Enzymatic Recovery of Elemental Palladium by Using Sulfate-Reducing Bacteria

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Worldwide usage of platinum group metals is increasing, prompting new recovery technologies. Resting cells of *Desulfovibrio desulfuricans* reduced soluble Pd^{2+} to elemental, cell-bound Pd^0 supported by pyruvate, formate, or H_2 as the electron donor without biochemical cofactors. Pd reduction was O_2 insensitive, opening the way for recycling and recovery of Pd under oxic conditions.

Many types of metal waste are produced from nonferrous industries such as mining and surface treatments; in general, physicochemical and biotechnological methods are available to treat these wastes. For most metals, global mineral reserves are substantial, and environmental protection, and not metal acquisition, is the major consideration in wastewater treatment. In contrast, the platinum group metals (PGM) are highly valuable, but their "clean" recovery from waste has not received high priority. The routine use of PGM, especially Pt and Pd, is increasing due their widespread and often obligatory use in automotive catalytic converters. With approximately 5 g of PGM per catalyst, the consumption of Pt and Pd together was 7×10^4 kg in 1994, with only 1×10^4 kg recovered (1).

Chemical treatments for reclaiming PGM are made difficult by their complex solution chemistry. Precipitation techniques are not readily applicable. Solvent extraction techniques have been developed with, for example, 8-hydroxyquinoline or tributyl phosphate (4, 8). Solvent extraction requires substantial plant investment and is costly, and the solvents may be toxic. Electrochemical recovery of PGM is feasible, but recovery of the thin metal film deposit from the electrode may limit industrial adoption.

Biological reduction of metals is well documented, for example, the enzymatically mediated bioreduction of hexavalent uranium to stable UO_2 (6, 15–19) by the sulfate-reducing bacterium *Desulfovibrio desulfuricans* and the iron-reducing strain *Geobacter metallireducens*, with the electron transport system responsible for reduction of U(VI) by *Desulfovibrio vulgaris* via cytochrome c_3. Involvement of hydrogenase activity in metal reduction by *Micrococcus lactilyticus* was implicated in early work with uranium (21), was confirmed for the obligate anaerobe *Clostridium pasteurianum* (with selenite used in this case [22]), and was attributed unequivocally to the hydrogenase 3 component of the formate hydrogenlyase complex of *Escherichia coli* for the reduction of Tc(VII) anaerobically (11, 12). Use of this facultatively anaerobic organism showed conclusively that Tc reductase (in this case hydrogenase 3) was sited in the cytoplasm and was under the control of the anaerobic switch protein FNR, upregulated upon shifting to anaerobiosis (11). Anaerobic Tc(VII) reduction was demonstrated also by *Shewanella putrefaciens*, *Geobacter metallireducens* (10), and *D. desulfuricans* (13); with the latter, H_2 consumption was observed during Tc(VII) reduction (13), and cells immobilized in a flow-through hollow-fiber reactor were more than 10 times more effective in Tc(VII) removal than were *E. coli* cells under similar conditions (14). The type strain *D. desulfuricans* ATCC 29577 was selected for use in the present study, with the aim of evaluating its potential for the bioreductive recovery of Pd for the following reasons. *D. desulfuricans* has high metal reductase activity via hydrogenase or cytochrome c_3, with broad metal specificity (Fe, Mn, U, Cr, and Tc [13, 15]). In addition, metal [Tc(VII)] reduction is unaffected by 100 mM NO_3^- (9), and the site of metal reduction and precipitation is the periplasm—a preferable, cell surface location for easy metal recovery. Although Pd^{2+} bioreduction to Pd^0 has received little or no attention previously, this phenomenon was noted in a preliminary study using unidentified environmental isolates incubated anaerobically; however, the incubation took place over a period of >1 week, unattractive for bioprocess use (2).

This study used cells of *D. desulfuricans* ATCC 29577 (American Type Culture Collection) grown for 2 days (30°C) in Postgates's medium C (20) in sealed bottles under N_2, harvested by centrifugation in the bottles, washed under N_2 in 20 mM MOPS (morpholinepropanesulfonic acid)-NaOH buffer, (pH 7), and resuspended to a biomass density of 0.5 g liter^{-1} in MOPS buffer (13). Aliquots (2.5 ml) were transferred to 12-ml serum bottles sealed with butyl rubber stoppers and supplemented with sodium pyruvate or formate (25 mM) or with H_2 replacing the N_2 in the headspace. $Pd(NH_3)_4Cl$ was added to 0.5 mM, and the cultures were incubated at 30°C versus cell-free controls, controls consisting of heat-killed cells, or live cells supplied with no added electron donor. A further control used 0.5 mM Cu^{2+} (an inhibitor of periplasmic hydrogenase [5]) for 10 min during preincubation, followed by a wash in buffer and challenge with Pd^{2+} as before. In some experiments,

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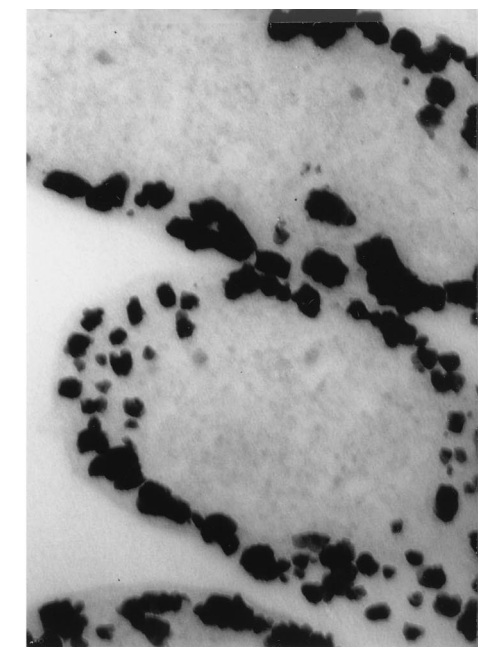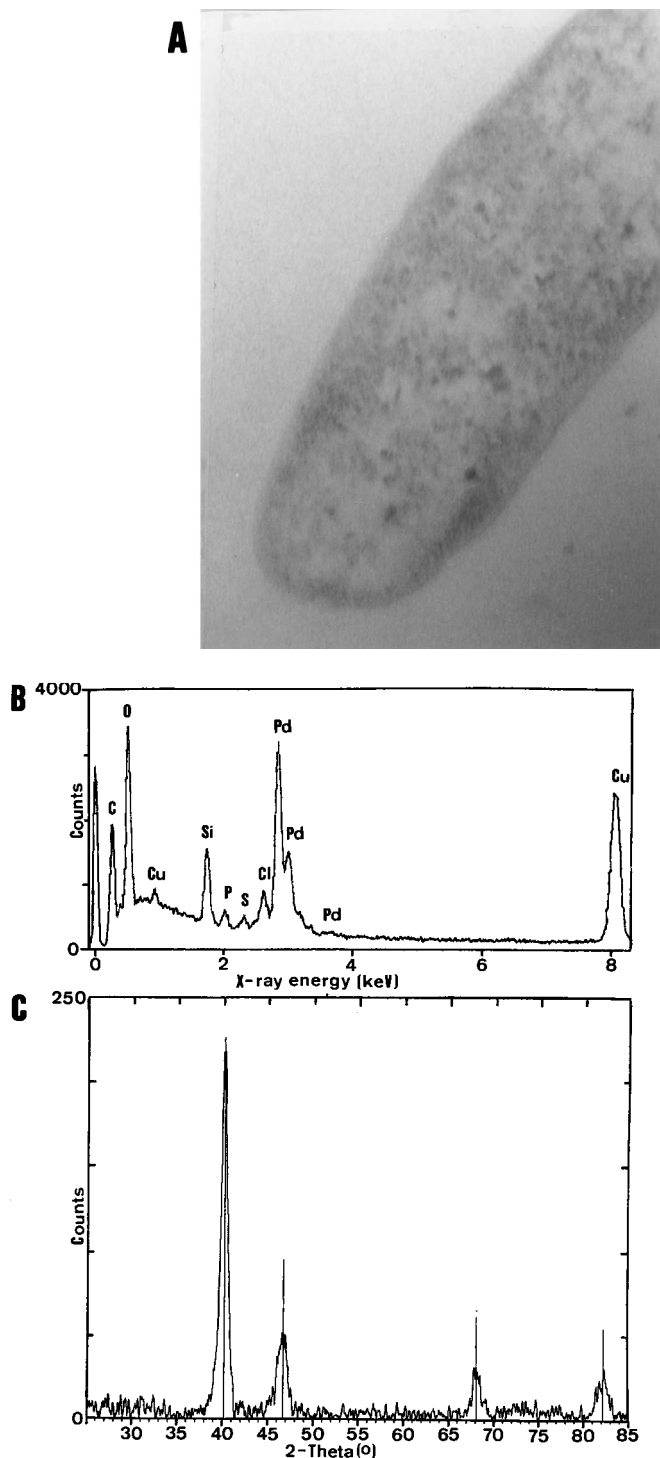
TABLE 1. Pd^{2+} removal from resting cell suspensions

| Cell treatment | Rate of Pd^{2+} removal (nmol min^{-1} mg of protein^{-1})[a] | | | |
| | 0.5 mM Pd^{2+} | | 2.0 mM Pd^{2+} | |
	I	II	I	II
N_2 sparged	79.4	85.5	168.5	201.0
Air sparged	67.8	75.6	168.5	208.0

[a] *D. desulfuricans* was grown and resuspended in solutions supplemented with 0.5 or 2.0 mM Pd^{2+}, as described in the text. I and II are independent experiments using separate batches of cells. Losses in activity: I (0.5 mM Pd^{2+}), 15%; II (0.5 mM Pd^{2+}), 12%; I (2.0 mM Pd^{2+}), 0%; II (2.0 mM Pd^{2+}), 0%.

FIG. 1. Pd accumulation by *D. desulfuricans* ATCC 29577. Cultures were grown and challenged with Pd^{2+}, as described in the text. (A) Electron microscopy of Pd-loaded biomass. Magnifications, ×72,000. (Left) Cells unchallenged with Pd or challenged in the absence of an electron donor. (Right) Cells challenged with Pd for 24 h with H_2 as the electron donor. (B) Individual cell-bound crystalline deposits from the cells shown in panel A were examined by energy-dispersive X-ray microanalysis, with peaks corresponding to the X-ray emission energies of Pd. (C) X-ray diffraction spectrum of dried Pd-loaded biomass (solid line) compared to the reference database for metallic Pd (vertical lines).

distilled water, fixed, sectioned, and examined under the electron microscope as described previously (11, 13). Areas of electron-opaque deposit were examined by energy-dispersive X-ray microanalysis (11, 13), with peaks sought corresponding to the X-ray emission energies of Pd. For X-ray diffraction analysis, Pd-loaded biomass was air dried, washed in chloroform-methanol (1:1, vol/vol) and then acetone, and air dried. X-ray diffraction spectra were obtained as described previously (3) and compared to the reference database for metallic Pd.

Initial tests using resting cells of *D. desulfuricans* supplied with either pyruvate or formate (under N_2) or hydrogen as electron donors in outgassed anaerobic cell suspensions with $Pd(NH_3)_4Cl$ removed Pd^{2+} (Table 1) and showed 100% depletion of dissolved Pd^{2+} from solution after 24 h and a black precipitate, detectable after 10 min and heavy after 24 h, which was not seen with hydrogen- or formate-supplemented solutions alone (no biomass) or suspensions of killed (autoclaved) cells supplied with an electron donor, with live cells lacking an electron donor, or with Cu-treated cells. Under the electron microscope, Pd-unchallenged cells or cells with no electron donor had no electron-opaque areas (Fig. 1A, left), but the Pd-challenged cells showed an array of cell surface-localized electron-opaque deposits (Fig. 1A, right) with sizes of approximately 50 nm. Examination of specimen microareas by energy-dispersive X-ray analysis (single-point deposits) showed that the material contained Pd (Fig. 1B). Further analysis of dried, bulk preparations of sample with X-ray powder diffraction analysis gave a spectrum (Fig. 1C) with peaks corresponding exactly to those of elemental Pd^0 (Fig. 1C, vertical lines). It was concluded that the Pd^{2+} had been reduced enzymatically by the bacteria to elemental Pd^0. Use of hydrogen as the

cell suspensions (5 ml) were sparged with either air or N_2 (30 min) prior to incubation under H_2 with 0.5 mM or 2 mM Pd^{2+}, as before. Timed samples were removed, and residual Pd^{2+} levels in supernatants were determined by differential pulse voltammetry (Metrohm 693 VA polarographic processor with a sweep from -450 mV to -900 mV; sweep rate, 60 mV s^{-1}) in a carrier of 0.1 M NH_4Cl–0.1 M NH_4OH (pH 9.0).

For electron microscopy and solid-state analysis, samples (100 μl) were withdrawn after 24 h, washed twice in double-

electron donor implicates the harnessing of hydrogenase with, possibly, cytochrome c_3 activity to Pd reduction, in accordance with the periplasmic localization of many hydrogenases in the sulfate-reducing bacteria and their inhibition by Cu^{2+}. Unlike with *E. coli* (11), the lack of molecular genetic studies on *D. desulfuricans* makes confirmation with specific deletion mutants difficult, but the involvement of hydrogenase activity is strongly implicated.

Throughout this study, resting cells were used to avoid possible precipitation of Pd^{2+} as the sulfide via H_2S production from the use of sulfate as the terminal electron acceptor. Biomass growth requires oxidizable substrate (e.g., lactate), while low biomass content and "clean" Pd^0 are preferable for easy metal recovery. The use of growth-decoupled cells deserves additional notice. Although microbial reductase activities have been harnessed successfully in other areas of waste remediation (e.g., in denitrification processes), it has not been easy to decouple oxidoreductase activity from biomass growth due to the need for the recycling of metabolic cofactors such as NADH (7). The use of molecular hydrogen can circumvent this requirement and opens up possible applications of hydrogenases in biotechnology. In all cases so far, hydrogenase activity has been confined to strictly anaerobic bacteria or facultatively anaerobic bacteria under anaerobic conditions. In final tests, we sparged the cultures with air prior to testing for Pd reduction. Although O_2 is lethal to many sulfate-reducing bacteria, the precipitation of Pd was largely unaffected (Table 1). This opens the way to the application of these organisms without implementation of special anaerobic facilities following biomass pregrowth; preliminary tests using a flat PTFE (Teflon) membrane with Pd^{2+} on the flow side and H_2 supplied on the back side of a suspension of *D. desulfuricans* gave a black precipitate of Pd^0 within minutes.

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