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Use of *Desulfovibrio* and *Escherichia coli* Pd-nanocatalysts in reduction of Cr(VI) and hydrogenolytic dehalogenation of polychlorinated biphenyls and used transformer oil

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Abstract

BACKGROUND *Desulfovibrio* spp. biofabricate metallic nanoparticles (e.g. 'Bio-Pd') which catalyse the reduction of Cr(VI) to Cr(III) and dehalogenate polychlorinated biphenyls (PCBs). *Desulfovibrio* spp. are anaerobic and produce H_2S, a potent catalyst poison, whereas *Escherichia coli* can be pre-grown aerobically to high density, has well defined molecular tools, and also makes catalytically-active 'Bio-Pd'. The first aim was to compare 'Bio-Pd' catalysts made by *Desulfovibrio* spp. and *E. coli* using suspended and immobilised catalysts. The second aim was to evaluate the potential for Bio-Pd-mediated dehalogenation of PCBs in used transformer oils, which preclude recovery and re-use.

RESULTS Catalysis via Bio-Pd$_{D.\ desulfuricans}$ and Bio-Pd$_{E.\ coli}$ was compared at a mass loading of Pd:biomass of 1:3 via reduction of Cr(VI) in aqueous solution (immobilised catalyst) and hydrogenolytic release of Cl^- from PCBs and used transformer oil (catalyst suspensions). In both cases Bio-Pd$_{D.\ desulfuricans}$ outperformed Bio-Pd $_{E.\ coli}$ by ~3.5-fold, attributable to a ~3.5-fold difference in their Pd-nanoparticle surface areas determined by magnetic measurements (Bio-Pd$_{D.\ desulfuricans}$) and by chemisorption analysis (Bio-Pd$_{E.\ coli}$). Small Pd particles were confirmed on *D. desulfuricans* and fewer, larger ones on *E. coli* via electron microscopy. Bio-Pd$_{D.\ desulfuricans}$-mediated chloride release from used transformer oil (5.6 ± 0.8 μg mL^{-1}) was comparable to that observed using several PCB reference materials.

CONCLUSIONS At a loading of 1:3 Pd: biomass Bio-Pd$_{D.\ desulfuricans}$ is 3.5-fold more active than Bio-Pd$_{E.\ coli}$, attributable to the relative catalyst surface areas reflected in the smaller nanoparticle sizes of the former. This study also shows the potential of Bio-Pd$_{D.\ desulfuricans}$ to remediate used transformer oil.

Keywords: chromium (VI), polychlorinated biphenyl, sulphate-reducing bacteria, *Desulfovibrio Escherichia coli*, palladium catalyst

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INTRODUCTION

Problematic environmental industrial contaminants, e.g. heavy metals and chlorinated organic compounds, can occur in the same ecosystem.[1,2] Conventional methods for removing Cr(VI) from water (e.g.[3]) are relatively expensive and/or require addition of chemicals. Cr(VI)-waters could be treated by bioreduction to Cr(III);[4] various microorganisms are capable of this reduction e.g.[5-10] including sulphate-reducing bacteria (SRB).[11,12] The latter can also reduce various other metals[13] including precious metals (see[14,15]) at the expense of formate or H_2.

Chlorinated aromatic molecules are environmentally-important contaminants, e.g. pentachlorophenol is found in wood preservatives (often with Cr), while polychlorinated biphenyls (PCBs) have historical uses, e.g. as insulators and plasticisers.[16] PCBs also occur in used transformer oils, complicating oil disposal or recovery. Environmental biodegradation of PCBs is slow[17] and their long-term persistence is problematic.[18] The recent use of polybrominated diphenyl ethers (PBDEs) as flame retardants is causing their environmental accumulation and these may be similarly persistent.[19] A tenfold accumulation of a more recent alternative, tris(chloroisopropyl) phosphate (TCPP), was found in groundwater over a 10 year period.[20] The ability of zero-valent iron to promote reductive dehalogenation of PCBs was potentiated by incorporation of Pd(0),[21,22] which prompted this investigation.

Some SRB can reductively dehalogenate chlorinated aromatic compounds by ill-defined metabolic mechanisms.[17,23] Microbial approaches to the co-treatment of chlorophenols and Cr(VI) have been described[8] but biological systems require physiologically compatible conditions. A robust alternative, SRB cells coated with Pd particles (Bio-Pd), reduced Cr(VI)[24,25] and also reductively dehalogenated chlorinated aromatic compounds,[26] PBDEs[19] and TCPP.[20] The latter (hydrogenolysis reactions) accords with the known activity of Pd(0) as a hydrogenolytic catalyst.[27]

Previous studies on the use of Bio-Pd for catalysis utilised SRB but other bacteria able to couple hydrogen or formate oxidation to Pd(II) reduction can make catalytically active Bio-Pd and, indeed, other precious metal bionanocatalysts (for review see[15]), a new area of biotechnology which is burgeoning since the initial reports.[24,26]

E. coli provides a potentially useful alternative to SRB since this organism provides ready molecular tools for strain improvement,[28] it does not produce H_2S (a powerful catalyst poison) and cells can be grown aerobically to high cell density and then placed anoxically for upregulation of hydrogenase activity for metal reduction.

The aim of this study was to compare the catalytic efficacy of Bio-$Pd_{D.\ desulfuricans}$ and Bio-$Pd_{D.\ vulgaris}$ with the corresponding Bio-Pd made on *E. coli* using two environmentally-relevant, independent criteria: reduction of Cr(VI) to Cr(III) in a flow-through system using gel-immobilised palladised cells and liberation of chloride ion from polychlorinated biphenyls in a batch system. With respect to potential applications in resource efficiency in a climate of increasing oil prices and dwindling supplies, used transformer oils could be reprocessed but the presence of high levels of PCBs currently limits their re-use. The second aim of the study was to show the potential for Bio-Pd-mediated decontamination of waste transformer oil.

MATERIALS AND METHODS

Organisms and growth conditions

Desulfovibrio vulgaris NCIMB 8303 and *D. desulfuricans* NCIMB 8307 were grown anaerobically as described previously.[12,26] Cultures were harvested after 24 h (OD_{600} 0.7 ± 0.2). *E. coli* MC4100 was grown as described previously (24 h; 30 °C;[29]) in 1 L closed anoxic bottles (OD_{600} at harvest = 0.8). Cells were washed in isotonic saline (8.5 g L^{-1} of NaCl) and stored under oxygen-free nitrogen (OFN; 4 °C) as concentrates in 20 mM MOPS/ NaOH buffer, pH 7, until use (within 24 h). The biomass concentration was estimated by converting the OD_{600} to dry cell weight using a previously determined calibration.

Preparation of Bio-Pd on bacteria

The required volume of 2 mM Na_2PdCl_4 (98 %, Aldrich Chemicals, UK) solution (to pH 2 with HNO_3), was placed in a 200 mL serum bottle sealed with a butyl-rubber stopper. Cells were added (final mass ratio of dry cells:Pd was 3:1), left at 30 °C for 1 h for Pd(II) biosorption and then sparged with H_2 (15 min) to deposit Pd(0) on the cell surface (25% Pd by mass). Removal of Pd(II) from solution was monitored by loss of the yellow colour spectrophometrically, cross-validated using the $SnCl_2$ method, and polarographically.[30] Pd-coated cells (Bio-Pd) were recovered by centrifugation (3800 g, 10 min); no residual Pd(II) remained in the supernatant by assay. The pellet was washed once with distilled H_2O and three times with acetone,

dried (60 °C) to constant weight and ground to a fine powder to make the final Bio-Pd catalyst.

Transmission electron microscopy

Pd-loaded bacteria were rinsed twice with distilled water, fixed in 2.5 % (w/v) aqueous glutaraldehyde, washed once in distilled water then 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 step) washed twice in propylene oxide (15 min), embedded in epoxy resin (degassed under vacuum for 30 min) and left to polymerize (24 h; 60 ˚C). Sections (~100 nm) were cut, placed onto a copper grid and viewed with a JEOL 120CX2 transmission electron microscope (TEM); accelerating voltage 80 kV.

Determination of catalyst surface area

mg mL^{-1} agar (mass of Pd was 25% by weight)

Catalytic testing of the Bio-Pd

For testing suspensions of Bio-Pd against Cr(VI) reaction mixtures were set up in 12 mL serum bottles. Bio-Pd (5 mg) or Bio-Pd beads (2 mL, 2.5 mg Bio-Pd^0 mL agar gel^{-1}) were suspended in 5 mL solution comprising 20 mM MOPS/NaOH buffer and 25 mM each of sodium formate (electron donor), sodium acetate, and sodium citrate (to chelate the Cr(III) product), with 500 μM sodium chromate, pH 7. For anaerobic tests, reaction mixtures were degassed with oxygen free nitrogen (OFN) for 10 min before addition of chromate. Samples were withdrawn periodically (under OFN for anaerobic tests) via a syringe, and supernatants were analysed for residual Cr(VI) using diphenylcarbazide.[12,19,31]

For continuous Cr(VI) reduction tests, Bio-Pd agar beads (4 mL) were placed in a glass column (i.d. 12 mm, length 10 cm). Solution (500 μM sodium chromate and 25 mM each of sodium formate, citrate and acetate in 20 mM MOPS/NaOH buffer, pH 7) was passed upwards (flow rate: 13.2 mL h^{-1}). The outflow was analysed for residual Cr(VI) using diphenylcarbazide as above. Controls

Release of chloride from PCBs in waste transformer oil

Waste transformer oil was provided by C-Tech Innovation, Capenhurst, UK. It contained ~ 2.5 mg mL^{-1} of PCBs, (mixture of arochlors 60, 42 and 54: analysis by H_2b Ltd., Capenhurst. UK). The oil was extracted into tetrahydrofuran at C-Tech Innovation to give a solution comprising 978 ppm PCBs (analysis H_2b, Capenhurst, UK). The final PCB concentration in the test (set up as for pentachlorobiphenyl) was 9.78 ppm; the arochlor extraction efficiency (not tested) was assumed to be the same for all arochlors.

CO chemisorption was used to estimate the active particle size of Bio-$Pd_{E.coli}$ using a Micromeritics Autochem II Chemisorption Analyser equipped with a thermal conductivity detector (TCD). Samples (0.5 g) were slowly heated (10 °C min^{-1}) to 100 °C under an H_2 atmosphere (50 mL min^{-1}) and allowed to cool and stabilise at 40 °C. Pulses of CO (loop volume = 0.5389 mL) were applied to the sample until consistent TCD peak sizes were obtained, indicating that metallic particles had reached CO saturation. The determined active Pd(0) surface area (m^2 g^{-1} Pd metal) was used to calculate the average nanoparticle size using 'Autochem II 2920V2.00' software supplied with the Chemisorption Analyser.

Immobilisation of Bio-Pd preparations

Immobilisation of Bio-Pd in agar beads was as described previously.[31] The catalyst loading was 7.5 mg Bio-Pd(0) cm^{-3} agar; Pd content was 1.87 contained agar beads only. Previous studies had shown that live or killed cells removed negligible Cr(VI) in the absence of Pd(0) under these conditions.[24]

For testing reductive dehalogenation Bio-Pd (2 mg) or Pd prepared by chemical reduction under H_2 (2 mg) was placed in 12 mL serum bottles in 20 mM MOPS-NaOH buffer pH 7.0 and chlorinated aromatic compound (Table 1) was added in 1 ml of hexane carrier and shaken well (the nominal concentration was as shown in Table 1; the actual concentration chlorinated aromatic compound in the aqueous phase was not determined). Bottles were outgassed with OFN (15 min) and tests were initiated via the addition of sodium formate (aq.) (to 10 mM, pH 7.3; final volume was 10 mL) and re-shaken. Chloride release was estimated in withdrawn samples by spectrophotometric assay of Cl^- ion in the aqueous supernatant fraction using the colorimetric mercury (II) thiocyanate method[32] with NaCl as the standard. The range of sensitivity was 0.5-100 μg mL^{-1} Cl^-. For these tests the washing procedures in the preparation of the Bio-Pd used high purity distilled water to ensure a low background level of Cl^-, and Analar grade reagents.

Table 1 Chlorinated aromatic compounds used in this study

Short title	Systematic title	Nominal concentration
2-chlorophenol		5 mM
pentachlorophenol		5 mM
PCB# 28	2,4,4'- trichlorobiphenyl	0.31 mM
PCB# 52	2,2',5,5'-tetrachloropbiphenyl	0.27 mM
PCB# 101	2,2',4,5,5'-pentachlorobiphenyl	0.12 mM
PCB# 118	2,3',4,4',5'-pentachlorobiphenyl	0.12 mM
PCB# 138	2,2',3,4,4',5',-hexachloropbiphenyl	0.11 mM
PCB# 153	2,2',4,4',5,5' –hexachlorobiphenyl	0.11 mM
PCB# 180	2,2',3,4,4',5,5'- heptachlorobiphenyl	0.05 mM

RESULTS AND DISCUSSION

Deposition of Pd by *Desulfovibrio* spp. and *E. coli*

Examples of Pd-loaded cells of the *Desulfovibrio* and *E. coli* are shown in Fig. 1 a-c. Following nucleation of Pd(0) in the biosorption step the growing Pd clusters erupt through the outer membrane where they form outgrowing nanoparticles. Fig. 1 shows the locations of nano-Pd(0) deposits; their identity as Pd was confirmed using energy dispersive X-ray microanalysis and X-ray powder diffraction analyses as described by Deplanche et al.[33] with identical results for all preparations (not shown). The total loading of Pd(0) onto the cells was the same throughout, hence the pattern and size of Pd particles would reflect the distribution and cellular content of the hydrogenases. Given that *Desulfovibrio* has significantly more levels of hydrogenase than *E. coli*[34] and, hence more potential Pd-deposition sites, the same amount of Pd distributed around many sites should result in a greater number of smaller deposits, given that Pd removal proceeds to completion. Fig. 1 a,b confirms that Pd(0) deposition is widespread in *D. desulfuricans* and *D. vulgaris*, with small crystals deposited uniformly and no difference between the two *Desulfovibrio* strains but it is less widespread on *E. coli* cells (Fig. 1c), which show fewer, larger crystals at the same loading (Pd:biomass 1:3 by mass). Fig. 1 shows that (apart from the different hydrogenase activities of the two genera: above) the preparations also differed in that nearly all cells of *Desulfovibrio* participated in Pd(0) deposition (Fig. 1a,b) but only some *E. coli* cells did so (Fig 1c). The reason for this was not examined (e.g. this was possibly attributable to metal toxicity to the cells and/or hydrogenases) but for a given Pd(0) loading fewer participating cells would also result in fewer, larger crystals as seen in Fig 1c.

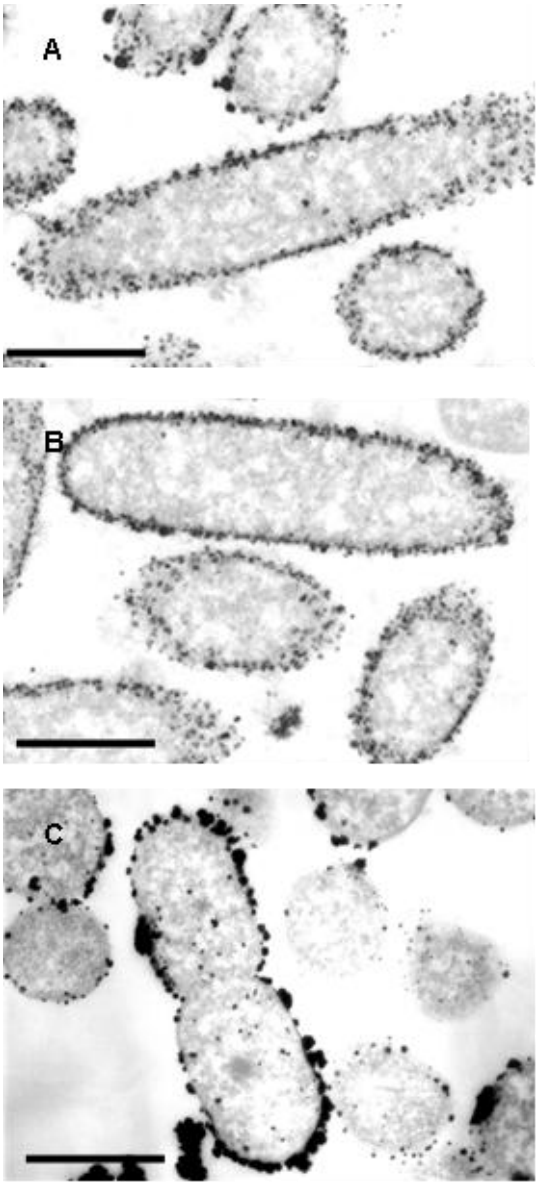

Fig. 1. Deposits of Pd(0) on the surface of *D. desulfuricans* (a), *D. vulgaris* (b) *E. coli* strain MC4100 (c). Cells were exposed to Pd(II) as described in Materials and Methods. The biomass: Pd ratio was 3:1 (see text). Bars are 1 μm.

Table 2. Effect of Bio-Pd bacterial source on Cr(VI) reduction by Bio-Pd in batch suspensions of Bio-Pd.

Bacterial strain	Initial rate[a] of Cr(VI) reduction (nmol Cr(VI) reduced h^{-1} mg Bio-Pd^{-1})	
	Reduction in air	Reduction under OFN[b]
Bio-Pd(0) (*D. vulgaris*)	152 ± 7	175 ± 32
Bio-Pd(0) (*D. desulfuricans*)	166 ± 22	168 ± 15
Bio-Pd(0) (*E. coli* MC4100)	91 ± 15	115 ± 16

Bio-Pd samples (prepared as described in Materials and Methods and shown in Fig. 1) were challenged with 500 μM Cr(VI), 25 mM each of formate, acetate, and citrate, 20 mM MOPS/ NaOH, pH 7. Data are means ± SEM from 3 experiments.

[a]Initial rates were taken over the first 1.5 h as gradients from linear regions of the appropriate graphs.

[b]OFN = Oxygen Free Nitrogen

Catalytic activity of Bio-Pd made on surfaces of *Desulfovibrio* spp. and *E. coli*

To establish whether anaerobic conditions were required for catalytic activity of Bio-Pd once formed under H_2, preliminary Cr(VI) reduction tests were carried out in air with batch suspensions of Bio-Pd. This established that exposure to O_2 did not affect the catalytic activity (at P= 0.95; Table 2).

When Bio-Pd was immobilised in agar ~ 20% of the Cr(VI) was removed onto the Bio-Pd in suspensions using gel beads, attributable to absorption by the immobilisation matrix (no biological activity was present since the catalyst was dried and ground); little Cr(VI) was removed by Bio-Pd alone.

Further tests used flow-through reactors fixed at the flow rate giving 50% reduction of Cr(VI) by the best strain (Bio-$Pd_{D.\ desulfuricans}$), with the activities of the other Bio-Pds expressed relative to this (Fig. 2). Columns containing Bio-$Pd_{D.\ desulfuricans}$ and Bio-$Pd_{D.\ vulgaris}$ reduced Cr(VI) comparably (~50 % Cr(VI) reduction at 24 h, declining to 20% after 96h). Columns containing Bio-$Pd_{E.\ coli\ MC4100}$ reduced Cr(VI) poorly (10-15 % removal of Cr(VI) after 24 h). At 24 h (Fig. 2) the difference between Cr(VI) reduction by Bio-$Pd_{Desulfovibrio\ spp.}$ and Bio-$Pd_{E.\ coli}$ was ~3.5-fold.

Reductive dehalogenation tests against pentachlorobiphenyl, PCBs and transformer oil extract used Bio-$Pd_{D.\ desulfuricans}$ since it was shown previously that this was more effective in reductive dehalogenation of PCBs than Bio-$Pd_{D.\ vulgaris.}$[26] The control was Pd(0) reduced chemically from solution under H_2 (Chem-Pd) which gave negligible chloride release from all compounds except 2-chlorophenol and 2,4,4' trichlorobiphenyl (Table 3). Pd-free bacteria gave no Cl^- release, confirming the stability of the dried cells with respect to chloride release in the hexane suspension. Table 3 shows that the relative catalytic efficacy of the two Bio-Pds was dependent on the substrate used, e.g. for pentachlorophenol they gave a similar activity, whereas Bio-$Pd_{E.\ coli}$ was ineffective against PCB# 52, PCB# 118, PCB# 138, PCB# 153 and PCB#180, with low activity seen against PCB# 28 and with no apparent relation to the degree or positioning of the chloride substituents (c.f. Table 1). For the compounds supporting Cl^- release by Bio-$Pd_{E.\ coli}$ the median value for superiority of the Bio-$Pd_{D.\ desulfuricans}$ was 3.5-fold (Table 3). The median takes into account the large range of comparative values. The transformer oil, being an average of several arochlors, is more representative of real samples and, similarly, Bio-$Pd_{D.\ desulfuricans}$ was 3.5-fold higher than Bio-$Pd_{E.\ coli}$ against the PCB mixture present in the used oil (Table 3).

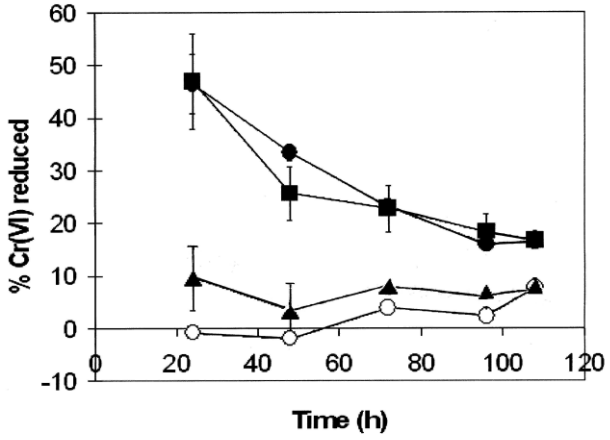

Fig. 2. Continuous Cr(VI) reduction by Bio-Pd immobilized in agar (loading 7.5 mg cm^{-3}). Columns were challenged at a flow rate of 13.2 mL h^{-1} with 500 μmol dm^{-3} sodium chromate in the presence of electron donor (see Materials and Methods). Bio-Pds were: ●, *D. vulgaris*; ■, *D. desulfuricans*; ▲, *E. coli* MC4100; ○ agar beads (no Bio-Pd). Error is ± SEM from 3 experiments.Where no errors are shown these were within the dimensions of the symbols.

Table 3 Release of chloride from chlorinated aromatic compounds by Bio-Pd$_{D.\ desulfuricans}$ and Bio-Pd$_{E.\ coli}$

Compound	Chloride released (μg mL^{-1})*			
	Chem-Pd(0)	Bio-Pd$_{D.\ desulfuricans}$	Bio-Pd$_{E.\ coli}$	
2-chlorophenol	5.9 ± 2.4.	144.4 ± 11.2	47.2 ± 6.4	3.05
pentachlorophenol	0.2 ± 1.5	18.8 ± 6.4	13.6 ± 6.4	1.38
PCB# 28	2.8 ± 0.5	52.8 ± 10.8	2.8 ± 0.4	18.8
PCB# 52	0.2 ± 0.0	4.0 ± 0.4	NS	
PCB#101	0.02 ± 0	15.6 ± 4.0	4.4 ± 0.4	3.54
PCB#118	NS	17.2 ± 5.2	NS	
PCB# 138	NS	4.8 ± 0.4	NS	
PCB# 153	0.3 ± 0	10.4 ± 2.4	NS	
PCB# 180	0.2 ± 0	9.6 ± 3.2	NS	
Transformer oil extract	0.01 ± 0	5.6 ± 0.8	1.6 ± 0	3.5

Palladised cells were challenged with chlorinated aromatic compounds in hexane-in-water suspensions, with palladium loaded at 1:3 ratio to biomass. Data are means ± SEM from 3 experiments using different Bio-Pd preparations. * Released chloride was determined after 1 h for 2-chlorophenol and pentachlorophenol and 24 h for the PCBs. NS: No significant chloride detected. Note that for the 2-chlorophenol, 2,2',4,5,5' pentachlorobiphenyl and the transformer oil extract the Bio-Pd$_{D.\ desulfuricans}$ was 3-3.5 fold more effective than the Bio-Pd$_{E.\ coli}$

It is shown that Bio-Pd$_{D\ desulfuricans}$ promoted a 3-3.5-fold increase of Cl^- released as compared to Bio-Pd$_{E.\ coli}$ for 2-chlorophenol, pentachlorobiphenyl and transformer oil extract (Table 3), i.e. the difference between them was similar to that seen in the Cr(VI) reduction test using immobilised cells (above). Taking the immobilised cell Cr(VI) tests and the PCB Cl^- release tests together, it is clear that Bio-Pd (at 1:3 ratio of Pd:biomass; 25% loading) made on *Desulfovibrio* gives a ~3.5-fold better Pd-catalyst than that made on *E. coli*. The electron microscopy data (Fig 1) suggested that, due to the smaller particle sizes, the surface area of the Bio-Pd$_{D.\ desulfuricans}$ would be greater than that of Bio-Pd$_{E.\ coli}$.

Comparison of catalytic nanoparticles

The nanoparticle size of Bio-Pd$_{D.\ desulfuricans}$ was calculated previously from magnetic measurements as 17.6 nm and 14.7 nm for two preparations.[35] A mean value of 16.15 nm was assumed. The Bio-Pd$_{E.\ coli}$ was not sufficiently active magnetically to facilitate a similar analysis. Instead the chemisorption method was used, which gave an active particle diameter calculated for Bio-Pd$_{E.\ coli}$ as 30.04 nm (metallic surface area was 4.15 m^2/g Pd). Assuming that the surface area of a sphere is $4\Pi r^2$ the corresponding particle surface areas were calculated as 2835.3 and 819.5 nm^2 respectively, i.e. the estimated surface area was 3.5-fold greater for the Bio-Pd(0)$_{D.desulfuricans}$ which is in agreement with the difference in catalytic activities (above). It can therefore be deduced that the catalytic activity is related to the surface area but this is not the only factor involved; other studies have shown that by removal of specific hydrogenases the catalytic activity of Bio-Pd can be manipulated in both *E. coli*[33] and *Desulfovibrio fructosovorans*;[36] in the latter case the most highly active Bio-Pd catalyst was located on the inner membrane with few if any discrete nanoparticles visible.[37]

Future prospects: potential applications of Bio-Pd(0) to industrial wastes and decontamination

The use of a Pd-based catalyst for treatment of environmental contaminants (e.g. by doping of Fe-catalyst as suggested by Korte et al.[21,22] would appear economically unattractive. However, a 'dirty' catalyst can be used, bypassing the costs of precious metal refining and using biorecycled scrap for this purpose. Bacteria biorecovered precious metals from processing wastes,[38] spent automotive catalyst leachates[20,39] and electronic scrap[40] and biorecovered material was catalytically active.[15,41] Taking into account the costs of landfilling wastes and the high costs associated with hazardous waste disposal, the economic feasibility of this approach becomes apparent. The use of spent biomass from

other processes as nanocatalyst support (e.g. [28,42]) would also mitigate against the costs of biomass waste disposal and hence the overall cost. Although the release of chloride from used transformer oil was limited in this study further investigations would be justified as a potential clean route to oil recovery for re-use, given the rapidly rising price of crude oil.

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