



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

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Manuscripts

1 A novel aerobic mechanism for reductive palladium

2 biomineralization and recovery by *Escherichia coli*

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

26 anaerobically-grown *E. coli* cultures. The potential implications for Pd(II) recovery
27 and bioPd catalyst fabrication are discussed.

29 **Keywords:** palladium nanoparticles, *Escherichia coli*, biomineralization.

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 H₂S, 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 oxydans* (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

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

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2
3 76 elucidate reaction mechanisms under aerobic and anaerobic conditions. The enzymes
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5 77 potentially involved in the bioreduction of palladium by *E. coli* under the latter
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7 78 conditions are the nickel-dependent hydrogenase enzymes Hyd-1, Hyd-2, and Hyd-3,
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9 79 and the formate dehydrogenase molybdoenzymes FDH-N, and FDH-H. Another
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11 80 molybdoenzyme, FDH-O, is expressed under both aerobic and anaerobic conditions.
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13 81 A possible role for FDH-O is to allow bacteria to adapt rapidly to a sudden shift from
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15 82 aerobic respiration to anaerobiosis, before FDH-N has been produced in sufficient
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17 83 amounts to continue formate metabolism (Abaibou et al. 1995). Hyd-1, Hyd-2, FDH-
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19 84 O, and FDH-N are membrane-bound and periplasmically oriented, whereas Hyd-3
20
21 85 and FDH-H are subunits of the formate hydrogenlyase (FHL) complex, an
22
23 86 intracellular enzyme complex that is also membrane-bound but which faces into the
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25 87 cytoplasm. The mechanisms responsible for the formate-dependent bioreduction by
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27 88 anaerobically-grown cultures of *E. coli* have been studied, showing that the
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29 89 hydrogenase enzymes Hyd-1 and Hyd-2 are mainly responsible for Pd(II)
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31 90 bioreduction (Deplanche et al. 2010). In a study of formate-dependent Pd(II)
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33 91 bioreduction by *Desulfovibrio fructosovorans*, the deletion of the periplasmic
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35 92 hydrogenases caused the Pd(0) nanoparticles to be relocated to the cytoplasmic
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37 93 membrane site of the remaining hydrogenases, indicating that the periplasmic
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39 94 hydrogenases are at least partially involved (Mikheenko et al. 2008).
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47 96 The growth yield of anaerobic cultures is lower than that of aerobic cultures, and for
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49 97 economic production at scale a method of growth of high biomass density is required.
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51 98 When using anaerobic cultures there is also the cost of supplementing with sodium
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53 99 fumarate and glycerol. The dual aims of this study are to establish whether *E. coli*
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56 100 cells grown aerobically are capable of manufacturing bioPd and to identify the
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enzyme(s) responsible for such metal reduction. A move away from the need for anaerobic growth would simplify the preparation of high levels of active biomass for catalyst production at industrial scale.

Methods

Bacterial growth

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

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

Reduction of Pd(II) to produce bioPd on bacteria

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

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

Use of mutants to determine electron transfer pathway to Pd(II)

In order to investigate the possible role of the aerobic formate dehydrogenase (FDH-O) and other hydrogenase/formate dehydrogenase enzymes in the reduction of Pd(II) by aerobically-grown cells of *E. coli*, the rates of reduction by six different additional strains (Table 1) were compared by measuring the Pd(II) remaining in solution by ICP-MS. The strains were ‘palladised’ as above, and rates of reduction/removal compared to those in a series of controls: killed cells (MC4100), cell-free suspension, and live cells (MC4100) unsupplemented with formate.

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

X-ray diffraction (XRD) analysis

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

151 from 5-70 degrees 2theta in steps of 0.2 degrees, with a count time of 2 seconds per
152 step.
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154 *Extended X-ray absorption fine structure (EXAFS)*
155 Aliquots of the cell/Pd/formate suspension were taken at times 0 and 30 min, and 1, 3
156 and 4 h from the addition of formate, and frozen immediately in liquid nitrogen. The
157 direct reduction of Pd(II) to Pd(0) was demonstrated using EXAFS, performed at the
158 European Synchrotron Radiation Facility (ESRF), in Grenoble, France. The samples
159 were transported to the synchrotron at ESRF on dry ice, where they were thawed and
160 injected immediately into sample holders, before freezing once more in liquid
161 nitrogen and placing into the beam. X-ray absorption data were collected on beamline
162 BM29 at the Pd K-edge in the energy range 24 200 – 24 900 eV. Data were recorded
163 at low temperature (77 K) and under vacuum to reduce the thermal Debye-Waller
164 factor and prevent oxidation. A Si(III) double crystal monochromator was used,
165 calibrated with a Pd foil, and the spectra were collected in fluorescence mode using a
166 13-element solid-state detector. A reference spectrum of a palladium foil was
167 recorded in transmission mode on station 9.3 at the SRS Daresbury. The data were
168 background subtracted and the EXAFS spectra fitted in DL_Excurv
169 (<http://www.cse.scitech.ac.uk/cmng/EXCURV/>) using full curved wave theory
170 (Gurman et al., 1984).
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172 *Transmission electron microscopy (TEM) and energy dispersive X-ray spectroscopy*
173 *(EDS)*
174 Following Pd(II) reduction, cells were stored at 10°C overnight. The cell pellets were
175 then rinsed twice with deionised water, fixed in 2.5% (wt/vol) glutaraldehyde,

centrifuged for 5 min at 16 000 g, resuspended in 1.5 ml of 0.1 M cacodylate buffer (pH 7) and stained in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7 (60 min). Cells were dehydrated using an ethanol series (70, 90, 100, 100, 100% dried ethanol, 15 min each) and washed twice in propylene oxide (15 min, 9500 g). Cells were embedded in epoxy resin and the mixture was left to polymerise (24 h; 60°C). Sections (100-150 nm thick) were cut from the resin block, placed onto a copper grid and viewed with a JEOL 1200CX2 TEM, accelerating voltage 80 keV. EDS was performed on electron-dark areas, to confirm the presence of palladium.

Results

Palladisation of E. coli BL21(DE3)

The pH of the aerobically-grown liquid culture was between 7.7-7.9, indicating that there was not extensive production of organic acids due to overflow metabolism. Oxygen saturation measurements showed that the liquid culture was 72% saturated following 24 h of incubation, indicating that it was not oxygen-limited. After harvesting, the cells were able to couple the reduction of Pd(II) to the oxidation of formate, indicated by the rapid formation of a black precipitate, tentatively identified as Pd(0) (Figure 1). ICP-MS analysis confirmed complete removal of Pd(II) from solution within 45 min, and the presence of crystalline Pd(0) was confirmed using XRD in this, but not in the heat-killed cells control where the cells removed substantial Pd(II) abiotically. An increase in metal biosorption by heat killed biomass as compared to live cells is well documented (Machado et al. 2009; Parameswari et al. 2009) and was attributed to loss of membrane integrity to reveal additional intracellular metal binding sites (Machado et al. 2009).

201 *Extended X-ray absorption fine structure (EXAFS)*

202 The nature of the Pd associated with the biomass was assessed further using X-ray
203 absorbance spectroscopy. The features in the corresponding EXAFS spectra (Figure 2)
204 are due to the wave-like nature of the photoelectron, which is released from the atom
205 with increasing energy and scattered from surrounding atoms with new waves being
206 emitted. With increasing photon energy, the interference between the waves alternates
207 between constructive and destructive, which leads to oscillations in the spectrum.
208 Examining these oscillations gives information on the number, species and distance of
209 the surrounding atoms. As seen in Figure 2, the samples taken at times 0 and 30 min,
210 which contain Pd(II), have identical EXAFS spectra. The samples taken at 60 min
211 onwards are identical to the Pd(0) foil control, which indicates that only Pd(0) was
212 present. Reduction of the Pd(II) to Pd(0) was therefore confirmed to be complete in
213 less than 30 min, as confirmed by ICP-MS analysis.

215 *Use of mutants to determine electron transfer pathway to Pd(II)*

216 Aerobic cultures of the parental strains MC4100 and BW25113 and the strain which
217 lacked all hydrogenases (JW2682) removed Pd(II) identically with no residual Pd(II)
218 detected after 30 min (Figure 3). Removal of the hydrogenase enzymes had no effect
219 on the rate of palladium removal from solution, confirming that these hydrogenases
220 have no role in the aerobic reduction of Pd(II). The FDH-O-negative strain JW3865
221 reduced Pd(II) within 1 h, and the FDH-O/FDH-N-negative strain FTD128 within 2 h.
222 Strain MC4100 $\Delta moaA$, lacking all molybdoenzymes, reduced the palladium within 7
223 h. These results indicate the likely involvement of the FDH-O enzyme in the
224 reduction of Pd(II) by aerobically-grown *E. coli* using formate, although other Mo-
225 containing enzymes must also be involved given the impaired metal reduction noted

with the Δmoa mutant. Controls containing no biomass showed no abiotic reduction of Pd(II) using formate (Figure 1B), although a brown precipitate was seen in the no-formate control. The X-ray powder diffraction pattern did not show the presence of any peaks characteristic of Pd(0) in this precipitate, indicating that it was probably amorphous and non-crystalline. Time zero on Figure 3 is the point at which formate was added, following 1 h of incubation to allow biosorption of the Pd(II) to the cells; hence the abiotic Pd(II) removal by killed cells (Figure 1) was apparent at the time of formate addition with no evidence for further Pd(II) reduction.

Transmission electron microscopy (TEM)

TEM images of thin sections of cells showed that with all strains the reduced palladium was precipitated predominantly in the extracellular matrix of the cultures (Figure 4), although it appears that the nanoparticles may be associated with the outer membrane of the cells. Energy dispersive X-ray spectroscopy (EDS) confirmed the presence of palladium in these precipitates.

Discussion

The results from this study demonstrate that it is possible for aerobically-grown cultures of *E. coli* to reduce Pd(II) enzymatically, with no need to remove oxygen from the experimental system during the bioreduction step. Autoclaved control experiments indicate that Pd(II) bioreduction in these cultures is enzymatic, with reduction of palladium not occurring in the absence of viable cells irrespective of the length of incubation. The major enzymes shown to be involved include the formate dehydrogenases FDH-O and FDH-N, although bioreduction still occurs in strains without these enzymes albeit at a much lower rate. Other molybdoenzymes must

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3 251 therefore be involved. The strain that lacked all molybdoenzymes did however still
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5 252 reduce the palladium, although this took 7 h, compared with less than 30 min by the
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7 253 wild-type strains. Hydrogenases, implicated as the dominant Pd(II) reductases in other
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10 254 experimental systems grown under anaerobic conditions (Deplanche et al. 2010;
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12 255 Mikheenko et al. 2008), are not expressed in aerobically grown cultures, and their
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14 256 lack of involvement was evident as the strain lacking hydrogenase enzymes reduced
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16 257 palladium at the same rate as the wild-type strains in this study.
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21 259 Furthermore, whichever biological system is responsible for the aerobic bioreduction
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23 260 of Pd(II), there seems to be little impact on the site of Pd(0) deposition. The location
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25 261 of the bioreduced Pd(0) in our experiments is almost always extracellular, although
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27 262 often associated with the outer membrane of the cells. This is particularly the case
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30 263 with the MC4100 *ΔmoaA* strain (which lacks all molybdoenzymes), in which the
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32 264 majority of the Pd(0) nanoparticles are closely associated with the outer membrane
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34 265 (Figure 4E). One conclusion that may be drawn from this is that whilst cells that lack
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36 266 the formate dehydrogenases are still capable of reducing Pd(II), when all of these
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38 267 enzymes are missing a cellular component associated with the outer membrane may
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40 268 be responsible. Furthermore, this formate oxidation activity is much weaker than that
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43 269 seen with the strains containing formate dehydrogenases, where Pd(II) reduction is
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45 270 more rapid. It is possible however that following the initial enzymatic reduction of a
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47 271 small percentage of the Pd(II), the Pd(0) nanoparticles formed may themselves be
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49 272 responsible for catalysing the reduction of the remainder of the Pd(II) (Yong et al.
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51 273 2002), which would mean that only a minor, initial biological input is required.
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Although the formate dehydrogenase enzyme systems implicated in Pd(II) bioreduction by *E. coli* are periplasmic, the majority of the reduced Pd(0) precipitates outside the cell. It is possible that an electron shuttle system exists similar to that found in *Shewanella oneidensis* (von Canstein et al. 2008) that is as yet undiscovered in *E. coli*. It is also possible that the first Pd(0) nanoparticles to form breach the outer membrane, and themselves form an electron conduit for further Pd(II) reduction outside the cell. The pH of these experiments is also higher than others where Pd(0) nanoparticles accumulated in the periplasm (Redwood et al. 2008), which could indicate the higher biosorption of cationic metal to the outer membrane and extracellular polymeric substances, which are then not able to enter the periplasm. The influence of a higher pH in the location of the Pd(0) may be confirmed by the observation that Pd(0) nanoparticles were located on the cell surface of *D. desulfuricans* when bioreduction of Pd(II) was performed at pH 7 (Yong et al. 2002).

In conclusion, this study has demonstrated the presence of a novel biological mechanism responsible for the bioreduction of Pd(II) in aerobically-grown cultures of *E. coli*, catalysed mainly by molybdenum-containing enzyme systems. Subsequent studies will investigate the catalytic activity and selectivity of the Pd(0) nanoparticles produced under aerobic conditions in a range of industrially important reactions. If active, this new form of bioPd has the advantage over that produced by anaerobic culture as it is easier to produce at high yield, from increased biomass levels associated with aerobic growth. There is also no requirement for additional processing steps to remove H₂S (produced by SRB systems), and the use of formate instead of hydrogen gas means that the procedure is less hazardous and more controllable. The advantages of this more scalable method of synthesis would need to be considered

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3 300 against any alterations in activity/selectivity of the resulting catalyst (versus synthetic
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5 301 and other bioPds), using a cost-benefit analysis. Importantly, identification of the
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7 302 specific enzymatic process(es) involved in the biomanufacture of bioPd is the first
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9 303 step towards application of the tools of synthetic biology for ‘designer catalyst’
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11 304 production for specific applications.
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16 306 In a geomicrobiological context, this study shows that aerobic cells of *E. coli* restrict
17
18 307 the deposition of Pd(0) to locations outside the cell. However in both *D. desulfuricans*
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20 308 (grown anaerobically) and *Bacillus benzeovorans* (grown aerobically) intracellular
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22 309 depositions of small Pd-nanoparticles were observed at the expense of both hydrogen
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24 310 and formate (JB Omajali, IP Mikheenko, ML Merroun, J Wood and LE Macaskie, in
25
26 311 press) and, notably, were also seen in *E. coli* grown anaerobically (LE Macaskie, A
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28 312 Williams, R Priestley and J Courtney, unpublished). This raises questions about
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30 313 potential biochemical ‘trafficking’ pathways of Pd(II), the possibility of Pd(II) efflux
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32 314 by aerobic (but not anaerobic) cells and, following from that, the possibility of
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34 315 biogeochemical cycling of this element.
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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/Al₂O₃ and bio-Pd in the hydrogenation of 2-pentyne. Chemical
347 Engineering Science 65:282-290.
348

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, Søjberg 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

- 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.

398

399 Harrad S, Robson M, Hazrati S, Baxter-Plant VS, Deplanche K, Redwood MD,

400 Macaskie LE. 2007. Dehalogenation of polychlorinated biphenyls and

401 polybrominated diphenyl ethers using a hybrid bioinorganic catalyst. *Journal of*

402 *Environmental Monitoring* 9:314-318.

403

404 Hennebel T, Verhagen P, Simoen H, De Gusseme B, Vlaeminck SE, Boon N,

405 Verstraete W. 2009a. Remediation of trichloroethylene by bio-precipitated and

406 encapsulated palladium nanoparticles in a fixed bed reactor. *Chemosphere* 76:1221-

407 1225.

408

409 Hennebel T, Simoen H, De Windt W, Verloo M, Boon N, Verstraete W. 2009b.

410 Biocatalytic dechlorination of trichloroethylene with bio-palladium in a pilot-scale

411 membrane reactor. *Biotechnology and Bioengineering* 102:995-1002.

412

413 Lloyd JR. 2003. Microbial reduction of metals and radionuclides. *FEMS*

414 *Microbiology Reviews* 27:411-425.

415

416 Lloyd JR, Pearce CI, Coker VS, Pattrick RA, van der Laan G, Cutting R, Vaughan DJ,

417 Paterson-Beedle M, Mikheenko IP, Yong P, Macaskie LE. 2008. Biomineralization:

418 linking the fossil record to the production of high value functional materials.

419 *Geobiology* 6:285-297.

420

421 Lovley DR. 2006. Microbial fuel cells: novel microbial physiologies and engineering

422 approaches. *Current Opinion in Biotechnology* 17:327-332.

- 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

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

450

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

454

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

458

459 Søjberg LS, Gauthier D, Lindhardt AT, Bunge M, Finster K, Skrydstrup T, Meyer RL.
460 2009. Bio-supported palladium nanoparticles as a catalyst for Suzuki-Miyaura and
461 Mizoroki-Heck reactions. Green Chemistry 11:2041-2046.

462

463 Studier FW, Moffatt BA. 1986. Use of bacteriophage T7 RNA polymerase to direct
464 selective high level expression of cloned genes. Journal of Molecular Biology
465 189:113-130.

466

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

470

- 471 von Canstein H, Ogawa J, Shimizu S, Lloyd JR. 2008. Secretion of flavins by
472 *Shewanella* species and their role in extracellular electron transfer. Applied and
473 Environmental Microbiology 74:615-623.
474
- 475 Wood J, Bodenes L, Bennett J, Deplanche K, Macaskie LE. 2010. Hydrogenation of
476 2-butyne-1,4-diol using novel bio-palladium catalysts. Industrial & Engineering
477 Chemistry Research 49:980-988.
478
- 479 Yong P, Rowson N., Farr JPG, Harris IR, Macaskie, LE. 2002. Bioreduction and
480 biocrystallization of palladium by *Desulfovibrio desulfuricans* NCIMB 8307.
481 Biotechnology and Bioengineering 80:369-379.
482
- 483 Yong P, Paterson-Beedle M, Mikheenko IP, Macaskie LE. 2007. From bio-
484 mineralisation to fuel cells: biomanufacture of Pt and Pd nanocrystals for fuel cell
485 electrode catalyst. Biotechnology Letters 29:539-544.
486
487
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Table 1. *E. coli* strains used to determine biological involvement in the reduction of palladium (II) using formate as the electron donor.

| Strain | Genotype | Phenotype | Reference |
|----------------------|--|--|----------------------------|
| BL21(DE3) | <i>F2 ompT gal dcm lon hsdS_B(r_B⁻ m_B⁻) λ(DE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5])</i> | Wild type strain commonly used for recombinant protein expression. | (Studier and Moffatt 1986) |
| MC4100 | <i>F- ΔlacU169 araD139 rpsL150 relA1 ptsF rbs flbB5301</i> | Parental strain for FTD128 and Δ <i>moaA</i> . | (Casadaban and Cohen 1979) |
| BW25113 | <i>lacI^f rrbB_{T14} ΔlacZ_{WJ16} hsdR514 ΔaraBAD_{AH33} ΔrhaBAD_{LD78}</i> | Parental strain for JW2682 and JW3865. | (Datsenko and Wanner 2000) |
| FTD128 | As MC4100, with in-frame deletion in the <i>fdhE</i> gene. | FDH-O & FDH-N negative. | (Luke et al. 2008) |
| JW2682 | As BW25113, with in-frame deletion of the <i>hypF</i> gene. | Deficient in all hydrogenases. | (Baba et al. 2006) |
| JW3865 | As BW25113, with in-frame deletion of the <i>fdoG</i> gene. | FDH-O negative. | (Baba et al. 2006) |
| MC4100 Δ <i>moaA</i> | As MC4100, disruption of the <i>moaA</i> gene. | Deficient in all molybdoenzymes | This study. |

Fig. 1. (A) Complete reduction of Pd(II) to Pd(0) by an aerobically-grown culture of *E. coli*. Both bottles contain cells resuspended in 20 mM MOPS buffer at pH7.6, and 1 mM sodium tetrachloropalladate (total volume 25 ml). This image was taken 45 min after the addition of formate to the bottle on the right. (B) Reduction by *E. coli* MC4100 and by controls showing no abiotic reduction of Pd(II). Controls used were killed (autoclaved) cells and cell-free suspension. Soluble Pd(II) in the supernatant was measured using ICP-MS. ▲ = MC4100; ■ = no cells; ♦ = killed cells.

Fig. 2. EXAFS data showing the presence of Pd(II) at 0 and 30 min (bottom two traces), and Pd(0) at 1, 3 and 4 h (ascending series). The top trace is palladium foil.

Fig. 3. Pd(II) reduction by six different strains of *E. coli*, using formate as the electron donor. Soluble Pd(II) in the supernatant was measured using ICP-MS. ♦ = BW25113; □ = JW2682; ▲ = JW3865; Δ = MC4100 $\Delta moaA$; ■ = MC4100; ◇ = FTD128. Data points for BW25113, JW2682 and JW3865 are mean values of triplicates, with standard error shown.

Fig. 4. TEM of thin sections of aerobically grown cells showing extracellular palladium; (A) MC4100, inset BL21; (B) BW25113, inset BL21 (no Pd); (C) FTD128; (D) JW2682; (E) MC4100 $\Delta moaA$; (F) JW3865. Scale bar (A) = 100 nm; (B)-(F) = 500 nm; insets = 1 μ m.

Figure 1

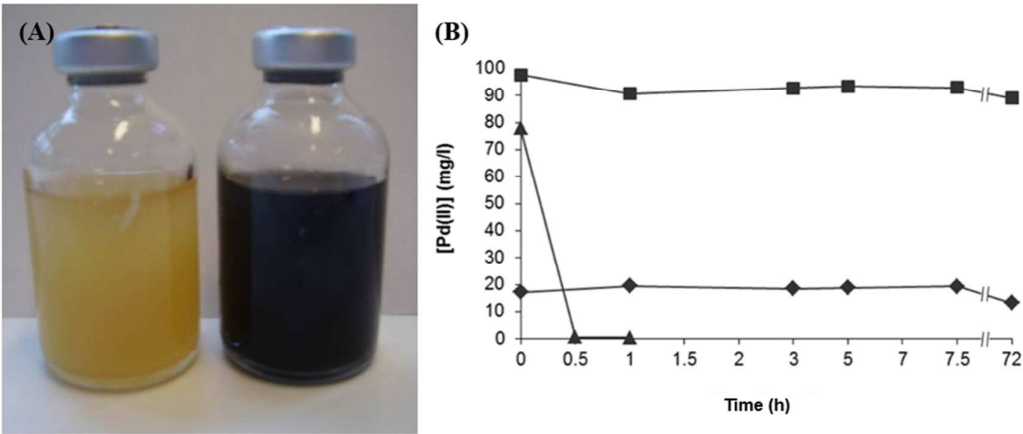


Figure 2

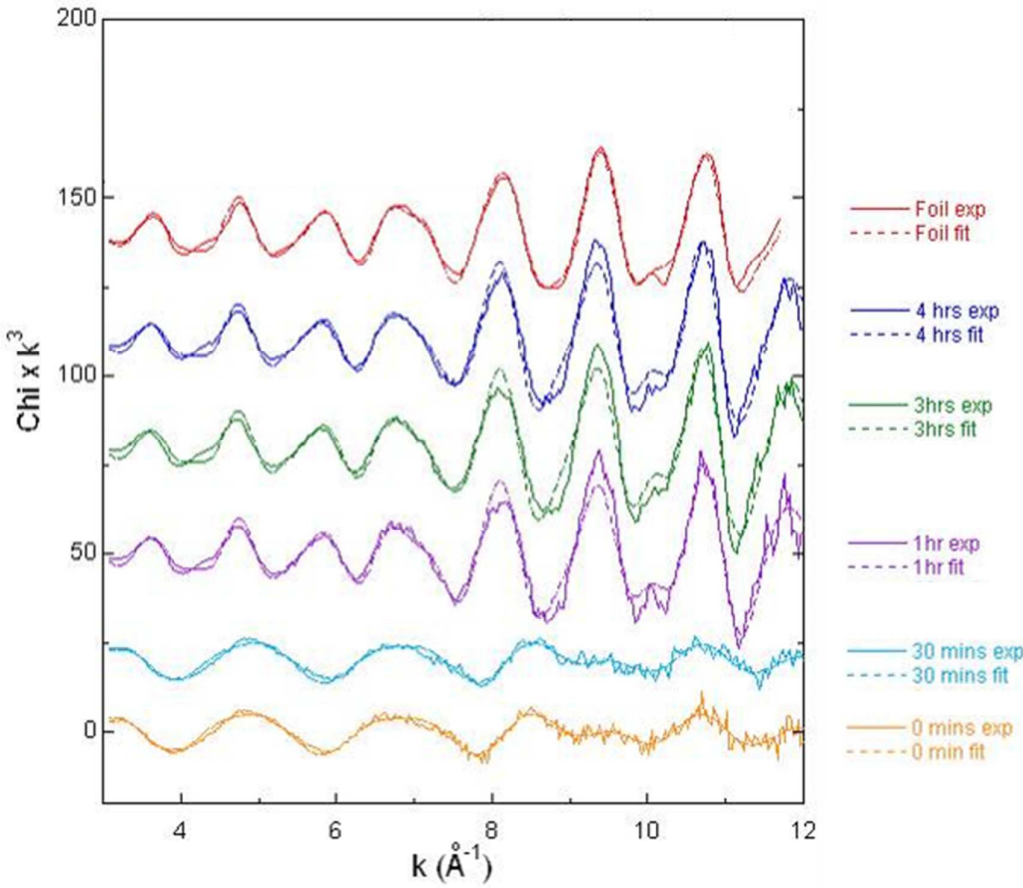


Figure 3

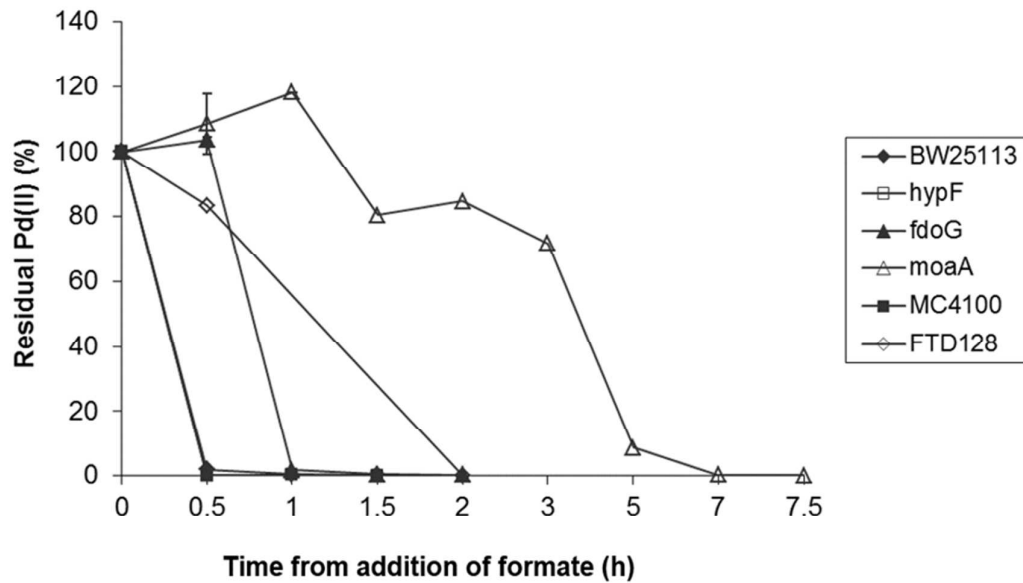


Figure 4

