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ORIGINAL PAPER

Selective Oxidation of Benzyl-Alcohol over Biomass-Supported Au/Pd Bioinorganic Catalysts

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Abstract We report a novel biochemical method based on the sacrificial hydrogen strategy to synthesise bimetallic Au/Pd nanoparticles supported on bacterial cells. The synergistic effect of Au/Pd over monometallic preparations was demonstrated in the oxidation of benzyl alcohol. The bioinorganic catalysts outperformed a commercial Pd catalyst (5% Pd/C) showing no deactivation and high selectivity towards benzaldehyde.

Keywords Bioreduction · Core/shell · *Escherichia coli* · Gold · Palladium · Selective oxidation

1 Introduction

The selective oxidation of primary alcohols to aldehydes is an important process of the fine chemicals synthesis industry. Aldehydes are often used as platform chemicals, high value components or as synthesis intermediates, for example in the perfume industry. Under mild conditions and in the presence of an aqueous base, the oxidation of alcohols using supported Au nanoparticles usually leads to the corresponding acid but not aldehyde. Studies by Edwards et al. [1] showed that supported Au–Pd alloys were effective in the direct synthesis of H_2O_2 by promoting the formation of hydroperoxy species, which are also key intermediates in the oxidation of alcohols. Subsequently, studies by Enache et al. [2, 3] on selective oxidation of benzylalcohol showed that TiO_2-supported Au–Pd nanocrystals (2.5/2.5% w/w) were highly active and selective for benzaldehyde as compared to monometallic Au and Pd catalysts and confirmed the synergistic effect of Pd addition to supported Au catalysts.

Recent studies on the biorecovery of precious metals (Ag, Au, Pd, Pt) have demonstrated the ability of some bacteria to precipitate metallic ions from pure solutions (i.e. solutions of metal salts) as well as from secondary source leachates [4–7] with formation of catalytically active mixed metallic mixtures [8] that can have enhanced catalytic activity as compared to monometallic biomaterials [9]. The bioreduction of Pd(II) by *Desulfovibrio desulfuricans* and *Escherichia coli* leads to the formation in the bacterial periplasmic space of a population of metallic nanoparticles (NPs) that exhibit remarkable catalytic activity in a wide variety of reactions [10–14]. Similarly, the bioreduction of $HAuCl_4$ produces biomass-bound Au NPs of varying size (5–50 nm depending on the pH of the precursor solution used, [7] that exhibit a catalytic behaviour similar to chemically prepared Au/C catalysts in the selective oxidation of glycerol to glyceric acid [15]. From this, we postulated that the combination of Au with biomass-supported Pd NPs could result in the formation of supported Pd/Au bimetallic structures with novel or increased catalytic properties.

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2 Experimental Section

2.1 Organisms and Culture Conditions

Escherichia coli MC4100 (provided by Professor J. A. Cole, University of Birmingham, UK) was maintained aerobically at 30 °C on nutrient agar plates (Oxoid Ltd, Basingstoke Hampshire, UK). Precultures (10% inoculum (v/v) from a mid-exponential phase culture grown anaerobically in nutrient broth No. 2 (NB (Oxoid, UK) containing 50 mM sodium formate) were grown overnight at 37 °C in NB No. 2 under anaerobic respiratory conditions (NB No. 2 supplemented with 0.4% sodium fumarate (w/v) and 0.5% glycerol (v/v), final concentrations) to maximize hydrogenase expression [16]. *D. desulfuricans* ATCC 29577 was routinely cultured as described previously [17] in Postgate C medium [18]. For catalyst preparation, cultures were grown in a similar way in 2 L Durham bottles almost filled to the brim with medium (nutrient broth No.2) and sealed with rubber stoppers. Mid-logarithmic phase cultures (OD_{600} = 0.5–0.7) were harvested by centrifugation (12,000×*g*, 15 min), washed three times in 100 mL of degassed MOPS-NaOH buffer (20 mM, pH 7.2), resuspended in 50 mL of the same buffer and stored at 4 °C as concentrated cell suspensions until use, usually the next day. Cell concentration (mg mL^{-1}) was determined by reference to a predetermined OD_{600} to dry weight conversion.

2.2 Pd(II) and Au(III) Solutions

For the preparation of Pd-coated cells (bioPd), an aqueous Pd(II) solution (2 mM, to pH 2.3 with 0.01 M HNO_3) was made by dissolving an appropriate amount of sodium tetrachloropalladate (Na_2PdCl_4, Sigma-Aldrich, Poole, UK). Similarly, aqueous Au(III) solutions (1 mM, to pH 2.3 with 0.01 M HNO_3) used in reduction studies were made by dissolving hydrogen tetrachloroaurate ($HAuCl_4$ nH_2O), Sigma-Aldrich, Poole, UK) in distilled water.

2.3 Assay of Soluble Au(III) and Pd(II)

Removal of Au(III) from test solutions was monitored by the thiamine-phloxine assay [19] as described previously [7]. During bioPd manufacture, complete removal of Pd(II) from solution was confirmed by assaying cell/Pd mixtures supernatants for residual Pd(II) by the spectrophotometric $SnCl_2$ method [20].

2.4 Manufacture of Pd/Au Bioinorganic Catalysts

First, cells were palladized as follows. A known volume of concentrated resting cell suspension (see above) was transferred anaerobically into 200 mL serum bottles and an appropriate volume of degassed 2 mM Pd(II) solution was added so that the final ratio (weight of Pd:dry weight of cells) was 1:38.5 to give a loading of 2.5% (w/w) Pd (or as otherwise stated) on biomass. Cells/Pd mixtures were left to stand for 30 min at 30 °C before H_2 was sparged through the suspension (200 $mLmin^{-1}$) for 20 min. During H_2 sparging, the colour of the cell/Pd mixtures went from yellow to grey, indicating the reduction of cell surface-bound Pd(II) into Pd(0). Complete removal of Pd(II) from supernatants was confirmed by the $SnCl_2$ assay (see below). Next, bioPd suspension was allowed to settle overnight under gravity, recovered by centrifugation (12,000×*g*, 15 min), washed three times in distilled water, resuspended in distilled water and mixed with Au(III) solution so that the final ratio of Au(III) solution to bioPd water suspension was 4:3 (v/v). The bioPd suspension was degassed (20 min) and transferred anaerobically in an appropriate volume of H_2-saturated Au(III) solution (by sparging H_2 in the solution 30 min at 200 $mLmin^{-1}$) so that the final ratio of Pd:Au was 1:1, 4:1 or 1:4 by mass. For this, the bioPd/Au(III) mixture was allowed to react overnight in a rotary shaker (150 rpm, 30 °C) and supernatants were assayed for residual Pd(II) and Au(III) (see below) to ensure the complete removal of both metal species. The final material was recovered as above, washed three times in dH_2O, once in acetone and left to dry in air in a small volume of acetone.

2.5 Electron Microscopy and Energy Dispersive X-ray Analysis (EDX) of Pd/Au Loaded Biomass

Following metal challenge, pellets of metal-loaded bacteria were prepared for transmission electron microscopy (TEM). Preparations were rinsed twice with distilled water, fixed in 2.5% (wt/vol) glutaraldehyde, centrifuged, 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, 9,500×*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. Environmental scanning electron microscopy (ESEM) was carried out on dried powders on a Philips XL30 ESEM-FEG, accelerating voltage 10 kV, using the backscattering detector to allow for visualisation of metallic nanoparticles on the biomass.

2.6 Benzyl Alcohol Oxidation

The oxidation of benzyl alcohol was initially performed in a 250 mL round bottom flask fitted with a high efficiency

condenser and heater/stirrer plate. The catalyst (150 mg) was dispersed in 30 mL benzyl alcohol. The reactor was pressurised to 1 bar with air (200 mL min^{-1}), and held at 90 °C with a thermostat. The reaction was initiated by stirring (250 rpm). Samples were withdrawn periodically through a sampling valve and analysed using GC (see below). Subsequent experiments were performed in a 50 mL Parr batch reactor loaded with 25 mL benzyl alcohol and 90 mg catalyst. The reactor was sealed and allowed to reach the desired temperature (90 °C) before pressurizing with O_2 (1 bar). The pressure was maintained at a constant value by continually feeding O_2 Samples were periodically removed using a sample valve, filtered (0.2 μm) and then analyzed using a Fisons GC8000/MD800 GC/MS versus commercial standards.

3 Results and Discussion

3.1 Characterisation of Au/Pd Bioinorganic Catalysts Using ESEM, TEM and EDX

Cells were palladized (bioPd) and subsequently used to reduce Au(III) from $HAuCl_4$ in the presence of H_2. Upon addition of bioPd, the colour of the H_2-saturated Au(III) solution rapidly changed from yellow to dark red, indicating the formation of colloidal Au(0). The characteristic pink-purple colour of colloidal gold solutions is due to excitation of surface plasmon vibrations in gold nanoparticles [21, 22] and is a strong indication of nano-Au. Figure 1 shows representative ESEM (backscattering detector) and TEM micrographs of *E. coli* cells following successive bioreduction of Pd(II) and Au(III). The total metal loading for electron microscopy was set at 10% (5% of each metal) by mass to facilitate the visualisation of the NPs (Fig. 1). Cells exhibited complete coverage of both the cell surface and the periplasmic space with some cells showing a small number of intracellular particles. Although some large clusters were observed (Fig. 1a), the majority of NPs exhibited remarkable monodispersity. EDX analysis of selected areas shows the presence of both Au and Pd on the biomass (Fig. 1a, inset). A study on the characterisation of the bimetallic catalyst using a combination of surface and bulk probing techniques showed an important degree of metal–metal coordination and indicated the formation of Au/Pd core/shell NPs where surface-exposed Pd atoms decorate a core of Au atoms [23]. Similar results have been reported in studies where the sacrificial hydrogen strategy was used to generate the Pd/Au NPs [24, 25]. In these published methods, the construction of core/shell structures is facilitated by the sequential reduction of Pd(II) followed by Au(III). However, using chemical reduction methods, it is often necessary to use strong reducing agents (e.g. sodium borohydride) and passivate the Pd seeds generated in the preliminary step to prevent their agglomeration in order to avoid non-uniform deposition of Au NPs. The bioreductive method described here does not require such compounds as the NPs are stabilised by the cellular envelope, possibly through coordination to cell wall molecules.

3.2 Benzyl Alcohol Oxidation over Mono- and Bimetallic Bioinorganic Catalysts

Preliminary experiments were carried out in air to investigate the effect of the addition of Au to Pd bioinorganic catalysts (bioPd). Results of benzyl alcohol oxidations using monometallic (Au, Pd, 5% metal on *E. coli* by weight) and bimetallic (Au/Pd, 2.5/2.5% loading) bionanocatalysts are shown in Fig. 2. For all catalysts, the overall conversion was low and generally inferior to values available from the

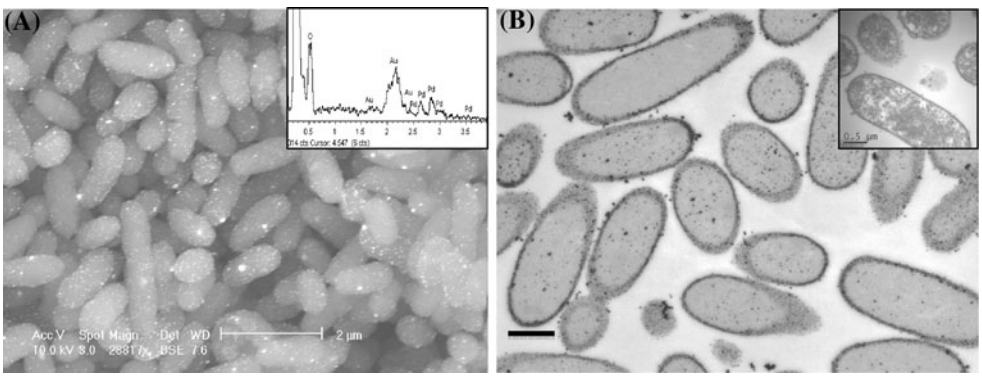

Fig. 1 Electron microscopy of metallized cells of *E. coli* MC4100. **a** ESEM of cells of *E. coli* MC4100 following the sequential reduction of Pd(II) and Au(III) (5/5% Pd/Au on biomass w/w). EDX analysis of a selected area of the preparation is shown in *inset*. **b** TEM of a similar preparation showing electron opaque metallic NPs immobilised on the cell surface with larger intra-cellular aggregates. Untreated cells are shown in *inset*, *scale bars* are 500 nm

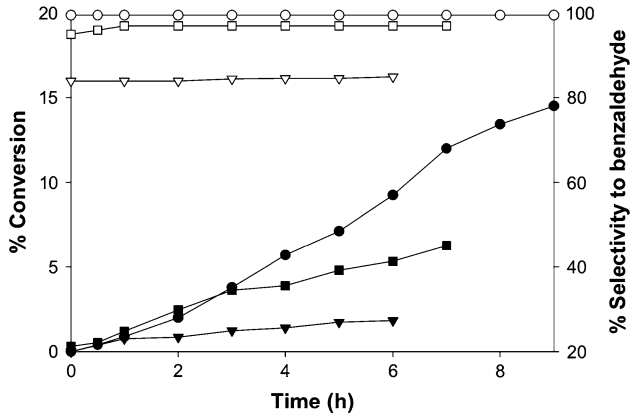

Fig. 2 Oxidation of benzyl alcohol in air over mono- (Au, Pd) and bimetallic (Au/Pd) biogenic catalysts. The mass of metal for each catalyst was set to 5% (w/w) and the Au:Pd ratio in the bimetallic catalyst was 1:1. Conversion (*closed symbols*) and selectivity to benzaldehyde (*open symbols*) over Au (*black inverted triangle*), Pd (*black square*) and Au/Pd (*black circle*) bionanocatalysts. For each catalyst, two independent batches were tested

literature for benzyl alcohol oxidations using supported metal catalysts (carbon or metal oxides supports) [2, 3]. This was especially true for bioAu which showed less than 5% substrate conversion after 8 h. However, using bioAu, selectivity to benzaldehyde was > 80% which compared well with 5% Au/TiO_2 catalysts [2]. The monometallic Pd(0) biocatalyst (bioPd) was more active and more selective than bioAu with respective values of 6.5% conversion and 98% selectivity after 7 h of reaction. Such high selectivity to benzaldehyde from Pd catalysts in this reaction was not observed with commercially available 5% Pd/C catalysts (see later). The lower activity of Au/C over Pd/C catalysts has been reported previously and it is suggested that Au NPs, when supported on carbon instead of an oxide are not able to extract the hydride from the alcoholic function [26]. Other studies on hydrogenations over bioPd have shown similarly high product selectivity [13, 14] and, although the reason(s) behind the increased selectivity of bioPd preparations are still unclear, low conversion yields suggest mass transfer limitations, i.e. some Pd nanoparticles may not be accessible to the reaction substrates. Intracellular (and to an extent periplasmic) Pd clusters, as well as those passivated via outer membrane fragments may display reduced "available" reactive surface. Results obtained for Pd/Au bionanocatalysts in this reaction show that the addition of Au to bioPd leads to a positive synergistic effect between both metals. For the same overall mass of metal (5% by mass), catalyst activity at 6 h was increased two- and three-fold when compared to bioAu and bioPd respectively. Moreover, selectivity to benzaldehyde was total and constant over 9 h, i.e. no post-conversion to other products such as toluene and benzyl benzoate was observed.

3.3 Influence of the Bacterial Strain and Metal:Metal Ratio

Previous studies on hydrogenations using bioPd showed that the choice of bacterial strain used to manufacture the metallic NPs had a strong influence on the activity of the catalyst [27]. In particular, the specific activity and cellular location of hydrogenase enzymes is thought to be a key parameter affecting the formation of Pd NPs. Two independent studies have shown that the deletion of specific isomers of this enzyme affected the catalytic activity of bioPd preparations [9, 16]. To investigate the effect of the cellular support on the formation of bimetallic NPs, two bacterial strains (*E. coli* and *D. desulfuricans*) were used to manufacture the bimetallic catalyst. A commercial monometallic Pd catalyst (5% Pd/C, Johnson Matthey, UK) was used for comparison. Results of benzyl alcohol oxidations in a Parr autoclave using O_2 are summarised in table 1. Both bioinorganic catalysts performed better than the commercial catalyst showing both increased substrate conversion and selectivity to benzaldehyde after 180 min of reaction. The 5% Pd/C catalyst showed a fast initial rate but the conversion remained constant at 15–180 min suggesting that the Pd/C catalyst suffered from deactivation. The formation of both toluene and benzoic acid was observed by GC analysis and this led to a decreased selectivity to benzaldehyde (~70%). In contrast, the biogenic catalysts retained activity and high (>95%) selectivity to benzaldehyde with benzyl-benzoate being the main side-product by GC analysis. Conversion did not exceed 40% but no attempt to optimise the reaction conditions was carried out. No significant support effect was observed; both *D. desulfuricans* and *E. coli* catalysts showed ~35% conversion and 95% selectivity to benzaldehyde after 180 min. A more detailed investigation of the effect of the bacterial support during the synthesis of mono and bimetallic NPs is ongoing in our laboratory and will be reported in full at a later date.

Table 1 Benzyl-alcohol oxidation in oxygen over various catalysts

Catalyst	Reaction time			
	15 min		180 min	
	Conv.	Sel.	Conv.	Sel.
2.5/2.5% Au/Pd *E. coli*	NT	NT	35%	96%
2.5/2.5% Au/Pd *D. desulfuricans*	8%	77%	38%	95%
1/4% Au/Pd *E. coli*	7%	86%	27%	92%
4/1% Au/Pd *E. coli*	6%	79%	12%	87%
5% Pd/C	25%	70%	25%	70%

Overall conversion and selectivity to benzaldehyde are expressed as percentages of starting material after 15 and 120 min of reaction

NT not tested

Next, we investigated the effect of varying the Au:Pd ratio on the activity of the bimetallic catalysts (Table 1). Doping the bioPd(0) catalysts with a small amount of Au (4% Pd/1% Au catalysts) was sufficient to observe the synergistic effect between Au and Pd. High selectivity to benzaldehyde (92%) was retained at conversion levels slightly lower than the 2.5/2.5% Au/Pd catalyst (= 30%). Bimetallic catalysts mainly composed of Au doped with a small amount of Pd (1% Pd/4% Au) showed a marked decrease in activity and, in this respect, confirmed the results of experiments carried out in air (Fig. 2) and are in agreement with previous studies [28].

4 Conclusions

In conclusion, we report for the first time the synthesis and catalytic activity of bimetallic Au/Pd NPs manufactured using bacterial cells. The biogenic catalysts outperform a commercial Pd catalyst showing excellent selectivity to benzaldehyde in selective oxidations of benzyl alcohol in the absence of solvent and without a base. We suggest that the development of biological routes of synthesis of metallic NPs for application in catalysis is a viable, economic and clean alternative to traditional chemical methods.

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