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Nanoparticles of palladium supported on bacterial biomass: New re-usable heterogeneous catalyst with comparable activity to homogeneous colloidal Pd in the Heck reaction☆

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ABSTRACT

The Heck coupling of iodobenzene with ethyl acrylate or styrene was used to assess the catalytic properties of biogenic nanoparticles of palladium supported upon the surface of bacterial biomass (bioPd), this approach combining advantages of both homogeneous and heterogeneous catalysts. The biomaterial was comparably active or superior to colloidal Pd in the Heck reaction, giving a final conversion of 85% halide and initial rate of 0.17 mmol/min for the coupling of styrene and iodobenzene compared to a final conversion of 70% and initial rate of 0.15 mmol/min for a colloidal Pd catalyst under the same reaction conditions at 0.5 mol.% catalyst loading. It was easily separated from the products under gravity or by filtration for reuse with low loss or agglomeration. When compared to two alternative palladium catalysts, commercial 5% Pd/C and tetraalkylammonium-stabilised palladium clusters, the bioPd was successfully reused in six sequential alkylations with only slight decreases in the rate of reaction as compared to virgin catalyst (initial rate normalised for g Pd decreased by 5% by the 6th run with bioPd catalyst cf. a decrease of 95% for Pd/C). A re-usable Pd-catalyst made cheaply from bacteria left over from other processes would impact on both conservation of primary sources via reduced metal losses in industrial application and the large environmental demand of primary processing from ores.

1. Introduction

The catalytic properties of palladium in C—C bond-forming reactions have been well known for many years and the development of both homo- and heterogeneous palladium-based catalysts still receives a great deal of attention (e.g. [1–6]). The use of nanoparticulate palladium in catalysis has been the source of much interest, due to its improved activity over the bulk metal and the very low loadings required to achieve acceptable rates of reaction (e.g. 0.001–0.1 mol.% loading [7,8]). Much of the focus involves the use of complexes or stabilisers to prevent the nanoparticles (NPs) from aggregating to form larger, less active particles or palladium-black precipitate. A traditional approach for transition metal NP preparation involves the use of a metal salt, reductant and a molecular stabiliser. In this method, electropositive NPs are surrounded by a layer of anions (e.g. chloride) which is, in turn, surrounded by a layer of bulky cations, such as tetra-N-alkylammonium [9]. This arrangement is reversed under certain circumstances, for example with imidazolium-based stabilisers where cations surround the NPs [10]. Other approaches used to prevent agglomeration include pincer-type Pd^{4+} complexes [11] (which are generally considered as reservoirs of palladium clusters rather than the actual active catalyst), functionalised ionic liquids [12], supports such as carbon nanotubes [13], silica [14] or alumina, [15,16] and the use of polyvinyl pyrole as a stabiliser [17]. Ionic liquids have been shown to be particularly useful in Heck couplings, serving as both solvent for the reaction and a stabiliser of metal nanoparticles [18,19].

Although these methods can generate stable, catalytically active, palladium clusters of narrow size distributions they generally involve multi-step synthesis, starting from palladium salts, and there is often a compromise between high activity and stability. For example, surfactant-stabilised colloids of palladium particles are more stable when used as sterically hindered cations but, as the metal is more effectively shielded from substrates, the reaction rate is adversely affected. Importantly, stabilised Pd-nanoparticles

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behave essentially as homogeneous catalysts (i.e. in the same phase as the reactants) and hence are difficult or impossible to recover economically.

A new approach, immobilisation of metallic NPs on bacterial surfaces (see [20–22] for reviews), combines the advantages of NPs (high surface area and reactivity) with micron-sized supporting entities (bacterial cells) that present a NP array which is recoverable under gravity and also reduce potential environmental and health risks associated with handling free NPs. This approach gives high-activity catalysts [23] or, in the case where a bacterial self-adhered biofilm is used as support, the option to use an immobilised catalyst in a stable, continuous flow-through system [24–26] while still retaining the nanoparticle format and advantages. Individual bacterial-Pd NPs are held in a stable conformation by their location at the cell surface held within a thin coat of residual biomaterial [27] which prevents the agglomeration problems that beset traditional NP manufacture while still permitting substrate access and fast reaction rates. Importantly, the use of waste bacteria from primary fermentation processes as the means to make immobilised Pd catalysts for (e.g.) fuel cell [28] or hydrogenation [29] catalysts points the way to future green chemistry via biotechnology.

In this communication we report the preparation of NPs of palladium supported upon cells of *Desulfovibrio desulfuricans* (bioPd) and their use as a recoverable catalyst for one of the most important carbon—carbon bond forming reactions, the Heck reaction, which is a generic model for platform chemical synthesis.

The use of bacterial biomass also offers an environmentally benign, cost effective and straightforward route to supported nanoparticulate palladium catalysts. The support itself (bacterial cells killed during the palladisation process) is non-toxic and biodegradable. Furthermore, the precious metal can be acquired from secondary sources (such as used catalysts or electronic scrap) and bioconverted to active catalyst [16,30]. The cell-bound metal new catalyst from waste can be reclaimed by settlement under gravity and then destroying the biomass via incineration or indeed acid hydrolysis to recover soluble metal for further use.

The Heck reaction is usually catalysed by soluble Pd-complexes. The coupling of iodobenzene with two alkenes, ethyl acrylate and styrene, is used in this study as a test reaction to evaluate the catalytic potential of the bioPd catalyst in parallel with catalytic tests using a conventional heterogeneous catalyst (5% Pd on carbon) and nanoparticles of palladium stabilised as colloids by tetraalkylammonium salts. These colloidal palladium clusters were shown to be highly active in carbon-carbon coupling reactions and are relatively simple to prepare from $Pd^{(II)}$ complexes [31–34]

Although both homogeneous and heterogeneous palladium catalysts are active in the Heck reaction, it is now generally agreed that the reaction is catalysed by solubilised palladium particles, either released from complexes/colloids or leached from the surface of an insoluble support. For example Pröckl et al. [7] monitored the concentration of palladium in solution during the Heck coupling of iodobenzene with styrene catalysed by heterogeneous palladium catalysts supported on alumina and titania, finding a clear correlation between conversion and the amount of solubilised metal. Notably, below the reaction temperature, very little metal was found in solution, with correspondingly no conversion, whereas at 140 °C approx. 30% of the palladium was leached from the support, accompanied by an increase in conversion, during which virtually all the halide reacted; subsequently the soluble palladium concentration returned to zero and conversion ceased. A large number of studies and reviews on the nature of the active catalyst in Heck couplings exist in the literature, such as those by De Vries, Jones, Dupont and Gómez [18,33,35,36]. Most authors come to the same conclusion that, atleast for reaction temperatures above 120 °C, a dynamic equilibria exists between nanoparticulate palladium (catalyst precursor) and dissolved molecular palladium (actual catalyst).

Thathagar et al. [37] provided corroborative evidence using a divided membrane reactor (5 nm pores). Reactants and an insoluble base were added the front-side whilst the catalyst (Pd clusters of ~14 nm in diameter) was added to the back-side. Initially no product was detected in either side but thereafter product rapidly accumulated in the side of the reactor containing base, hence proving that the Heck coupling of iodobenzene and butyl acrylate was catalysed by Pd species of <5 nm leached from the surface of larger clusters. It was not clear, however, whether these species were Pd^0 atoms released from defect sites or soluble Pd^{II} species formed after oxidative addition of PhI. In related work, Gómez et al. carried out Heck couplings in a continuous flow membrane reactor at room temperature with palladium nanoparticles stabilised by oxazolinyl-phosphite ligands. Their results proved that the actual active catalyst was leached molecular palladium species containing oxazolinyl-phosphite ligands [36]. For heterogeneous catalysts it is possible that not only the morphology of the palladium particles but also their position on the support and the nature of the material upon which they are supported affect the reaction rate and selectivity. The acidity of the support affects the electronic environment of the supported metal; changing from a basic support, such as magnesia, to an acidic one, such as silica, can lead to substantial differences in product selectivity [38]. Carbonaceous supports are generally acidic due to the presence of phenolic and carboxylic groups on their surfaces. In a similar way, the Gram negative bacterial surface [39] comprises carboxyl-rich extracellular polymeric materials (EPM) located exterior to the outer membrane layer (and usually crosslinked via Ca(II) bridges) and, beneath this, a peptidoglycan layer comprising a gel of n-acetyl glucosamine and n-acetyl muramic acid containing both amine groups for Pd(II) coordination and carboxyl groups for support; both carboxyl and amine groups were confirmed as implicated in the binding of Pd(II) within the bacterial cell surface layers [40] prior to the formation of Pd-NPs following nucleation at these loci.

As in alkene hydrogenation/isomerisation, arylation occurs on co-ordinatively unsaturated corner atoms and adatoms of supported palladium, rather than metal atoms located within the face or edge [38]. Therefore, if the metal particles are small and irregular in shape, they will have a greater proportion of corner and adatoms which would lead to an increase in catalytic activity. As biogenic Pd-NPs are only a few nanometers in diameter [23,41] good activity in the Heck reaction is anticipated.

The first objective of this study was to compare the efficacy of bioPd and more traditional Pd-catalysts in model Heck reactions with respect to the reaction rate and stability in reuse. Since bio-Pd can be regarded as an intermediate between heterogeneous and homogeneous catalysts with the attributes of both [23] the ability to recover and re-use the catalyst formed the second objective of the work which, if successful, has the potential to reduce reliance on primary sources and hence the environmental damage of primary ore refining [30], while also providing an application for waste bacteria [28,29] which could otherwise generate CH4 (a potent greenhouse gas) via landfill.

2. Materials and Methods

2.1. Preparation of bioPd and chemical Pd(0) catalysts

Desulfovibrio desulfuricans NCIMB 8307 was as described previously [20,23]. For bioPd manufacture, cells were grown in 2 L of medium inoculated using an overnight preculture (10% (v/v)) grown in the same way. Mid-logarithmic phase cultures (OD_{600} = 0.5–0.7) were harvested by centrifugation (12,000 g,

15 min), washed three times in 100 mL of MOPS–NaOH buffer (20 mM, pH 7.2), resuspended in 50 mL of the same buffer and stored at 4 °C as concentrated cell suspensions until use, usually the next day. Cell concentration (mg/mL) was determined by correlation to a pre-determined OD_{600} to dry weight conversion.

Bacteria were palladised as follows. A known volume of the concentrated resting cell suspension was transferred under N_2 into 200 ml serum bottles and an appropriate volume of degassed 2 mM Pd(II) solution was added so that the final ratio (weight of Pd:dry weight of cells) was 1:19 to give a final loading of 5% Pd on biomass, or as otherwise stated. Cells/Pd mixtures were left to stand (30 min, 30 °C) with occasional shaking to promote biosorption of Pd(II) complexes before H_2 was sparged through the suspension for at least 10 min (0.2 atm) to reduce cell surface-bound Pd(II) to Pd(0) (complete removal of Pd(II) onto cells was confirmed by assay). The Pd(0)-coated biomass was harvested by centrifugation (3000 g, 10 min, 25 °C) and washed three times in distilled water (dH_2O). The black precipitate was washed in copious amount of acetone and left to dry in air overnight. BioPd powder was finely ground in a mortar and tested for catalytic activity without any further processing.

For chemical Pd catalysts NanoPd colloids stabilised by tetrabutyl ammomium bromide were prepared as described by Reetz and Maase [31]. 5% Pd on carbon (Pd/C) catalyst was as described previously [23].

2.2. Pd(II) solution and assay of Pd(II)

Pd(II) solution (2 mM, pH 2.3) was made by dissolving an appropriate amount of sodium tetrachloropalladate (Na_2PdCl_4, Sigma–Aldrich, Poole, UK) in 0.01 M HNO_3.

Following addition of cells to the Pd(II) solution (above), the concentration of residual Pd(II) ions in sample supernatants was monitored at all stages of bioPd preparation using the spectrophotometric method of Charlot [42] using timed samples (1 ml) withdrawn and separated (12,000 g, 4 min, IEC Centra M bench centrifuge). Sn(II) reagent was made by dissolving 29.9 g of $SnCl_2$ powder into 500 ml of concentrated HCl. For Pd(II) assay, 200 μl of sample was added to 800 μl of $SnCl_2$ solution (1.5 ml plastic cuvette) and A_{463} was determined after 1 h against a blank prepared in the same way. The system obeyed Beer's law over the range 5–80 ppm Pd(II). The assay method was validated polarographically [43] (see Mikheenko, 2004) and by analysis of reference and selected test samples by a commercial laboratory (H2b, Capenhurst, UK).

2.3. Separation of bioPd nanoparticles from bacteria

To separate Pd NPs of approximately 4 to 12 nm in size from larger clusters associated with bacterial cells, 5 ml of bioPd suspension in water was combined with an equal volume of phenol:chloroform:isoamyl alcohol 25:24:1 mixture (Sigma–Aldrich), blended on a vortex mixer for 3 min, transferred into a dividing funnel and left for 1 h for separation. A gray layer of protein-bound nano-Pd was formed (confirmed by protein assay, in parallel to extracts of Pd-unsupplemented cells) on the interphase of the biphasic aqueous-organic mixture. This protein-bound nano-Pd was separated and re-suspended in distilled water. The yield was too low to permit catalytic testing but the samples were examined under electron microscopy.

2.4. Transmission electron microscopy (TEM) of Pd-loaded bacteria and bioPd NPs

Pellets of Pd-loaded bacteria (10% w/w Pd on biomass) were rinsed twice with distilled water, fixed in 2.5% (wt/vol) glutaraldehyde, centrifuged, resuspended in 1.5 ml of 0.1 M cacodylate buffer (pH 7) and stained in 1% osmium tetroxide in 0.1 M phosphate buffer, pH 7 (60 min) for transmission electron microscopy (TEM). Cells were dehydrated using an ethanol series (70, 90, 100, 100, 100% dried ethanol, 15 min each) and washed twice in propylene oxide (15 min, 9500 g). Cells were embedded in epoxy resin and the mixture was left to polymerize (24 h; 60 °C). Sections (100–150 nm thick) were cut from the resin block, placed onto a copper grid and viewed with a JEOL 1200CX2 TEM; accelerating voltage 80 kV. Individual separated bioPd NPs (see below) were viewed using a Philips TECNAI F20 transmission electron microscope operating at 200 kV. The TEM samples were prepared by dropping 5 μl of suspension of protein-bound nano-Pd particles onto the TEM grid and drying it in air.

2.5. X-ray powder diffraction analysis

Powdered samples of Pd-loaded biomass, prepared as described above, were used for XRD analysis. X-ray powder diffraction patterns were acquired as described by [44] Creamer et al. using a Siemens D5005 diffractometer using monochromatic high-intensity CuKα radiation (λ = 0.1240598 nm). The powder pattern was compared to references in the Joint Committee for Powder Diffraction Studies (JCPDS) database. The particle size of the cell-bound Pd NPs was estimated using Scherrer's equation [45].

2.6. Evaluation of catalytic activity via Heck coupling in solvents

The arylation and alkenylation of alkenes over a palladium catalyst (C—C bond formation; Heck coupling) has the overall reaction scheme:

The reaction scheme for the Heck coupling of phenyl iodide (PhI) and styrene and PhI and ethyl acrylate as used in this study is:

The general procedure for the catalytic tests was as follows: a 50 mL two neck round bottom flask equipped with condenser and magnetic stirrer was charged with phenyl halide (10 mmol), base (15 mmol), DMF (or as stated; 40 mL) and Pd catalyst under nitrogen. The mixture was heated to 120 °C, the olefin (ethyl acrylate or styrene; 15 mmol) was added and the mixture was stirred (750 rpm, 120 °C) for up to 5 h under N_2. To determine reaction completion and rate samples were removed periodically for analysis by high-performance liquid chromatography using a Dionex Summit HPLC (with Chromeleon software) with a Summit p580 quaternary low-pressure gradient pump, Summit UVD 170s UV/vis multichannel detector with analytical flow cell, and a Phenomenex Luna 10u C18 (2) column, 250 mm × 4.6 i.d. A flow rate of 1 mL/min was used with a solvent gradient of 100% water to 100% acetonitrile (MeCN) over 40 min, held for 10 min, and then back to 100% water for 10 min.

3. Results and discussion

3.1. Examination of bioPd samples by electron microscopy and X-ray diffraction

At a loading of 1:19 individual nanoparticles on biomass are difficult to discern. Samples loaded to 1:9 (25% by weight) showed that whereas individual native cells were uniform in appearance (Fig. 1a), their palladised counterparts (Fig. 1b) showed electron small opaque deposits identified as Pd(0) by energy dispersive X-ray microanalysis (not shown) and with (at a loading of 1:19) a population of nanoparticles of Pd(0) identified by X-ray powder diffraction [44] with a nanoparticle size calculated in this study of ~3.1 nm using the Scherrer equation (J. Omajali and L.E. Macaskie, unpublished) which is in agreement with the ~4.7 nm

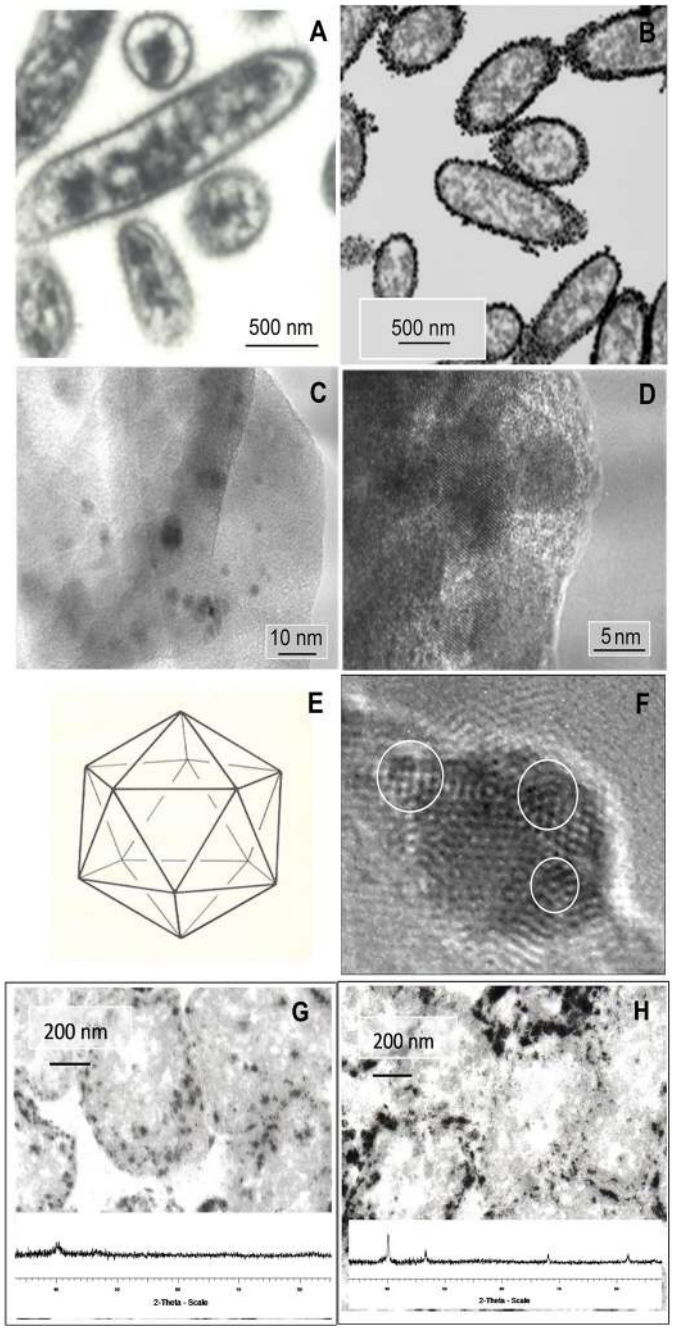

Fig. 1. Cells of *D. desulfuricans before* (A) and after (B) palladisation. In order to visualise the small nanoparticles (arrowed) the cells were loaded to 1:9 (Pd:biomass; 25% mass Pd(0)). Bar is 500 nm. Photos courtesy of P. Yong. (C and D) Single Pd-nanoparticles within extracted protein matrix. At the higher magnification (D) detail becomes apparent (E) icosahedron geometry. (F) A single Pd-nanoparticle extracted from the cells. (G) Fresh, dried 5 wt.% bioPd with XRD pattern inset. (H) Dried 5 wt.% bioPd after 6 uses in the Heck reaction with XRD pattern inset.

reported previously by [23] Creamer et al. using a magnetic method.

Examination of a single bioPd nanoparticle (Fig. 1c) gave a size by measurement of ~5 nm. Fast Fourier transformation and analysis of reflexes showed that particles have a fcc crystal structure. Values for the (1 1 1) and (2 0 0) planes and angles show distortion of up to 10% compared to the theoretical values for bulk palladium. Analysed particles are probably icosahedral. Further structural studies of the bio-Pd crystals will be reported in full at a later date but it is important to note that additional crystal planes were visible superimposed on the surface (circled) that show a potentially larger number of key sites than those potentially available from a perfect geometry.

3.2. Comparison of bioPd and chemical catalysts in the Heck reaction

An initial series of experiments used both colloid-stabilised and biomass-supported nanoparticulate palladium, to determine the optimum reaction conditions for the Heck coupling of iodobenzene with two model substrates, ethyl acrylate (Table 1) and styrene (Table 2). Initially sodium acetate was used as a base but, since this resulted in low halide conversion, triethylamine was used for all subsequent reactions.

The initial survey using ethyl acrylate (Table 1) showed that 25% mass bio-Pd generally compared well against the colloidal nanoparticles with reaction rates similar to those obtained with the colloidal nanoparticles. Pd-free biomass gave no conversion (Table 1). When styrene was substituted for ethyl acrylate (Table 2) bioPd (25%) was similarly comparable to the colloidal Pd in terms of final conversion, while decreasing the Pd loading to 5% by mass gave enhanced conversion (Table 2). The respective initial rates for colloidal Pd, bioP (25%) and bioPd (5%) were 28, 25 and 30 mmol/min/g catalyst.

It has been previously reported that using low catalyst loadings can benefit reaction rates of carbon-carbon coupling reactions [8] and that increasing the amount of catalyst above a critical point actually reduces turnover number and turnover frequency. This is attributed to the increased agglomeration of the nanoparticles into larger, less active clusters. [8]. Therefore, the experiments in this study were carried out using varying catalyst loadings of 2, 1 and 0.5 mol.% relative to moles of halide. In the styrene coupling with colloidal catalyst, all the catalyst loadings gave similar initial rates and final conversions (Table 2). In the ethyl acrylate coupling with colloidal catalyst, the optimal loading was found to be 1 mol.% catalyst (Table 1). It is proposed that a catalyst loading of 1 mol.%, under the experimental conditions used here, uses the optimal amount of palladium to supply optimal catalyst surface area. For the bio-Pd catalyst (25% by mass), there was little change in rate when lowering the loading from 1 to 0.5 mol.%, possibly suggesting a higher stability against agglomeration of the biomass-supported particles than the colloid stabilised particles, but this was not tested.

These results confirm that bioPd is an active catalyst for the Heck coupling and that the biomass acts as support material. Possible modifications of the catalytic activity via the biochemical 'tethers' were not investigated or precluded. Inspection of Fig. 1C shows that while one edge of the Pd-NP is clearly defined the other is not, such that the limit of the NP is difficult to delineate and the edge region may form an additional reactive site(s) in addition to the surface features circled. A previous study [46] using bioPd as the reductant for Au(III) clearly suggested the formation of soluble Pd(II); Pd was observed in a shell around Au(0) and also outside the Au/Pd core-shell NPs, in the surrounding matrix. In accordance with the literature, a mechanism that relied on cycling between Pd(0), Pd(II) and back to Pd(0) was suggested (see [46] Deplanche et al., 2012). In the published study the growing NP 're-recruited' Pd under H_2. The exact mechanism of Pd cycling with respect to the biomatrix contribution in the Heck reaction remains to be established.

3.3. The effect of solvent on the Heck conversion

In a second series of tests, various solvents of varying polarity and coordination strength were assessed for the coupling reactions, using a catalyst loading of 0.5 mol.% palladium (Table 3). Of these, the amine dimethyl formamide (DMF) gave the highest activities,

Table 1
Heck coupling of iodobenzene and ethyl acrylate after 4 h.

Catalyst	Catalyst loading (mol.%)	Base	Final conversion (%)	Initial rate (mmol/min/g Pd)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	1	NaOAc	52.4	4.6 (0.051 mmol/min)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	0.5	NEt_3	88.0	29 (0.16 mmol/min)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	1	NEt_3	99.5	24 (0.26 mmol/min)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	2	NEt_3	96.8	9.0 (0.20 mmol/min)
BioPd (25 wt.% Pd)	0.5	NEt_3	95.5	28 (0.15 mmol/min)
BioPd (25 wt.% Pd)	1	NEt_3	93.5	11(0.12 mmol/min)
			98.7 (double base)	26 (0.29 mmol/min)
			98.7 (double olefin)	21 (0.23 mmol/min)
Non-metallalised Biomass	–	NEt_3	<1	

Initial rates are for first 30 min of reaction and are normalised for the mass of catalyst present (values in brackets are rates not normalised for amount of catalyst); NaOAc: Sodium acetate; NEt_3: triethylamine. Double base: twice the amount of triethylamine was added. Double olefin: twice the amount of ethyl acrylate was added

Table 2
Heck coupling of iodobenzene and styrene after 5 h.

Catalyst	Catalyst loading (mol.%)	Base	Final conversion (%)	Initial rate (mmol/min/g Pd)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	1	NaOAc	52.8	8.3 (0.091 mmol/min)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	0.5	NEt_3	69.9	28 (0.15 mmol/min)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	1	NEt_3	71.0	14 (0.15 mmol/min)
Pd $(Bu)_4NOAc$ (~33 wt.% Pd)	2	NEt_3	67.9	7.7 (0.17 mmol/min)
BioPd (25 wt.% Pd)	0.5	NEt_3	74.6	25 (0.14 mmol/min)
BioPd (25 wt.% Pd)	1	NEt_3	73.4	12 (0.13 mmol/min)
BioPd (5 wt.% Pd)	0.5	NEt_3	84.9	30 (0.17 mmol/min)

Initial rates are for first 30 min of reaction and are normalised for the mass of catalyst present (values in brackets are rates not normalised for amount of catalyst); NaOAc: sodium acetate; NEt_3: triethylamine.

Table 3
Heck coupling of iodobenzene and ethyl acrylate in various solvents after 2 h.

Catalyst	Solvent	Final conversion (%)	Initial rate (mmol/min/g Pd)
NanoPd colloids	DMF	81.74	29 (0.16 mmol/min)
5 wt.% bioPd	DMF	96.42	57 (0.31 mmol/min)
NanoPd colloids	MeCN	6.89	0.67 (0.0037 mmol/min)
5 wt.% bipod	MeCN	2.03	0.091 (0.00050 mmol/min)
NanoPd colloids	DMSO	16.60	29 (0.16 mmol/min)
5 wt.% bipod	DMSO	47.79	49 (0.27 mmol/min)
NanoPd colloids	Toluene	4.38	0.72 (0.0040 mmol/min)
5 wt.% bipod	Toluene	5.83	1.5 (0.0083 mmol/min)
NanoPd colloids	Ethyl acetate	1.74	0.59 (0.0032 mmol/min)
5 wt.% bipod	Ethyl acetate	0	0

Initial rates are for first 30 min of reaction and are normalised for the mass of catalyst present (values in brackets are rates not normalised for amount of catalyst); DMF: dimethyl formamide; MeCN: acetonitrile; DMSO: dimethyl sulfoxide. Catalyst loading was 0.5 mol.% Pd (c.f. Table 1)

with all other solvents leading to unacceptably low conversions. This is in agreement with the published work involving palladium-catalysed Heck coupling [47]. Notably, the rate of reaction in DMSO was higher when using bio-Pd than the colloid-stabilised particles, with a final conversion of 48% compared to 17% (Table 3), while in DMF the conversion was >95% with bio-Pd as compared to only 82% using the colloidal material.

The solvent selection may affect reaction rates by altering the degree of palladium nanoparticle solubilisation (assuming this to be the rate-limiting step). In their study of heterogeneous palladium catalysts for the Heck reaction, Kohler et al. [47] found that nonpolar solvents promoting a low amount of palladium leaching, such as THF and toluene, gave low reaction rates for the Heck coupling of bromoacetophenone and styrene. However, the solvent selection was found to be only one of several factors affecting the degree of palladium leaching. The reaction temperature and wt.% palladium content of the supported catalyst were also found to strongly influence leaching and hence activity in the Heck reaction [47].

3.4. Recovery and re-use of the bioPd(0) and chemical catalysts

BioPd (5%) as used in the coupling of iodobenzene and ethyl acrylate was recovered and recycled to determine if the catalyst maintained its activity over multiple reaction cycles. At the end of each test, the bioPd was filtered from the mixture, washed and dried as before then reused in a further five successive reactions. The catalyst remained active in all tests with only a small decrease

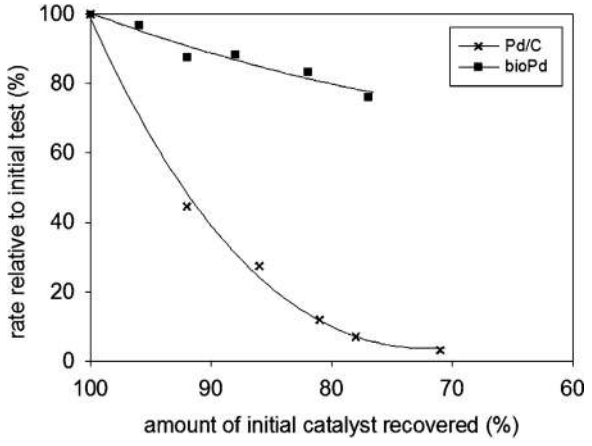

Fig. 2. Initial reaction rate of halide conversion relative to first run vs amount of initial catalyst recovered for six sequential Heck couplings with bioPd and Pd/C.

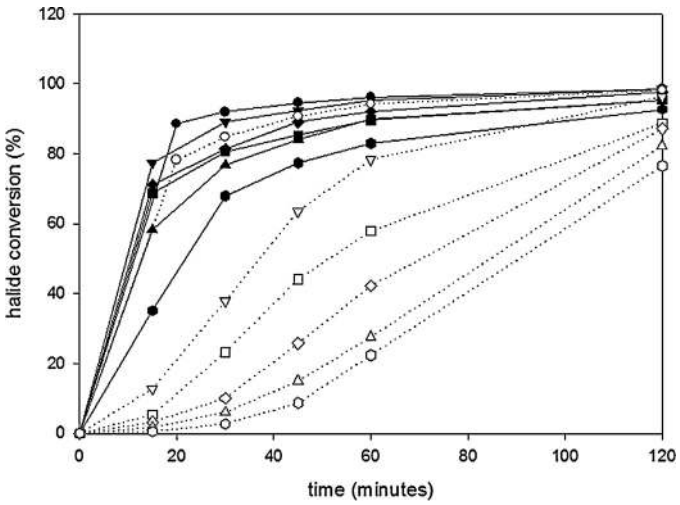

Fig. 3. Halide conversion versus time in each sequential experiment (PhI and ethyl acrylate) for bioPd(0) (filled symbols) and Pd/C (open symbols) catalysts. Sequential experiments were as follows: 1 (●), 2 (▼), 3(■), 4 (♦), 5 (▲) 6 (⬣).

in rate (Fig. 2) with a linear relationship between the reductions in the rate, relative to the initial rate of the first reaction. The reaction mixture was analysed by ICP after removal of the solid by filtration, with a negligible amount of Pd found, indicating that the slight decrease in rate over the tests was not due to leaching of palladium from the support.

A commercial 5% Pd/carbon catalyst was also tested for comparison (Figs. 2 and 3) Although still active during the sixth test, the series of reactions showed a greater decrease in overall reaction rate with, notably, a delay before onset of halide conversion with the recycled material, progressively increasing with the number of cycles (Fig. 3). The mass of catalyst lost in each recycling step was higher for Pd/C than for bio-Pd (Table 4). However, this seems unlikely to be the only reason for the fall in activity as the amount of material lost was not proportional to the fall in activity. To confirm this, initial rates after 30 min of reaction were normalised for total amount of palladium present in each test. Using bioPd the rate was little affected when normalised for the mass of palladium. However, the normalised initial rate decreased with each successive test when using the Pd/C catalyst (Table 4). Hence, the difference between the two systems is not solely due to greater mechanical losses of Pd/C catalyst during the recycling steps.

The correlation between the loss of catalyst material in each recycling step and the decrease in catalytic activity was not strong ($r^2 = 0.87$).

Another possible reason for the decrease in conversion found with the Pd/C catalyst is the difference in the metal dispersion over the support and the resulting average metal particle diameter. Although the morphology of the bio-NPs was not examined in great detail (see above and Fig. 1), the larger palladium particles in the Pd/C will have (by virtue of their large size alone) a lower proportion of corner and adatoms which are responsible for the catalysis. It is likely that the chemical Pd on carbon progressively agglomerates during each reaction. Increased metal particle size, due to agglomeration or ripening during reprecipitation of the palladium onto the support after catalysis, is a likely explanation for the decrease in activity over the 6 successive tests [47]. The Pd/C catalyst is expected to deactivate by this method at a faster rate than the bio-Pd catalyst as the average metal particle diameter is much greater for the fresh catalyst and the effect is therefore exaggerated. In related work, Kohler et al. [47] found that the average particle size of palladium supported on activated carbon increased by an order of magnitude, from 2.4 to 23 nm, after only a single use in the Heck reaction. Also, these larger particles aggregated to from grape-like structures, reducing the dispersion of metal over the support and leading to formation of an 'egg shell' catalyst. When used for a third sequential Heck coupling, the recycled catalyst gave an even lower reaction rate, indicating a further reduction in available metal surface area on the Pd/C, although XRD size data was not reported [47].

In contrast, a sample of used bioPd catalyst after 6 sequential catalytic tests was analysed by X-ray diffraction; the average Pd diameter had increased ~5-6-fold to 20 nm after 6 successive uses (not shown). This confirms that biomass-supported nanoparticles are more resistant to agglomeration/growth than those supported on activated carbon. The stabilisation using the biological support is suggested to arise from the fact that the nanoparticles grow within the cell membrane, eventually erupting through it when they reach a sufficient size. As a part of the particles are rooted within the residual biomass structure this may restrict reordering of the metal over the support. Other studies using Pt on carbon catalysts have shown the influence of functional groups on the carbon support [see 48] and it is likely that the residual contributes in a similar way, e.g. via surface oxygen-containing functional groups (for example carboxyl groups) that may act as anchoring points for metallic nanoparticles, limiting their growth [48]. In this context, cells of *Desulfovbrio fructosovorans* containing periplasmic hydrogenases showed corresponding localisation of small nanoparticles of bioPd within the N-acetyl muramic acid-rich matrix of the periplasmic gel layer, while a mutant stripped of these hydrogenases relocated its Pd(0) to the inner membrane site of the residual hydrogenases with correspondingly larger Pd-NPs visible associated with the lipid membrane layers [49].

It should be noted that although it is possible to prepare particles of palladium on carbon supports with a size range comparable to that of the bio-Pd, the methods involved, such as vapour grafting [50], require more extreme conditions and specialist equipment compared to that required for growing cultures of bacteria. Other work has shown that bacteria recycled from other fermentations can provide a cheap source of biomass for palladisation to make active catalyst [28,29].

Separation and reuse of the tetrabutylammonium bromide Pd-colloids was also attempted by filtration. Although the material

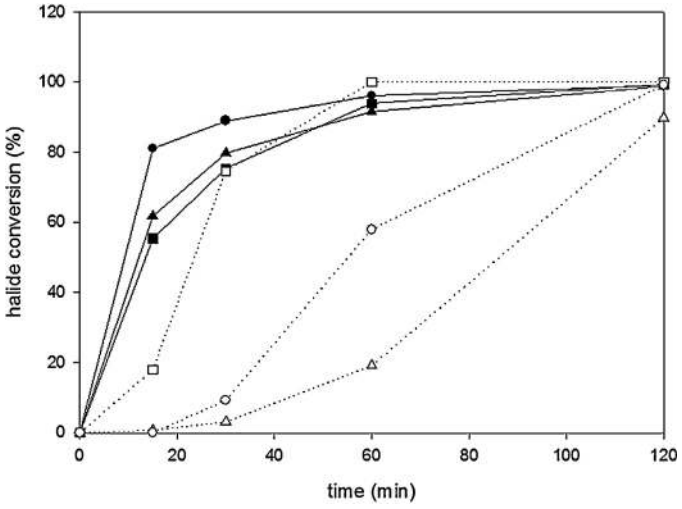

Fig. 4. Halide conversion versus time (PhI and ethyl acrylate) in sequential experiments 1 (filled symbols) and 2 (open symbols) for colloidal Pd catalyst at loadings of: 33 wt.% Pd colloids, 1 mol.% loading: (■); 33 wt.% Pd colloids, 0.5 mol.% loading (▲); 80 wt.% Pd colloids, 0.5 mol.% loading (●).

Table 4
Summary of catalysts activities after recovery and on re-use.

Catalyst	Sequential test	Final conversion (%)	Mass recovered per run (%)	Total mass recovered (%)	Initial rate (mmol/min/g Pd)
5% bioPd	1	99	96	96 (106 mg)	56 (0.31 mmol/min)
	2	99	95	92 (101 mg)	56 (0.30 mmol/min)
	3	96	96	88 (97 mg)	53 (0.27 mmol/min)
	4	98	93	82 (90 mg)	56 (0.27 mmol/min)
	5	95	94	77 (85 mg)	57 (0.26 mmol/min)
	6	93	–	–	53 (0.22 mmol/min)
5% Pd/carbon	1	98	92	92 (101 mg)	51 (0.28 mmol/min)
	2	97	94	86 (95 mg)	25 (0.13 mmol/min)
	3	89	94	81 (89 mg)	16 (0.075 mmol/min)
	4	87	95	78 (85 mg)	7.6 (0.034 mmol/min)
	5	82	92	71 (78 mg)	4.7 (0.020 mmol/min)
	6	77	–	–	2.3 (0.0090 mmol/min)
33 wt.% Pd colloids	1	99	6		24 (0.13 mmol/min)
	2	90	–		ND
33 wt.% Pd colloids	1	99	16	16	23 (0.13 mmol/min)
(1 mol.% catalyst)	2	99	–		ND
80 wt.% Pd colloids	1	99	27	27	27 (0.15 mmol/min)
	2	99	–		ND

Initial rates are for first 30 min of reaction and are normalised for the mass of catalyst present (values in brackets are rates not normalised for amount of catalyst); ND: not determined.

remained active in the coupling reaction during its second use, the conversion with time was reduced substantially in the second test (Table 4; Fig. 4) and the mass of material recovered was so low that further use in a third test was not practical.

4. Conclusion

Nanoparticles of palladium supported on the surface of bacterial biomass (bio-Pd) are active, recyclable catalysts for the Heck coupling of ethyl acrylate and iodobenzene in dimethylformamide. The bio-Pd activity compared well to that of a commercial Pd/C catalyst and colloidal palladium clusters stabilised by tetralkylammonium salts. Final conversions of halide and initial rates of reaction with the bioPd were generally equal to or superior than both Pd/C and colloidal Pd. The bio-Pd can be easily separated from liquid-phase products and reused, with only a small decrease in activity over 6 successive tests (5% decrease in initial rate when normalised for Pd). Based on X-ray diffraction data of used catalyst, the degree of metal particle growth or aggregation was reduced using the biological support material as opposed to conventional supports such as activated carbon. Thus, this biogenic supported-catalyst offers a viable and economic alternative to 'once-through' homogeneous palladium complexes, which are more difficult to separate from products, and also conventional heterogeneous palladium catalysts, such as palladium on carbon, which deactivate more rapidly under the conditions used. As well as giving greater economy and higher efficiency, the biogenic material reduces the need for purification of the product stream from contaminating catalyst and also demand on primary resources.

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