Section: Biofuels and Environmental Biotechnology

Biorefining of precious metals from wastes - an answer to manufacturing of cheap nanocatalysts for fuel cells and power generation via an integrated biorefinery?

Ping Yong, Iryna P. Mikheenko, Kevin Deplanche, Mark D. Redwood, Lynne E. Macaskie*

Unit of Functional Bionanomaterials, School of Biosciences, The University of Birmingham, Birmingham, B15 2TT, UK

Purpose of work

We have examined the feasibility of using palladium, being recovered microbiologically from industrial processing waste, for the manufacture of a bio-fuel cell for power production.

Abstract

Bio-manufacturing of nano-scale palladium was achieved via enzymatically-mediated deposition of Pd from solution using *Desulfovibrio desulfuricans*, *Escherichia coli* and *Cupriavidus metallidurans*. Dried 'Bio-Pd' materials were sintered, applied onto carbon papers and tested as anodes in a proton exchange membrane (PEM) fuel cell for power production. At a Pd(0) loading of 25% by mass the fuel cell power using Bio-Pd_{*D. desulfuricans*} (positive control) and Bio-Pd_{*E. coli*} (negative control) was ~140 and ~ 30 mW respectively. Bio-Pd_{*C. metallidurans*} was intermediate between these with a power output of ~ 60 mW. An engineered strain of *E. coli* (IC007) was previously reported to give a Bio-Pd that was >3-fold more active than Bio-Pd of the parent *E. coli* MC4100 (i.e. a power output of > 110 mW). Using this strain, a mixed metallic catalyst was manufactured from an industrial processing waste. This 'Bio-precious metal' ('Bio-PM') gave ~68% of the power output as commercial

Pd(0) and ~50% of that of Bio-Pd_{*D. desulfuricans*} when used as fuel cell anodic material. The results are discussed in relation to integrated bioprocessing for clean energy.

Key words Metal recovery, Fuel cell catalyst, Palladium, *Desulfovibrio desulfuricans*, *Escherichia coli*

Introduction

Proton-exchange membrane fuel cells (also described as polymer electrolyte fuel cells: PEM-FC; Larminie and Dicks 2000; Litster and McLean 2004), have been identified as a hydrogen-based future technology to power motor vehicles and generators, and for small stationary applications. The PEM-FC membrane electrode assembly (MEA) comprises precious metal-based electro-catalysts (anode and cathode) and a proton conducting membrane between them. H_2 is supplied to the anode, and O_2 (i.e. air) to the cathode (Litster and McLean 2004; Schmittinger and Vahidi, 2008). In the presence of precious metal (PM) catalyst (usually platinum), H_2 is oxidised at the anode; the released protons traverse the proton exchange membrane to the cathode and are reunited with the electrons and oxygen (from air) to form water as the only product. The electrons, via an electrical circuit, power a load; electric current is generated since the membrane is not electrically conductive (Larminie and Dicks 2000; Litster and McLean 2004).

Although the most efficient type of fuel cell, PEM-FCs require a clean source of H_2 (Larminie and Dicks, 2000). For commercial economic operation a fuel cell should operate for 5 years but the current operational lifetime may be as low as ~167 days (Aki et al. 2005). Commercial H_2 contains traces of CO, a potent catalyst poison. It is feasible to produce CO-free bio- H_2 by fermentation, and electricity was produced by a PEM-FC fuelled by a bio- H_2 stream from *E. coli* (Macaskie et al. 2005).

The performance of a PEM-FC is also sensitive to a range of operating parameters in the MEA including the active catalyst surface area and catalyst particle size, with metallic nanoparticles (NP) commonly used (Ivanchev 2008; Schmittinger and Vahidi 2008). However even with avoidance of catalyst poisoning and effective NP production, the cost and availability of precious metals (PM) will ultimately limit the application of PEM-FC into the hydrogen economy as sustainable energy production replaces fossil fuels.

Bio-derived PM nanoparticles are alternative fuel cell quality catalysts (Dimitriadis et al. 2007; Yong et al. 2007), the dual biosynthesis and nanoparticle-supporting functions overcoming the agglomeration problems that can beset conventional, non-biological methods of nanoparticle catalyst manufacture (see Creamer et al. 2007). Catalytically active Pd(0) nanoparticles were manufactured by *Desulfovibrio desulfuricans* and also by *E. coli* (Deplanche, 2008; Orozco et al 2010), prompting the possibility to utilise waste *E. coli* biomass from biohydrogen production as the precursor to a fuel cell catalyst (Orozco et al. 2010). Metallic NP synthesis is achieved by using hydrogenases (Mikheenko et al. 2008;

Deplanche et al. 2010), expressed maximally under fermentative conditions (H_2 synthetic direction), to split H_2 (catabolic direction) and reduce, as electron acceptors, Pd(II) and Pt(IV) ions to Pd(0) and Pt(0) NPs.

In addition to *D. desulfuricans* and *E. coli* (Mikheenko et al. 2008; Deplanche and Macaskie 2008; Deplanche et al. 2010), other organisms with high hydrogenase activity, like *Shewanella* (De Windt et al., 2005) and *Ralstonia* (Ludwig et al. 2009), can also reduce Pd(II) (Deplanche 2008). *Cupriavidus* (formerly *Ralstonia*) *metallidurans* can biomineralise gold by various mechanisms including its reduction to Au(0) (Reith et al. 2009). The first objective of this study was to compare the activity of a PEM-FC anode made with Pd(0) manufactured by *C. metallidurans* (Bio-Pd_{*C. metallidurans*}) with that of Bio-Pd_{*D. desulfuricans*} and Bio-Pd_{*E. coli*}.

Use of bio-manufacturing, unlike many physico-chemical manufacturing methods, is intrinsically scalable technology but is limited ultimately by the high cost of precious metals (PMs). Previous studies showed that PMs can be bio-recovered from wastes and secondary sources with the resulting mixed-metal deposits on the bacterial cells being catalytically active (Mabbett et al. 2006; Murray et al. 2007). Therefore the second objective was to evaluate the activity of a PEM-FC made using PMs recovered from an industrial processing waste by a strain of *E. coli* previously shown to have potential for both H_2 production and for use as a FC catalyst (Orozco et al. 2010).

Materials and Methods

Preparation of bacteria

E. coli IC007, a derivative of *E. coli* MC4100, provided by Prof F. Sargent (University of Dundee, UK) was as described previously (Orozco et al. 2010). *E. coli* was cultured anaerobically at 37 °C in nutrient broth no. 2 supplemented with 0.4% (w/v) sodium fumarate and 0.5% (v/v) glycerol. *Desulfovibrio desulfuricans* NCIMB 8307 was cultured anaerobically in Postgate C medium at 30°C (Yong et al. 2002; 2007). *Cupriavidus metallidurans* CH34 (NCIMB14178) was cultured anaerobically at 30 °C in tryptone soy broth (TSB). Cultures were grown in sealed 2 l Durham bottles with anaerobic conditions promoted by degassing under vacuum (30 min) and bubbling O_2 -free N_2 (OFN 30 min). Growing cultures ($OD_{600} = 0.5-0.7$) were harvested by centrifugation, washed three times

in 100 ml degassed MOPS/NaOH buffer (20 mM, pH 7.2), resuspended in the same buffer (1/20 of the original volume) and stored at 4°C under N₂ for up to 2 days before use.

Bio-deposition of Pd(0) and precious metals (PM)

The concentrated cells were resuspended anaerobically (room temperature) in dilute HNO₃ (pH 2, pre-sparged with OFN) containing 2 mM Na₂PdCl₄ or 1/500 dilution of an industrial reprocessing waste solution (to pH 2.3 and Pt: 2.8 mM; Pd: 1.9 mM and Rh: 0.14 mM; Degussa Ltd, Germany) to give ~25% Pd/cell dry weight (w/w), respectively. The suspension was left for 2 h to allow biosorption of metal (much of the hydrogenase activity was retained for this duration: I. Mikheenko, unpublished) then H₂ was bubbled through the solution for ~30 min. Complete Pd(II) reduction was confirmed as previously described (Yong et al. 2002) after the resulting black precipitate settled under gravity.

Examination of metal crystals by transmission electron microscopy (TEM) and X-ray powder diffraction (XRD)

Bacterial cells without or with Pd(0)-precipitates ('Bio-Pd') were washed twice in deionised water, fixed with 2.5% (w/v) glutaraldehyde (4°C, 1 h), sectioned, stained with uranyl acetate and lead citrate and examined under a TEM (Jeol JEM-1200 EX electron microscope accelerating voltage 80 kV). The identity of the deposited metals as Pd(0) was confirmed using X-ray powder diffraction as previously described (Yong et al. 2002).

Pd and PM catalyst preparation, electrode preparation and fuel cell testing

Bio-Pd or Bio-PM (made from the precious metal waste as above) samples were centrifuged, washed with water three times and then with acetone, dried at room temperature and placed into 10 ml alumina ceramic crucibles. The crucibles were put in a program- controlled furnace,

with the temperature raised from room temperature to 700 °C within 7 h gradually and held at 700 °C for a further hour. The sintered samples were cooled in the furnace to room temperature. Sintered Bio-Pd/PM samples (20 mg, as metal) were each mixed with 80 mg pure activated carbon powder (BDH Chemicals Ltd, England). Nafion perfluorinated ion-exchange resin (0.2 ml; 10 % (w/v) in water, Sigma-Aldrich) and 0.2 ml water were added into each sample. The sample was mixed well and applied evenly by hand on to 16 cm² Teflon treated carbon paper (Fuel Cell Scientific, USA), and dried at room temperature.

Power generation was tested using the laboratory made Bio-Pd and Bio-PM electrodes and parallel electrodes made using commercial Pd powder (Aldrich, UK) as anodes using a commercial fuel cell testing unit as described previously (Yong et al. 2007). Current (A) and voltage (V) were measured and recorded for variable resistance (R) as described by Voigt et al. (2005).

Results

Formation of bio-Pd by bacterial cells

Electron micrographs of the bacteria without added Pd(II) are shown in Fig 1 (A, C, E, G). After introduction of H₂ and soluble Pd(II), the metal was reduced to black deposits on the cells (Fig. 1B,D,F,H). The identity as Pd(0) was confirmed by X-ray powder diffraction. Pd(0) on cells of *D. desulfuricans* was visible as homogeneous small nano-particles (Fig. 1B) while that on the two *E. coli* strains was similar (fewer, larger particles). Cells of *C. metallidurans* appeared to show Pd(0) deposits (Fig. 1H) of size intermediate between those on the cells of *D. desulfuricans* (Fig. 1B) and *E. coli* (Fig.1 D, F).

Activity of FC anodes made with Bio-Pd

Preliminary tests using electrodes made with Bio-Pd using native dried palladised biomasses without sintering gave negligible electricity output, probably due to the poor conductivity of biological materials. However, after sintering Bio-Pd (and also Bio-Pt; P. Yong, unpublished work) catalysts performed better than commercial Pd(0) and comparably (Bio-Pt) to fuel cell

grade Pt black (Yong et al. 2007). Note that the electrode area was only 16 cm²; commercial PEMs are much larger and commercially are employed as ‘stacks’ for power output in the region of ~1kW (Wang et al. 2005).

Preliminary tests showed that Bio-anodes made using a 5% mass loading of Pd(0) (Orozco et al. 2010) gave approximately half the power output ($54 \pm 10\%$; pooled data for all the strains) as anodes made with 25% mass loading of Pd(0) and the latter was adopted for the fuel cell studies (Fig. 2). A previous study (Orozco et al. 2010) showed high activity of Bio-Pd_{*D. desulfuricans*} (comparable to commercial materials) and this was taken as the positive control in this study. Conversely, Bio-Pd_{*E. coli* MC4100} had only about ~ 10% of this activity (Orozco et al. 2010) and was taken as the negative control for the evaluation of Bio-Pd_{*C. metallidurans*}. The maximum power output of the latter was intermediate between the controls (~140 and ~29 mW respectively: Fig. 2) at ~61 mW (Fig 2C; summarised in Table 1).

For Bio-Pd_{*C. metallidurans*} the activity was comparable to that of bio-Pd_{*E. coli* MC4100} at 5% Pd-loading (not shown) and was also enhanced by ~4-fold at 25% Pd(0) loading whereas the bio-Pd_{*E. coli* MC4100} was only increased by 2-fold (18 mW at 5% loading (Orozco et al. 2010); 29 mW at 25% Table 1). It is apparent that use of *C. metallidurans* confers no advantage in terms of the catalytic activity of its Bio-Pd and this strain was not considered further.

A previous study using 5% mass loading of Pd(0) (Orozco et al. 2010) showed the mutant *E. coli* IC007 produced Bio-Pd giving a maximum power output of ~56 mW as compared to ~18 mW for the parent strain MC4100, i.e. an increase of ~ 3-fold for reasons which are still unclear. In comparison, this study (25% mass loading of Pd(0)) showed the respective maximum power outputs of Bio-Pd_{*E. coli* MC4100} and Bio-Pd_{*E. coli* IC007} to be 29 and 114 mW (Fig. 2 D,E), i.e. an increase of ~ 4-fold by use of the mutant strain. Hence, increasing the mass loading of Pd(0) from 5% to 25% enhances the activity of the mutant strain Bio-Pd more than that of the parent strain MC4100.

A notable feature of the low-activity Bio-Pd_{*E. coli* MC4100} and for Bio-Pd_{*C. metallidurans*} (Fig. 2C,D) the maximum power output was sustained over a wide voltage range whereas for Bio-Pd_{*D. desulfuricans*} and Bio-Pd_{*E. coli* IC007} (Fig 2B,E) the maximum power output was observed as a sharp peak at $V = \sim 0.4$ (Fig.2). Hence these two active electrodes gave a higher current than the ‘low active’ electrodes when the V was decreased. Apparently the mutation(s) imposed in strain IC007 (Fig. 2E) yield a pattern of Bio-Pd behaviour more consistent with that of *D. desulfuricans* (Fig. 2B) than that of *E. coli* MC4100 (Fig. 2C) and the profile and power output was almost identical to that of the commercial Pd(0) (Fig. 2A,E). A comparison of the electron micrographs of strains MC4100 and IC007 (Fig. 1D,F) revealed no apparent

morphological differences in the appearance of their respective Pd(0) deposits. A high resolution TEM study is clearly warranted, in conjunction with examination of possible pleiotrophic effects of the mutations imposed on strain IC007, as noted by Orozco et al. (2010).

Activity of electrode made using waste precious metals solution

Since *E. coli* strain IC007 gave a power output profile from its Bio-Pd similar to that of Bio-Pd_{*D. desulfuricans*} (Fig. 2) and was only compromised by ~20% in terms of the maximum power output as compared to the positive control ($P_{\max}$ of 114 and 140 mW respectively, Table 1) and since *E. coli* biomass residual from hydrogen production is desirable in terms of process efficiency (Orozco et al. 2010) strain IC007 was used for estimation of power output using metallised cells that had recovered Pd, Pt and Rh from a commercial processing waste. All three metals were removed from solution and the composition of the material was therefore assumed to be Pd (6.6%), Pt (17.9%) and Rh (0.5%) but this was not tested. The power output profile of the Bio-PM material in the FC is shown in Fig 2 E; significant power was generated, at ~68% of commercial finely-divided Pd(0) (Table 1). It is noteworthy that the sharp peak of power output at ~0.4V was absent and in this respect the Bio-PM_{*E.coli*IC007} (Fig. 2F) resembled the poorly performing Bio-Pd_{*E.coli*MC4100}. (Fig. 2D).

Discussion

In the PEM FC the power output relies on the catalyst activity but can also be affected by the electrode preparation method, e.g. by the formation of catalyst layer, gas diffusion layer and electrode design (Litster and McLean 2004). Such commercial methods are proprietary; hence, a positive and negative control for this study was fabricated using our standard method (see Materials and Methods) together with a commercial catalyst material for comparison. A fixed composition of metal powder, activated carbon, Nafion, and water was applied by hand in 16 cm² electrode preparations. In this way a previous study showed that bio-Pt_{*D. desulfuricans*} gave a power output identical to commercial fuel cell Pt catalyst and the outputs of both were ~15% less than the proprietary intact FC, indicating the extent to which our method differs from the proprietary method (Yong et al. 2007). However our method was reproducible; for all except

Bio-Pd_{*C. metallidurans*} standard deviations of within 2 mW were observed between three independent fuel cells with each strain (Fig. 2)

Consistent with tests in other, chemical, catalyses (Creamer et al. 2007; Deplanche, 2008; Deplanche et al. 2007;) Bio-Pd_{*D. desulfuricans*} gave good power generation (Yong et al., 2007). However the requirement for obligately anaerobic growth, the need to grow biomass specially, and the need to remove all traces of H₂S (a powerful catalyst poison) from the biomass product would make the use of *D. desulfuricans* unattractive in large scale manufacturing of fuel cell catalyst. Previous attempts to use Bio-Pd_{*E. coli*} were unsuccessful, with only a low power output reported (Orozco et al. 2010) despite that the chemical catalytic activity of bio-Pd_{*D. desulfuricans*} and Bio-Pd_{*E. coli*} was comparable (Deplanche 2008). A strain of *E. coli* (IC007) developed with the initial aim of increasing hydrogen production was unexpectedly found to make a Bio-Pd with enhanced FC anode activity (Orozco et al. 2010). As noted previously (Orozco et al. 2010) the activity using Bio-Pd_{*E. coli* IC007} was enhanced by several-fold (Fig. 2; Table 1). A previous study (Creamer et al. 2007) showed that the optimal Pd loading onto biomass for chemical catalysis was 5% by mass. However, regardless of the bacterial strain used, the activity of electrodes made from 5% of Pd/biomass (w/w) samples was ~ half than these made from 25% of Pd/biomass (w/w). This may be attributable to the proportional residual (cell ashes) from the samples with 5% Pd after sintering being much greater than these with 25% Pd, possibly giving a greater resistance. However this would not account for the different activities of the two *E. coli* strains Bio-Pd_{MC4100} and Bio-Pd_{IC007}, at the same Pd(0) loading by mass; an in-depth TEM study of the progression of the bio-materials during the sintering process is in progress.

In conclusion, bio-manufacture of fuel cell-grade precious metal catalysts is achieved using resting cells of *D. desulfuricans* or the *E. coli* mutant IC007. Electrodes from the latter gave up to 81% of maximum power generation compared to these from bio-Pd of *D. desulfuricans*, and ~ 115% of maximum power generation compared to those from a commercial Pd catalyst (maximum power: ~99 mW). A Bio-PM (precious metal) catalyst made directly from an industrial reprocessing solution by *E. coli* IC007 made into a FC anode gave ~ 68% of the power generation as pure Pd(0). This ground-breaking study paves the way to use FC catalysts made directly from precious metal waste solutions using waste biomass from biohydrogen production, with the consumption of the OAs downstream into further hydrogen (Redwood et al. 2008) ‘closing the loop’.

The concept of an ‘integrated biorefinery’ to convert agri-food wastes and precious metal wastes into power is shown in Fig. 3. Light-driven photofermentation and waste supply

may be intermittent whereas power demand may be constant; for this reason a hydrogen store is inserted before the PEM FC to hold the hydrogen and deliver it as required, decoupling the rates of hydrogen production and consumption. In this context, preliminary studies have shown the effective uptake of bio-hydrogen into a chemical store from a fermenter outflow at ~ 1 bar, and later discharge of H_2 for power production (M.D. Redwood and A. Bevan, unpublished. This will be reported in full in a later publication.

Further study is needed to optimise the power generation by Bio-PM directly from a greater variety of precious metal wastes. In this context, PMs recovered from acidic leachate from metallic scraps (Murray et al. 2007) illustrates the possibility to bypass commercial metal reprocessing. The integrated biorefinery shown in Fig. 3 is currently under construction.

Acknowledgments

This work was supported by the Royal Society (Industrial Fellowship and a Brian Mercer Senior Award for Innovation to LEM) and by BBSRC (Grant nos BB/C516195/2 and BB/E003788/1) and EPSRC (Grant nos EP/D05768X/1 and EP/E034888/1). The authors wish to thank Professor K. Kendall and Dr B.G. Pollet (Department of Chemical Engineering, University of Birmingham) for helpful discussions.

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Legends to Figures

Fig 1. Cells of *D. desulfuricans* (A, B), *E. coli* MC4100 (C, D), *E. coli* IC007. (E, F) and *C. metallidurans* (G, H) viewed under TEM. A, C, E, and G: Pd-unchallenged cells. B, D, F and H: Pd(II) (25% of Pd to Biomass, w/w) was reduced to Pd(0) after reduction under H₂. Bars show 500 nm.

Fig.2. The output current and power by fuel cells tested with anodes made (as described in Materials and Methods) from commercial finely divided Pd (A) and Bio-Pd from 25% Pd/dry cells (w/w) of *D. desulfuricans* (B), *C. metallidurans* (C), *E. coli* MC4100 (D), *E. coli* IC007 (E) and 25% PM/dry cells of *E-coli* IC007 (F). PM: an industrial PM reprocessing (Degussa Ltd, Germany) solution diluted 1/500 for use and containing a mixture of Pt (2.8 mM), Pd (1.9 mM) and Rh (0.14 mM). ■: Power (Watts); ♦ Current (Amps). Data are from three experiments; standard deviations generally (< 2 mW) where not shown were within the dimensions of the symbols

Fig. 3. The concept of the ‘integrated biorefinery’. Production of biohydrogen from food wastes has been shown by many authors previously. An integrated bioprocess for biohydrogen production (dark upstream fermentation and downstream photofermentation) was described by Redwood et al (2009) in comparison with other biohydrogen-producing systems. Electrodialytic (ED) separation of organic acids is employed to generate biohydrogen streams from the two bioreactors. These streams are pooled with the H₂ generated via electrolysis of water within the ED cell, into a hydrogen store. The waste biomasses from fermentation can be used (Orozco et al. 2010) to recover catalytically-active precious metals from waste streams (i.e. this study) and also leachates made from PM scrap (Creamer et al. 2006; Mabbett et al. 2006; Murray et al. 2007). The PEM fuel cell is made using a Bio-Pd anode (this study) and biohydrogen is fed (Macaskie et al. 2005) to generate 68 mW of power per 16 cm² anode (Table 1). This compares with 170 mW obtained with commercial Pt fuel cell catalyst and also Bio-Pt_{*D. desulfuricans*} (Yong et al. 2007) and suggests that an improvement of 2.5-fold is needed for the Bio-Pd_{IC007} to become competitive with a commercial PEMFC. Note however that optimisation studies have not yet been done and also that bio-H₂ does not contain CO, which reduces FC operational life using commercial hydrogen.

Fig. 1

Fig. 2

Fig. 3

Table 1. The maximum output power generation by electrodes made from Bio-Pds and Bio-Precious Metals made from waste

Bio-Pd	Maximum power output (mW)
Bio-Pd _{<i>D. desulfuricans</i>}	141.4
Bio-Pd _{<i>E. coli</i> MC4100}	29.3
Bio-Pd _{<i>C. metallidurans</i>}	61.6
Bio-Pd _{<i>E. coli</i> IC007}	115.2
Bio-PM _{<i>E. coli</i> IC007}	67.7

Pd(0) or precious metals were loaded onto biomass at 25% by weight (see text). Maximum power output of commercial -Pd: ~99 mW. Sample size: $n > 3$, standard deviations < 2 mW.

Data are shown as $P_{\max}$ (mW).