

Extracellular Palladium Nanoparticle Production using *Geobacter sulfurreducens*

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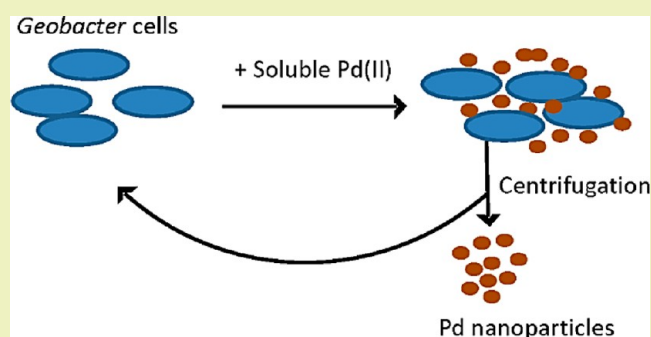
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S Supporting Information

ABSTRACT: Sustainable methods are needed to recycle precious metals and synthesize catalytic nanoparticles. Palladium nanoparticles can be produced via microbial reduction of soluble Pd(II) to Pd(0), but in previous tests using dissimilatory metal reducing bacteria (DMRB), the nanoparticles were closely associated with the cells, occupying potential reductive sites and eliminating the potential for cell reuse. The DMRB *Geobacter sulfurreducens* was shown here to reduce soluble Pd(II) to Pd(0) nanoparticles primarily outside the cell, reducing the toxicity of metal ions, and allowing nanoparticle recovery without cell destruction that has previously been observed using other microorganisms.

Cultures reduced 50 ± 3 mg/L Pd(II) with 1% hydrogen gas (v/v headspace) in 6 h incubation tests [100 mg/L Pd(II) initially], compared to 8 ± 3 mg/L (10 mM acetate) without H₂. Acetate was ineffective as an electron donor for palladium removal in the presence or absence of fumarate as an electron acceptor. TEM imaging verified that Pd(0) nanoparticles were predominantly in the EPS surrounding cells in H₂-fed cultures, with only a small number of particles visible inside the cell. Separation of the cells and EPS by centrifugation allowed reuse of the cell suspensions and effective nanoparticle recovery. These results demonstrate effective palladium recovery and nanoparticle production using *G. sulfurreducens* cell suspensions and renewable substrates such as H₂ gas.

KEYWORDS: Nanoparticles, Biosynthesis, Sustainable, *Geobacter*



■ INTRODUCTION

Palladium is a precious metal commonly used in catalytic converters of automobiles to lower emissions of harmful pollutants, as well as in refining, electronics, dentistry, and catalysis.^{1,2} Palladium consumption has outpaced production rates in the past decade,² increasing the need for more effective and sustainable methods for its recovery from waste streams. Bulk palladium recovery processes commonly involve dissolution of the metal using acids at high temperatures following separation of spent catalysts from other waste products. Strong reductants, such as sulfur dioxide or sodium borohydride, are then used to generate bulk insoluble palladium.³ The production of palladium nanoparticle catalysts (0.1–100 nm in diameter)⁴ has become favored over bulk production in order to take advantage of the unique physicochemical properties of these nanosized particles.⁵ Traditional chemical methods of palladium nanoparticle synthesis requires use of strong reducing agents, as well as ligand stabilizing polymers and capping agents to control particle size and dispersion.⁶ These processes generate undesirable waste streams that need to be avoided.

There is growing interest in using microbiological methods to precipitate metals out of waste streams.⁷ The major advantages of using bacteria is that the cells are the reducing

agents, eliminating the need for chemical reducing agents (except for substrates for the bacteria), and the cells self-regulate particle growth so that they form only nanosized particles. This strategy for nanoparticle synthesis avoids utilization of chemical reductants such as sodium borohydride⁴ and expensive polymeric capping agents and stabilizers.⁸ The catalytic activity of biologically supported palladium nanoparticles for contaminant reduction has been compared to commercially available catalysts with mixed conclusions.^{6,9,10} The formation of palladium nanoparticles on cell surfaces in high dry cell weight to palladium ratios has led to a decrease in catalytic activity due to poisoning by sulfur compounds contained in cell proteins,⁶ making separation of nanoparticles from cells important to maintain catalytic activity.

Biological metal precipitation has been studied using various pure cultures,⁷ including *E. coli*,¹¹ fermentative bacteria,¹² and dissimilatory metal reducing bacteria (DMRB). Most of the work utilizing DMRB has focused on the *Shewanella* and *Desulfovibrio* genera.^{13,14} The primary route for nanoparticle synthesis by DMRB is through hydrogenases. In *Desulfovibrio*

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spp., different hydrogenases have been found in the periplasm, cytoplasm, and cytoplasmic membrane and have been shown to act as the nucleation and initiation point for metal reduction,¹⁵ with subsequent nanoparticle growth via autocatalytic reduction.¹⁴ The nanoparticles formed in the periplasm and outer-membrane are naturally stabilized and capped with the native polymers, inhibiting aggregation and uncontrolled growth of the particles. Extracellular reduction also occurs in *Desulfovibrio* spp. during reduction of Au(III), but the mechanism, although not fully understood, is believed to differ from that of palladium.^{12,16} Outer membrane *c*-type cytochromes have also been shown to be a site for Pd(0) reduction in *Shewanella oneidensis*.¹⁷ *Shewanella* and *Desulfovibrio* spp. can precipitate a broad range of metals (Fe, Mn, U, Cr, Te, Tc, Pd)^{18,19} using various electron donors (e.g., formate, pyruvate, or hydrogen). In addition, both *Desulfovibrio desulfuricans* (200 mg/L) and *S. oneidensis* (1000 mg/L) can reduce high concentrations of soluble palladium to nanoscale particles. Nanoparticle formation by *Desulfovibrio* occurs primarily in the periplasm, which has the disadvantage of inactivating the cells by protruding through and rupturing the cell membrane, necessitating a new culture of bacteria for each reduction cycle.¹⁴ Intracellular and periplasmic nanoparticle production also require additional procedures to access catalytically active sites.²⁰ The response of different microorganisms to palladium and other precipitated metals varies. *E. coli*, for example is able to perform enantioselective deracemization reactions after being challenged with soluble palladium (~100 mg/L)¹¹ and fermentative bacteria are able to produce hydrogen after reduction of soluble palladium (50 mg/L).²¹ Additionally, *Shewanella* cell suspensions have remained culturable after being challenged with low levels of soluble palladium (10 mg/L),¹³ but cell culturability at elevated levels of palladium have not been tested. The viability of *Desulfovibrio* cells after palladium reduction has not been reported.

Geobacter are DMRB that can reduce solid and insoluble metals extracellularly via multiple outer membrane cytochromes (e.g., OmcB, OmcC, OmcS, OmcF, OmcZ)^{22,23} or a *c*-type cytochrome that is excreted into solution.²⁴ *Geobacter* spp. also have multiple NiFe hydrogenase complexes (two membrane-bound periplasmic and two cytoplasmic hydrogenases)²⁵ with one essential periplasmic hydrogenase (Hyb) for respiration with hydrogen.²⁶ These microbes can reduce a variety of metals,²⁷ including U(VI) to U(IV)²⁸ and amorphous Fe(III) to magnetite. Magnetite formed by *G. sulfurreducens* has been used as a nanoscale support for a palladium catalyst.²⁹ *G. sulfurreducens* has been shown to reduce Ag(I) to Ag(0) nanoparticles primarily via outer membrane *c*-type cytochromes during acetate oxidation and fumarate reduction.³⁰ Silver reduction occurred extracellularly with no evidence of reduction in the periplasm of the cells,³⁰ suggesting that cells could remain viable, allowing for reuse of the cell suspension and recovery of nanoparticles without rupturing the cells. Recently, Pd(0) nanoparticle formation by *G. sulfurreducens* was shown for Cr(VI) reduction with formate as the electron donor, but only in the presence of a mediator (AQDS).³¹ The ability of *Geobacter* spp. to reduce palladium directly, or to use H₂ for metal nanoparticle formation, has not previously been examined.

The extent of Pd(II) reduction to Pd(0) nanoparticles was examined here using *G. sulfurreducens* with hydrogen or acetate as electron donors and the presence or absence of an alternate electron acceptor (fumarate). The potential for reusing the cell

suspensions in successive reduction cycles was also examined following particle recovery by simple centrifugation. The location of the particles (intracellular versus extracellular) was examined using electron microscopy.

MATERIALS AND METHODS

Culturing Methods. *G. sulfurreducens* PCA was obtained from frozen stocks (-80 °C) and cultured in ATCC medium 1957 with 30 mM acetate at 30 °C. Cells were anaerobically washed twice with ATCC medium 1957 (no electron donor or acceptor) and resuspended in sterilized anaerobic (80% CO₂/ 20% N₂) serum bottles at an OD₆₁₀ of 0.5 ± 0.05.

Location of Palladium Reduction. Samples were collected and fixed in a 0.1 M sodium cacodylate buffer containing 2.5% glutaraldehyde and 1.5% paraformaldehyde in a 1:10 ratio of sample to fixative solution, and stored at 4 °C. Samples for TEM analysis were prepared by either pelleting the cells (4500 g for 10 min) before fixation or by gravitational settling of the cells in fixative. Samples were analyzed with an environmental scanning electron microscope (E-SEM) (FEI Quanta 200) equipped with an electron dispersive X-ray spectroscopy (EDS) detector to confirm that the extracellular precipitates were palladium. Samples were also analyzed with a transmission electron microscope (TEM) (JEOL JEM 1200 EXII) to capture images of the extracellular palladium nanoparticles on the cell surface. Nanoparticle size was estimated by counting particles in TEM images using the Image J software. Samples were analyzed with a Live/Dead BacLight Bacterial Viability Kit (Invitrogen, USA) to determine the relative amount of cells that were alive (based on having intact outer membranes) after palladization using an epifluorescent microscope (Olympus BX61).

To investigate the extent of extracellular palladium reduction, samples were fixed as described above and embedded in Eponate resin after staining with osmium tetroxide and uranyl acetate and dehydrated in an ethanol and acetone series. The embedded sample was ultramicrotomed (Leica EM UC6 Microtome) into 70 nm slices and imaged using TEM.

Palladium Reduction Tests. To determine the optimal time for palladium reduction and nanoparticle formation, *G. sulfurreducens* cell suspensions and abiotic controls were incubated in 56 mL culture bottles (26 mL headspace) for 6, 12, and 24 h (30 °C) at a Pd(II) concentration of 100 mg/L. Culture incubation time was set to 6 h to prevent excessive abiotic palladium reduction (Supporting Information Figure S11). To determine the mass of palladium that *G. sulfurreducens* could reduce, cultures were incubated for 6 h in solutions containing 5, 10, 50, 100, and 200 mg/L of Pd(II). The electron donors tested included hydrogen (headspace concentrations of 1, 2.5, and 5% v/v) and sodium acetate (10 mM). Sodium tetrachloropalladate (Na₂PdCl₄, Sigma-Aldrich, USA) was added to the cultures as the electron acceptor in all tests and sodium fumarate (40 mM) was added as an alternate electron acceptor in some tests with acetate as the electron donor. Negative controls were run for each condition using fresh, sterile medium. Killed controls for testing palladium removal were prepared by autoclaving cell suspensions at 121 °C for 15 min. Any pressure in the serum bottles was released prior to addition of the electron donor. The reuse of cell suspensions examined using 2.5% H₂ in the headspace was also tested with sodium acetate (1 mM) as a carbon source. Tests were run in duplicate with abiotic controls. Cell suspensions reused in multiple reduction cycles were centrifuged, washed once with ATCC medium 1957 (no electron donor or acceptor) and then resuspended in fresh medium after each test. Subsequent tests were then conducted as above, with the indicated electron donor and 100 mg/L Pd(II) for 6 h at 30 °C.

Palladium Analysis and Recovery. Soluble palladium (duplicates) was determined by filtering samples through 0.22 μm pore diameter filters followed by analysis using inductively coupled plasma emission spectrometry (ICP-AES) (Perkin-Elmer Optima 5300). Samples were acidified with 1 M nitric acid to prevent precipitation prior to analysis.

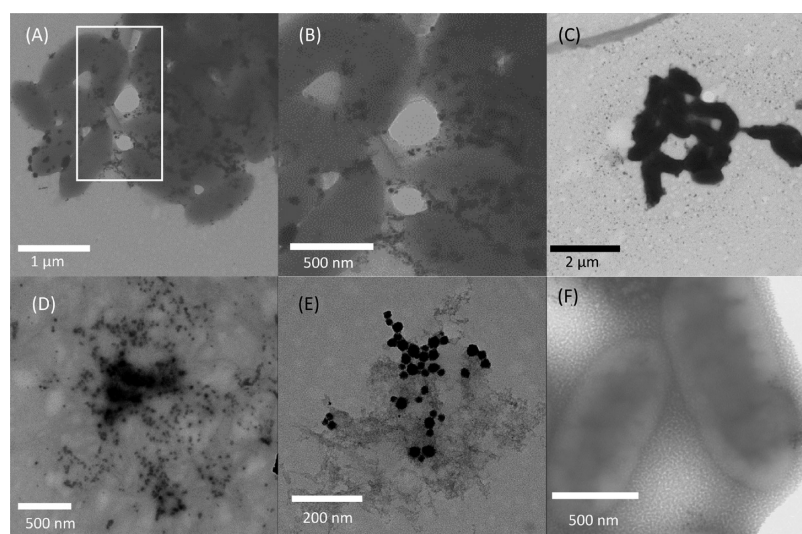


Figure 1. TEM images of palladium reduction with hydrogen as the electron donor: (A) palladium nanoparticles on the surface *G. sulfurreducens* and near the cell (expanded in part B); (C) nanoparticles in the solution surrounding the cells; (D) magnification of particles in solution surrounding cells; (E) particles in centrifugation pellet; (F) autoclaved biomass with palladium as the sole electron acceptor exhibiting limited nanoparticle formation compared to viable biomass.

Particulate palladium recovery was examined using centrifugation and filtration. Cell suspensions were divided into three fractions: the supernatant containing no cells (fraction 1), the cell fraction (fraction 2), and the pellet that could not be resuspended by vortexing (fraction 3). These fractions were obtained by first pelleting cells by centrifugation (4500 g) for 10 min. The supernatant (fraction 1) was decanted and centrifuged at 12 000g for 2 h to concentrate suspended nanoparticles. The liquid was then placed in an oven set to 650 °C for 2 h to remove any organics and determine the mass of suspended palladium. Samples were stored at 4 °C prior to further analysis. The cell fraction (fraction 2) was then resuspended in bicarbonate buffer (described above) and filtered through 0.65 μm pore diameter centrifugal filters (Millipore, US) at 10 000g for 10 min in to test the efficacy of filtration on the separation of palladium nanoparticles from cells. The filtrate was centrifuged for an additional 2 h at 12 000g to pellet any remaining particles and stored at 4 °C. The pellet that could not be resuspended by vortexing (fraction 3) was removed from the centrifuge tube, pyrolyzed at 650 °C for 2 h to remove organics, and weighed to determine the mass of palladium. A small portion (~200 μL) of these samples was taken to be analyzed by TEM to determine the size and morphology of the particles.

RESULTS AND DISCUSSION

Extracellular Palladium Nanoparticle Formation. Palladium reduced with *G. sulfurreducens* cultures incubated with headspace hydrogen of 1 and 2.5% (v/v) and 10 mM acetate with fumarate produced nanoparticles on the surface of cells and in the EPS. Nanoparticles formed in solution were smaller and had less variation (14 ± 3 nm in diameter) than those closely associated with cells (25 ± 11 nm) (Figure 1), in agreement with previous reports for nanoparticle production with *Desulfovibrio* and *Shewanella*.^{13,32} Particles associated with the surface of cells were confirmed to be palladium using EDS (Supporting Information Figure SI2). Controlling palladium particle size and preventing aggregation is desirable because active surface area is maximized compared to larger, aggregated particles (1–10 μm) produced via abiotic reduction with H₂. There was little evidence of aggregation of the nanoparticles based on TEM images, highlighting the importance of palladium reduction in the presence of stabilizing ligands in the exopolymer matrix. Abiotic reduction of palladium occurred

at an appreciable rate when the headspace H₂ (v/v) was above 2.5%, resulting in the growth of large and nondispersed particles. Aggregates were apparent in the solution and microscopic analysis indicated the presence of many particles in the size range of 1–10 μm (Supporting Information Figure SI3).

Killed cells also removed palladium from solution (see below), but only a few extracellular nanoparticles were observed in TEM images (Figure 1). The middle of the killed cells appeared dark relative to living cells in palladium reduction cultures, which indicated intracellular palladium accumulation. Palladium adsorption to biomass, and subsequent reduction by H₂, was likely the dominant mechanism of palladium removal in killed cell cultures. The internal reduction of palladium by autoclaved cells was observed in cross sections of resin embedded cells (Figure 2). Cells were ruptured during the sterilization process allowing palladium to diffuse into the cells and reduced. Metal adsorption to biomass is well-known and can be used as a method of metallic ion removal from solution.³³ Dead biomass produced less nanoparticles because the extracellular and outer-membrane bound proteins were inactivated, inhibiting the normal cellular mechanisms of nanoparticle generation. Though removal of metals from waste streams by dead biomass is a viable option, live cells are better suited for concurrent removal and nanoparticle production that can be used for downstream applications.

Cross sections of resin embedded cells from live suspensions fed 1% H₂ showed primarily extracellular reduction (Figure 2). Some periplasmic and intracellular reduction by *G. sulfurreducens* was also observed. It is possible that periplasmic reduction occurred due to oversaturation of extracellular reduction sites, allowing soluble palladium to diffuse into the periplasmic space where it could have been reduced via periplasmic hydrogenases.¹¹ Previously, periplasmic reduction of Ag(0) nanoparticles was not observed using *G. sulfurreducens*.³⁰ However, the Pd(II) concentration here was five times higher than the Ag(I) concentration in the previous study.

Palladium nanoparticle formation in solution was more evident when cells were gravity settled instead of centrifuged

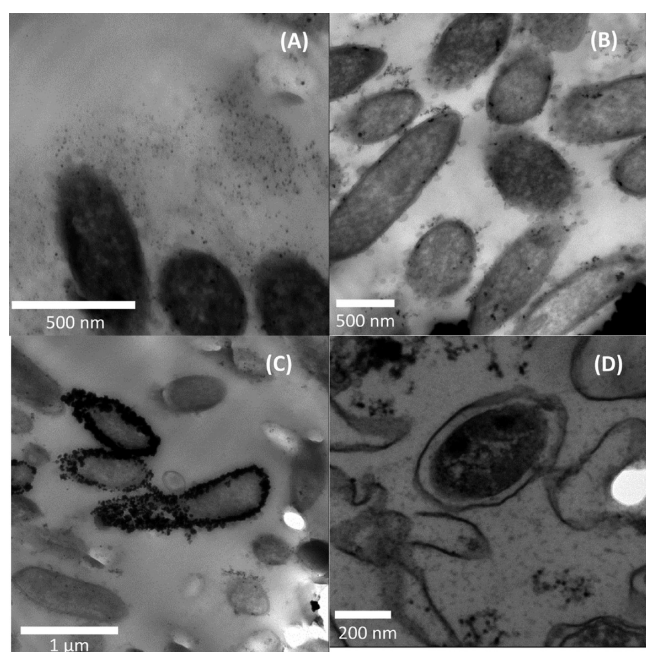


Figure 2. TEM images of ultramicrotomed cell pellet. (A) Extracellular reduction is seen in the space between the cells. (B) Cells exhibit some periplasmic and intracellular reduction. (C) A few cells exhibit buildup of palladium particles that cause cell death. (D) Killed cells fed hydrogen with increased intracellular palladium reduction due to membrane rupturing.

before TEM imaging (Figure 1). Cells that were settled in fixative prior to imaging exhibited a noticeable increase in the amount of extracellular nanoparticles relatively far away (~ 200 nm) from cells. When cells were centrifuged before imaging, observed palladium nanoparticles were all very close to the cell surface (~ 20 nm). Centrifugation of cells to concentrate them before TEM imaging causes nanoparticles formed in solution to stick to the cell aggregates, which misrepresents the distribution of nanoparticles in different locations.

Palladium Reduction with Hydrogen or Acetate.

Hydrogen was an effective electron donor for palladium reduction in cultures and controls. *G. sulfurreducens* cultures removed 51 ± 1 mg/L of a 100 mg/L Pd(II) solution, and 68 ± 1 mg/L of a 200 mg/L Pd(II) solution with 1% H_2 (v/v) in the headspace (Figure 3). Removal increased to 59 ± 1 mg/L [100 mg/L Pd(II)] when the headspace hydrogen was increased to 2.5% (v/v). Abiotic palladium reduction increased in the presence of high concentrations of H_2 (Supporting Information Figure SI4). Pd(II) removal in abiotic controls was greater than in suspensions of *G. sulfurreducens* when H_2 concentrations were increased above 2.5% (v/v). However, the sizes of these particles could not be confined to the nanoparticle size range, and therefore, they became much larger (tens of micrometers) than the bio-palladium nanoparticles, which is undesirable for downstream applications as less catalytic surface area is exposed.

Palladium removal by *G. sulfurreducens* using H_2 is low compared to other DMRB (Table 1). *Desulfovibrio* spp. were able to use hydrogen as an electron donor to remove 200 mg/L of soluble palladium in 30 min (*D. vulgaris*)⁹ to 80 min (*D. fructosivorans*)³⁴ compared to 75% removal of a 50 mg/L solution by *G. sulfurreducens* in 360 min. *S. oneidensis* MR-1 was able to use H_2 as an electron donor to reduce 90% of a 50 mg/L

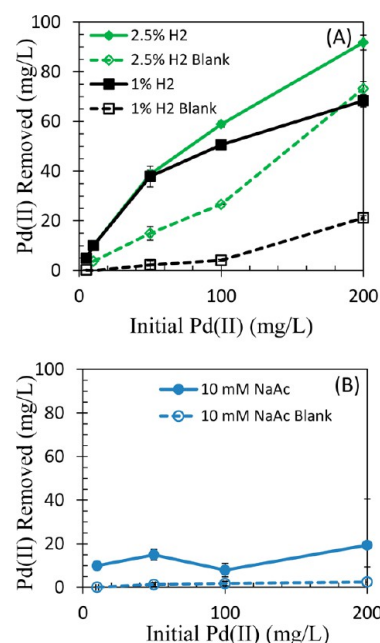


Figure 3. Palladium removal by *G. sulfurreducens* with (A) hydrogen or (B) acetate as the electron donor. Acetate was not an effective electron donor. Hydrogen was effective, but concentrations higher than 2.5% (v/v) in the headspace gave a large amount of abiotic reduction that confounded the amount of biological palladium removal.

L Pd(II) solution. However, the reduction reaction was allowed to proceed overnight, making the palladium removal rates by *S. oneidensis* MR-1 similar to those obtained in this study.

Acetate was ineffective as an electron donor for palladium reduction. The maximum palladium removal of *G. sulfurreducens* cultures fed sodium acetate (10 mM) was 15 ± 3 mg/L using a 50 mg/L Pd(II) solution (Figure 3). Removal decreased to 8 ± 3 mg/L Pd(II) when soluble palladium concentration was increased to 100 mg/L Pd(II). Cultures fed acetate and incubated with palladium as the sole electron acceptor produced few nanoparticles, as seen in TEM images (Figure 4). Cross sections of resin embedded cells fed acetate showed an increase of periplasmic palladium reduction compared to cells fed hydrogen (Figure 4). Cell suspensions in a 100 mg/L Pd(II) solution given no electron donor removed a similar amount of palladium (6 ± 2 mg/L) as those fed acetate.

The inability of *G. sulfurreducens* to use acetate (its preferred electron donor) as an organic electron donor under anaerobic conditions for palladium reduction is different from other species of DMRB (Table 1). *S. oneidensis* MR-1 can effectively use organic electron donors such as formate, lactate, ethanol, and pyruvate, and *D. vulgaris* was able to use formate to reduce palladium.

Killed cells removed a significant portion of the soluble palladium in solution, in agreement with previous studies.^{10,12,35} In the presence of hydrogen, killed *G. sulfurreducens* cells removed 35 ± 1 mg/L (1% headspace H_2) and 42 ± 5 mg/L (2.5% headspace H_2) of a 100 mg/L Pd(II) solution.

Palladium Reduction with Fumarate. The addition of fumarate, as an alternate electron acceptor to acetate fed *G. sulfurreducens* cultures did not increase palladium reduction. Palladium removal was similar (8 ± 1 mg/L) upon the addition of fumarate to cultures with 100 mg/L Pd(II) in solution. No palladium reduction occurred in abiotic controls with acetate and fumarate. However, extracellular nanoparticles were

Table 1. Palladium Removal by Various Bacterial Species

organism	cell OD ₆₁₀	Pd(II) concentration (mg/L)	reduction time (min)	electron donor	removal (%)	cells recycled	ref
<i>Desulfovibrio desulfuricans</i>	0.7	200	2	H ₂	40	N	14
				formate	100		
<i>Desulfovibrio vulgaris</i>	0.5	200	30	H ₂	100	N	9
<i>Desulfovibrio fructosivorans</i>		200	80	H ₂	100	N	34
<i>Shewanella oneidensis</i> MR-1	2	50	overnight	H ₂	90	N	10
				formate	95		
				pyruvate	75		
				ethanol	75		
<i>Geobacter sulfurreducens</i>	0.5	100	360	H ₂	50	Y	this study
		50		H ₂	75	Y	
		100		acetate	8	Y	

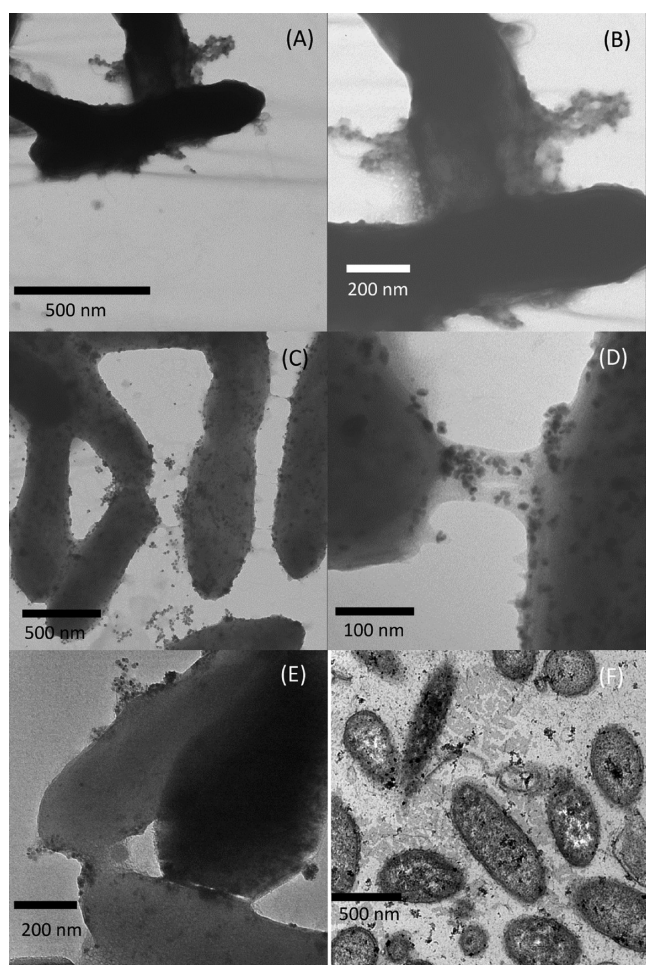


Figure 4. TEM images of palladium reduction with acetate as the electron donor. (A–B) Palladium as the sole electron acceptor. (C–D) Palladium and fumarate as electron acceptors. (E) Viable cells with no electron donor and palladium as the sole electron acceptor exhibiting similar amounts of palladium reduction as suspensions containing acetate without fumarate. (F) Ultramicrotomed cells fed acetate with palladium as the sole electron acceptor. More periplasmic reduction occurs compared to cells fed hydrogen.

produced with fumarate addition instead of intracellular accumulation (Figure 4), similar to that observed in previous experiments on the reduction of soluble Ag(I) to Ag(0) nanoparticles in the presence of fumarate.³⁰

The limited ability of *G. sulfurreducens* to reduce palladium with acetate as the electron donor suggests that the major

pathway of Pd(II) reduction is via hydrogenase activity. However, there are no extracellular hydrogenases identified in the genome of *G. sulfurreducens*.²⁶ With acetate as the electron donor, the expected mechanism of extracellular Pd(II) reduction is by cytochromes, but little extracellular reduction occurs with palladium as the sole electron acceptor, implying that soluble palladium could not be effectively used as a terminal electron acceptor. In tests with H₂ as the electron donor, palladium adsorbed to acid functional groups in the EPS are reduced to palladium nanoparticles, as shown in a similar test using carboxymethylcellulose as the ligand for nanoparticle formation.³⁶ However, acetate is unable to act as a reductant in the EPS, leading to a decrease in palladium reduction. When fumarate is present as the terminal electron acceptor, a portion of the electrons taken from acetate oxidation could be redirected to cytochromes for extracellular palladium reduction during respiration as a detoxification mechanism. The addition of fumarate increased nanoparticle formation but did not increase the rate of palladium reduction, implying that acetate oxidation is the rate limiting step. The precise biological mechanism explaining the difference in palladium reduction by *G. sulfurreducens* when using hydrogen or acetate as the electron donor remains unclear.

Recycling of Cell Suspensions. Cultures were reused in successive reduction cycles for each electron donor with 100 mg/L of Pd(II) as the electron acceptor (Figure 5). Palladium removal was lower after the first cycle, but the percent removal was relatively stable thereafter. With 1% H₂ as the electron donor, 50 ± 1 mg/L of Pd(II) was removed during the initial

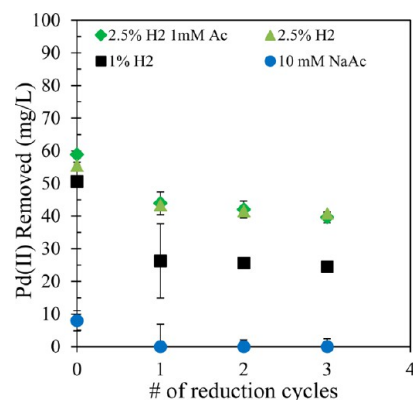


Figure 5. Successive cycles of palladium reduction by *G. sulfurreducens*. There is a decrease in efficacy between the first two cycles, but steady reduction between cycles 1 and 3 showing that cells are reusable. Cultures with acetate as the electron donor could not be reused.

cycle, and 26 ± 4 mg/L was removed during the next three cycles. Palladium removal increased to 55 ± 1 mg/L followed by 42 ± 1 mg/L for successive cycles with 2.5% H₂. Intracellular palladium accumulation in some cells in suspension, observed in the TEM images of embedded cells, could disrupt normal cellular processes and explain the drop in palladium removal after the first cycle.

The addition of low acetate concentrations (1 mM) as a carbon source for cell maintenance during reduction with 2.5% H₂ enhanced removal (58 ± 1 mg/L initially and 42 ± 3 mg/L for successive cycles). However, cultures with 10 mM acetate as the sole electron donor reduced only 8 ± 3 mg/L Pd(II) during the first cycle, and there was no Pd(II) reduction in any of the three following cycles demonstrating acetate was an ineffective electron donor for Pd(II) reduction. Suspensions of *G. sulfurreducens* can be supplemented with acetate to provide a carbon source to support cellular maintenance while using a different electron donor (such as H₂) for palladium reduction, as acetate is ineffective as the sole electron donor for palladium reduction.

Palladium Recovery. Centrifugation was effective for palladium nanoparticle recovery from cell suspensions because nanoparticles were relatively far from the cell surfaces. Roughly $63 \pm 5\%$ of the initial soluble palladium added to the *G. sulfurreducens* cultures (1% H₂ in the headspace) was recovered as palladium particles by centrifugation. The centrifugation supernatant (fraction 1) contained $13 \pm 3\%$ (0.4 ± 0.1 mg) of the initial palladium as palladium particles and the fraction of the cell pellet that could not be resuspended by vortexing (fraction 3) contained $50 \pm 1\%$ (1.5 ± 0.3 mg) of the initial soluble palladium as particles. The remaining palladium remained attached to the cells or was lost in the solution. The palladium that remains attached to cells or inside the periplasm can be recovered once cell death and lysis occurs, allowing the palladium to be separated via centrifugation and recovered along with the palladium in solution. A continuous process would need to be employed to maintain an active, steady cell suspension. These results suggest that further optimization of nanoparticle separation and recovery via centrifugation is warranted to increase the overall efficiency of the process.

Filtration was ineffective at separating nanoparticles from cells. No palladium particles were detected in the cell fraction filtered by $0.65 \mu\text{m}$ centrifugal filters (fraction 2). Filtration was a less effective separation technique because the EPS (and any nanoparticles in the EPS) was likely trapped by the filter material instead of passing through. Additionally, nanoparticles still attached to the cells or reduced inside the cells would not be passed through the filter.

■ ASSOCIATED CONTENT

● Supporting Information

EDS spectra and additional microscope images. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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