ACS Catalysis

RESEARCH ARTICLE

pubs.acs.org/acscatalysis

Engineering a Biometallic Whole Cell Catalyst for Enantioselective Deracemization Reactions

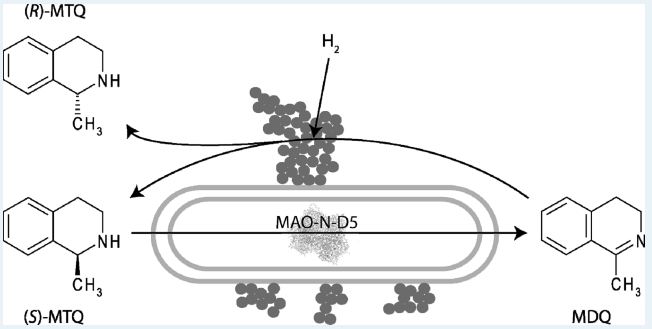
Joanne M. Foulkes,[†] Kirk J. Malone,[‡] Victoria S. Coker,[†] Nicholas J. Turner,[‡] and Jonathan R. Lloyd*[,†]

[†]Williamson Research Centre for Molecular Environmental Science and School of Earth, Atmospheric and Environmental Sciences, University of Manchester, Oxford Road, Manchester M13 9PL, U.K.

[‡]School of Chemistry, Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester M1 7DN, U.K.

Supporting Information

ABSTRACT: The ability of microbial cells to synthesize highly reactive nanoscale functional materials provides the basis for a novel synthetic biology tool for developing the next generation of multifunctional industrial biocatalysts. Here, we demonstrate that aerobic cultures of *Escherichia coli*, genetically engineered to overproduce a recombinant monoamine oxidase possessing high enantioselectivity against chiral amines, can be augmented with nanoscale Pd(0) precipitated via bioreduction reactions. The result is a novel biometallic catalyst for the deracemization of racemic amines. This deracemization process is normally achieved by discrete sequential oxidation/reduction steps using a separate enantiomer-specific biocatalyst and metal catalyst, respectively. Here, use of *E. coli* cultures carrying the cloned monoamine oxidase gene and nanoscale bioreduced Pd(0) particles was used successfully for the conversion of racemic 1-methyltetrahydroisoquinoline (MTQ) to (*R*)-MTQ, via the intermediate 1-methyl-3,4-dihydroisoquinoline, with an enantiomeric excess of up to 96%. There was no loss of catalyst activity following the five rounds of oxidation and reduction, and importantly, there was minimal loss of palladium into the reaction supernatant. This first demonstration of a whole cell biometallic catalyst mixture for "single-pot", multistep reactions opens up the way for a wide range of integrated processes, offering a scalable and highly flexible platform technology.



KEYWORDS: palladium catalyst, *Escherichia coli*, biocatalysis, biotransformation, deracemization

The fabrication of functional biominerals by microbial cells offers the potential to revolutionize the synthesis of nanomaterials. These biosynthetic routes are potentially highly scalable and work at ambient pressures and temperatures in the absence of toxic compounds such as capping agents, and their properties can be fine-tuned using genetic or physiological manipulations.[1] In several examples, waste materials can be used as the feedstock for biomineral synthesis, offering a green chemistry solution to nanomaterial synthesis.[2] Functional biominerals produced recently by microbial routes include precious metal catalysts,[2,3] nanomagnets for hyperthermic cancer treatment,[4] quantum dots,[5] bioremediation agents,[6] silver-based antimicrobials,[7] and SERS probes.[8] The model organisms used for these processes are all amenable to manipulations using the rapidly advancing tools of synthetic biology. Thus, they have the potential to form the basis of complex multifunctional systems when integrated with the microbial host's cellular physiology.

A particularly promising area for this type of approach is the development of multifunctional biocatalysts. The application of recombinant whole cell biocatalysts is an established technology for a broad range of industrial conversions, including notable examples such as the reduction of ketones to alcohols using ketoreductases[9] and also the asymmetric synthesis of chiral amines using transaminases.[10]

The requirement for enantiomerically pure chiral amines in the pharmaceutical and agrochemicals industries has led to the development of asymmetric transformations, dynamic kinetic resolutions (DKR), and deracemization reactions using biocatalysts that can efficiently generate products in up to 100% yield and 100% ee.[11] Previous work has reported the use of lipases with a carbon-supported palladium catalyst for the DKR of racemic amines.[12] However, this method takes up to 5 days at 60 °C to give moderate yields and generates unwanted byproduct. Lipases are suitable for use with primary amines and some secondary amines, but cannot be applied to tertiary amines.[13] By contrast, monoamine oxidase-N (MAO-N), has attracted attention as a result of its enantioselectivity toward the *S* form of the primary amine α-methyl-benzylamine.[14] MAO-N is a tetrameric flavoprotein that catalyzes the oxidative deamination of terminal amine groups.[15] The use of this enzyme for the deracemization of racemic chiral amines is based on the creation of imine intermediates by oxidation of the *S* enantiomer. The addition of a chemical reductant, such as ammonia borane or palladium

Received: August 2, 2011
Revised: September 17, 2011
Published: September 22, 2011

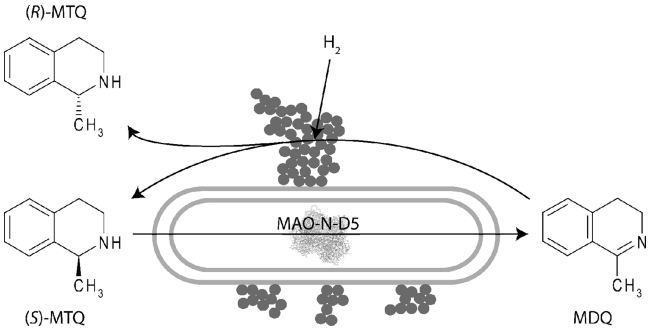


Figure 1. The cyclic deracemization of MTQ via the imine MDQ using palladized biocatalyst with the *mao-N-D5* insert.

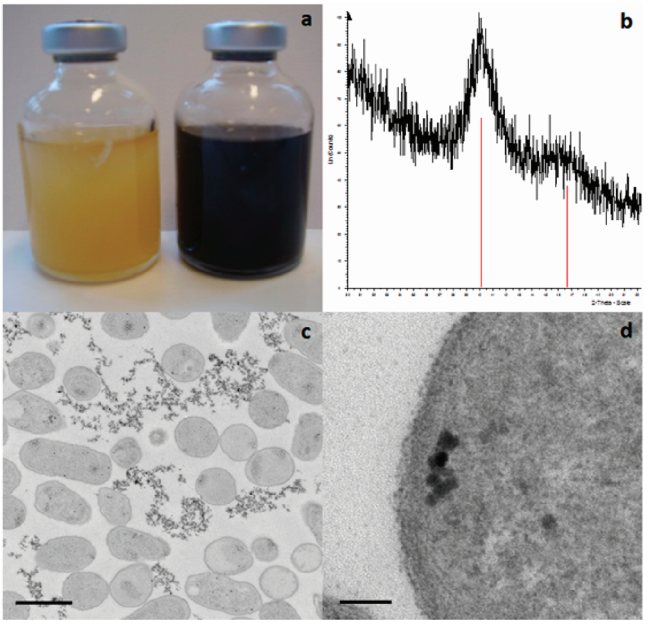


Figure 2. (a) Biomass with palladium(II) (left), and reduced palladium (right); (b) XRD showing the presence of palladium in the microbial cultures supplied with Pd(II) and hydrogen as the electron donor; (c) TEM of thin sections of aerobically grown cells showing extracellular palladium (scale bar =1 μm); (d) TEM of a cell showing intracellular nanoparticles associated with the inner surface of the plasma membrane (scale bar =50 nm).

formate/hydrogen, results in the nonselective conversion of the imine back to the racemic amine. After several oxidation/reduction cycles, a very high enantiomeric excess and yield of the *R* enantiomer is achieved.

The directed evolution of MAO-N from *Aspergillus niger* has led to the generation of enzymes with enhanced activity and specificity toward primary,[11] secondary,[16] and tertiary amines.[13] The advantage of directed evolution as a method for improving enzymes for specific applications is that no prior knowledge of enzyme structure is required because screening is carried out purely on the basis of the desired functional properties.[17] After several cycles of mutagenesis and screening, a variant enzyme was identified with activity toward the secondary amine 1-methyltetrahydroisoquinoline (MTQ)[16] and the tertiary amine *N*-methyl-2-phenylpyrrolidine.[13] The latter variant has five point mutations in the gene, giving rise to the alterations Ile246Met/Asn336Ser/Met348Lys/Thr384Asn/Asp385Ser in the product. This enzyme is designated MAO-N-D5.[13,18]

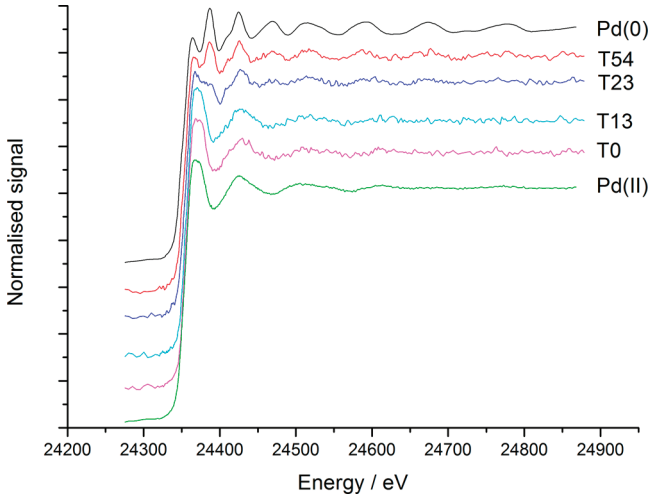


Figure 3. EXAFS spectra showing reduction of Pd(II) to Pd(0) within 1 h (in situ experiment). Times shown are in minutes.

It has been known for more than a century that a range of specialist prokaryotes can reduce metals by using them as the terminal electron acceptor during anaerobic respiration.[19] These microbial activities can be used in the bioremediation of land contaminated with toxic metals and also in the reclamation of potentially valuable precious metals.[1] For example, the obligately anaerobic sulfate-reducing bacterium *Desulfovibrio desulfuricans* has been shown to reduce soluble Pd(II) to insoluble Pd(0), which is precipitated in the periplasm as nanoparticles.[20] This property has allowed the use of the palladized organism (known as Bio-Pd) directly in a broad range of reactions, showing good activity compared with a commercially available carbon-supported palladium catalyst for the dehalogenation of chlorophenol, polychlorinated biphenyls, and polybrominated diphenyl ethers.[21,22] Here, we outline how Bio-Pd can be produced without the use of strict anaerobic culture or complicated washing steps to remove sulfide formed by the sulfate-reducing bacterium (which can poison palladium catalysts) by using the facultative anaerobe *Escherichia coli* grown under aerobic conditions. *E. coli* is also easily transformed with plasmid DNA and, hence, can be used to express foreign genes, making this organism ideal for use as a biocatalyst in a one-pot reaction utilizing recombinant enzymes such as MAO-N-D5 in combination with Bio-Pd . The aim of this study was to demonstrate for the first time the coupled activity of a functional biomineral catalyst and enzyme in a novel engineered catalyst, in this case for the deracemization of racemic amines, providing a prototype for multistep transformations and other hybrid biometallic systems (Figure 1).

RESULTS AND DISCUSSION

Palladization of *E. coli* BL21. To develop a multifunctional, biometallic, whole-cell catalyst for deracemization reactions, washed aerobically grown *E. coli* BL21 cells containing the cloned gene expressing the enzyme MAO-N-D5 were palladized by challenging with 1 mM Pd(II) and hydrogen gas as an electron donor. The cells were coated in a black precipitate within a few minutes (Figure 2a), indicating the reduction of Pd(II) to insoluble particles of Pd(0).[20] Pd(II) did not reduce to Pd(0) in bottles that were not purged with H_2. The black precipitate was analyzed by

dx.doi.org/10.1021/cs200400t |*ACS Catal.* 2011, 1, 1589–1594

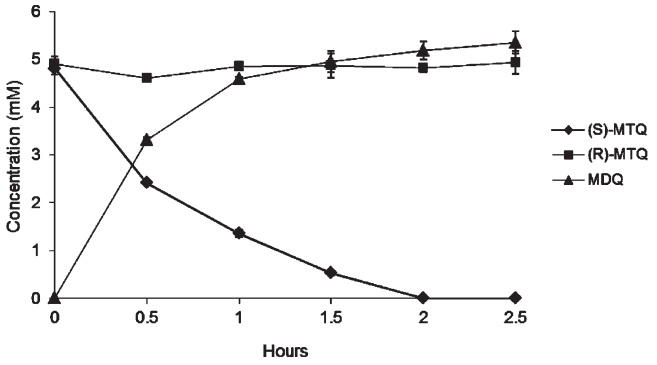


Figure 4. The oxidation of (*S*)-MTQ to MDQ within 2 h at 37 °C by *E. coli* transformed with the *mao-N-D5* gene insert.

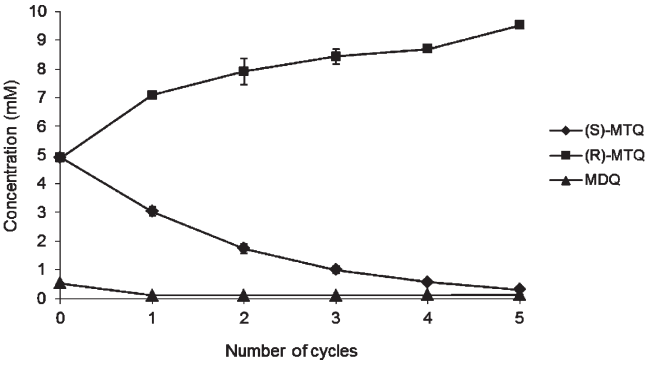


Figure 6. The deracemization of MTQ, giving an ee of 96% (*R*)-MTQ (five cycles of air alternated with five cycles of hydrogen).

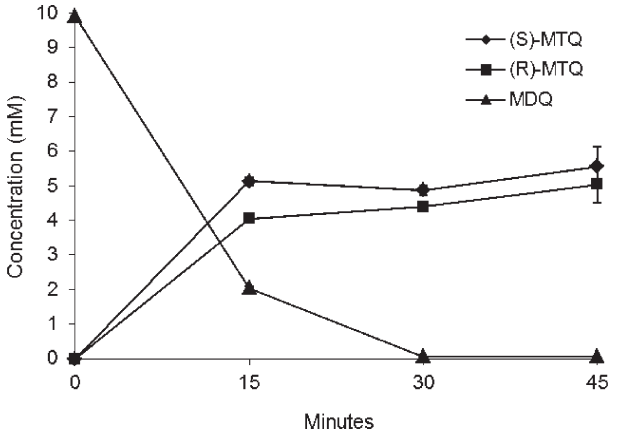


Figure 5. The reduction of MDQ to racemic MTQ within 30 min at 37 °C, by palladized *E. coli* using hydrogen gas as the electron donor.

X-ray diffraction to confirm the presence of elemental palladium (Figure 2b), although the signal was weak due to the nanoparticulate nature of the sample. The size of the particles was calculated to be ~1 nm in diameter, as measured using the Scherrer equation.

TEM images of thin sections of cells showed that the reduced palladium was precipitated predominantly in the extracellular matrix of the cultures (Figure 2c). Occasionally, larger nanoparticles (~10 nm) were seen inside the cell, possibly located on the inner surface of the plasma membrane (Figure 2d). Energy-dispersive X-ray spectroscopy (EDS) confirmed the presence of palladium in these electron-dense nanoscale precipitates.

QEXAFS analysis at the Swiss Light Source showed that the Pd(II) is reduced to Pd(0) with no evidence of intermediate species (Figure 3). However, the reduction of the Pd(II) was slower than under normal laboratory conditions, as hydrogen could not be continuously supplied due to the necessity of vacating the experimental area during use of synchrotron radiation on the beamline. Hydrogen was therefore supplied for only 30 s between measurements.

Substrate Oxidation and Reduction. When *E. coli* transformed with the *mao-N-D5* gene insert was added to 10 mM racemic MTQ and incubated at 37 °C, all of the *S* enantiomer of the MTQ was oxidized to MDQ within 2 h (Figure 4).

When palladized untransformed *E. coli* was added to 10 mM MDQ and 70 mM sodium formate and incubated at 37 °C, all of the MDQ was reduced to racemic MTQ within 1 h. Subsequent experiments using hydrogen gas showed complete reduction of MDQ within 30 min (Figure 5). Thus, both the oxidative and reductive steps required for the deracemization of chiral amines such as MTQ were active in the same culture of recombinant, palladized *E. coli* developed in this study.

Integration of Processes for Deracemization. Despite the rapid conversion of the starting materials in both the oxidation and reduction experiments, when initially combined into one system and incubated for 24 h, deracemization did not proceed beyond the initial oxidation step. This was due to the unexpected formation of 1-methylisoquinoline (MIQ) from MDQ by further chemical oxidation. However, production of MIQ was not observed when hydrogen gas was used instead of formate as the electron donor for the reduction step, possibly indicating that hydrogen drives the bioreduction of MDQ at a faster rate than formate. To avoid the potentially hazardous mixing of air and hydrogen in the reaction vessel, oxidation and reduction steps were performed as separate cycles. On the basis of the results from the separate oxidation and reduction reactions, each oxidation step was maintained for 2 h, and each reduction step was 45 min, to allow each reaction to proceed to completion. After four cycles of oxidation and reduction, the *R* enantiomer was present in an enantiomeric excess (ee) of 89%. The hypothetical value at this stage after four cycles of oxidation and reduction is a 96.875% ee. Increasing the experiment from four to five cycles increased the ee to 96% (Figure 6), which would be the standard required for commercial application.

When cells used in a 5-cycle biotransformation were collected by centrifugation and washed with potassium phosphate buffer and used in further oxidation- and reduction-only experiments, it was found that the activity of both the MAO-N-D5 enzyme and the palladium was the same as that prior to the biotransformation. Thus, both the recombinant enzyme and Bio-Pd were stable for multiple rounds of biotransformation.

Comparison of Activity with Commercially Available Palladium. When the performance of the palladized cells was compared with that of recombinant nonpalladized cells with an equivalent amount of conventional Pd (carbon with 10% palladium) in a biotransformation of five cycles of oxidation and reduction, there was a 97% conversion to (*R*)-MTQ by both the Bio-Pd and the conventional Pd. However, the total mass of the final product (*R*)-MTQ was low, at 85% of the expected total produced for the Bio-Pd and 75% for the conventional Pd. Washing the cell pellets three times with methyl-*tert*-butyl ether recovered the (*R*)-MTQ from the Bio-Pd cell pellet; however, 10% of the missing (*R*)-MTQ was still not recoverable at this stage from the conventional-Pd cell pellet.

dx.doi.org/10.1021/cs200400t |*ACS Catal.* 2011, 1, 1589–1594

The third wash for the conventional Pd samples continued to yield (*R*)-MTQ, indicating retention of the amine through adsorption onto the carbon support, which was difficult to recover through washing. The recovery of product from the Bio-Pd was 100%, with no further (*R*)-MTQ recovered after the second wash. With the additional material taken into account from the washing of the cell pellets, both the Bio-Pd and the conventional Pd showed a 92% conversion to (*R*)-MTQ. However, the yield was 10% lower than expected for the conventional Pd. In this respect, Bio-Pd is superior to conventional Pd for use in deracemization reactions due to the comparative ease of product recovery.

Measuring Loss of Palladium into the Reaction Supernatant. Although centrifugation was sufficient to remove all Bio-Pd nanoparticles from the reaction supernatant, visible particles of conventional Pd remained in the supernatant after centrifugation and required filtering to remove. The ICP-MS results also showed that more palladium was lost to the supernatant when using conventional-Pd than with Bio-Pd. The Bio-Pd reaction supernatant contained 0.17 mg L^{-1} palladium, whereas the conventional-Pd reaction supernatant contained 0.24 mg L^{-1}.

CONCLUSIONS

Recent advances in the field of synthetic biology allow us to anticipate the possibility of creating a "designer" biometallic catalyst, engineered precisely to the requirements of the desired chemical transformation. This study has shown that it is already possible to engineer a biometallic catalyst mixture capable of one-pot deracemization reactions.

Although previous studies investigating the bioreduction of palladium have used anaerobic cultures, this study has demonstrated that palladium reduction is possible with aerobic cultures of *E. coli*, using hydrogen as the electron donor. Although Pd(II) is reduced by hydrogen without the presence of bacteria, there is likely to be a biological mechanism for Pd(II) reduction in this culture, due to the significantly increased time taken for reduction to complete in cell-free controls and killed (autoclaved) cell controls (based on visual interpretation), as also seen by others.[23] The aerobic expression of hydrogenases known to mediate Pd(II) reduction in anaerobic cultures[24] is one possibility that is being investigated in our laboratory, or the expression of another macromolecular system with the capability to catalyze hydrogen oxidation and Pd(II) reduction. The use of aerobic cultures for the production of biometallic catalysts is preferable, due to increased cell yield; higher yields of recombinant proteins;[25] and, in the case of SRB, no production of H_2S, which can poison metallic catalysts.[26] TEM images show the presence of elemental palladium as extracellular nanoparticles, associated with the cell surface and possibly with exopolysaccharides. It may be this association that prevents the removal of the palladium nanoparticles during washing of the biocatalyst. Occasionally nanoparticles were seen inside the cell, possibly located on the inner surface of the plasma membrane. QEXAFS data confirms the reduction of Pd(II) directly to Pd(0), without the production of intermediate species.

Palladized *E. coli* transformed with the *mao-N-D5* plasmid is capable of performing both oxidation and reduction reactions required for the deracemization reactions studied here. However, it was not possible to perform both reactions simultaneously, with aerobic cells supplied with formate as an electron donor for the Pd-catalyzed reaction, due to the formation of the unwanted product MIQ under these conditions. However, this product was not seen when hydrogen was used instead of formate as the electron donor in the reduction reaction. The use of hydrogen required the two reactions to be performed separately but in the same reaction vessel, in a cyclical manner. This simple approach should be amenable for use in a broad range of reactor configurations, including those using high densities of cells immobilized on a suitable support for easy reuse. Because the two reactions are not run simultaneously, each discrete reaction step can be optimized fully, with prolonged activity for the catalyst demonstrated in this study.

When the performance of enzymatically produced Bio-Pd was compared with that of commercially available "conventional Pd" in a biotransformation of five cycles of oxidation and reduction, both were found to adsorb the reactants and products of the biotransformation. However, the conventional Pd adsorbed around 10% more than the Bio-Pd (1 mM of the potential 10 mM final product in our study), indicating that the amine could not be desorbed from the catalyst, even with multiple washing steps. It was also found that the conventional Pd was present in the reaction supernatant at higher levels than the Bio-Pd and had to be removed by an additional filtration step, complicating downstream processing. The ease of removal of Bio-Pd from the reaction mixture indicates both reduced contamination of the products with palladium and improved recyclability of the catalyst. The reuse of Bio-Pd cells following a five-cycle biotransformation confirmed that the activity of these cells was the same as in previous experiments.

In conclusion, we have demonstrated that it is possible to create a biometallic catalyst that expresses MAO-N as a model oxidase enzyme in palladized cells and can perform both oxidation and reduction reactions, leading to a 96% deracemization of a secondary amine, without the addition of a separate chemical reductant and with limited contamination or adsorption of the reaction products by the catalyst. This method has a broad potential for simplifying or improving other enzyme/inorganic catalyst combinations, not being specific to either *E. coli* or palladium, and could be potentially useful for a range of industrial and biotechnological applications. This study, therefore, illustrates the exciting potential of applying the rapidly evolving synthetic biology tool kit available to biotechnologists to engineer biocatalysts and novel nanoscale biomineral precipitates in a single organism for multistep catalysis.

EXPERIMENTAL SECTION

Transformation of *E. coli* BL21 with pET-16b Plasmid Containing the *mao-N-D5* Insert. *Strains and Plasmid. E. coli* BL21 cells were obtained from Invitrogen. The pET16-b plasmid containing the *mao-N-D5* insert and an ampicillin resistance gene was obtained from Dr. Andrew Ellis at the Manchester Interdisciplinary Biocenter. Bacterial isolates were stored at −80 °C, and the plasmid was stored at −20 °C.

Transformation. Cells were transformed according to the supplier's instructions, using approximately 100 ng of plasmid. Transformed BL21 cells were subcultured onto LB agar plates containing 100 μg mL^{-1} ampicillin and incubated overnight at 37 °C before storage at 4 °C for a maximum of 1 week.

Palladization of *E. coli* BL21. The method used was adapted from that used by Lloyd and Macaskie.[20]

Starter Cultures. A 50 mL portion of LB broth in a 500 mL Erlenmeyer flask was inoculated with a single isolated colony of the transformed *E. coli* and incubated overnight at 37 °C, shaking at 180 rpm.

Aerobic Cultures. An 11 mL starter culture was added to 99 mL LB broth containing 100 μg mL^{-1} ampicillin in a 1 L

Erlenmeyer flask. Flasks were incubated for 24 h at 37 °C, shaking at 180 rpm. An oxygen saturation of 72% was measured using an Oakton D06 Acorn Series dissolved oxygen meter.

Palladization. Each culture was divided between two 50 mL Falcon tubes (supplied by Fisher) and washed three times in 20 mL MOPS−NaOH (morpholinepropanesulfonic acid) buffer, 20 mM at pH 7.6 after centrifugation for 20 min at 2500*g*. Cell pellets were adjusted to a mass of 250 mg, based on the wet cell pellet, and resuspended in MOPS−NaOH to a volume of 1 mL. Two tubes of each culture were resuspended in 25 mL of buffer with 1 mM sodium tetrachloropalladate (supplied by Alfa Aesar) in 30 mL bottles sealed with butyl rubber stoppers (two bottles per culture). Bottles were incubated in the dark at 30 °C for 1 h for the Pd(II) to biosorb to the cells.[21] One of each of the two different cultures was then purged with H_2 for 5 min, with H_2 allowed to fill the headspace to promote the enzymatic reduction of the Pd(II). The final loading of bioreduced, elemental Pd(0) in the biomass was approximately 1% by mass. The remaining two bottles were H_2-negative controls.

X-ray Diffraction (XRD) Analysis. The cells were coated in a black precipitate within a few minutes in bottles containing cells as Pd(II) purged with H_2. The palladized cells settled out, leaving a clear, colorless supernatant. The black precipitate was washed once in acetone and air-dried before investigation by XRD. The measurements were performed on a Bruker D8 Advance diffractometer, using Cu Kα1 radiation. The samples were scanned from 5 to 70° 2θ in steps of 0.2°, with a count time of 2 s/step.

Transmission Electron Microscopy (TEM) and Energy Dispersive X-ray Spectroscopy (EDS). Sections of the palladized cells were provided by the EM facility in the Faculty of Life Sciences, University of Manchester. The bacteria were fixed in primary fixative (2.5% glutaraldehyde and 4% formaldehyde in 0.1 M sodium cacodylate buffer at pH7.4) and secondary fixative (1% osmium tetroxide and 1.5% potassium ferrocyanide in 0.1 M sodium cacodylate buffer at pH 7.4) and dehydrated in a graded acetone series (30 min each in 50%, 70%, 90% and three changes at 100%) before embedding in TAAB LV medium epoxy resin. EDS was performed on electron-dark areas to confirm the presence of palladium.

Quick-Scanning Extended X-Ray Absorption Fine Structure (QEXAFS). The reduction of Pd(II) to Pd(0) was analyzed by QEXAFS in an in situ reduction experiment on beamline BL21 at the Swiss Light Source (SLS) synchrotron facility. Cells were grown aerobically as described previously, frozen, and transported to the SLS on dry ice. After thawing, the cells were resuspended in 25 mL of 20 mM MOPS−NaOH buffer (pH 7.6) and challenged with 1 mM Pd(II), in a 5 cm tube placed longitudinally in the beam. The tube was purged with hydrogen for 30 s at 10 min intervals until the sample had turned completely black. Scans were performed at a rate of 4 scans/min for 10 min at a time. X-ray absorption data were collected in the energy range 23 970−25 727 eV. Data were recorded in transmission in fast-scanning mode, each run accumulating 118 spectra over 10 min. The spectra were isolated, the data range was cut to 22 210−24 900 eV, and the number of points in each spectrum was reduced (and signal-to-noise improved) by averaging adjacent groups of points to a grid finely spaced over the Pd K-edge and after the edge evenly spaced in k-space. The individual spectra were summed into 10 groups of 10 and 1 group of 8 for each run. This was repeated until all spectra had been processed.

The summed data were background-subtracted in the Daresbury program EXBACK.

Substrate Oxidation and Reduction. *Oxidation.* Frozen transformed cells (250 mg mL^{-1} in 20 mM MOPS−NaOH buffer at pH 7.6) were thawed and added to 8 mL of 12.5 mM racemic MTQ, from a stock solution made up in 0.1 M potassium phosphate buffer at pH 7.6. Potassium phosphate buffer (1 mL) was added to bring the volume of the reaction mixture to 10 mL. The vessel used was a 120 mL gas bottle sealed with a butyl rubber stopper, and the reaction was allowed to proceed in air at 37 °C, with shaking at 225 rpm. Samples were taken as required by removing 550 μL in triplicate, centrifuging for 20 min at 2500*g*, and storing 500 μL of the supernatant at 20 °C before extraction with methyl-*tert*-butyl ether (MTBE).

Reduction with Sodium Formate As the Electron Donor. Frozen palladized untransformed cells (250 mg mL^{-1} in 20 mM MOPS−NaOH buffer at pH 7.6, with 1% Pd loading by mass) were thawed and added to 8 mL of 12.5 mM MDQ (1-methyl-3,4-dihydroisoquinoline, supplied by Acros Organics), from a stock solution made up in 0.1 M potassium phosphate buffer at pH 7.6. Sodium formate (1 mL) was added to a final concentration of 70 mM, giving a total reaction volume of 10 mL. The conditions of incubation and MTBE extraction were the same as for the oxidation step.

Reduction with Hydrogen As the Electron Donor. The procedure was carried out as above, with 1 mL of 0.1 M potassium phosphate buffer replacing the sodium formate in the reaction mixture. The reaction volume was 10 mL, in a 120 mL gas bottle, providing a 110 mL headspace. The bottle was sparged with hydrogen for 2 min before incubating and extracting as previously.

HPLC Analysis. The samples were resuspended in 200 μL of isohexane prior to testing by normal phase chiral HPLC on an Agilent 1200 chromatograph, using a Daicel Chiralpak AD-H 4.6 mm × 250 mm column at a flow rate of 1 mL min^{-1} for 20 min at 40 °C with a mobile phase of 88% isohexane, 2% ethanol, and 10% isohexane containing 0.5% diethylamine. The analytes were detected by UV−vis spectroscopy at wavelengths of 220 nm (MTQ) and 254 nm (MDQ) (see Supporting Information Figure 1). The error (RSD) was 2.8%.

Integration of Processes for Deracemization. *Deracemization with Sodium Formate As the Electron Donor.* The two reactions were combined by using palladized transformed cells in the reaction mixture and with the addition of 70 mM sodium formate (final concentration). The bottles were incubated as above.

Deracemization with Hydrogen As the Electron Donor. The two reactions were combined by using palladized transformed cells in the reaction mixture. Oxidation and reduction steps were performed as separate cycles by flushing the vessel alternately with air or hydrogen. The reaction vessel was a 120 mL gas bottle with a 10 mL reaction volume, providing a 110 mL headspace. The vessel was first flushed with nitrogen to avoid the mixing of air and hydrogen. Each oxidation step was 2 h long, and each reduction step was 45 min. Samples were taken at the end of each reduction step for four cycles. The deracemization took a total of 11 h, with intervention for the flushing of gases at the end of each oxidation and each reduction step (eight interventions in total). Increasing the deracemisation step from four to five cycles required an additional two interventions for the flushing of gases, over a total of 13 h and 45 min.

Comparison of Activity with Commercially Available Palladium. The activity of the biometallic catalyst was compared with nonpalladized cells and commercially available 10% palladium on carbon (conventional Pd), supplied by Sigma Aldrich. The biotransformation was carried out over five cycles of air and hydrogen.

Measuring Loss of Palladium into the Reaction Supernatant. To determine whether palladium was leached into the reaction supernatant, contaminating the products, the supernatant was obtained by centrifugation of 1 mL at 15 682*g* for 5 min before digestion with an equal volume of 70% nitric acid for 24 h. Samples were then diluted to 2% nitric acid and filtered before analysis for the presence of palladium using the ICP-MS Agilent 7500cx.

■ ASSOCIATED CONTENT

Supporting Information. Additional data, including details of HPLC analysis, controls for palladium reduction with hydrogen, reuse of the biocatalyst, and a comparison of Bio-Pd with conventional Pd. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Phone: +44 (0)161 275 7155. Fax: +44 (0)161 306 3961. E-mail: jon.lloyd@manchester.ac.uk.

■ ACKNOWLEDGMENT

The authors thank the staff in the EM facility in the Faculty of Life Sciences, The University of Manchester for their assistance, and the Wellcome Trust for equipment grant support to the EM facility. We also thank Dr. John Charnock of the University of Manchester for analysis of QEXAFS data, and Dr. Valentin Koehler of the University of Basel for assistance with HPLC analysis.

■ REFERENCES

(1) Lloyd, J. R.; Pearce, C. I.; Coker, V. S.; Pattrick, R. A. D.; Van Der Laan, G.; Cutting, R.; Vaughan, D. J.; Paterson-Beedle, M.; Mikheenko, I. P.; Yong, P.; Macaskie, L. E. *Geobiology* **2008**, *6* (3), 285–297.
(2) Macaskie, L. E.; Mikheenko, I. P.; Yong, P.; Deplanche, K.; Murray, A. J.; Paterson-Beedle, M.; Coker, V. S.; Pearce, C. I.; Cutting, R.; Pattrick, R. A. D.; Vaughan, D.; van der Laan, G.; Lloyd, J. R. *Hydrometallurgy* **2010**, *104* (3−4), 483–487.
(3) Coker, V. S.; Bennett, J. A.; Telling, N. D.; Henkel, T.; Charnock, J. M.; van der Laan, G.; Pattrick, R. A. D.; Pearce, C. I.; Cutting, R. S.; Shannon, I. J.; Wood, J.; Arenholz, E.; Lyon, I. C.; Lloyd, J. R. *ACS Nano* **2010**, *4* (5), 2577–2584.
(4) Coker, V. S.; Telling, N. D.; van der Laan, G.; Pattrick, R. A. D.; Pearce, C. I.; Arenholz, E.; Tuna, F.; Winpenny, R. E. P.; Lloyd, J. R. *ACS Nano* **2009**, *3* (7), 1922–1928.
(5) Pearce, C. I.; Coker, V. S.; Charnock, J. M.; Pattrick, R. A. D.; Mosselmans, J. F. W.; Law, N.; Beveridge, T. J.; Lloyd, J. R.. *Nanotechnology* **2008**, *19*, (15), -.
(6) Cutting, R. S.; Coker, V. S.; Fellowes, J. W.; Lloyd, J. R.; Vaughan, D. J. *Geochim. Cosmochim. Acta* **2009**, *73* (14), 4004–4022.
(7) Suresh, A. K.; Pelletier, D. A.; Wang, W.; Moon, J. W.; Gu, B. H.; Mortensen, N. P.; Allison, D. P.; Joy, D. C.; Phelps, T. J.; Doktycz, M. J. *Environ. Sci. Technol.* **2010**, *44* (13), 5210–5215.
(8) Jarvis, R. M.; Law, N.; Shadi, L. T.; O'Brien, P.; Lloyd, J. R.; Goodacre, R. *Anal. Chem.* **2008**, *80* (17), 6741–6746.
(9) Moore, J. C.; Pollard, D. J.; Kosjek, B.; Devine, P. N. *Acc. Chem. Res.* **2007**, *40* (12), 1412–1419.
(10) Savile, C. K.; Janey, J. M.; Mundorff, E. C.; Moore, J. C.; Tam, S.; Jarvis, W. R.; Colbeck, J. C.; Krebber, A.; Fleitz, F. J.; Brands, J.; Devine, P. N.; Huisman, G. W.; Hughes, G. J. *Science* **2010**, *329* (5989), 305–309.
(11) Alexeeva, M.; Enright, A.; Dawson, M. J.; Mahmoudian, M.; Turner, N. J. *Angew. Chem., Int. Ed.* **2002**, *41* (17), 3177–3180.
(12) Choi, Y. K.; Kim, M. J.; Ahn, Y.; Kim, M. J. *Org. Lett.* **2001**, *3* (25), 4099–4101.
(13) Dunsmore, C. J.; Carr, R.; Fleming, T.; Turner, N. J. *J. Am. Chem. Soc.* **2006**, *128* (7), 2224–2225.
(14) Alexeeva, M.; Enright, A.; Dawson, M. J.; Mahmoudian, M.; Turner, N. J. *Angew. Chem., Int. Ed.* **2002**, *41* (17), 3177–3180.
(15) Schilling, B.; Lerch, K. *Biochim. Biophys. Acta* **1995**, *1243* (3), 529–537.
(16) Carr, R.; Alexeeva, M.; Dawson, M. J.; Gotor-Fernandez, V.; Humphrey, C. E.; Turner, N. J. *ChemBioChem* **2005**, *6* (4), 637–639.
(17) Alexeeva, M.; Carr, R.; Turner, N. J. *Org. Biomol. Chem.* **2003**, *1*, 4133–4137.
(18) Atkin, K. E.; Reiss, R.; Turner, N. J.; Brzozowski, A. M.; Grogan, G. *Acta Crystallogr., Sect. F: Struct. Biol. Cryst. Commun.* **2008**, *64*, 182–185.
(19) Lloyd, J. R. *FEMS Microbiol. Rev.* **2003**, *27* (2−3), 411–425.
(20) Lloyd, J. R.; Yong, P.; Macaskie, L. E. *Appl. Environ. Microbiol.* **1998**, *64* (11), 4607–4609.
(21) Baxter-Plant, V.; Mikheenko, I. P.; Macaskie, L. E. *Biodegradation* **2003**, *14* (2), 83–90.
(22) De Windt, W.; Aelterman, P.; Verstraete, W. *Environ. Microbiol.* **2005**, *7* (3), 314–325.
(23) Yong, P.; Rowson, N. A.; Farr, J. P. G.; Harris, I. R.; Macaskie, L. E. *Biotechnol. Bioeng.* **2002**, *80* (4), 369–379.
(24) Deplanche, K.; Caldelari, I.; Mikheenko, I. P.; Sargent, F.; Macaskie, L. E. *Microbiology SGM* **2010**, *156*, 2630–2640.
(25) Krause, M.; Ukkonen, K.; Haataja, T.; Ruottinen, M.; Glumoff, T.; Neubauer, A.; Neubauer, P.; Vasala, A. *Microb. Cell Fact.* **2010**, *9*, 11.
(26) Redwood, M. D.; Deplanche, K.; Baxter-Plant, V. S.; Macaskie, L. E. *Biotechnol. Bioeng.* **2008**, *99* (5), 1045–1054.