N are membrane-bound and periplasmically oriented, whereas Hyd-3
85 and FDH-H are subunits of the formate hydrogenlyase (FHL) complex, an
86 intracellular enzyme complex that is also membrane-bound but which faces into the
87 cytoplasm. The mechanisms responsible for the formate-dependent bioreduction by
88 anaerobically-grown cultures of *E. coli* have been studied, showing that the
89 hydrogenase enzymes Hyd-1 and Hyd-2 are mainly responsible for Pd(II)
90 bioreduction (Deplanche et al. 2010). In a study of formate-dependent Pd(II)
91 bioreduction by *Desulfovibrio fructosovorans*, the deletion of the periplasmic
92 hydrogenases caused the Pd(0) nanoparticles to be relocated to the cytoplasmic
93 membrane site of the remaining hydrogenases, indicating that the periplasmic
94 hydrogenases are at least partially involved (Mikheenko et al. 2008).
95
96 The growth yield of anaerobic cultures is lower than that of aerobic cultures, and for
97 economic production at scale a method of growth of high biomass density is required.
98 When using anaerobic cultures there is also the cost of supplementing with sodium
99 fumarate and glycerol. The dual aims of this study are to establish whether *E. coli*
100 cells grown aerobically are capable of manufacturing bioPd and to identify the

For Peer Review Only

101 enzyme(s) responsible for such metal reduction. A move away from the need for
102 anaerobic growth would simplify the preparation of high levels of active biomass for
103 catalyst production at industrial scale.
104

105 **Methods**

106 *Bacterial growth*

107 Starter cultures: 50 ml LB broth in a 500 ml Erlenmeyer flask was inoculated with a
108 single isolated colony of the *E. coli* strain under investigation and incubated
109 aerobically (37°C, shaking at 180 rpm for 18 h).
110

111 Aerobic cultures: An 11 ml starter culture was added to 99 ml LB broth in a 1 L
112 Erlenmeyer flask. Flasks were incubated for 24 h (37°C, 180 rpm) to produce
113 stationary phase 'resting' cells. The pH of the cells after 24 h incubation was
114 measured to determine that organic acids had not been produced that would otherwise
115 lower the pH considerably (Vasala et al. 2006). Oxygen saturation of a 5 ml aliquot of
116 the broth culture was measured immediately after 24 h of incubation using an Oakton
117 D06 Acorn Series dissolved oxygen meter.
118

119 *Reduction of Pd(II) to produce bioPd on bacteria*

120 The aerobically grown liquid culture was divided between two 50 ml Falcon tubes
121 and washed three times in 20 ml MOPS-NaOH (morpholinepropanesulfonic acid)
122 buffer, 20 mM at pH7.6 after centrifugation for 20 min at 2500 *g*. Cell pellets were
123 adjusted to a mass of 250 mg wet pellet weight, and resuspended in the MOPS-NaOH
124 buffer to a volume of 1 ml. One tube of 250 mg wet weight cells was resuspended in
125 22.5 ml MOPS-NaOH buffer with 1 mM sodium tetrachloropalladate in a 30 ml bottle

For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

126 sealed with a butyl rubber stopper. The bottle was incubated in the dark at 30°C for 1
127 h for the Pd(II) to biosorb to the cells (Baxter-Plant et al. 2003). 2.5 ml 10 mM
128 sodium formate was then added to the bottle to initiate bioreduction of the Pd(II).
129

130 *Use of mutants to determine electron transfer pathway to Pd(II)*
131 In order to investigate the possible role of the aerobic formate dehydrogenase (FDH-O)
132 and other hydrogenase/formate dehydrogenase enzymes in the reduction of Pd(II) by
133 aerobically-grown cells of *E. coli*, the rates of reduction by six different additional
134 strains (Table 1) were compared by measuring the Pd(II) remaining in solution by
135 ICP-MS. The strains were 'palladised' as above, and rates of reduction/removal
136 compared to those in a series of controls: killed cells (MC4100), cell-free suspension,
137 and live cells (MC4100) unsupplemented with formate.
138

139 All strains except BL21(DE3) were from the culture collection of Professor Frank
140 Sargent at the College of Life Sciences, University of Dundee. Strain BL21(DE3) was
141 obtained from Invitrogen, Paisley, UK. Strain MC4100 Δ*moaA* was created by
142 disruption of the *moaA* gene which encodes the molybdenum cofactor biosynthesis
143 protein A, using the method of Datsenko and Wanner (2000) whereby PCR products
144 are used to disrupt the gene of choice by recombination using the plasmid-borne
145 phage λ Red recombinase.
146

147 *X-ray diffraction (XRD) analysis*
148 The black precipitates were washed once in acetone and air dried, before analysis by
149 X-ray diffraction (XRD). The measurements were performed on a Bruker D8
150 Advance diffractometer, using Cu K alpha1 radiation. The samples were scanned

For Peer Review Only

from 5-70 degrees 2theta in steps of 0.2 degrees, with a count time of 2 seconds per step.

Extended X-ray absorption fine structure (EXAFS)

Aliquots of the cell/Pd/formate suspension were taken at times 0 and 30 min, and 1, 3 and 4 h from the addition of formate, and frozen immediately in liquid nitrogen. The direct reduction of Pd(II) to Pd(0) was demonstrated using EXAFS, performed at the European Synchrotron Radiation Facility (ESRF), in Grenoble, France. The samples were transported to the synchrotron at ESRF on dry ice, where they were thawed and injected immediately into sample holders, before freezing once more in liquid nitrogen and placing into the beam. X-ray absorption data were collected on beamline BM29 at the Pd K–edge in the energy range 24 200 – 24 900 eV. Data were recorded at low temperature (77 K) and under vacuum to reduce the thermal Debye-Waller factor and prevent oxidation. A Si(III) double crystal monochromator was used, calibrated with a Pd foil, and the spectra were collected in fluorescence mode using a 13-element solid-state detector. A reference spectrum of a palladium foil was recorded in transmission mode on station 9.3 at the SRS Daresbury. The data were background subtracted and the EXAFS spectra fitted in DL_Excurv (http://www.cse.scitech.ac.uk/cmg/EXCURV/) using full curved wave theory (Gurman et al., 1984).

Transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy (EDS)

Following Pd(II) reduction, cells were stored at 10°C overnight. The cell pellets were then rinsed twice with deionised water, fixed in 2.5% (wt/vol) glutaraldehyde,

For Peer Review Only

1
2
3 176 centrifuged for 5 min at 16 000 *g*, resuspended in 1.5 ml of 0.1 M cacodylate buffer
4
5 177 (pH 7) and stained in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7 (60 min).
6
7 178 Cells were dehydrated using an ethanol series (70, 90, 100, 100, 100% dried ethanol,
8
9 179 15 min each) and washed twice in propylene oxide (15 min, 9500 *g*). Cells were
10
11 180 embedded in epoxy resin and the mixture was left to polymerise (24 h; 60°C).
12
13 181 Sections (100-150 nm thick) were cut from the resin block, placed onto a copper grid
14
15 182 and viewed with a JEOL 1200CX2 TEM, accelerating voltage 80 keV. EDS was
16
17 183 performed on electron-dark areas, to confirm the presence of palladium.
18
19 184
20
21 185 **Results**
22
23 186 *Palladisation of E. coli BL21(DE3)*
24
25 187 The pH of the aerobically-grown liquid culture was between 7.7-7.9, indicating that
26
27 188 there was not extensive production of organic acids due to overflow metabolism.
28
29 189 Oxygen saturation measurements showed that the liquid culture was 72% saturated
30
31 190 following 24 h of incubation, indicating that it was not oxygen-limited. After
32
33 191 harvesting, the cells were able to couple the reduction of Pd(II) to the oxidation of
34
35 192 formate, indicated by the rapid formation of a black precipitate, tentatively identified
36
37 193 as Pd(0) (Figure 1). ICP-MS analysis confirmed complete removal of Pd(II) from
38
39 194 solution within 45 min, and the presence of crystalline Pd(0) was confirmed using
40
41 195 XRD in this, but not in the heat-killed cells control where the cells removed
42
43 196 substantial Pd(II) abiotically. An increase in metal biosorption by heat killed biomass
44
45 197 as compared to live cells is well documented (Machado et al. 2009; Parameswari et al.
46
47 198 2009) and was attributed to loss of membrane integrity to reveal additional
48
49 199 intracellular metal binding sites (Machado et al. 2009).
50
51 200
52
53
54
55
56
57
58
59
60

For Peer Review Only

1
2
3 201 *Extended X-ray absorption fine structure (EXAFS)*
4
5 202 The nature of the Pd associated with the biomass was assessed further using X-ray
6
7 203 absorbance spectroscopy. The features in the corresponding EXAFS spectra (Figure 2)
8
9 204 are due to the wave-like nature of the photoelectron, which is released from the atom
10
11 205 with increasing energy and scattered from surrounding atoms with new waves being
12
13 206 emitted. With increasing photon energy, the interference between the waves alternates
14
15 207 between constructive and destructive, which leads to oscillations in the spectrum.
16
17 208 Examining these oscillations gives information on the number, species and distance of
18
19 209 the surrounding atoms. As seen in Figure 2, the samples taken at times 0 and 30 min,
20
21 210 which contain Pd(II), have identical EXAFS spectra. The samples taken at 60 min
22
23 211 onwards are identical to the Pd(0) foil control, which indicates that only Pd(0) was
24
25 212 present. Reduction of the Pd(II) to Pd(0) was therefore confirmed to be complete in
26
27 213 less than 30 min, as confirmed by ICP-MS analysis.
28
29 214
30
31 215 *Use of mutants to determine electron transfer pathway to Pd(II)*
32
33 216 Aerobic cultures of the parental strains MC4100 and BW25113 and the strain which
34
35 217 lacked all hydrogenases (JW2682) removed Pd(II) identically with no residual Pd(II)
36
37 218 detected after 30 min (Figure 3). Removal of the hydrogenase enzymes had no effect
38
39 219 on the rate of palladium removal from solution, confirming that these hydrogenases
40
41 220 have no role in the aerobic reduction of Pd(II). The FDH-O-negative strain JW3865
42
43 221 reduced Pd(II) within 1 h, and the FDH-O/FDH-N-negative strain FTD128 within 2 h.
44
45 222 Strain MC4100 Δ*moaA*, lacking all molybdoenzymes, reduced the palladium within 7
46
47 223 h. These results indicate the likely involvement of the FDH-O enzyme in the
48
49 224 reduction of Pd(II) by aerobically-grown *E. coli* using formate, although other Mo-
50
51 225 containing enzymes must also be involved given the impaired metal reduction noted
52
53
54
55
56
57
58
59
60

For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

226 with the Δ*moa* mutant. Controls containing no biomass showed no abiotic reduction
227 of Pd(II) using formate (Figure 1B), although a brown precipitate was seen in the no-
228 formate control. The X-ray powder diffraction pattern did not show the presence of
229 any peaks characteristic of Pd(0) in this precipitate, indicating that it was probably
230 amorphous and non-crystalline. Time zero on Figure 3 is the point at which formate
231 was added, following 1 h of incubation to allow biosorption of the Pd(II) to the cells;
232 hence the abiotic Pd(II) removal by killed cells (Figure 1) was apparent at the time of
233 formate addition with no evidence for further Pd(II) reduction.
234
235 *Transmission electron microscopy (TEM)*
236 TEM images of thin sections of cells showed that with all strains the reduced
237 palladium was precipitated predominantly in the extracellular matrix of the cultures
238 (Figure 4), although it appears that the nanoparticles may be associated with the outer
239 membrane of the cells. Energy dispersive X-ray spectroscopy (EDS) confirmed the
240 presence of palladium in these precipitates.
241
242 **Discussion**
243 The results from this study demonstrate that it is possible for aerobically-grown
244 cultures of *E. coli* to reduce Pd(II) enzymatically, with no need to remove oxygen
245 from the experimental system during the bioreduction step. Autoclaved control
246 experiments indicate that Pd(II) bioreduction in these cultures is enzymatic, with
247 reduction of palladium not occurring in the absence of viable cells irrespective of the
248 length of incubation. The major enzymes shown to be involved include the formate
249 dehydrogenases FDH-O and FDH-N, although bioreduction still occurs in strains
250 without these enzymes albeit at a much lower rate. Other molybdoenzymes must

For Peer Review Only

1
2
3 251 therefore be involved. The strain that lacked all molybdoenzymes did however still
4
5 252 reduce the palladium, although this took 7 h, compared with less than 30 min by the
6
7 253 wild-type strains. Hydrogenases, implicated as the dominant Pd(II) reductases in other
8
9 254 experimental systems grown under anaerobic conditions (Deplanche et al. 2010;
10
11 255 Mikheenko et al. 2008), are not expressed in aerobically grown cultures, and their
12
13 256 lack of involvement was evident as the strain lacking hydrogenase enzymes reduced
14
15 257 palladium at the same rate as the wild-type strains in this study.
16
17
18 258
19
20 259 Furthermore, whichever biological system is responsible for the aerobic bioreduction
21
22 260 of Pd(II), there seems to be little impact on the site of Pd(0) deposition. The location
23
24 261 of the bioreduced Pd(0) in our experiments is almost always extracellular, although
25
26 262 often associated with the outer membrane of the cells. This is particularly the case
27
28 263 with the MC4100 Δ*moaA* strain (which lacks all molybdoenzymes), in which the
29
30 264 majority of the Pd(0) nanoparticles are closely associated with the outer membrane
31
32 265 (Figure 4E). One conclusion that may be drawn from this is that whilst cells that lack
33
34 266 the formate dehydrogenases are still capable of reducing Pd(II), when all of these
35
36 267 enzymes are missing a cellular component associated with the outer membrane may
37
38 268 be responsible. Furthermore, this formate oxidation activity is much weaker than that
39
40 269 seen with the strains containing formate dehydrogenases, where Pd(II) reduction is
41
42 270 more rapid. It is possible however that following the initial enzymatic reduction of a
43
44 271 small percentage of the Pd(II), the Pd(0) nanoparticles formed may themselves be
45
46 272 responsible for catalysing the reduction of the remainder of the Pd(II) (Yong et al.
47
48 273 2002), which would mean that only a minor, initial biological input is required.
49
50
51 274
52
53
54
55
56
57
58
59
60

For Peer Review Only

1
2
3 275 Although the formate dehydrogenase enzyme systems implicated in Pd(II)
4
5 276 bioreduction by *E. coli* are periplasmic, the majority of the reduced Pd(0) precipitates
6
7 277 outside the cell. It is possible that an electron shuttle system exists similar to that
8
9 278 found in *Shewanella oneidensis* (von Canstein et al. 2008) that is as yet undiscovered
10
11 279 in *E. coli*. It is also possible that the first Pd(0) nanoparticles to form breach the outer
12
13 280 membrane, and themselves form an electron conduit for further Pd(II) reduction
14
15 281 outside the cell. The pH of these experiments is also higher than others where Pd(0)
16
17 282 nanoparticles accumulated in the periplasm (Redwood et al. 2008), which could
18
19 283 indicate the higher biosorption of cationic metal to the outer membrane and
20
21 284 extracellular polymeric substances, which are then not able to enter the periplasm.
22
23 285 The influence of a higher pH in the location of the Pd(0) may be confirmed by the
24
25 286 observation that Pd(0) nanoparticles were located on the cell surface of *D.*
26
27 287 *desulfuricans* when bioreduction of Pd(II) was performed at pH 7 (Yong et al. 2002).
28
29 288
30
31 289 In conclusion, this study has demonstrated the presence of a novel biological
32
33 290 mechanism responsible for the bioreduction of Pd(II) in aerobically-grown cultures of
34
35 291 *E. coli*, catalysed mainly by molybdenum-containing enzyme systems. Subsequent
36
37 292 studies will investigate the catalytic activity and selectivity of the Pd(0) nanoparticles
38
39 293 produced under aerobic conditions in a range of industrially important reactions. If
40
41 294 active, this new form of bioPd has the advantage over that produced by anaerobic
42
43 295 culture as it is easier to produce at high yield, from increased biomass levels
44
45 296 associated with aerobic growth. There is also no requirement for additional processing
46
47 297 steps to remove H_2S (produced by SRB systems), and the use of formate instead of
48
49 298 hydrogen gas means that the procedure is less hazardous and more controllable. The
50
51 299 advantages of this more scalable method of synthesis would need to be considered
52
53
54
55
56
57
58
59
60

For Peer Review Only

against any alterations in activity/selectivity of the resulting catalyst (versus synthetic and other bioPds), using a cost-benefit analysis. Importantly, identification of the specific enzymatic process(es) involved in the biomanufacture of bioPd is the first step towards application of the tools of synthetic biology for 'designer catalyst' production for specific applications.

In a geomicrobiological context, this study shows that aerobic cells of *E. coli* restrict the deposition of Pd(0) to locations outside the cell. However in both *D. desulfuricans* (grown anaerobically) and *Bacillus benzeovorans* (grown aerobically) intracellular depositions of small Pd-nanoparticles were observed at the expense of both hydrogen and formate (JB Omajali, IP Mikheenko, ML Merroun, J Wood and LE Macaskie, in press) and, notably, were also seen in *E. coli* grown anaerobically (LE Macaskie, A Williams, R Priestley and J Courtney, unpublished). This raises questions about potential biochemical 'trafficking' pathways of Pd(II), the possibility of Pd(II) efflux by aerobic (but not anaerobic) cells and, following from that, the possibility of biogeochemical cycling of this element.

Acknowledgement

The authors would like to thank Dr Victoria Coker, Prof Richard Pattrick, and Dr John Charnock of the University of Manchester for assistance with EXAFS.

Funding

This study was supported by a grant from the Biotechnology and Biological Sciences Research Council (BBSRC).

For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

325 **References**

326 Abaibou H, Pommier J, Benoit S, Giordano G. Mandrandberthelot MA. 1995.
327 Expression and characterization of the *Escherichia coli fdo* Locus and a possible
328 physiological role for aerobic formate dehydrogenase. Journal of Bacteriology
329 177:7141-7149.
330
331 Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita
332 M, Wanner BL, Mori H. 2006. Construction of *Escherichia coli* K-12 in-frame,
333 single-gene knockout mutants: the Keio collection. Molecular Systems Biology 2:
334 Article no. 2006.0008.
335
336 Baxter-Plant V, Mikheenko IP, Macaskie LE. 2003. Sulphate-reducing bacteria,
337 palladium and the reductive dehalogenation of chlorinated aromatic compounds.
338 Biodegradation 14:83-90.
339
340 Beauregard D, Yong P, Macaskie LE, Johns ML. 2010. Using non-invasive magnetic
341 resonance imaging (MRI) to assess the reduction of Cr(VI) using a biofilm-palladium
342 catalyst. Biotechnology and Bioengineering 107:11-20.
343
344 Bennett JA, Creamer NJ, Deplanche K, Macaskie LE, Shannon IJ, Wood J. 2010.
345 Palladium supported on bacterial biomass as a novel heterogeneous catalyst: A
346 comparison of Pd/Al_2O_3 and bio-Pd in the hydrogenation of 2-pentyne. Chemical
347 Engineering Science 65:282-290.
348

For Peer Review Only

349 Bennett JA, Mikheenko I, Deplanche K, Shannon IJ, Wood J. & Macaskie LE. 2013.
350 Nanoparticles of palladium supported on bacterial biomass: new re-usable
351 heterogeneous catalyst with comparable catalytic activity to homogeneous colloidal
352 palladium in the Heck reaction. Applied Catalysis B Environmental 140-141:700-707.
353

354 Bunge M, Søbjerg LS, Rotaru AE, Gauthier D, Lindhardt AT, Hause G, Finster K,
355 Kingshott P, Skrydstrup T, Meyer RL. 2010. Formation of palladium (0)
356 nanoparticles at microbial surfaces. Biotechnology and Bioengineering 107:206-215.
357

358 Casadaban MJ, Cohen SN. 1979. Lactose genes fused to exogenous promoters in one-
359 step using a Mu-*lac* Bacteriophage: *in vivo* probe for transcriptional control sequences.
360 Proceedings of the National Academy of Sciences of the United States of
361 America 76:4530-4533.
362

363 Chidambaram D, Hennebel T, Taghavi S, Mast J, Boon N, Verstraete W, van der
364 Lelie D, Fitts JP. 2010. Concomitant microbial generation of palladium nanoparticles
365 and hydrogen to immobilize chromate. Environmental Science & Technology
366 44:7635-7640.
367

368 Creamer NJ, Mikheenko IP, Yong P, Deplanche K, Sanyahumbia D, Wood J,
369 Pollmann K, Merroun M, Selenska-Pobell S, Macaskie LE. 2007. Novel supported Pd
370 hydrogenation bionanocatalyst for hybrid homogeneous/heterogeneous catalysis.
371 Catalysis Today 128:80-87.
372

For Peer Review Only

373 Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in
374 *Escherichia coli* K-12 using PCR products. Proceedings of the National Academy of
375 Sciences of the United States of America 97:6640-6645.
376
377 De Windt W, Aelterman P, Verstraete,W. 2005. Bioreductive deposition of palladium
378 (0) nanoparticles on *Shewanella oneidensis* with catalytic activity towards reductive
379 dechlorination of polychlorinated biphenyls. Environmental Microbiology 7:314-325.
380
381 Deplanche K, Caldelari I, Mikheenko IP, Sargent F, Macaskie LE. 2010. Involvement
382 of hydrogenases in the formation of highly catalytic Pd(0) nanoparticles by
383 bioreduction of Pd(II) using *Escherichia coli* mutant strains. Microbiology 156:2630-
384 2640.
385
386 Deplanche K, Bennett JA, Mikheenko IP, Omajali J, Wells AS, Meadows PE, Wood J,
387 Macaskie LE. 2014. Catalytic activity of biomass supported palladium nanoparticles:
388 influence of the biological component in catalytic efficacy and potential applications
389 in green synthesis of fine chemicals and pharmaceuticals. Applied Catalysis B
390 Environmental 147:651-665.
391
392 Gauthier D, Søbjerg LS, Jensen KM, Lindhardt AT, Bunge M, Finster K, Skrydstrup
393 T, Meyer RL. 2010. Environmentally benign recovery and reactivation of palladium
394 from industrial waste by using Gram-negative bacteria. Chemsuschem 3:1036-1039.
395
396 Gurman SJ, Binsted N, Ross I. 1984. A rapid, exact curved-wave theory for EXAFS
397 calculations. Journal of Physics C: Solid State Physics 17:143-151.

For Peer Review Only

Harrad S, Robson M, Hazrati S, Baxter-Plant VS, Deplanche K, Redwood MD, Macaskie LE. 2007. Dehalogenation of polychlorinated biphenyls and polybrominated diphenyl ethers using a hybrid bioinorganic catalyst. Journal of Environmental Monitoring 9:314-318.

Hennebel T, Verhagen P, Simoen H, De Gusseme B, Vlaeminck SE, Boon N, Verstraete W. 2009a. Remediation of trichloroethylene by bio-precipitated and encapsulated palladium nanoparticles in a fixed bed reactor. Chemosphere 76:1221-1225.

Hennebel T, Simoen H, De Windt W, Verloo M, Boon N, Verstraete W. 2009b. Biocatalytic dechlorination of trichloroethylene with bio-palladium in a pilot-scale membrane reactor. Biotechnology and Bioengineering 102:995-1002.

Lloyd JR. 2003. Microbial reduction of metals and radionuclides. FEMS Microbiology Reviews 27:411-425.

Lloyd JR, Pearce CI, Coker VS, Pattrick RA, van der Laan G, Cutting R, Vaughan DJ, Paterson-Beedle M, Mikheenko IP, Yong P, Macaskie LE. 2008. Biomineralization: linking the fossil record to the production of high value functional materials. Geobiology 6:285-297.

Lovley DR. 2006. Microbial fuel cells: novel microbial physiologies and engineering approaches. Current Opinion in Biotechnology 17:327-332.

For Peer Review Only

423

424 Luke I, Butland G, Moore K, Buchanan G, Lyall V, Fairhurst SA, Greenblatt JF,
425 Emili A, Palmer T, Sargent F. 2008. Biosynthesis of the respiratory formate
426 dehydrogenases from *Escherichia coli*: characterization of the FdhE protein. Archives
427 of Microbiology 190:685-696.

428

429 Mabbett A, Sanyahumbi D, Yong P, Macaskie LE. 2006. Biorecovered precious
430 metals from industrial wastes: Single-step conversion of a mixed metal liquid waste to
431 a bioinorganic catalyst with environmental application. Environmental Science &
432 Technology 40:1015-1021.

433

434 Machado MD, Janssens S, Soares HMVM, Soares EV. 2009. Removal of heavy
435 metals using a brewers' yeast strain of *Saccharomyces cerevisiae*: advantages of using
436 dead biomass. Journal of Applied Microbiology 106:1792-1804.

437

438 Mertens B, Blothe C, Windey K, De Windt W, Verstraete W. 2007. Biocatalytic
439 dechlorination of lindane by nano-scale particles of Pd(0) deposited on *Shewanella*
440 *oneidensis*. Chemosphere 66:99-105.

441

442 Mikheenko IP, Rousset M, Dementin S, Macaskie LE. 2008. Bioaccumulation of
443 palladium by *Desulfovibrio fructosivorans* wild-type and hydrogenase-deficient
444 strains. Applied and Environmental Microbiology 74:6144-6146.

445

446 Orozco RL, Redwood MD, Yong P, Macaskie LE. 2010. Towards an integrated
447 system for bio-energy: hydrogen production by *Escherichia coli* and use of

For Peer Review Only

palladium-coated waste cells for electricity generation in a fuel cell. Biotechnology Letters 32:1837-1845.

Paramewsari E, Lakshmanan A, Thilagavathi T. 2009. Effect of pretreatment of blue green algal biomass on biosorption of chromium and nickel. Journal of Algal Biomass Utilisation 1:9-17.

Redwood MD, Deplanche K, Baxter-Plant VS, Macaskie LE. 2008. Biomass-supported palladium catalysts on *Desulfovibrio desulfuricans* and *Rhodobacter sphaeroides*. Biotechnology and Bioengineering 99:1045-1054.

Søbjerg LS, Gauthier D, Lindhardt AT, Bunge M, Finster K, Skrydstrup T, Meyer RL. 2009. Bio-supported palladium nanoparticles as a catalyst for Suzuki-Miyaura and Mizoroki-Heck reactions. Green Chemistry 11:2041-2046.

Studier FW, Moffatt BA. 1986. Use of bacteriophage T7 RNA polymerase to direct selective high level expression of cloned genes. Journal of Molecular Biology 189:113-130.

Vasala A, Panula J, Bollok M, Illmann L, Halsig C, Neubauer P. 2006. A new wireless system for decentralised measurement of physiological parameters from shake flasks. Microbial Cell Factories 5:8.

For Peer Review Only

von Canstein H, Ogawa J, Shimizu S, Lloyd JR. 2008. Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer. Applied and Environmental Microbiology 74:615-623.

Wood J, Bodenes L, Bennett J, Deplanche K, Macaskie LE. 2010. Hydrogenation of 2-butyne-1,4-diol using novel bio-palladium catalysts. Industrial & Engineering Chemistry Research 49:980-988.

Yong P, Rowson N., Farr JPG, Harris IR, Macaskie, LE. 2002. Bioreduction and biocrystallization of palladium by *Desulfovibrio desulfuricans* NCIMB 8307. Biotechnology and Bioengineering 80:369-379.

Yong P, Paterson-Beedle M, Mikheenko IP, Macaskie LE. 2007. From bio-mineralisation to fuel cells: biomanufacture of Pt and Pd nanocrystals for fuel cell electrode catalyst. Biotechnology Letters 29:539-544.

For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

495 **Table 1.** *E. coli* strains used to determine biological involvement in the reduction of

496 palladium (II) using formate as the electron donor.

Strain	**Genotype**	**Phenotype**	**Reference**
BL21(DE3)	*F2 ompT gal dcm lon* $hsdS_B(r_B^- m_B^-)$ λ(DE3 [*lacI lacUV5-T7 gene 1 ind1 sam7 nin5*])	Wild type strain commonly used for recombinant protein expression.	(Studier and Moffatt 1986)
MC4100	*F- ΔlacU169 araD139 rpsL150 relA1 ptsF rbs flbB5301*	Parental strain for FTD128 and Δ*moaA*.	(Casadaban and Cohen 1979)
BW25113	$lacI^q$ $rrbB_{T14}$ $\Delta lacZ_{WJ16}$ *hsd*R514 $\Delta araBAD_{AH33}$ $\Delta rhaBAD_{LD78}$	Parental strain for JW2682 and JW3865.	(Datsenko and Wanner 2000)
FTD128	As MC4100, with in-frame deletion in the *fdhE* gene.	FDH-O & FDH-N negative.	(Luke et al. 2008)
JW2682	As BW25113, with in-frame deletion of the *hypF* gene.	Deficient in all hydrogenases.	(Baba et al. 2006)
JW3865	As BW25113, with in-frame deletion of the *fdoG* gene.	FDH-O negative.	(Baba et al. 2006)
MC4100 Δ*moaA*	As MC4100, disruption of the *moaA* gene.	Deficient in all molybdoenzymes	This study.

497

498

499

500

501

502

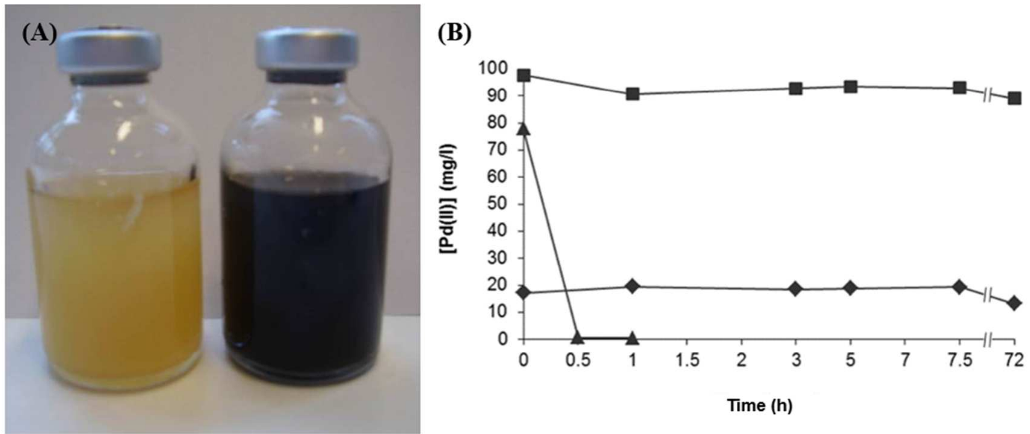
For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

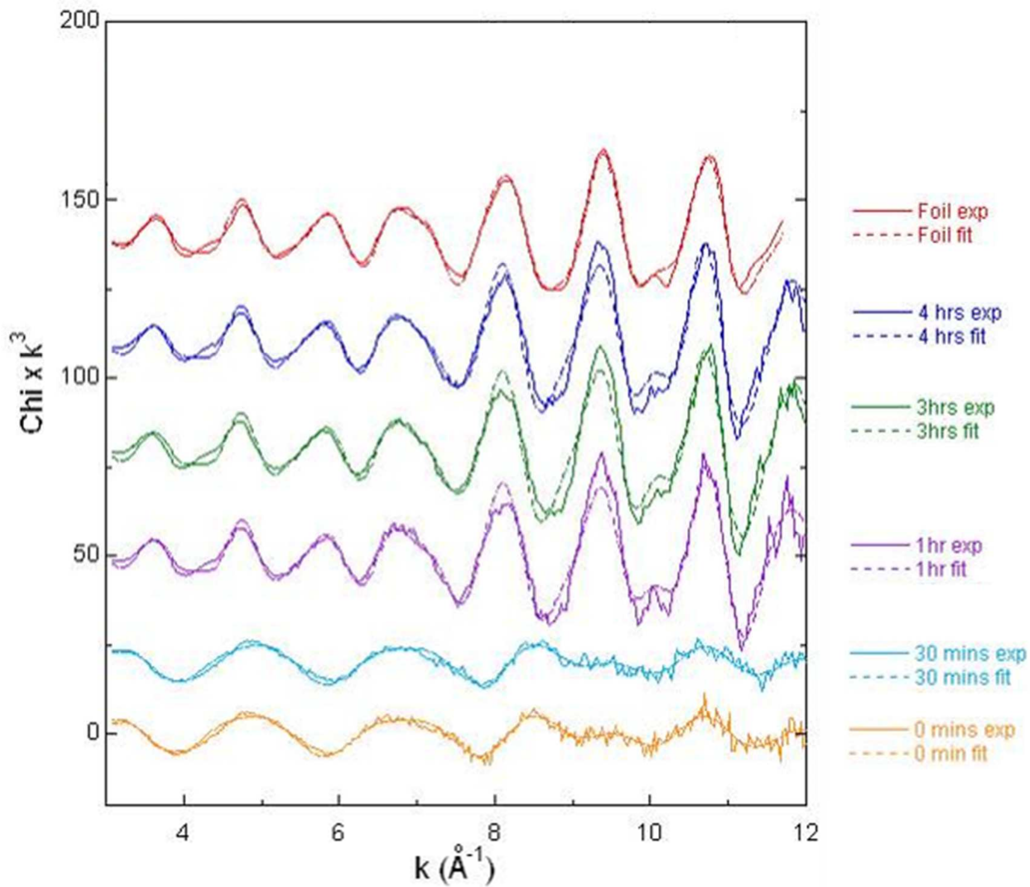
503 **Fig. 1.** (A) Complete reduction of Pd(II) to Pd(0) by an aerobically-grown culture of
504 *E. coli*. Both bottles contain cells resuspended in 20 mM MOPS buffer at pH7.6, and
505 1 mM sodium tetrachloropalladate (total volume 25 ml). This image was taken 45 min
506 after the addition of formate to the bottle on the right. (B) Reduction by *E. coli*
507 MC4100 and by controls showing no abiotic reduction of Pd(II). Controls used were
508 killed (autoclaved) cells and cell-free suspension. Soluble Pd(II) in the supernatant
509 was measured using ICP-MS. ▲ = MC4100; ■ = no cells; ♦ = killed cells.
510
511 **Fig. 2.** EXAFS data showing the presence of Pd(II) at 0 and 30 min (bottom two
512 traces), and Pd(0) at 1, 3 and 4 h (ascending series). The top trace is palladium foil.
513
514 **Fig. 3.** Pd(II) reduction by six different strains of *E. coli*, using formate as the electron
515 donor. Soluble Pd(II) in the supernatant was measured using ICP-MS. ♦ = BW25113;
516 □ = JW2682; ▲ = JW3865; Δ = MC4100 Δ*moaA*; ■ = MC4100; ◊ = FTD128. Data
517 points for BW25113, JW2682 and JW3865 are mean values of triplicates, with
518 standard error shown.
519
520 **Fig. 4.** TEM of thin sections of aerobically grown cells showing extracellular
521 palladium; (A) MC4100, inset BL21; (B) BW25113, inset BL21 (no Pd); (C)
522 FTD128; (D) JW2682; (E) MC4100 Δ*moaA*; (F) JW3865. Scale bar (A) = 100 nm;
523 (B)-(F) = 500 nm; insets = 1µm.
524
525
526
527

For Peer Review Only

1
2
3 528 Figure 1
4
5
6
7
8
9
10
11
12
13
14
15
16
17

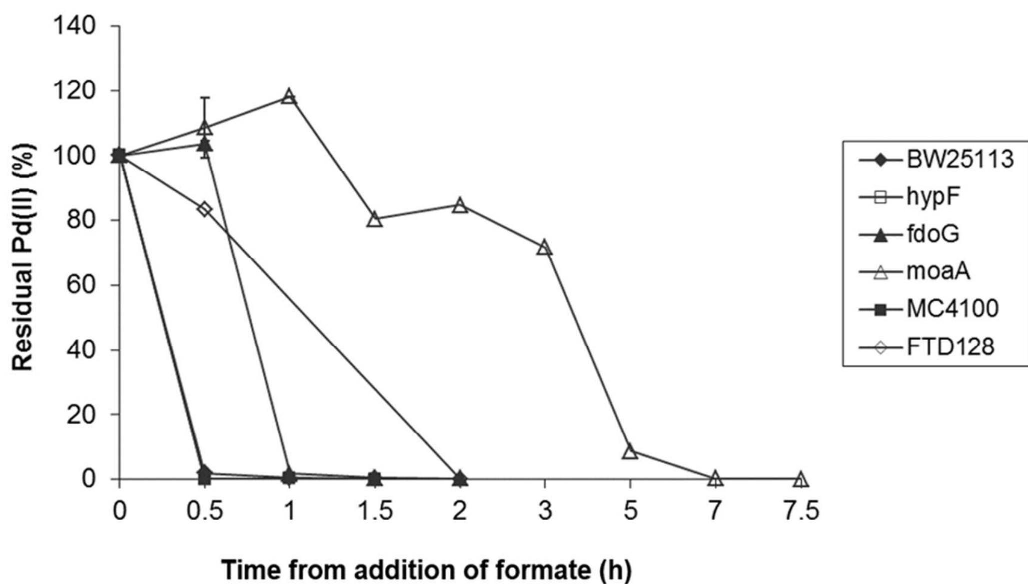


18 529
19
20 530
21
22 531
23
24
25 532 Figure 2
26



27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55 533
56
57 534
58
59
60

1
2
3 535 Figure 3
4

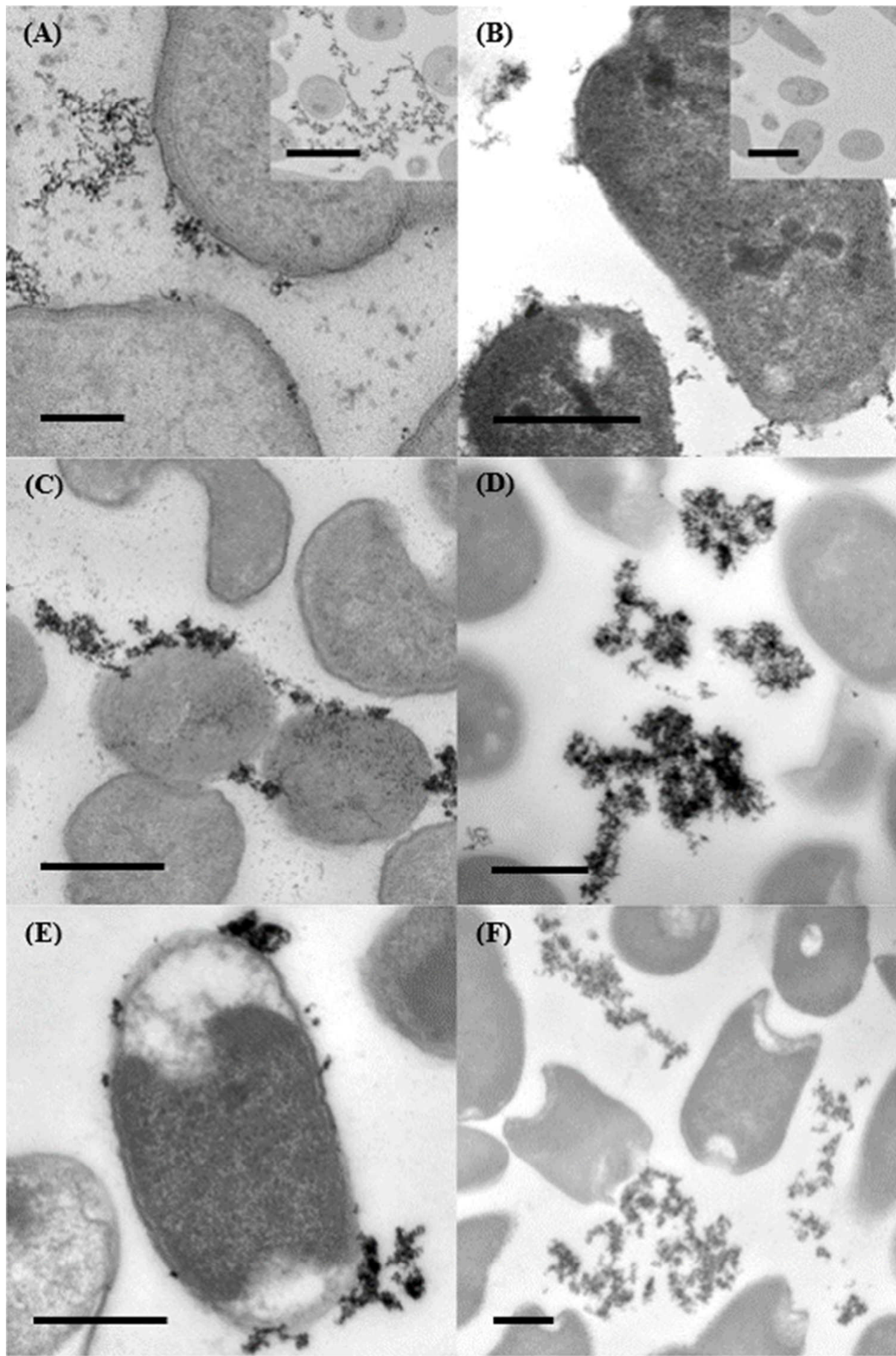


536
537
538
539
540
541
542
543
544
545
546
547
548
549
550
551

For Peer Review Only

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

552 Figure 4



553

554