Biomass-supported palladium catalysts on Desulfovibrio desulfuricans and Rhodobacter sphaeroides.

Redwood, Mark; Deplanche, Kevin; Baxter-Plant, VS; Macaskie, Lynne

DOI:
10.1002/bit.21689

Biomass-supported palladium catalysts on *Desulfovibrio desulfuricans* and *Rhodobacter sphaeroides*

Mark D. Redwood, Kevin Deplanche, Victoria S. Baxter-Plant[1], Lynne E. Macaskie[2]

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

[1]Deceased

[2]*Correspondence to* L.E. Macaskie

School of Biosciences, The University of Birmingham. Edgbaston Birmingham,

B15 2TT, UK

Tel (44)-121-414-5889

Fax (44)-121-414-5925

e-mail L.E.Macaskie@bham.ac.uk

Keywords: catalysis; palladium; chlorophenols; polychlorinated biphenyl; PCB; reductive dehalogenation; *Desulfovibrio desulfuricans*; *Rhodobacter sphaeroides*

ABSTRACT

A *Rhodobacter sphaeroides*-supported palladium catalyst ("*Rs*-Pd(0)") was compared with a *Desulfovibrio desulfuricans*-supported catalyst ("*Dd*-Pd(0)") and with unsupported palladium metal particles made by reduction under H_2 ("Chem-Pd(0)"). Cell surface-located clusters of Pd(0) nanoparticles were detected on both *D. desulfuricans* and *R. sphaeroides* but the size and location of deposits differed among comparably loaded preparations. These differences may underlie the observation of different activities of *Dd*-Pd(0) and *Rs*-Pd(0) when compared with respect to their ability to promote hydrogen release from hypophosphite and to catalyse the reductive dehalogenation of chlorinated aromatic compounds. *Dd*-Pd(0) was more effective in the reductive dehalogenation of polychlorinated biphenyls (PCBs), whereas *Rs*-Pd(0) was more effective in the initial dehalogenation of pentachlorophenol (PCP) although the rate of chloride release from PCP was comparable with both preparations after 2 h. The extent of dechlorination of PCBs was related to their aqueous solubility in the case of *Rs*-Pd(0) and to the localisation of the chlorine substitutions at the *para* positions on the aromatic rings in the case of *Dd*-Pd(0).

INTRODUCTION

The application of *Rhodobacter sphaeroides* in the recovery of palladium from solution as supported metallic nanoparticles, and in catalysis using the resulting palladized biomass was investigated in comparison with Pd(0) biomanufactured by *Desulfovibrio desulfuricans*. The use of sulfate-reducing bacteria (SRB) for palladium catalyst production is limited by the need for washing to remove H_2S (the product of dissimilatory sulfate metabolism), which is a potent catalyst poison. *R. sphaeroides* and related purple non-sulfur (PNS) bacteria can exhibit intrinsically high resistance to various metallic species, for example chromate, tellurite, selenite and rhodium sesquioxide (Borsetti et al., 2003; Kessi et al., 1999; Moore and Kaplan, 1992; Nepple et al., 2000; Van Praag et al., 2002; Yamada et al., 1997).

Many bacteria can reduce metallic ions to lower valence species and, sometimes, to the metallic state by dissimilatory metal reduction, *via* which simple organic substrates are oxidised and metals can act as the primary or sole terminal electron acceptor (Lloyd, 2003; Lloyd et al., 2003; 2005). In the case of SRB, the ability to grow *via* dissimilatory metal reduction is described in only one strain to date (Tebo and Obraztsova, 1998) although many examples of metal reduction are documented (Lovley, 1993; Lloyd, 2003; Lloyd et al., 2003; 2005). In PNS bacteria metal reduction is broadly considered to be a mechanism of detoxification to permit growth in the presence of metallic ions (Borsetti et al., 2003; Kessi et al., 1999; Moore and Kaplan, 1992; Nepple et al., 2000; Van Praag et al., 2002; Yamada et al., 1997) and hence different mechanisms could underlie reductive metal precipitation. The first objective of this study was to compare the pattern of Pd(0) deposition by *D. desulfuricans* and *R. sphaeroides*.

Palladium is extracted from mixed ores with other platinum group metals (PGM), for use in automotive catalysts, jewellery, dental amalgams and as a catalyst in various in industrial reactions (e.g. hydrogenations). Efficient use of PGM and their recovery are essential due to the increasing price of precious metals and finite ore resources. (Kendal, 2006) Palladium can be reclaimed from wastes (e.g. spent automotive catalytic converters and other scrap) using *aqua regia* to oxidize Pd(0) to Pd(II) in the form of the $[PdCl_4]^{2-}$ anion to create a Pd(II)-rich leachate (Mabbett et al., 2006; Yong et al., 2003). In order to regenerate Pd(0), a reducing step is required and the rate of reduction of Pd(II) was significantly increased in the presence of cells of *Desulfovibrio* spp. (Yong et al., 2002b, 2003). In this process the cells became 'palladized' (coated with a layer of Pd(0) particles) and dry palladized biomass (Bio-Pd(0)) was shown to be an active catalyst using various test reactions (Mabbett et al.,

2001; Mikheenko, 2004; Yong et al., 2002a; 2002b, 2003) The catalytic activity was seen also in 'Bio-Pd(0)' sourced from waste leachate (Mabbett et al., 2006).

In the process of Bio-Pd(0) catalyst preparation, Pd(II) initially biosorbs onto bacterial cells (de Vargas et al., 2004), coordinating to amine groups (de Vargas et al., 2005), followed by reduction to Pd(0) mediated by cellular hydrogenase activity (Mikheenko, 2004; Mikheenko et al., 2005). On provision of excess reductant (e.g. H_2 or formate) crystal growth continues autocatalytically (i.e. *via* the ability of Pd(0) to catalyse reduction of Pd(II) abiotically (Mabbett et al., 2004; Yong et al., 2002a)) until reduction to Pd(0) is complete (Yong et al., 2002b). Hence, Bio-Pd(0) of a known Pd:biomass loading can be produced by reducing a known mass of Pd(II) in the presence of a known mass of cells.

Bio-Pd(0) was a more active catalyst than Pd(0) powder reduced chemically under H_2 (Chem-Pd(0)) and this was attributed to the small Pd(0) particle size (Yong et al., 2002b), including a subpopulation of Pd(0) nanoparticles (~5 nm), detected magnetically (Mikheenko, 2004; Mikheenko et al., 2005). The Bio-Pd(0) can thus be described as a bionanocatalyst.

Biomass-supported palladium catalysts could have potential environmental applications. For example, Bio-Pd(0) prepared using *D. desulfuricans* catalysed the reduction of toxic Cr(VI) to Cr(III) (Mabbett et al., 2001; 2006) and was also effective in the reductive dehalogenation of chlorinated aromatic compounds (Baxter-Plant et al., 2003; Mabbett et al., 2001), which are a group of problematic environmental contaminants. For example, pentachlorophenol (PCP) is used as a wood preservative and as a pesticide, while highly toxic polychlorinated biphenyls (PCBs), which were industrially prevalent due to their stability and thermal properties, persist in the environment.(Anon., 1999) Microbial degradation of chlorinated aromatic compounds occurs slowly in the environment because the chloride substitutions protect the aromatic rings sterically from microbial oxygenase attack (Lee and Carberry, 1992). Use of Bio-Pd(0) could overcome this limitation and, indeed, Bio-Pd(0)-mediated reductive dehalogenation of PCBs produced less heavily substituted intermediate species and biphenyl (Macaskie and Harrad., 2005; Windt et al., 2005).

Microbial cell surface chemistry may influence the initial biosorption and subsequent enzymatically-mediated reduction of Pd(II), thus affecting the patterning and hence, potentially, the catalytic properties of the resultant Bio-Pd(0). Thus, within a single strain (*D. fructosovorans*) a mutant deficient in its periplasmic hydrogenases relocated its Pd(0) deposits to the cytoplasmic membrane-location of the remaining hydrogenases (Mikheenko et al., 2005; Rousset et al., 2006) with the effect of reducing the catalytic activity against chlorobiphenyl and tetrachlorobiphenyl by ~25% (Skibar et al.,

2005). The second objective of this work was to compare the Pd(0) localisation and catalytic efficacy of *R. sphaeroides*-supported Bio-Pd(0) ("*Rs*-Pd(0)") and *D. desulfuricans*-supported Bio-Pd(0) ("*Dd*-Pd(0)"), with respect to the promotion of H_2 release from hypophosphite and the reductive dehalogenation of chlorinated aromatic compounds.

MATERIALS AND METHODS

Microorganisms and culture conditions

Desulfovibrio desulfuricans (NCIMB 8307) was maintained and cultured as described previously (Yong et al. 2002b). *Rhodobacter sphaeroides* O.U.001 (DSMZ 5864) was held in stock at -80 ºC (in 15 % glycerol v/v), revived on nutrient agar (30 ºC) and cultured in filled sealed bottles under fluorescent illumination (39.5 µM photons m^{-2} s^{-1} measured using a PAR light meter SKP200, Skye Instruments Ltd.) at 30 ºC using SyA medium (Hoekema et al., 2002).

Determination of dry weight

Biomass concentration (mg dry weight mL^{-1}) was calculated from the optical density (660 nm) with reference to a conversion factor, determined in triplicate by recording optical densities from dense cultures after various dilutions in deionised H_2O. Cultures were washed twice in isotonic saline (8.5 g L^{-1} of NaCl, pH 7) (2400 x *g*, 20 min, 4 ºC, 50 mL) before drying at 60 ºC to constant mass.

Preparation of Bio-Pd(0)

The procedure was based on that described previously (Mabbett et al., 2001; Yong et al., 2002b). Bacterial cells were harvested from the mid-logarithmic phase of growth by centrifugation (11900 x *g*, 10 min), resuspended in a small volume of sterile buffer (20 mM sodium morpholinepropanesulfonic acid (MOPS)-NaOH (pH 7)) and used immediately. The *R. sphaeroides* suspensions were kept in the dark. Analytical grade reagents were used throughout to minimise the chloride background in chloride release assays.

Aliquots of cell concentrate and Pd(II) solution (Na_2PdCl_4;Sigma) were mixed to produce the desired mass ratio. For example, in order to produce Bio-Pd(0) loaded at 25 % Pd(0) (w/w), 0.1 g Pd(II) and 0.3 g cell dry weight were mixed in 0.01 M HNO_3, pH 2. Mixtures (50-100 mL) were sealed in 100 mL serum bottles with butyl rubber stoppers and aluminium tear seals, degassed under vacuum (5 min), sparged with oxygen-free N_2 (10 min) and incubated statically (30 ºC, 60 min) to

allow biosorption of Pd(II) before sparging with H_2 (15 min) after which complete removal of Pd(II) was confirmed by assay (below). The preparations were harvested by centrifugation, washed three times in sterile MOPS buffer (above) and once in acetone and dried at 60 ºC. Chemically reduced Pd(0) (Chem-Pd(0)) was prepared in parallel without bacterial cells and with complete Pd(II) reduction requiring 60 min under H_2. Dried material was ground using a pestle and mortar before catalytic testing. For each type of catalyst, 3 independent batches were prepared and tested; data are means ± SEM.

Assay of Pd(II)

Before harvesting palladized biomass, complete reduction of Pd(II) was confirmed by reading the A_{420} of sample supernatants in a variable wavelength spectrometer (Ultraspec III, Pharmacia Biotech). This assay method was validated previously using the $SnCl_2$ method, and polarographically (Mikheenko, 2004).

Electron microscopy

Samples of palladized biomass were washed as above, omitting the acetone wash, and prepared for examination of cell sections by transmission electron microscopy (TEM) as described previously (Baxter-Plant et al., 2003).

Evaluation of catalytic activity by the hypophosphite test reaction

The method was developed from that described previously (Yong et al., 2002b). For assay, each reaction contained 0.5 mg Pd(0) as a variable mass of total material dependent upon the relative Pd(0) and biomass fractions. The preparations were suspended in 10 mL of 100 g L^{-1} NaH_2PO_2 buffered with MOPS-NaOH (0.5 M, pH 8), at 25 ºC. After the onset of gas release the volume of H_2 generated over 30 min was measured by displacement of water using an inverted measuring cylinder. The pH of the reaction mixtures was unchanged after 30 min.

Assay for catalytic dehalogenation of chlorinated aromatic compounds

Dehalogenation of chlorophenols and polychlorinated biphenyls (PCBs) by Bio-Pd(0) was demonstrated previously (Baxter-Plant et al., 2003). As an electron donor for catalysis in test reactions, formate was preferred over H_2 since its concentration in the aqueous phase can be more accurately

controlled; metallic Pd(0) on the bacteria catalyses the cleavage of HCOOH to CO_2 and H_2 and then effects the homolytic fission of H_2, holding highly reactive $H^{\bullet}$ within the Pd crystal (Rhodin, 1979). The substrates tested are shown in Table 1. Chlorophenols were from Aldrich (Fancy Rd, Poole, Dorset BH12 4QH UK) and PCBs from QMX Laboratories Ltd. (Bedford St, Thaxted, Essex CM6 2PY UK). Substrates were used at equivalent concentrations of chloride; the nominal molar concentrations were as shown in Table 1.

For assay, each reaction contained 2 mg of test catalyst (i.e. total material: Pd(0) and biomass component, Pd:biomass loadings as specified), resuspended in 9 ml sterile MOPS-NaOH buffer (20 mM, pH 7) and 1 ml aromatic substrate in hexane carrier. After shaking and settling of the hexane-in-water suspension (5 min) a 1 mL sample was taken from the aqueous fraction. The reaction was initiated by the addition of 1 mL 1 M sodium formate (pH 7), the suspension was shaken and further samples were taken from the aqueous phase at suitable intervals, centrifuged (13000 x *g*, 4 min), and supernatant was transferred into cuvettes. Reductive dehalogenation was monitored by the release of chloride, as determined by the mercury (II) thiocyanate method (Mendam et al., 2000). A standard curve was prepared using NaCl in MOPS buffer. Assay interference by the organic components and spontaneous chloride release from biomass or aromatic substrate was excluded using catalyst-free controls and aromatic substrate-free controls supplemented with hexane alone. In the case of PCB 138 negligible chloride release was observed (see later) and this served as a negative control to show that PCB-in-hexane did not promote spontaneous chloride release from the biomass.

RESULTS

Examination of the palladized biomass under electron microscopy

R. sphaeroides and *D. desulfuricans* were successfully palladized without modification to the procedure, as shown by the appearance of black Pd(0) deposits under TEM (Fig. 1). Both species formed Pd(0) deposits in the periplasm and occasional outgrowth structures, indicating a probable periplasmic initiation of Pd(II) reduction followed by Pd(0) crystal growth and eruption beyond the cell surface. The surface-located Pd(0) deposits (~ 20 nm) can be seen to include clusters of Pd(0) nanoparticles ~5-10 nm in diameter (by measurement). This is in accordance with the previous detection of a population of ~5 nm Pd(0) nanoparticles using magnetic measurements. (Mikheenko, 2004; Mikheenko et al., 2005)

Several differences between *Dd*-Pd(0) and *Rs*-Pd(0) were noted (Fig. 1). At a Pd(0) loading of 1 % (w/w) surface-located clusters of Pd(0) on *Rhodobacter* were infrequent but visible, whereas no clusters were visible on *Desulfovibrio*, suggesting that Pd(0) deposits were below the limit of detection. Nucleation sites are primarily on or near the hydrogenase itself as shown by analysis of mutants deficient in one of more of their hydrogenases (Mikheenko 2004; Skibar et al., 2005; Rousset et al., 2006). These results would suggest (assuming the hydrogenase activity of *Desulfovibrio* to be greater than the metal reductase of *Rhodobacter*; this was not tested) a potentially higher incidence of nucleation foci in *Desulfovibrio* than in *Rhodobacter*, leading to more deposits of smaller size, whereas fewer initiation sites in *Rhodobacter* would promote the formation of fewer, larger clusters derived from the same total mass of Pd(II). This was observed in *D. fructosovorans* where removal of the periplasmic hydrogenases gave larger Pd(0) deposits at the location of the remaining hydrogenase enzymes (Mikheenko et al., 2005). At a Pd(0) loading of 5 % Pd(0) clusters were visible in the periplasm and at the cell surface of *Desulfovibrio*, while *Rs*-Pd(0) clusters were retained periplasmically (Fig.1).

At a Pd(0) loading of 25 %, clusters of Pd(0) nanoparticles appeared smaller and fewer overall on *Rhodobacter* than on *Desulfovibrio* and, while the latter showed a relatively even dispersion, the *R. sphaeroides* cellular deposits were more heterogeneous, with occasional very large deposits (Fig. 1, inset) visible on approx. 1 in 20 cells. While the overall Pd:biomass ratio was controlled, this skewed distribution of particle sizes resulted in an overall lighter deposition of Pd(0) for the majority of the *R. sphaeroides* cells.

Evaluation of catalytic activity using the hypophosphite test

H_2 evolution from hypophosphite was used as an initial simple indicator of catalytic activity for various preparations (Fig. 2). Bio-Pd(0) loaded at 1 %, 5 % and 25 % Pd(0) w/w was tested alongside Chem-Pd(0) (100 % Pd(0) w/w) and non-palladized biomass (0 % Pd(0) w/w). No catalytic activity was seen at 0 % and 1 % Pd(0) loading (Fig. 2). Bio-Pd(0) preparations loaded at 5 % or 25 % on *Rhodobacter* or *Desulfovibrio* were significantly more catalytically active than Chem-Pd(0) ($P = 0.99$). The highest rate of H_2 release was seen using *Dd*-Pd(0) loaded at 25 % Pd(0) w/w, showing more than four times the rate using Chem-Pd(0) and approximately double the rate for the corresponding *Rs*-Pd(0). Whether loaded at 5 % or 25 % Pd(0) w/w, *Dd*-Pd(0) was significantly more active than *Rs*-Pd(0) ($P = 0.99$). The Pd(0) loading (5 % or 25 %) did not affect catalytic activity of *Rs*-Pd(0), while for *Dd*-Pd(0) the

mean rate was reduced by 37 % at the lower Pd(0) loading; this difference was significant at P= 0.90. A Pd(0) loading of 25 % was chosen for subsequent work.

Evaluation of catalytic activity by reductive dehalogenation of chlorinated aromatic compounds

Rs-Pd(0), *Dd*-Pd(0) and Chem-Pd(0) were tested for their ability to catalyse reductive dechlorination of polychlorinated biphenyls (PCBs) and chlorophenols (2-chlorophenol and pentachlorophenol) using formate as the electron donor. No chloride release was promoted by non-palladized biomass or by any Pd(0) in the absence of electron donor.

Chloride release from polychlorinated biphenyls (PCBs)

The hypophosphite test indicated that at 25 % Pd(0) loading *Dd*-Pd(0) was 2.3-fold more effective than *Rs*-Pd(0) (Fig. 2). However, this relationship was not maintained in the case of PCBs (Table 2) and there was no consistent comparator between *Dd*-Pd(0) and *Rs*-Pd(0). In no case did the catalytic activity of *Rs*-Pd(0) exceed that of *Dd*-Pd(0) and only *Dd*-Pd(0) was able to liberate chloride from PCBs 118 and 153. The cases where no significant chloride release was detected (Table 2) serve as negative controls to confirm lack of chloride release in a system containing all of the ingredients, ruling out the appearance of chloride from anywhere except the dehalogenation of PCBs. Chem-Pd(0) was unable to dehalogenate markedly any PCB except PCB28 (2,4,4'-trichlorobiphenyl) which was the most water-soluble of the PCBs used (Makino, 1998) and was also the congener most susceptible to attack by both types of Bio-Pd(0).

As discussed previously (Baxter-Plant et al., 2003) the susceptibility of the substrate to attack was related to its aqueous solubility, with the water solubility and the amenability to reductive dehalogenation decreasing generally with an increasing number of chlorine substitutions. For example, the difference between chloride release between the most and least soluble PCBs (PCBs 28 and 180 respectively: Table 1) was 5.5-fold in the case of *Dd*-Pd(0) and 3.1-fold in the case of *Rs*-Pd(0) (Table 2). Since the activity of Bio-Pd(0) was related to the availability of substrate in the aqueous phase (see discussion) chlorophenols, which have a higher aqueous solubility, were investigated.

Chloride release from 2-chlorophenol (2-CP) and pentachlorophenol (PCP)

Constant rates of chloride release from 2-CP were observed over 60 min, with no delay before onset, using all three catalysts (not shown). The highest chloride release (36.1 mg L^{-1} h^{-1}) was observed using *Dd*-Pd(0) (Table 2). The corresponding rates using *Rs*-Pd(0) and Chem-Pd(0) were 53 % and 33 % of this, respectively. On a mass of Pd(0) basis the differences between Bio-Pd(0) preparations and Chem-Pd(0) can be corrected by a factor of 4, i.e. the chloride release for *Dd*-Pd(0) and *Rs*-Pd(0) were 12.2 and 6.5 times higher than for Chem-Pd(0), respectively.

Table 2 shows the chloride released from pentachlorophenol after 1 hour, at which point the only extensive dehalogenation was observed using *Rs*-Pd(0) and this was comparable to that seen using 2-CP (above). For *Dd*-Pd(0) and Chem-Pd(0), the onset of chloride release occurred only after 1-2 hours, while *Rs*-Pd(0) catalyzed a similar overall extent of dechlorination, but with the first chloride release detected within 40 min (Fig. 3). After 2 hours the release of chloride by all three preparations was comparable (Fig. 3). On the basis of chloride release per mass of Pd(0) after 2h, both Bio-Pd(0) preparations were over four times as active as Chem-Pd(0). The release of chloride via Chem-Pd(0) ceased after 4 h whereas the Bio-Pd(0) preparations continued to liberate chloride at slower, broadly comparable rates. One important observation was the biphasic behaviour of chloride release by the Bio-Pd(0)s over the first 2 h. *Rs*-Pd(0) showed an initially rapid Cl^- release followed by a constant slower rate, while with *Dd*-Pd(0) the converse applied. No significant differences were apparent between the Bio-Pd(0)s after 2 h.

DISCUSSION

The cell-surface localization of Pd(0) deposits was similar on cells of *R. sphaeroides* and *D. desulfuricans* at 5 % and 25 % loading (Fig. 1). A periplasmic origin for Pd(0) crystals in *D. desulfuricans* was shown previously to derive from an involvement of periplasmic hydrogenases in the reduction of sorbed Pd(II) (Mikheenko et al., 2005) and hydrogenase activity was confirmed throughout the incubation period by assay (Mikheenko 2004). The TEM study (Fig. 1) provided evidence for a similar origin for Pd(0) crystals in the periplasm of *R. sphaeroides* but the specific involvement of hydrogenase(s) was not tested. It has been suggested that the intracytoplasmic membrane-associated photosynthetic apparatus of *Rhodobacter* spp. could provide an abundant source of reductant during photoheterotrophic metabolism and, in the case of tellurium, intracellular needle-

like granules were visible (Borsetti et al., 2003). However in this study, resting cells were exposed to Pd(II) in the dark and cytoplasmic deposits of Pd(0) were not evident (Fig. 1).

Use of biomass as a support in catalyst preparation augmented catalytic activity, which indicates a high availability of palladium catalytic surface due to the small crystal sizes (see Fig. 1 and Yong et al., 2002b). A further contributing factor may be the increased dispersion of the material and stabilisation of nanoparticles on the biomass, overcoming the natural tendency of nanoparticles to agglomerate. Inspection of Fig. 1 (e.g. *Dd*-Pd(0), 5%) shows that the Pd(0) deposits were composed of smaller clusters of sizes measured at ~ 3-10 nm which is in accordance with a subpopulation of nanoclusters of size ~ 5nm as calculated from magnetic measurements (Mikheenko, 2004; Mikheenko et al., 2005).

Different chlorinated aromatic compounds have different industrial applications and environmental occurrences and a catalyst targeting a specific compound could be potentially beneficial. These studies show that in the case of PCBs the *Desulfovibrio*-supported catalyst was superior in all cases whereas, in the case of pentachlorophenol, although the activity of both biomass-supported catalysts was comparable overall after an extended period, the *Rs*-Pd(0) could offer an advantage for the treatment of waste streams where a rapid flow rate might require a short flow residence time. In contrast, for cases such as PCB or PCP-contaminated soils or soil washings, where the timescale may be less important, *Desulfovibrio*-supported catalysts would be more useful. The constant slow rate after 2 h (Fig. 3) suggests that factors other than catalytic activity *per se* become rate-limiting and other studies using atomic force microscopy have shown that 5 nm Pd(0) nanoparticles tend to foul with the PCB substrate and partial dehalogenation products, requiring a hexane wash to liberate these from the catalyst (I.P. Mikheenko unpublished).

The extent of dehalogenation varied greatly within the portfolio of 7 PCB congeners (Table 2), with varying amounts of chlorine substitution (Table 1). As described previously (Baxter-Plant et al., 2003), the aqueous solubility of PCBs is related inversely to the number of chlorine substitutions, with the consequence of a limited aqueous availability of the more heavily substituted species. Parameters such as the octanol-water partition coefficient (K_{ow}) and the solvent-accessible surface area (SAS), being related to water solubility, are used in the study of environmental fates of organic pollutants such as PCBs (Makino, 1998, 1999; Yeh and Hong, 2002). The chloride release catalysed by *Rs*-Pd(0) (Table 2) showed significant dependence upon K_{ow} ($F = 11.0$, $P = 0.99$) (Fig. 4B) and SAS ($F = 5.80$, $P = 0.97$), confirming the previous assertion (Baxter-Plant et al., 2003) that the PCB recalcitrance is

probably a consequence of low water solubility. However, no such relationship was observed for *Dd*-Pd(0) and Chem-Pd(0) ($F < 6.61$, $P > 0.95$) suggesting that aqueous solubility was not the principal factor involved for these preparations.

Given the high K_{ow} values of PCBs (Makino, 1998; 1999), nearly all of the PCB mass would be expected to remain in the organic phase. Accordingly GC-MS analysis of post-dehalogenation mixtures (*Dd*-Pd(0)) revealed an absence of chlorinated intermediates or of biphenyl in the aqueous phase (Baxter-Plant et al., 2004). Washing the catalyst with hexane gave 76 % recovery of expected products. Movement of PCB into the aqueous fraction containing the catalyst suspension, i.e the ability of Bio-Pd(0) to adsorb PCB in the aqueous phase may be the rate-limiting step; as PCB is sorbed and degraded more PCB would move across the solvent interface to maintain the partition equilibrium. In this case the low aqueous solubility of PCBs would not be expected to limit the overall extent of dehalogenation and other factors must be contributory in the case of *Dd*-Pd(0), which are independent of water solubility.

Analytical studies of PCB degradation revealed preferential cleavage of *para*-chlorine over *meta*- and *ortho*- substitutions in both chemical and biological systems (Bedard and Haberl, 1990; Korte et al., 2002; Mitoma et al., 2004; Pieper, 2005; Yak et al., 1999). After reclassifying the PCBs according to the amount of potentially available *para*-chlorine, a relationship was shown for *Dd*-Pd(0) ($F = 24.4$, $P = 0.998$) (Fig. 4A) but not for *Rs*-Pd ($F = 0.45$, $P = 0.45$). Previous work using *Dd*-Pd(0) (loaded at 50 % Pd(0) w/w) supports this hypothesis, as a comparable extent of dehalogenation was measured for 2,4,6-trichlorobiphenyl and 2,2',4,4',6,6'-hexachlorobiphenyl which have a similar proportion of *para*-chlorines but differ in aqueous solubility by 2 orders of magnitude (Baxter-Plant et al., 2003). Taken together, the occurrence of *meta*- and *ortho*-chlorines had no influence upon the extent of chloride released.

These results suggest that, in accordance with other published work (above), the positions of Cl substitutions are at the root of PCB recalcitrance. In these tests the low aqueous solubility limited the capacity for catalytic dehalogenation by *Rs*-Pd(0), although *Dd*-Pd(0) was implied as being more able to overcome PCB hydrophobicity and reveal an underlying preference for *para*-chlorine. These differences in catalytic properties may be attributable to subtle differences in size and patterning of Pd(0) deposits (Fig. 1), but the supporting biomass may also contribute. In this respect the formation of outer membrane vesicles (OMVs), apparently unrelated spatially to the occurrence of the Pd-nanoparticles but produced in response to Pd-exposure, was observed previously (I. Mikheenko and

L.E. Macaskie, unpublished; see Fig 1; open arrows). Formation of OMV 'blebs' in response to metal-stress was documented in *Pseudomonas putida* (Higham et al., 1986) and, overall, formation of OMVs is a common phenomenon in Gram-negative bacteria (Beveridge, 1999). *Pseudomonas putida* also formed OMVs as a mechanism of resistance to toluene, which was found associated with the vesicles, illustrating the high affinity of OMVs for hydrophobic molecules, which are the most toxic to microorganisms (Kobayashi et al., 2000). Since OMVs are composed of inner face phospholipid and outer face lipopolysaccharide (Beveridge, 1999; Ruiz and Silhavy, 2005) which are both amphipathic molecules (i.e. they contain both polar and non polar domains) it seems likely that the OMVs could interact with PCB molecules across the hexane-water interface. The lack of observable metal-induced OMVs in *R. sphaeroides* (Fig. 1) could explain the dependence upon aqueous solubility for PCB dehalogenation by *Rs*-Pd(0), whereas with *Dd*-Pd(0) OMV-mediated emulsification of PCBs can be suggested. Furthermore, partial disruption of the cell surface near the location of some of the Pd-particles was observed and it seems possible that outer membrane materials may also occur near to Pd-nanoparticles (e.g. *Dd*-Pd(0) 25% loading; Fig 1, arrowed). Previous studies reported that Bio-Pd(0) effected reductive dehalogenation under conditions where finely-divided 'chemical'-Pd(0) was ineffective (Baxter-Plant et al., 2003; 2004) and from the current observations we suggest a possible role for outer membrane materials in facilitating access of the Pd(0) catalyst to the hydrophobic PCB molecule.

Development of chemical and bioremediation approaches for contaminated soils will need to address the problem of PCB availability and it appears from the above that the use of *Dd*-Pd(0) may increase the target compound availability. The high expense of precious metals may mitigate against the use of palladised biomass but previous studies showed good catalytic activity for Bio-Pd(0) scavenged from mixed-metal waste leachates (Mabbett et al., 2006), indicating the possibility for an inexpensive catalyst which could be sourced from industrial precious metal wastes.

Acknowledgements

This work was supported by the BBSRC (grant No. RRAD09846 and studentship 10703) and by a Royal Society/BBSRC Industry Fellowship to Prof. L. E. Macaskie. The authors would like to thank Mrs L. Tomkins (Centre for Electron Microscopy, University of Birmingham) for assistance with electron microscopy. The electron micrograph of *D. desulfuricans* showing outer membrane vesicles

(Fig. 1 inset) was provided by Dr I. P. Mikheenko. This paper is dedicated to the memory of Dr 'Vic' Baxter-Plant who died tragically in May 2006.

References

Anon. 1999. Public health implications of exposure to polychlorinated biphenyls (PCBs). US Environmental Protection Agency, www.epa.gov/waterscience/fish/pcb99.pdf.

Baxter-Plant V, Mikheenko IP, Macaskie LE. 2003. Sulphate-reducing bacteria, palladium and the reductive dehalogenation of chlorinated aromatic compounds. Biodegradation 14:83-90.

Baxter-Plant VS, Mikheenko IP, Robson M, Harrad SJ, Macaskie LE. 2004. Dehalogenation of chlorinated aromatic compounds using a hybrid bioinorganic catalyst on cells of *Desulfiovibrio desulfuricans*. Biotechnol. Lett. 26:1885-1890.

Bedard DL, Haberl ML. 1990. Influence of chlorine substitution pattern on the degradation of polychlorinated biphenyls by 8 bacterial strains. Microb. Ecol. 20:87-102.

Beveridge TJ. 1999. Structures of gram-negative cell walls and their derived membrane vesicles. J. Bacteriol. 18:4725-4733.

Borsetti F, Borghese R, Francia F, Randi MR, Fedi S, Zannoni D. 2003. Reduction of potassium tellurite to elemental tellurium and its effect on the plasma membrane redox components of the facultative phototroph *Rhodobacter capsulatus*. Protoplasma 221:153-161.

de Vargas I, Macaskie LE, Guibal E. 2004. Biosorption of palladium and platinum by sulfate-reducing bacteria. J. Chem. Technol. Biotechnol. 79:49-56.

de Vargas I, Sanyahumbi D, Ashworth MA, Hardy CM, Macaskie LE. 2005. Use of X-ray photoelectron spectroscopy to elucidate the mechanism of palladium and platinum biosorption by *Desulfovibrio desulfuricans* biomass. In: Harrison STL, Rawlings DE, Petersen J, Editors; 2005 25th-29th September 2005; Cape Town, S. Africa. Published by 16th Int. Biohydrometallurgy Symp. Cape Town. ISBN: 1-920051-17-1. pp 605-616.

Higham DP, Sadler PJ, Scawen MD. 1986. Cadmium-binding proteins in *Pseudomonas putida* - pseudothioneins. Environ. Hlth. Persp. 65:5-11.

Hoekema S, Bijmans M, Janssen M, Tramper J, Wijffels RH. 2002. A pneumatically agitated flat-panel photobioreactor with gas re-circulation: anaerobic photoheterotrophic cultivation of a purple non-sulfur bacterium. Int. J. Hyd. En. 27:1331-1338.

Kendal T. 2006. Platinum Review. Published by Johnson Matthey Plc., www.platinum.matthey.com/publications.

Kessi J, Ramuz M, Wehrli E, Spycher M, Bachofen R. 1999. Reduction of selenite and detoxification of elemental selenium by the phototrophic bacterium *Rhodospirillum rubrum*. Appl. Environ. Microbiol. 65:4734-4740.

Kobayashi H, Uematsu K, Hirayama H, Horikoshi K. 2000. Novel toluene elimination system in a toluene-tolerant microorganism. J. Bacteriol. 182:6451-6455.

Korte NE, West OR, Liang L, Gu B, Zutman JL, Fernando Q. 2002. The effect of solvent concentration on the use of palladized-iron for the step-wise dechlorination of polychlorinated biphenyls in soil extracts. Environ. Sci. Technol. 22:343-349.

Lee SH, Carberry JB. 1992. Biodegradation of PCP enhanced by chemical oxidation pretreatment. Wat. Environ. Res. 64:682-689.

Lloyd JR. 2003. Microbial reduction of metals and radionuclides. FEMS Microbiol. Rev. 27:411-425.

Lloyd JR, Lovley DR, Macaskie LE. 2003. Biotechnological application of metal-reducing microorganisms. Adv. Appl. Microbiol. 53:85-119.

Lloyd JR, Anderson RT, Macaskie LE. 2005. Bioremediation of metals and radionuclides. In: Atlas RM, Philp J, Editors. Bioremediation: Applied microbial solutions for real world environmental clean-up. Washington DC: ASM Press. p 293-317.

Lovley DR. 1993. Dissimilatory Metal Reduction. Ann. Rev. Microbiol. 47:263-290.

Mabbett AN, Yong P, Baxter-Plant VS, Mikheenko IP, Farr JPG, Macaskie LE. 2001. Effective reduction and removal of Cr(VI) and reductive dehalogenation of chlorophenol by hybrid bioinorganic catalytic processes. In *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development* Eds V.S.T. Ciminelli & O Garcia, Elsevier, Amsterdam p 335-342.

Mabbett AN, Yong P, Peter J, Farr G, Macaskie LE. 2004. Reduction of Cr(VI) by palladized biomass of *Desulfovibrio desulfuricans* ATCC 29577. Biotechnol. Bioeng. 87:104-109.

Mabbett A, N., Sanyahumbi D, Yong P, Macaskie LE. 2006. Biorecovered precious metals from industrial wastes: single step conversion of mixed metal liquid waste to a bioinorganic catalyst with environmental application. Environ. Sci. Technol. 40:1015-1021.

Macaskie LE, Harrad SJ. 2005. Novel bionanocatalyst for degradation of polychlorinated biphenyls (PCBs). Final report BBSRC Project No E19998. Biotechnology and Biological Sciences Research Council UK.

Makino M. 1998. Prediction of n-octanol/water partition coefficients of polychlorinated biphenyls by use of computer calculated molecular properties. Chemosphere 37:13-26.

Makino M. 1999. Novel classification to predict relative gas chromatographic retention times and *n*-octanol/water partition coefficients of polychlorinated biphenyls. Chemosphere 39:893-903.

Mendam J, Denney RC, Barnes JD, Thomas M. 2000. Vogel's Textbook of Quantitative Chemical Analysis. Harlow: Longman.

Mikheenko IP. 2004. Nanoscale Palladium Recovery PhD Thesis: University of Birmingham, UK.

Mikheenko IP, Mikheenko PM, Dementin S, Rousset M, Macaskie LE. 2005. Nanoengineering of ferromagnetic palladium in hydrogenase negative mutants of *Desulfovibrio fructosovorans*. In: Harrison STL, Rawlings DE, Petersen J, Editors; 2005 25th-29th September 2005; Cape Town, S. Africa. Published by 16th Int. Biohydrometallurgy Symp. Cape Town. ISBN: 1-920051-17-1 pp. 605-616.

Mitoma Y, Uda T, Egashira N, Simion C, Tashiro H, Fan X. 2004. Approach to highly efficient dechlorination of PCDDs, PCDFs and coplanar PCBs using metallic calcium in ethanol under atmospheric pressure at room temperature. Environ. Sci. Technol. 38:1216.

Moore MD, Kaplan S. 1992. Identification of intrinsic high-level resistance to rare earth oxides and oxyanions in members of the class *Proteobacteria* - characterization of tellurite, selenite, and rhodium sesquioxide reduction in *Rhodobacter sphaeroides*. J. Bacteriol. 174:1505-1514.

Nepple BB, Kessi J, Bachofen R. 2000. Chromate reduction by *Rhodobacter sphaeroides*. Journal of Ind. Microbiol. Biotechnol. 25:198-203.

Pieper DH. 2005. Aerobic degradation of polychlorinated biphenyls. Appl. Microbiol. Biotechnol. 67:170-191.

Rhodin TN. 1979. The nature of the surface chemical bond. Rhodin TN, Ertl G, editors. Amsterdam: North-Holland Pub. Co.

Rousset M, Casalot L, de Philip P. Belaich A, Mikheenko IP, Macaskie LE 2006. Use of bacterium strains for the preparation of metallic biocatalysts, in particular for the preparation of palladium biocatalysts. European Patent Application No EP2006050942

Ruiz N, Silhavy TJ. 2005. Sensing external stress: watchdogs of the *Escherichia coli* cell envelope. Curr. Op. Microbiol. 8:122-126.

Skibar W, Macaskie LE, Pompe W, Rousset M, Selenska-Pobell S. 2005. Novel precious metal-based bionanocatalysts from scrap Final Report EU Contract GRD1-2001-40424. The European Commission, Brussels.

Tebo BM, Obraztsova AY. 1998. Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. FEMS Microbiol. Lett. 162:193-198.

Van Praag E, Degli Agosti R, Bachofen R. 2002. Selenite reduction and uptake hydrogenase activity in *Rhodospirillum rubrum*. Arch. Des Sci. 55:69-80.

Windt WD, Aelterman P, Verstraete W. 2005. Bioreductive deposition of palladium (0) nanoparticles on *Shewanella oneidensis* with catalytic activity towards reductive dechlorination of polychlorinated biphenyls. Environ. Microbiol. 7:314-325.

Yak HK, Wenclawiak BW, Cheng BW, Doyle JG, Wai CM. 1999. Reductive dechlorination of polychlorinated biphenyls by zerovalent iron and subcritical water. Environ. Sci. Technol. 33:1307.

Yamada A, Miyagishima N, Matsunaga T. 1997. Tellurite removal by marine photosynthetic bacteria. J. Mar. Biotechnol. 5:46-49.

Yeh M-F, Hong C-S. 2002. Octanol-water partition coefficients of non-*ortho*- and mono-*ortho*-substituted polychlorinated biphenyls. J. Chem. Eng. Data 47:209-215.

Yong P, Farr JPG, Harris IR, Macaskie LE. 2002a. Palladium recovery by immobilized cells of *Desulfovibrio desulfuricans* using hydrogen as the electron donor in a novel electrobioreactor. Biotechnol. Lett. 24:205-212.

Yong P, Rowson NA, Farr JPG, Harris IR, Macaskie LE. 2002b. Bioreduction and biocrystallization of palladium by *Desulfovibrio desulfuricans* NCIMB 8307. Biotechnol. Bioeng.80:369-379.

Yong P, Rowson NA, Farr JPG, Harris IR, Macaskie LE. 2003. A novel electrobiotechnology for the recovery of precious metals from spent automotive catalysts. Environ. Technol. 24:289-297.

Table 1. Chlorinated aromatic compounds used in catalytic dehalogenation testing

Compound		
Short title	Systematic name	Nominal concentration (mM)*
PCB 28	2,4,4'-trichlorobiphenyl	0.308
PCB 52	2,2',5,5'-tetrachlorobiphenyl	0.274
PCB 101	2,2',4,5,5'-pentachlorobiphenyl	0.123
PCB 118	2,3',4,4',5-pentachlorobiphenyl	0.123
PCB 138	2,2',3,4,4',5'-hexachlorobiphenyl	0.111
PCB 153	2,2',4,4',5,5'-hexachlorobiphenyl	0.111
PCB 180	2,2',3,4,4',5,5'-heptachlorobiphenyl	0.051
2-CP	2-Chrolorophenol	0.500
PCP	Pentachlorophenol	0.500

* Due to their low water solubility, the aromatic substrates were used as hexane in water suspensions. The concentration shown is that in the 10 mL of test mixture, the actual concentration in the aqueous phase was not determined. Hexane/water partition coefficients were not known.

Table 2: Chloride release from PCBs and chlorophenols

Compound (see Table 1)	Chloride release (mg L^{-1})		
	Dd-Pd(0)	*Rs*-Pd(0)	Chem-Pd(0)
PCB 28	13.18 ± 2.67	1.43 ± 0.71	5.62 ± 1.04
PCB 52	1.02 ± 0.05	1.04 ± 0.41	0.36 ± 0.04
PCB 101	3.93 ± 0.97	0.93 ± 0.27	no sig Cl^-
PCB 118	4.29 ± 1.32	no sig Cl^-	no sig Cl^-
PCB 138	1.18 ± 0.13	no sig Cl^-	no sig Cl^-
PCB 153	2.55 ± 0.56	no sig Cl^-	0.54 ± 0.02
PCB 180	2.39 ± 0.76	0.46 ± 0.23	0.27 ± 0.02
2-CP	36.14 ± 2.85	19.31 ± 0.80	11.85 ± 4.87
PCP	4.65 ± 1.60	17.38 ± 1.93	0.43 ± 2.98

* Data represent the increase in chloride concentration after 60 min for chlorophenols, and 24 hours for PCBs. Data are means ± standard errors from at least three independent experiments. Note that since the Pd:biomass ratio was 1:3, the data for Bio-Pd(0) can be multiplied by four for direct comparison with Chem-Pd(0) on a mass of Pd(0) basis.

no sig Cl^- : significant chloride was not detected. Assay sensitivity was 0.5-100 mg Cl^- L^{-1}.

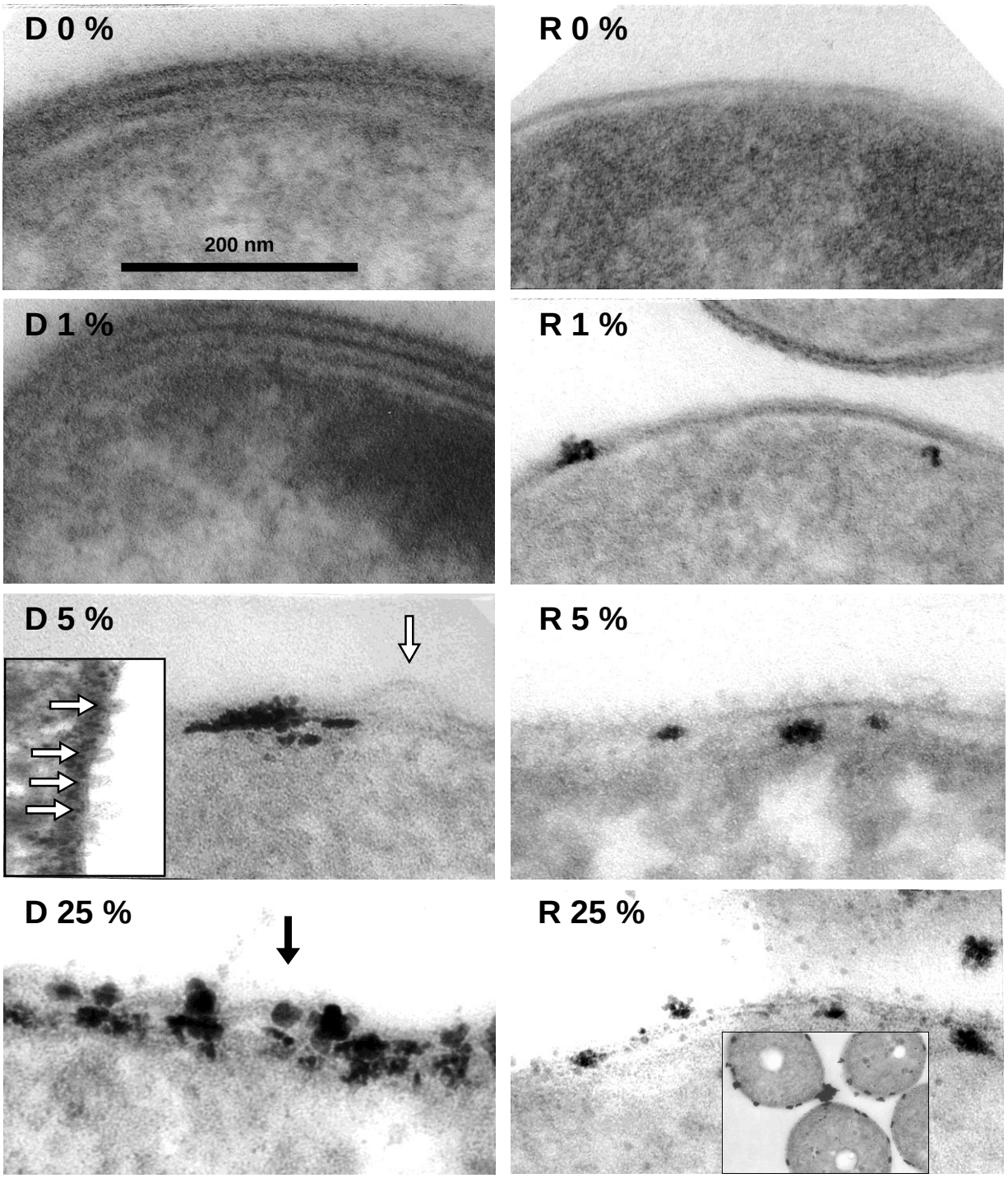

Figure 1. TEM sections of *D. desulfuricans* (D) and *R. sphaeroides* (R) palladized to 0 %, 1 %, 5 % and 25 % Pd(0) w/w. Scale-bar applies to all main frames. Inset: *D. desulfuricans*: formation of outer membrane vesicles during palladization; OMVs are indicated with open arrows. Filled arrow: Pd(0)-particle in association with outer membrane materials. Inset: *R. sphaeroides*: detail of occasional heavy cell surface deposit visible in approx. 1 in 20 cells.

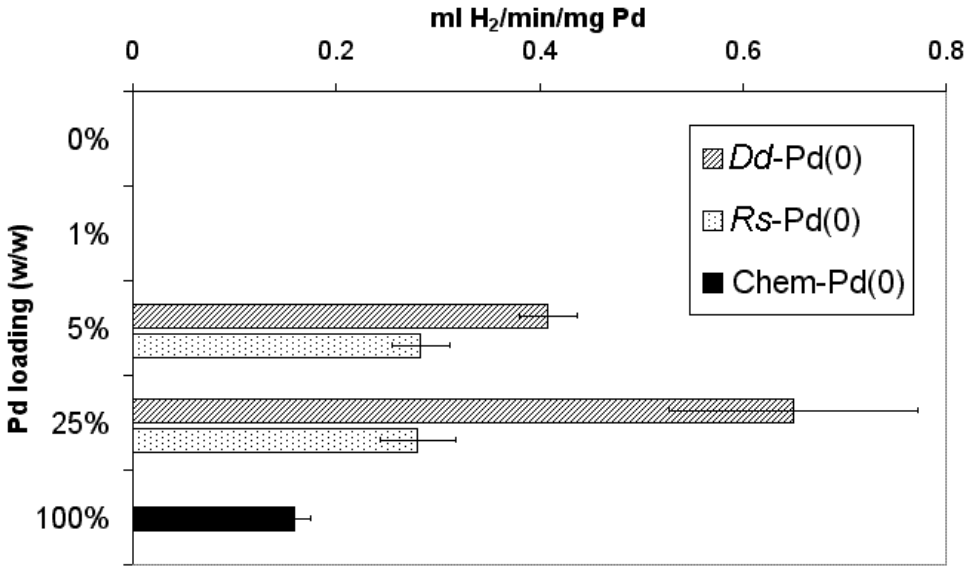

Figure 2. Rates of H_2 release via hypophosphite by various catalytic preparations. All reactions contained 0.5 mg Pd(0). Data are means and standard errors from four experiments. No H_2 was generated using unpalladized biomass or Bio-Pd(0) loaded at 1 % Pd(0) w/w.

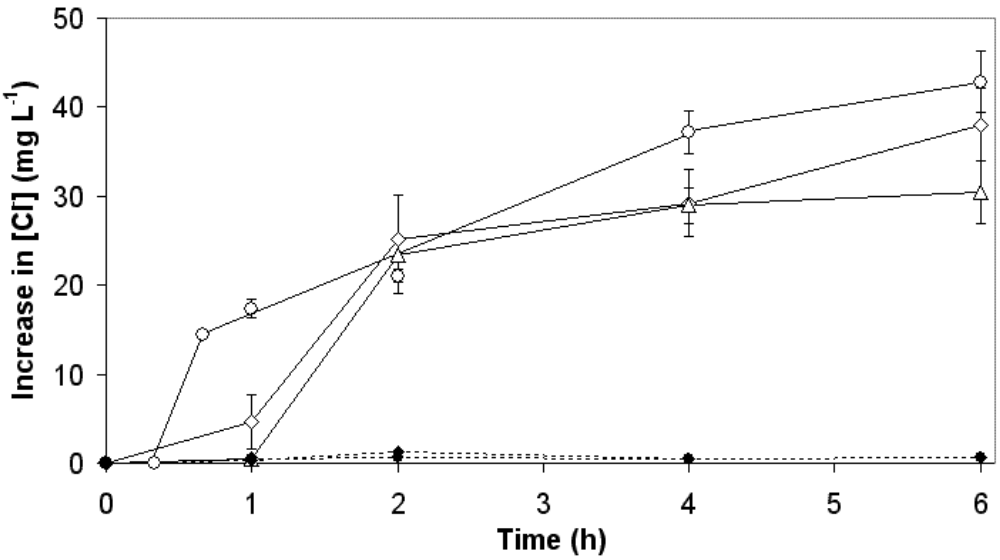

Figure 3. Reductive dehalogenation of pentachlorophenol (PCP). Pd(0) catalysts (solid lines): (◊) 25 % *Dd*-Pd(0) (○) 25 % *Rs*-Pd(0) (Δ) Chem-Pd(0). Pd(0)-free controls (dashed lines): (♦) *D. desulfuricans* biomass alone. (●) *R. sphaeroides* biomass alone. Data are means and standard errors from at least three independent experiments. Note that since the Pd:biomass ratio was 1:3, the data for Bio-Pd(0) can be multiplied by four for direct comparison with Chem-Pd(0) on a mass of Pd(0) basis.

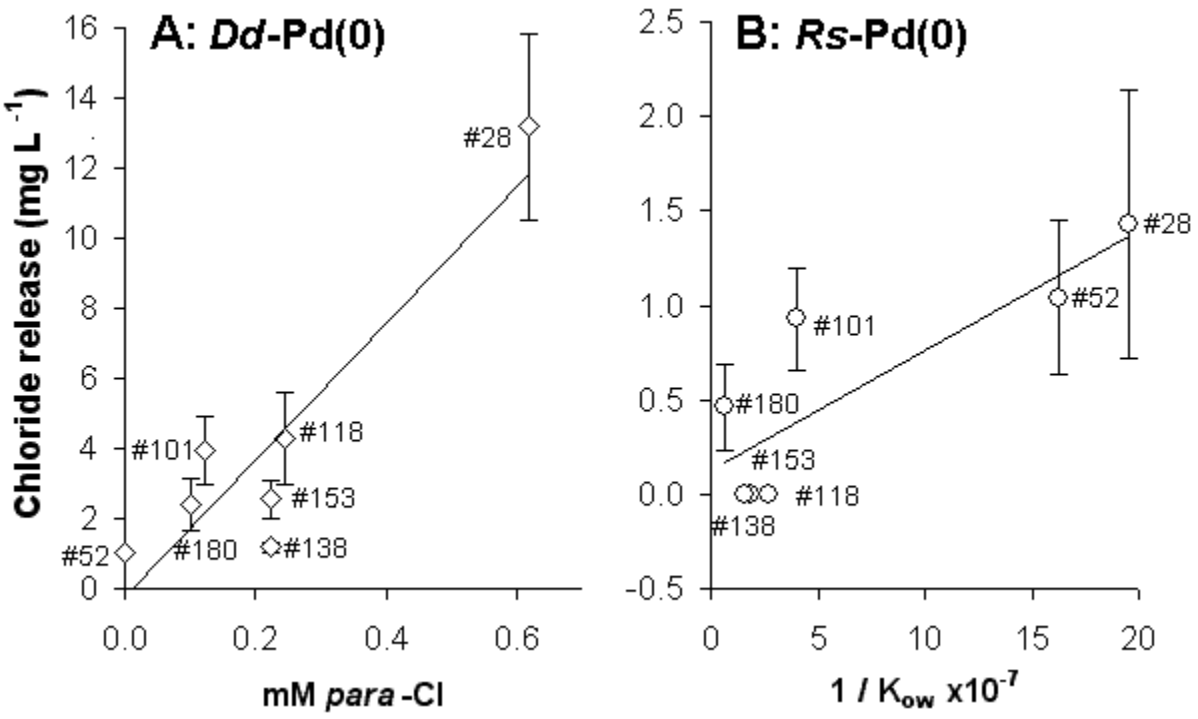

Figure 4. Dependence upon different factors for dehalogenation of PCBs by *Dd*-Pd(0) (A) and *Rs*-Pd(0) (B). Pd(0) loading was 25 % w/w. Data are means and standard errors from at least three independent experiments. $1/K_{ow}$ indicates aqueous solubility. logK_{ow} values were given by Makino (1998). The numbers shown relate to the PCBs shown in Table 1.