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Sulphate-reducing bacteria, palladium and the reductive dehalogenation of chlorinated aromatic compounds

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Abstract

The surfaces of cells of *Desulfovibrio desulfuricans*, *Desulfovibrio vulgaris* and a new strain, *Desulfovibrio* sp. 'Oz-7' were used to manufacture a novel bioinorganic catalyst via the reduction of Pd(II) to Pd(0) at the cell surface using hydrogen as the electron donor. The ability of the palladium coated (palladised) cells to reductively dehalogenate chlorophenol and polychlorinated biphenyl species was demonstrated. Dried, palladised cells of *D. desulfuricans*, *D. vulgaris* and *Desulfovibrio* sp. 'Oz-7' were more effective bioinorganic catalysts than Pd(II) reduced chemically under H_2 or commercially available finely divided Pd(0). Differences were observed in the catalytic activity of the preparations when compared with each other. Negligible chloride release occurred from chlorophenol and polychlorinated biphenyls using biomass alone.

Introduction

Polychlorinated biphenlys (PCBs) comprise two benzene rings linked at the C-1 carbon and substituted with 1–10 chlorine atoms in the ortho, meta and/or para positions to give a large class of compounds. PCBs were widely used industrially due to their excellent physical and chemical properties, including low water solubility, stability to oxidation, low vapour pressure, flame resistance, excellent dielectric properties and relative inertness (Kimbrough et al. 1989). Jensen (1966) initially reported the contamination of the environment by PCBs. Their extensive use has led to the widespread contamination of air, water, soil and sediments (Ericksen 1997) and their presence, persistence and recalcitrance are matters of concern.

PCBs and other chlorinated aromatic compounds such as chlorophenols can be biodegraded via two distinct microbial processes: aerobic oxidative degradation and anaerobic reductive dehalogenation. Oxidative degradation *per se* is not readily applicable to many chlorinated aromatic compounds since the chlorine substitution may block oxygenase attack (Wright et al. 1996; Weige et al. 2000), although the reduction of Fe (III) has been coupled to chlorobenzoate biooxidation (Kazumi et al. 1995). Anaerobic reductive dehalogenation of chlorinated aromatic compounds involves the removal of the chlorine substituents and their replacement by hydrogen by mechanisms which are still not clear. Mousa et al. (1998), Tiedje et al. (1993), Bak et al. (1986) and Häggblom et al. (1995) reported reductive dehalogenation of chlorinated aromatic compounds by anaerobic bacteria but the rate is usually low (e.g., 3.9×10^{-3} nmol/min/mg biomass using cell suspensions: Hartkamp-Commandeur et al. 1996) and the range of compounds attacked is limited (Häggblom et al. 1995; Aider et al. 1993).

Korte et al. (1997) reported an alternative approach using a palladised Fe(0) surface to act as a chemical reductant for polychlorinated biphenyls. This prompted investigation of the potential application of palladised biomass ('Bio-Pd(0)') as a bioinorganic catalyst for this purpose (Mabbett et al. 2001). Lloyd et al. (1998) reported that Pd(II) was reduced to Pd(0) on the surface of *Desulfovibrio desulfuricans* at the expense of hydrogen as the electron donor, with hydrogenase implicated as the mediating enzyme, while Yong et al. (2002a) demonstrated the application of this ap-

proach to the recovery of Pd(0) from industrial wastes containing soluble Pd(II). Hence wastes containing valuable platinum group metals can be recycled into a bioinorganic catalyst which can be used for remediation of another waste (in this case chlorophenol and PCB species) without the need for chemical reprocessing: a mixed base metal deposit biorecovered from liquid wastes was shown to be an effective catalyst in the reduction of Cr(VI) to Cr(III) (Mabbett 2002). This study compares palladised cells of three *Desulfovibrio* spp. as catalysts for the reductive dehalogenation of chlorophenol and PCB species. The rationale underlying this objective is that different strains will possess subtly different cell surface nucleation sites and/or hydrogenases and demonstrable differences in the catalytic activity of the palladium metal obtained on each strain could provide a good starting point to understanding the underlying mechanisms of Pd(0) patterning in the absence of well developed molecular methods for producing specific deletion mutants in sulphate-reducing bacteria.

Materials and methods

Organisms, and preparation of Pd catalysts

Desulfovibrio desulfuricans ATCC 29577 and *Desulfovibrio vulgaris* ATCC 29579 were from the American type culture collection. *Desulfovibrio* sp.'Oz-7' was obtained from a mine site in the Northern Territory, Australia, and was identified as a species of *Desulfovibrio* using 16s RNA homology (Mabbett & Macaskie, 2001). Bacteria were routinely cultured as previously described (Lloyd et al. 1998). The palladised biomass ('Bio-Pd(0)') was prepared using a known volume of a 2 mM solution of Na_2PdCl_4 (to pH 2.0 with 0.01 M HNO_3), placed in 50 ml butyl rubber sealed serum bottles and degassed using oxygen free nitrogen (15–20 min). Mid-logarithmic phase cell suspensions of *D. desulfuricans*, *D. vulgaris* and *Desulfovibrio* sp. 'Oz-7' were added to give a final ratio (wt of Pd: dry wt of cells) of 1 : 1 via a syringe under oxygen free nitrogen. The cell suspension was left for 1 h at 30 °C to allow initial biosorption of Pd(II) to the biomass surface. This was followed by sparging with H_2 (30 min) to allow reduction of Pd(II) to Pd(0) and fix the palladium onto the cells as Pd(0) (identified as base metal by X-ray powder diffraction analysis (Lloyd et al. 1998)). The recovered 'Bio-Pd(0)' was washed in distilled water three times and then acetone, dried in air to constant weight and ground. 'Chemical-Pd(0)' was prepared in parallel without the addition of cells, requiring 60 minutes under H_2 for the chemical reduction of Pd(II) to Pd(0). The reference material ('Ref-Pd(0)') was commercially available 39 μm powdered Pd(0) (99.9+ % pure, Aldrich Ltd.).

Reductive dehalogenation tests

Triplicate suspensions for each assay using 2–3 independent 'Bio-Pd(0)' preparations from separate *D. desulfuricans*, *D. vulgaris* and *Desulfovibrio* sp. 'Oz-7' cultures were used for all experiments. The 'Bio-Pd(0)' (2 mg $\pm$ 0.2 mg) was placed into 12 ml serum bottles. The chlorinated aromatic compound was then added to the required concentration in a carrier of degassed 20 mM MOPS/NaOH buffer pH 7.0 (10 ml). 'Biological' tests used washed fresh biomass (resting cells) of *D. desulfuricans*, *D. vulgaris* and *Desulfovibrio* sp.''Oz-7' added (to 0.5 mg/ml dry wt) in lieu of 'Bio-Pd(0)', while 'chemical' comparisons used 2 mg of 'chemical Pd(0) or 'Ref-Pd(0)' (2 mg of each; see above; note that the mass of Pd metal in the 'Bio-Pd(0)' used for tests was 50% of the 'Chemical-Pd(0)' or 'Ref-Pd(0)' since the biomass : Pd mass ratio was 1 : 1. Therefore the Pd comprised 50% of the total mass of the 'Bio-Pd(0)' catalyst). Experiments were initiated via the addition of 10 mM sodium formate (pH 7.3 $\pm$ 0.02). Where hydrogen was used, it was sparged for 5 min and experiments were initiated by addition of the chlorinated aromatic compound. In all cases the final volume was 10 ml. The H_2 was replenished to excess each time a sample was withdrawn. The reaction mixtures were agitated continuously to mix.

Dehalogenation of the chlorinated aromatic substrate was estimated in periodically withdrawn supernatant samples as release of chloride ion assayed spectrophotometrically by the mercury (II) thiocyanate method (Jeffrey et al. 1989) versus sodium chloride solution as standard. The washing procedures (above), and omission of Cl^- from all processing steps ensured a low background level of Cl^-, against which the Cl^- release was measured. The sensitivity of the method used was in the range 0.5–100 μg/ml Cl^-.

Chlorinated aromatic compounds

Chlorophenols were obtained from Sigma-Aldrich Co. Ltd., Fancy Road, Poole, Dorset, BH17 4QH, England. Polychlorinated biphenyls (PCBs):

4-chlorobiphenyl, 2,4,6-trichlorobiphenyl, 2,3,4,5-tetrachlorobiphenyl and 2,2′,4,4′,6,6′-hexachlorobiphenyl were obtained from Ultra-Scientific Inc., 250 Smith Street, North Kingstone, RI, 02852, USA. The chlorophenols were used as aqueous solutions diluted to the required concentration (usually 5 mM) in the medium. The PCBs were dissolved in hexane (1 mg/ml) and diluted to the required nominal concentrations (2.25 mM (80 μg/ml) as chloride; shaken to mix) as hexane suspensions in the medium. The ratio of PCB : Pd(0) was 4.2 : 1, 2 : 1, 1.6 : 1 and 1.3 : 1 respectively. The published solubilities of these PCBs in water (g/l at 20 °C) are, respectively, 1.46×10^{-3}, 2.39×10^{-4}, 1.39×10^{-5} and 2.27×10^{-6} (Li et al. 1992) but since no attempt was made to determine the extent of their partitioning into the aqueous phase in this comparative study the PCB : Pd(0) ratios are nominal values only.

Electron microscopy

The 'Bio-Pd(0)' was rinsed twice with distilled water (omitting the acetone wash and drying steps) and fixed in 2.5% (wt/vol) aqueous glutaraldehyde. The suspension was centrifuged, resuspended in 1.5 ml of Na-cacodylate buffer (0.1 M pH 7.2) and fixed in 1% osmium tetraoxide (60 min). The cells were dehydrated using ethanol (70, 90, 100, 100, 100% series; 15 min each step) and then washed twice for 15 minutes in propylene oxide and embedded in epoxy resin (degassed under vacuum for 30 min). The resin was then left for 24 hours at 60 °C to polymerise and sections (100–150 nm thick) were cut using a microtome, placed onto a copper grid and examined using a SJOEL 120CX2 Transmission Electron Microscope (TEM).

Results and discussion

Manufacture of bio-Pd(0) nanocatalyst

Example electron micrographs of Pd-loaded cells of *D. desulfuricans* are shown in Figure 1; similar results were obtained with *D. vulgaris* and *Desulfovibrio* sp. 'Oz-7'. The Pd(0) loading onto the biomass was obtained by calculation since the added bacterial mass and mass of Pd(II) were known and all of the Pd(II) was removed from solution by polarographic assay (Yong et al. 2002b) within the exposure period (biosorption and subsequent bioreduction under H_2). The concentration of Pd(II) in solution was determined spectrophotometrically with Sn(II) (Dasages, 1978). The extent of hydrogenase contribution is still conjectural; a pH of 2 during the biosorption step was found to be optimal in preliminary tests (probably being required to protonate biomass ligands in order to facilitate sorption of the $PdCl_4^{2-}$ species onto the polyanionic cell envelope); the stability of hydrogenase to low pH *in vivo* would be very difficult to measure. However, since the normal function of the enzyme is the cleavage of H_2 into $2H^+ + 2e^-$ it is likely that locally high concentrations of protons are found near the active site. Other tests showed that reduction of Pd(II) was sensitive to Cu(II) ions, a known inhibitor of hydrogenases (Lloyd et al. 1998). It is likely that the participation of hydrogenase is required to initiate ('seed') Pd(0) deposition on the cells; the reduction of Pd(II) to Pd(0) later becomes self-sustaining via the ability of crystalline solid Pd(0) to store and focus hydrogen as highly reactive $H^\bullet$, i.e., Pd(0) functions autocatalytically in the reduction of more Pd(II) (Yong et al. 2002b). In this respect the role of the hydrogenase is in the initiation of Pd(0) deposition and in 'directing' the cellular matrix template. Once the Pd-crystals are laid down the system behaves essentially chemically, with no contribution of cellular metabolic processes and with the reductive reaction catalysed by the Pd(0). Thus, the biomass behaves as a convenient immobilising matrix for the Pd(0) in a similar way to conventional supported metal catalysts.

Korte et al. (1997) documented the reductive dehalogenation of PCBs using a palladised Fe(0) surface. The H_2 liberated during chemical corrosion of Fe(0) and the entrapment of hydrogen within the Pd(0) matrix was thought by these authors to be to be the route of delivery of highly reactive $H^\bullet$ to the target PCB and this provides a rationale for $H^\bullet$ focusing via the 'Bio-Pd(0)' (Yong et al. 2002a, b). Thus the palladised biomass (Figure 1) is bifunctional, acting as both a support for the nascent Pd(0) nanocrystals and as a focus for the delivery of $H^\bullet$; effectively the Pd(0) loaded onto the bacteria behaves as a nanoscale version of the solid-state Pd-based alloy H-transfer membrane reactor described in Yong et al. (2002a). In addition to effecting homolytic fission of gaseous H_2, the Pd(0) catalyses the degradation of formate to release hydrogen, with the homolytic fission of nascent hydrogen within the crystal matrix (Rhodin et al. 1979). The electron donors used in this study were formate and hydrogen. Formate was routinely used since the accurate concentration was known and gas-liquid mass transfer limitations are avoided. In tests where hy-

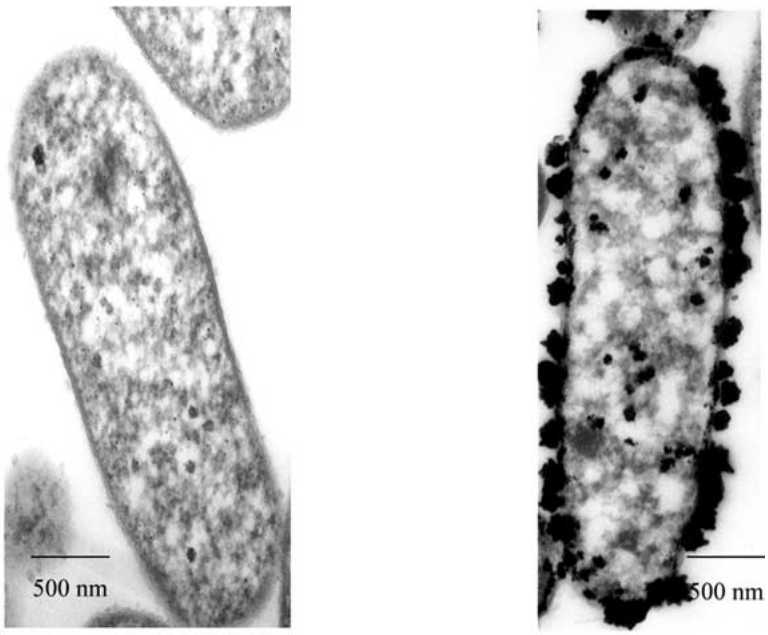

Figure 1. Cells of *Desulfovibrio desulfuricans* (a) without Pd(0) (unchallenged cells) and (b) loaded with Pd(0) at a dry Pd : biomass ratio of 1 : 1. Similar results were obtained using *D. vulgaris* and *Desulfovibrio* sp. 'Oz 7'.

drogen was used, it was maintained in excess in the headspace.

Dechlorination of chlorophenols

Initial tests used 5 mM 2-, 3- and 4-chlorophenol, with the rate of chloride release being slightly more rapid (~10%) using 2-chlorophenol, which was therefore used as the test substance for further study with 'Bio-Pd(0)' 1 : 1 (mass of Pd(0) : dry mass of cells) prepared from the three SRB strains and using formate as the electron donor. The controls included resting cells of the bacteria without bound Pd(0), Pd(0) prepared from Pd(II) by chemical reduction under H_2 and commercially available finely divided Pd(0).

Chloride release after 120 min was similar using chemical Pd(0), *D. vulgaris* and strain 'Oz-7' but was substantially higher, per mg of Pd(0), using *D. desulfuricans* (Figure 2). Chloride release catalysed by 'Chemical Pd(0)' was biphasic over 120 min for reasons that are not clear (note that reproducibility of data was within 5%) but the Pd(0) on the bacterial strains did not show this effect. No chloride liberation was promoted by any of the non-palladised bacteria with either electron donor (Figure 2). These results show that, overall, the catalytic efficacy of the *D. desulfuricans*, *D. vulgaris* and 'Oz-7'-based biomaterial against chlorophenol is equal to (*D. vulgaris*, 'Oz-7'), or higher than (*D. desulfuricans*) the chemical preparation (see Table 1). The extent of this superiority was related to the ratio of Pd(0) : cells; the lower the proportion of Pd, the higher the activity (I.P. Mikheenko, unpublished). It is not known whether the differences among the strains are a function of the cellular hydrogenase activity, the localisation of the enzyme, the cell surface support matrix or a combination these factors; this is currently under investigation. These studies are not trivial, largely due to the lack of well developed molecular biological methods in sulfate-reducing bacteria. In this respect *E. coli* could provide a better-defined model system and preliminary tests using *E. coli* have shown that this, too, can produce catalytically active Bio-Pd(0) which will form the basis of future tests.

Dechlorination of polychlorinated biphenyls

Little chloride release from PCBs was seen within 2 h, therefore the reaction time was increased to 24 h for these tests. Chloride release from 4-chlorobiphenyl, 2,4,6-trichlorobiphenyl and 2,3,4,5-tetrachlorobiphenyl using *D. desulfuricans* and *D. vul-*

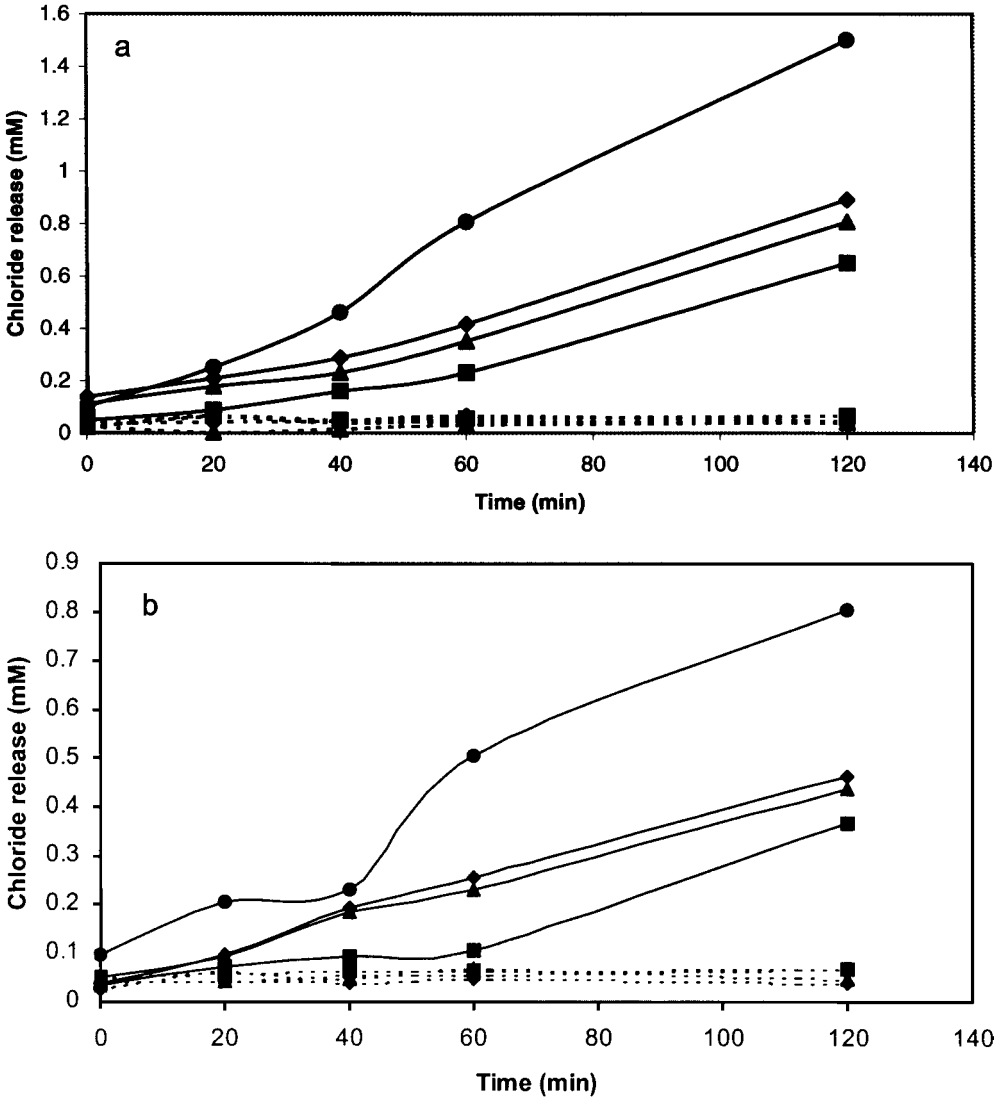

Figure 2. Reductive dehalogenation of 5 mM 2-chlorophenol using formate (a) or hydrogen (b) as the electron donor. 'Bio-Pd(0)', 'Chemical-Pd(0) and commercial reference material 'Ref-Pd(0)' were used (on a Pd(0) mass basis), and release of chloride was monitored as described in Materials and Methods. Solid lines show chloride liberation using: (●), *Desulfovibrio desulfuricans* 'Bio-Pd(0)'; (■), 'Chemical-Pd(0)'; (♦), *Desulfovibrio vulgaris* 'Bio-Pd(0)'; (▲), *Desulfovibrio* sp. 'Oz-7' 'Bio-Pd(0)'. Dashed lines are controls: (■), finely divided 'Ref-Pd(0)'; (●), *Desulfovibrio desulfuricans* cells alone (non-palladised); (♦), *Desulfovibrio vulgaris* cells alone (non-palladised); (▲), *Desulfovibrio* sp. 'Oz-7' cells alone (non-palladised). Data are means of two experiments, within 5% error.

Table 1. Rates of chloride release from 2-chlorophenol

	Chloride release (μmol/min/mg Pd)				
Compound	*D. desulfuricans* 'Bio-Pd(0)'	*D. vulgaris* 'Bio-Pd(0)'	*Desulfovibrio* sp. 'Oz-7' 'Bio-Pd(0)'	'Chemical-Pd(0)'	'Ref-Pd (0)'
2-chlorophenol	1.704 ± 0.12 (F) 0.879 ± 0.3 (H_2)	0.993 ± 0.26 (F) 0.505 ± 0.04 (H_2)	0.906 ± 0.17 (F) 0.466 ± 0.03 (H_2)	0.74 ± 0.62 (F) 0.393 ± 0.02 (H_2)	0.024 ± 0.015 (F) 0.03 ± 0.005 (H_2)

'Bio-Pd(0)' and its chemical counterpart were prepared as described in 'Materials and methods'. The loading of the Pd(0) onto the cells was at a mass ratio of 1 : 1 (Figure 1b) and samples were periodically removed for analysis of chloride released (electron donor was 10 mM formate (F) or hydrogen (H_2)). Data are means $\pm$ SEM from two experiments.

garis 'Bio-Pd(0)' was evaluated with formate as the electron donor; the dechlorination after 24 h and the corresponding rates (determined using 6 time points during 24 h) are shown in Tables 2 and 3. In each case *D. desulfuricans* and *D. vulgaris* cells alone (non-palladised biomass) showed negligible chloride release. In contrast to chlorophenol (above), the rate of chloride release from PCBs was greater throughout using 'Bio-Pd(0)' when compared with 'Chemical-Pd(0)' and 'Ref-Pd(0)'. Overall, the chloride release values after 24 h were similar for the mono-, tri- and tetra-chlorobiphenyls under attack with 'Bio-Pd(0)' derived from each organism, which is noteworthy, given that the water solubility of the PCBs becomes lower with increasing chloride substitution (nominally 2 orders of magnitude lower for the tetra- than for the mono-substituted compound: see above) and both 'Chemical-Pd(0)' preparations had approx. 3- to 4-fold less activity against the more highly substituted compound (Table 2), which suggests that the biological matrix facilitated access to the non-aqueous fraction. The possibility was considered that the recovered chloride was attributable to solubilisation of cellular materials by the hexane carrier. However no chloride release was promoted using cells challenged with an equivalent volume of hexane in the absence of PCBs. The relative molar proportion of the PCBs, for the mono-, tri- and tetra-substituted material was 1, 0.33 and 0.25, respectively. The applied concentrations were equivalent as chloride yet the Cl^- liberated after 24 h (not to completion) was approximately equal for each compound (Table 2).

The highest specific dechlorination rate was obtained using *D. desulfuricans* 'Bio-Pd(0)' with 2,3,4,5-tetrachlorobiphenyl (9 nmol/min/mg) where it was ∼30 times (on a mass of Pd(0) basis) greater than with 'Chemical-Pd(0)'. In the case of *D. vulgaris* 'Bio-Pd(0)' the corresponding increase was ∼6 times greater than with 'Chemical-Pd(0)' which was ∼2.5-fold slower than that obtained with the *D. desulfuricans* preparation (both at a 1 : 1 loading of Pd(0) : biomass). The choice of electron donor appeared to make little difference to the rate of chloride release (shown with each using 2,3,4,5- tetrachlorobiphenyl: Table 3) since Pd(0) can acquire hydrogen from formate (see above). It was assumed that H-availability is not rate-limiting, although this was not tested.

A final series of tests used 2,2′,4,4′,6,6′-hexachlorobiphenyl, where the rate of release of Cl^- promoted by the *D. desulfuricans* preparation was comparable to that from 2,4,6- trichlorobiphenyl despite the ca. 2 orders of magnitude lower water solubility of the former (Table 3; cf. above). At a nominal soluble concentration of 0.93 μmol/l and 0.006 μmol/l for 2,4,6-trichlorobiphenyl and 2,2′,4,4′,6,6′-hexachlorobiphenyl respectively, it is clear that the soluble Cl^- yield (8 nmol/min/mg Pd (0.183 mM in 24 h): see Table 3) substantially exceeds that nominally available in the aqueous phase and attack of the non-aqueous fraction is implied. However the water solubility increases with lower substitution (above) and since, as yet, it is not known whether 'Bio-Pd(0)'-mediated attack proceeds concurrently on all molecules or progressively on fewer, it is not possible to make these calculations without analysis of the dechlorination intermediates. The analytical studies form part of a follow-on study currently in progress.

Conclusions

The release of chloride ion from chlorophenols can be promoted using a new catalyst ('Bio-Pd(0)') which is superior to Pd(0) prepared by reduction under H_2 alone. Similarly, the reductive dehalogenation of polychlorinated biphenyls is achieved using 'Bio-Pd(0)' under conditions where 'Chemical-Pd(0)' is largely ineffective. The 'Bio-Pd(0)', when viewed via a transmission electron microscope, showed crystals whose size was too small to measure the whole population directly but other studies have showed the average crystal size to be ∼15 nm with a subpopulation of substantially smaller nanocrystals (Mikheenko et al. 2001). The catalysis may also be attributable to a 'templating' function of the cell surface; *Desulfovibrio desulfuricans* produced a more effective catalyst than two other SRB strains, which may be attributable to strain-specific differences in chemical composition, architecture and 3-D array of the functional groups supporting Pd(0) crystal nucleation and growth. In this respect other bacterial strains having hydrogenase activity, and a well understood genetic background, such as *E. coli*, may be good candidates for future study. The novelty of this approach lies in the fact that metabolic activity is only required for the initial manufacture of Pd(0) on the cells. Therefore, the use of this biomaterial in remediation is not hampered by a requirement for physiologically permissive conditions, which overcomes a major constraint of purely bioremediation processes.

Table 2. Extent of reductive dehalogenation of polychlorinated biphenyls after 24 hours

Compound	Water solubility of PCBs*	mM Cl^- in added PCB	Chloride release (mM)			
			D. desulfuricans 'Bio-Pd(0)'	*D. vulgaris* 'Bio-Pd(0)'	'Chemical- Pd(0)'**	'Ref-Pd(0)'
4-chlorobiphenyl	1.46×10^{-3}	2.25	0.217	0.104	0.051	0.031
2,4,6-trichlorobiphenyl	2.39×10^{-4}	2.25	0.183	NT	0.107	0.110
2,3,4,5-tetrachlorobiphenyl	1.39×10^{-5}	2.25	0.234	0.068	0.014	0.008

'Bio-Pd(0)' and its chemical counterpart were prepared as described in 'Materials and methods'. The loading of the Pd(0) onto the cells was at a mass ratio of 1 : 1 (Figure 1b) and samples were periodically removed for assay at 3–5 h intervals up to 24 h (electron donor was 10 mM formate). Data are means from two experiments with 5% error. NT: not tested.

* (g/l at 20 °C) (Li et al. 1992).

** Chloride release by 'Chemical-Pd(0)' was biphasic.

Table 3. Rates of chloride release from polychlorinated buphenyls

Compound	*D. desulfuricans* 'Bio-Pd(0)'	*D. vulgaris* 'Bio-Pd(0)'	'Chemical-Pd(0)'	'Ref-Pd (0)'
4-chlorobiphenyl	13.5 ± 1.2 (F)	5.8 ± 0.4 (F)	1.55 ± 0.6 (F)	0.95 ± 0.2 (F)
2,4,6-trichlorophenyl	8.3 ± 0.3 (F)	NT (F)	2.45 ± 0.2 (F)	2.45 ± 0.2 (F)
2,3,4,5-tetrachlorobiphenyl	9.3 ± 0.2 (F)	3.8 ± 0.5 (F)	0.3 ± 0.1 (F)	0.15 ± 0.1 (F)
	8.1 ± 0.5 (H_2)	NT (H_2)	NT (H_2)	NT (H_2)
2,2′4,4′,6,6′-hexachlorobiphenyl	8.12 ± 0.78 (H_2)	NT	1.58 ± 0.25 (H_2)	0.82 ± 0.75 (H_2)

'Bio-Pd(0)' and its chemical counterpart were prepared as described in 'Materials and methods'. The loading of the Pd(0) onto the cells was at a mass ratio of 1 1 (Figure 1b) and samples were periodically removed for analysis of chloride released (electron donor was 10 mM formate (F) or hydrogen (H_2)). Data are means ± SEM from three experiments. NT: not tested.

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