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Biomining of Pd nanoparticles using *Phanerochaete chrysosporium* as a sustainable approach to turn platinum group metals (PGMs) wastes into catalysts

Sophie Tarver, Daniel Gray, Konstantin Loponov, Diganta B. Das, Tao Sun, Maria Sotenko*

Department of Chemical Engineering, Loughborough University, Loughborough LE11 3TU, Leicestershire, United Kingdom

* Corresponding author, m.sotenko@lboro.ac.uk

Abstract

Due to the increasing demand for recovery of precious metals from waste streams, the role of fungus as a potential adsorbent is growing. Thus, this research aimed to develop a novel method for the biomining of palladium nanoparticles using *Phanerochaete chrysosporium* fungus. The adsorption of palladium onto the fungal mycelium was studied by adding palladium salt at 12-48 ppm to the fermentation medium following the fungal growth. Amide groups of chitin and proteins on the surface of the fungus are believed to coordinate palladium cations yielding Freundlich-type competitive adsorption isotherm. The adsorption capacity was found to reach 22-128 mgPd g⁻¹ of dried fungal biomass. Biomining of palladium from an aqueous solution led to the formation of extracellular well defined Pd nanoparticles of 10-14 nm confirmed by XPS, TEM and XRD analyses. The biologically synthesised nanoparticles were found to perform equally well to a Pd/C commercial catalyst in a Heck reaction of styrene and iodobenzene. This is the first reported use of *Phanerochaete chrysosporium* to synthesise palladium nanoparticles and confirmation of their resulting catalytic activity. The presented example paves the way for the development of fungi-based membranes to be used in the palladium recovery from industrial effluents.

1. Introduction

Biotechnology has recently found some novel non-traditional applications and bio-mineralization is one of those examples (Addadi & Weiner 2014; Rawlings et al. 2003; Weiner 2003; Gadd 2010). Bio-mineralization, i.e., the production of minerals or metal clusters via microorganisms, is especially useful in those cases where rare-earth elements or high value metals need to be recovered from industrial waste streams. There have been some biotechnological approaches developed with the main aim to recover platinum group metals, gold, silver and some lanthanides (Zhuang et al. 2015; Gherghe 2017; Das & Das 2013; Nanchaiah et al. 2016). Moreover, in those cases where metallic nanoparticles (NPs) are formed as the result of bio-mineralization process, one can achieve multiple benefits, such as the production of value-added products in addition to solving the environmental problem of water streams contaminated with otherwise toxic metals. Some examples include biotechnological methods for the production of antimicrobial AgNPs (Siddiqi et al. 2018; Saklani & Jain 2013), AuNPs used widely in bioimaging and labelling (Menon et al. 2017; Lengke et al. 2011; Zhao et al. 2013; Qin et al. 2018), bio-Pd catalytic nanoparticles (Deplanche et al. 2014; Bennett et al. 2013; Zhu et al. 2016) and even quantum dots (Qin et al. 2018; Mary et al. 2018). ‘Green’ biosynthesis methods for the production of nanoparticles offer some benefits in comparison to the traditional wet chemistry approaches such as mild reaction conditions and the use of environmentally friendly biocatalysts and non-toxic chemicals. Thus, the biotechnological methods eliminate the need for toxic and corrosive reducing agents, such as

sodium borohydride which is widely used in chemical methods and requires safe handling and disposal (ThermoFisher Scientific 2010). Moreover, chemical synthesis of metallic nanoparticles usually takes place in two steps, i.e., the initial formation of the nanoparticles followed by the addition of an encapsulating agent to prevent the nanoparticles from agglomeration (Cookson 2012). A biological method, however, does not require any encapsulating agents as the enzymes released by the microorganisms are believed to prevent the agglomeration of the nanoparticles (Sanghi et al. 2011; Zielonka & Klimek-Ochab 2017).

The majority of the reported biotechnological methods have been directed towards using *bacteria* for the synthesis of nanoparticles (Saklani & Jain 2013; Lengke et al. 2011; Menon et al. 2017; Narayanan & Sakthivel 2011; Guil-Guerrero et al. 2016; Edmundson et al. 2014; Cueva & Horsfall 2017). However, the bacterial nanoparticles are formed mainly intracellularly and the extraction of these nanoparticles leads to high processing costs. The costs are further increased by the required genetic modification of the available strains in order to overcome metal toxicity in bacteria. *Fungal* route, on the contrary, could offer some advantages in comparison to the bacterial methods. Firstly, when synthesized by fungi, the nanoparticles form extracellularly and therefore do not require the additional stage of biomass disruption to release the intracellular bacterial nanoparticles (Conesa et al. 2009). Secondly, fungal route does not require an electron donor eliminating the need for hydrogen atmosphere which is essential in bacterial processes (Deplanche et al. 2014; Bennett et al. 2013; Zhu et al. 2016). Thirdly, fungi were reported to have a higher tolerance to metal uptake and increased wall binding capacity in comparison to bacteria (Chen et al. 2009). Finally, fungi as the biological support for the nanoparticles offer an advantage of higher biomass yield and surface area due to their filamentous network structures. Because of all these advantages, the interest in biomineralization has recently shifted towards replacing bacteria with fungi to enable the bio-derivation of nanoparticles to be a promising alternative to chemical reduction methods (Saxena et al. 2014).

Despite the great interest in the fungal bio-mineralization, the range of the reported metals is still limited. The majority of the reports on fungal synthesis of nanoparticles is mainly devoted to the biomineralization of silver and gold (Saxena et al. 2014; Menon et al. 2017; Siddiqi et al. 2018). There seems to be no preference for a specific fungus in these processes although *Fusarium oxysporum*, *Aspergillus* genus and *Aspergillus niger* in particular are the most commonly used fungi (Moghaddam et al. 2015; Zielonka & Klimek-Ochab 2017; Kitching et al. 2015). The synthetic method could be based either on the use of mycelia-free fungal supernatant or co-incubation of the metal ions with the submerged fungal cultures, mainly after the fungal growth stage. The majority of the reported AuNPs and AgNPs are characterized as extracellular, spherical in shape and having high stability against agglomeration and a wide range of sizes from 5 to 100 nm (Moghaddam et al. 2015; Zielonka & Klimek-Ochab 2017; Kitching et al. 2015). Along with various bioactive compounds present in the medium and secreted by fungi, enzymes are regarded as the main stabilizing and reducing agents in the production of nanoparticles. Based on enzyme assays, Sanghi et al. attributed the fungus activity to laccase and ligninase in a reported synthesis of gold nanoparticles with *P. chrysosporium* (Sanghi et al. 2011). In addition to stabilizing effect, it was shown that the fungal extract possesses shape-forming properties in the synthesis of multi-shaped gold nanoparticles using a cell-free extract from *Rhizopus oryzae* fungus (Das et al. 2010).

Despite these advantages, the fungal route has not been used for platinum group metals (PGMs), which are known to be used in an incredibly wide range of applications, including catalytic converters, pharmaceuticals, diagnostic and bulk chemicals synthesis (Godwin et al. 2015; Heera

& Shanmugam 2015; Zhao et al. 2013; Siddiqi & Husen 2016). Unfortunately, the increasing demand for PGMs is accompanied by exhaustion of the current supply. According to Johnson-Matthey's most recent report, the worldwide supply deficit of palladium in 2017 was 629,000 oz and as a result the price of palladium rose by 51% (Johnson Matthey 2018). Despite the fact that one third of palladium is recycled, there is still a need to develop a cost-effective method to recover palladium from industrial effluents. Therefore, this study aimed to verify the ability of the fungus *P. chrysosporium* to biomineralise Pd from aqueous solutions with the main drive to further develop the fungi loaded membranes for the recovery of Pd from waste waters.

P. chrysosporium has demonstrated exceptional adsorption properties and has been previously widely used for the effective removal of heavy metals from waste waters (Yetis et al. 2000; Zhao et al. 2016; Marandi et al. 2008). It is hypothesized, that the advantages of the fungal synthesis of nanoparticles coupled with the fungal unique adsorptive properties will open new possibilities in developing novel metal remediating techniques based on the fungi loaded membranes. In the current paper, the adsorption of Pd from an aqueous solution was investigated; the obtained Pd nanoparticles were studied using various physico-chemical techniques such as X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM); the catalytic activity of the bio-synthesised palladium nanoparticles was also compared to that of a Pd/C catalyst in the Heck reaction as a model catalytic reaction of high industrial relevance.

2. Materials and Methods

2.1. Chemicals

Potassium hexachloropalladate (IV) (99%), 1000 mgL⁻¹ palladium atomic absorption spectroscopy (AAS) standard, mesitylene (98%), isopropanol (99.9%), dimethylformamide (99.8%, anhydrous), trimethylamine (>99.5%), styrene (>99%), iodobenzene (98%), 5% Pd/C commercial catalyst, potassium dihydrogen orthophosphate (99%), ammonium chloride (99%), magnesium sulphate (>99.5%), calcium chloride dihydrate (99%) were all purchased from Sigma Aldrich or Fischer Scientific and used without prior purification. Malt extract agar (CM0059) and D-glucose anhydrous were procured from Oxoid Ltd (UK). For the metal adsorption experiments, aqueous stock solutions of 1000 ppm palladium (IV) was prepared by dissolving 0.09360 g of potassium hexachloropalladate (IV) in 25 ml of distilled water.

2.2. Fungal cultures

An enriched spore solution of the white rot fungus *P. chrysosporium* stored at -80 °C in 20 % v/v glycerol stock solution was used as the inoculum. The fungus was re-cultured from the spore solution spread on the surfaces of malt extract agar plates in a static incubator (Stuart Scientific Orbital Incubator SI50) at 37 °C for 5 days. The spore solution was collected as the fresh inoculum by adding 5 ml of sterile distilled water into each of the Petri dishes and scraping the spores from the agar surfaces. The spore content was determined using BRAND™ Neubauer counting chamber (Fischer Scientific Ltd) after appropriate dilution. The broth for fungal culture was composed of 2.0 gL⁻¹ KH₂PO₄, 0.5 gL⁻¹ MgSO₄, 0.1 gL⁻¹ CaCl₂ with 10 gL⁻¹ glucose as carbon source and 0.12 gL⁻¹ NH₄Cl as nitrogen source and supplemented with 0.001 gL⁻¹ of thiamine using a filter-sterile stock solution (Yetis et al. 2000). The pH of the medium was adjusted to 4.5 prior to autoclaving at 121 °C for 15 min. The fungus was cultured in 50 ml sterile centrifuge tubes each with 19 ml broth inoculated with 1 ml fresh inoculum (5x10⁷ CFU mL⁻¹) in a shaking incubator at 37 °C and

80 rpm for 5 days. The cultures were conducted in triplicates and the fungal growth rate was determined to be 0.11 ± 0.01 g of dried biomass per L of the medium per day.

2.3. Adsorption of palladium by the fungal biomass

Pd stock solution (1000 ppm) was added into the fermentation medium *after* 5 days of the fungal culture under sterile conditions to adjust the concentration of palladium within the range of 12-48 ppm. The fungus was further cultured in the shaking incubator at 37 °C and 80 rpm for another 2 days. The Pd concentration in the fermentation medium was measured using AAS analysis during culture. After culture, the broth was centrifuged at 3500 rpm for 20 minutes using a Thermo Scientific Heraeus Labofuge 400 Centrifuge and the supernatant was discarded. The recovered fungal biomass was washed twice with 20 ml of 75 % v/v aqueous ethanol and finally freeze-dried using SciQuip S Christ Alpha 1-2 LD plus freeze dryer until constant weight. The amount of the adsorbed Pd (or sorption capacity) was expressed either in mg_{Pd} per g of dried biomass or in $\%W_{\text{Pd}}/W_{\text{biomass}}$ (W_{Pd} : palladium weight, W_{biomass} : weight of dried fungal biomass) and was obtained based on triplicate samples. The stock solution of Pd was also added into the freshly prepared fermentation medium free of the fungus or the fungi-free supernatant obtained from the broth after 5 days' fungal culture as the control experiments. A fungal culture without palladium salt was also carried out to verify the inhibitory effect of potassium hexachloropalladate on the fungal growth. The Freundlich model was applied for the adsorption experiments using the following correlation:

$$q = K_F C_{eq}^{1/n}$$

where q ($\%W_{\text{Pd}}/W_{\text{biomass}}$) is the palladium uptake by the biomass at equilibrium concentration of palladium C_{eq} (ppm) in the solution, K_F and n are Freundlich's constants of adsorption capacity and intensity accordingly (Maheswari et al. 2016).

2.4. Testing of bio-derived palladium nanoparticles in Heck reaction

Heck reaction was used as a model C-C coupling reaction to test the catalytic activity of Pd-loaded fungal biomass in comparison to commercial catalyst of 5 % Pd/C. The method was modified from (Fan et al. 2009). Briefly, in a 25 ml glass flask, 2 ml of the solvent (dimethylformamide, DMF) was added first, followed by the addition of 1.55 mmol of iodobenzene, 3.76 mmol of trimethylamine (TEA) and 0.854 mmol of mesitylene which was used as a GC internal standard. After the final addition of 2.37 mmol of styrene, the solution was then made up with DMF to obtain 5 ml of the final total volume of the reaction mixture. The molar ratios of styrene:iodobenzene, TEA:iodobenzene and mesitylene:iodobenzene were 1.5, 2.4 and 0.55 respectively. The catalyst concentration was taken in such quantities so as to obtain 0.5 % $\text{mol}_{\text{Pd}}/\text{mol}_{\text{styrene}}$. Thus, 0.0155g of 5% Pd/C and the appropriate amount of 4.9 % Pd/fungi was used in the experiment. After addition of the catalyst, the solution was deoxygenized by purging for 30 minutes with N_2 and the reaction was allowed to proceed at 120°C and 180 rpm for 3 hours on a hot stirrer plate. Gas chromatographic (GC) analysis was used to monitor the concentration of styrene in the reaction medium and for that 0.1 ml samples were withdrawn from the reaction medium at regular time intervals, mixed with 1ml of isopropanol and filtered through 0.2 μm PTFE filters for further analysis. A control experiment without any catalyst was also conducted for comparison purposes.

2.5. Analytical and characterization techniques

The morphology of the fungi was attained using Scanning Electron Microscope (SEM) (JEOL JSM-7800F). The dried fungal samples were mounted onto aluminum stubs using double-sided carbon tape, sputter coated with 80:20 of gold and palladium using a Quorum Q150RS machine and then monitored using SEM. The reduction of palladium was studied using X-ray photoelectron spectroscopy (XPS) on a K-Alpha XPS spectrometer (Thermo Fischer Scientific, UK). The X-ray source was Aluminium K-Alpha (1.486.68eV). The surface groups for the fungal biomass before and after the adsorption of palladium were studied using FTIR method in ATR mode (Shimadzu IR Tracer-100). A resolution of 2 cm⁻¹ was averaged over 48 scans. The composition of the Heck reaction samples was analysed using an Agilent Technologies gas chromatograph (7890A). Flame ionization detector (FID) detector was used with a Rtx-65TG column (0.1µm diameter, 30 m length and coated with a diphenyl dimethyl polysiloxane resin). The analysis parameters were as follows: 151.71mLmin⁻¹ flow rate, 50:1 split ratio and 1µL sample volume. A Varian SpectrAA 55B Atomic absorption spectrometer was used to determine the soluble palladium species in aqueous solutions. A calibration curve was plotted in the range of 0-100 ppm Pd using AAS standard solution. X-ray diffraction analysis (XRD) was used to determine the crystalline structure of the nanoparticles formed. The samples were analysed by PANalytical Empyrean X-ray diffractometer using Co K_α radiation ($\lambda=1.78901 \text{ \AA}$), the scanning range (2θ) was between 5°-90° angles with a scanning speed of 1.33° min⁻¹. It was operated at a voltage of 45 kV and 40 mA current. TEM images of the gold-coated samples of fungi were obtained using a JEM-2000FX Jeol 200 kV microscope equipped with Gatan Orius camera. The image analysis software ImageJ was used on TEM images to generate the particle size distribution of the palladium nanoparticles.

3. Results and discussion

3.1. Study of Pd adsorption by *P. chrysosporium*

The adsorption experiments using *P. chrysosporium* were conducted with a model aqueous solution of Pd (IV) prepared with K₂PdCl₆ salt. A simple procedure for the adsorption of palladium onto the fungi was used where the fungi were first cultured for 5 days prior to the addition of Pd (IV) salt to the fermentation medium. It is worth noting that, the addition of Pd⁴⁺ salt at a concentration of 12-48 ppm did not cause any inhibitory effect on the fungal growth as the final biomass yield in 7 days for both control and metal-added samples was 0.80±0.02 g L⁻¹. In contrary, previously it has been reported that tellurium and selenium were found to be inhibitory for the growth of *P. chrysosporium* at a concentration as low as 10 ppm indicating selectivity in the inhibitory effects of various metals onto the fungi (Espinosa-Ortiz et al. 2017). The selectivity in tolerance of *P. chrysosporium* towards various metals has been observed before where it demonstrated endurance to 200 ppm of lead but was inhibited by 16 ppm of cadmium (Zhao et al. 2016).

The adsorption of palladium onto the fungi was easily followed. Thus, during the adsorption experiments, the brown solution of Pd⁴⁺ gradually cleared, while the fungal mycelium changed from white to dark grey indicating the adsorption (and reduction) of palladium onto the fungi (Fig. 1, left, a-c). The gradual reduction in palladium concentration in the solution was also confirmed by AAS analysis. It is worth mentioning that one off experiment with Pd (II) salt was attempted but was accompanied by hydrolysis of Pd(NO₃)₂ upon addition to the fermentation medium which caused precipitation of insoluble Pd hydroxy species. Thus, a special attention needs to be paid to the state and coordination of the metal itself which might undergo modifications upon reaction with the components of the medium.

The curves expressing the adsorption kinetics of palladium uptake onto the fungus from an aqueous solution is shown in Fig. 1 (left). It has two regions with (1) fast adsorption rate within the first 5 hours where the concentration of palladium in the solution reduces by 40 % and (2) slow adsorption rate for the following 43 hours where the palladium removal efficiency reaches 81.5 %. The same two-staged process was previously observed for lead uptake by *P. chrysosporium* which was explained by fast surface adsorption and slow intracellular diffusion of the metal ions (Yetis et al. 2000). The adsorption isotherm and its linearised form built from the available data across various concentrations of the initial palladium (Fig. 1, right) indicate that the adsorption kinetics is best described by the Freundlich model, which, however hints that there is a competitive adsorption of other species (presumably, metals from the fermentation medium) onto the surface of the fungus. Overall, Langmuir isotherm is more commonly reported in the literature on adsorption properties of various fungi (Maheswari et al. 2016; Marandi et al. 2008).

Table 1 summarises the data from the adsorption experiments with different initial concentrations of palladium and shows that for the studied range of palladium concentration between 12-48 ppm, the final adsorption efficiency reaches 63.4-81.5 % where higher initial palladium content yielded somewhat higher adsorption efficiency.

It is worth noting, that the initial palladium content was kept low in the current set of experiments and optimized in regards to the fungal biomass yield. Within the studied range, the final palladium loading onto the fungal biomass increased with higher Pd content in the solution and ranged within 2.2-12.8 % $W_{Pd}/W_{biomass}$. This corresponds to 22-128 $mg_{Pd} g^{-1}_{biomass}$ which was determined using both AAS and XPS (XPS accuracy is somewhat lower as seen in Table 1). Similarly, the efficiency of removal attained by *P. chrysosporium* in extracting metals from waste waters has been studied in respect to lead, zinc, cadmium, selenium and tellurium ions and sorption capacity was reported to vary between 56-110 mg metal per g biomass dry weight (Yetis et al. 2000; Espinosa-Ortiz et al. 2017; Xu et al. 2016; Baldrian 2003; Zhao et al. 2016; Marandi et al. 2008).

In general, the adsorption of metallic ions by the fungal mycelium from the aqueous solution is a complex process and highly dependent on a range of parameters such as the medium pH, the composition of the broth, the fungal growth stage and the cells type (live, resting or dead) (Yetis et al. 2000; Baldrian 2003). Further studies are needed to further maximise the adsorption capacity of Pd onto the fungus.

3.2. Study of the Pd biomineralisation using physico-chemical techniques

As the morphological change to the fungal surfaces might be responsible for the adsorption of palladium, thus it was studied using FTIR. As shown in Fig. 2 (left), the difference between the FTIR-ATR spectra of *P. chrysosporium* with or without the treatment of palladium salt can be noticed. Overall, the interpretation of the FTIR data is problematic, because the fungal cell wall is composed of layers of chitin and glucans covered by proteins and the FTIR spectrum includes the combination and overlapping of characteristic bands of all these natural polymers and macromolecules (Haneef et al. 2017; Peter 2005). Moreover, chitin itself is aminoglucan which has the polysaccharide matrix of glucan with acetyl amino group replacing OH group on the glucose monomeric ring. Hence, chitin from glucan could be distinguished only by characteristic peaks at $1641 cm^{-1}$ and $1547 cm^{-1}$ which are attributed to Amide I (C=O stretching) and Amide II (N-H bending) of acetyl amino group; whereas the intensive peaks in $900-1200 cm^{-1}$ region are attributed to C-C, C-O stretching in polysaccharides and C-H stretching region in $2850-2926 cm^{-1}$ are common for chitin, proteins, glucans and even lipids (Fig. 2, left) (Ghimire et al. 2017;

Forfang et al. 2017; Pena et al. 2014; Gupta et al. 2011; Sastry M., Ahmad A. 2003; Iris et al. 2018). The peak intensity ratio of Amide I/Amide II falls from 2.0 to 1.6 from the control fungal sample to the Pd loaded fungi, indicating the disappearance of the C=O group in comparison to the N-H bond. Thus, Pd is thought to be coordinated by NCO ligand where electron density was distributed between N and O leaving N-H bond unaffected. Moreover, another specific band for chitin associated with Amide III (complex vibrations of NHCO group) at 1315 cm^{-1} reduced significantly in the Pd/fungi in comparison to neat fungi (Fig. 2) (Ghimire et al. 2017). It could be hypothesized that palladium cation initially was coordinated by acetylamino group of the chitin moieties. The amount of chitin, however, in the fungal cell wall is usually no more than 45 % with the rest being glucan and proteins (Peter 2005). Proteins, which cover the layers of glucan and chitin, have same Amide I and Amide II characteristic peaks as chitin and therefore are indistinguishable from the aminoglucan on the FTIR spectrum. The general action mechanism for fungus to absorb metals postulates that proteins are responsible for the formation of the nanoparticles (Sanghi et al. 2011; Syed & Ahmad 2012; Kitching et al. 2015). Yet, the role of chitin should not be dismissed as it was found to be an effective adsorbent for metal cations (Anastopoulos et al. 2017; Sağ 2002).

In addition to the high coordinating ability of the amides of the chitin polymer and proteins, the high adsorption capacity is also attributed to the high surface area of dense network of filamentous mycelia fungal hyphae as illustrated in the SEM image (Fig. 2, right), which was similar to the structures seen elsewhere (Vigneshwaran et al. 2006; Haneef et al. 2017). The average diameter of the dried fungal filaments is $1.3 \pm 0.1\text{ }\mu\text{m}$, which is expected to expand in culture medium and could provide high surface area for Pd adsorption. The data on the fungal specific surface area determined by the BET analysis are scarce, however the cotton-wool-like fibrous fungal mycelium has recently attracted much attention as a suitable alternative for the manufactured membranes and other advanced materials (Haneef et al. 2017).

The XRD and XPS analysis of the dried *P. chrysosporium* biomass after the adsorption experiments confirmed the presence and loading of palladium on the surface of the fungi (Fig. 3). The X-ray diffraction (XRD) pattern of the Pd/fungi sample showed intense Bragg peaks corresponding to the (111), (200) and (220) lattice planes of palladium at $2\theta = 41.2, 46.6$ and 67.4 degree (Fig. 3, left). The diffraction pattern shows a typical pattern for the face centered cubic (fcc) structure of palladium (JCPDS card no. 01-071-3757), this confirms the crystalline structure of Pd whereas the broad peak observed at $2\theta = 20.9$ degree is attributed to chitin. From the broadening of the peak at $2\theta = 67.4$ degree of Pd (200) and using the Debye/Scherrer equation, the size of the Pd nano particles was determined to be 11.3 nm, which showed good agreement with the data from the TEM analysis.

According to the XPS analysis, it appeared that not all of the palladium on the surface of the fungi had been reduced and peaks of Pd(IV), Pd(II) and Pd(0) were identified in the spectra at 343, 342 and 340 eV (Fig. 3, right) and both Pd $3d_{5/2}$ and Pd $3d_{3/2}$ could be seen (Tura et al. 1988). The ratio of all the species was Pd(0):Pd(II):Pd(IV) = 2:1:1.5 throughout the samples tested indicating that about 44 % of palladium was in a reduced state whereas the rest still being in a cationic form. The presence of Pd^{2+} indicated that the reduction of palladium undergoes two steps where the first step $[\text{PdCl}_6]^{4-} + 2e^- \rightarrow [\text{PdCl}_4]^{2-} + 2\text{Cl}^-$ is characterized by $E_0 = 1.288\text{ V}$ and is therefore a limiting stage whereas the second stage $[\text{PdCl}_4]^{2-} + 2e^- \rightarrow \text{Pd}(0) + 4\text{Cl}^-$ is less energy intensive with $E_0 = 0.591\text{ V}$. Some extracellular enzymes are believed to reduce the cations of metals and stabilize the nanoparticles according to literature (Saxena et al. 2014; Syed & Ahmad 2012; Zielonka &

Klimek-Ochab 2017). Thus, the synthesis of gold nanoparticles by *P. chrysosporium* was reported for both isolated fungal mycelium and fungus-free growth medium which were separately treated with the solution of HAuCl₄ at acidic pH (Sanghi, Verma and Puri, 2011). After monitoring the activity of laccase, lignin peroxidase and manganese peroxidase, the authors proposed enzymatic mechanism for the formation of Au NPs where carboxylic, amino and sulfhydryl (SH) groups of the fungal proteins/enzymes played a major role in the adsorption of Au³⁺ ions, which were further reduced by ligninase and laccase. A further study is needed to fully investigate the role of fungal reductases and other bioactive macromolecules in the metal ions reduction, however it is clear that the fungal biomineralization of palladium, unlike Pd-synthesizing bacteria, does not require the addition of reducing agents like hydrogen and formic acid, thus significantly reducing the costs for nanoparticle production (Deplanche et al. 2014; Bennett et al. 2013; Zhu et al. 2016).

TEM analysis (Fig. 4) visualised the palladium nanoparticles on the surface of *P. chrysosporium*, which followed a normal particle size distribution with a prevailing size between 10-14 nm. Some nanoparticles agglomerated; however, the identity of each individual palladium nanoparticle was maintained. Narrow size distribution and small particle size are essential for high catalytic activity of Pd nanoparticles. Previously, both narrow and broad range in the size of the bio-derived particles have been reported. Thus, Ahmad *et al.* obtained Ag nanoparticles of 5-15 nm and claimed that capping of the nanoparticles with the fungal proteins prevented agglomeration in an experiment with *Fusarium oxysporum* (Sastry M., Ahmad A. 2003). Similar narrow range was reported by Bhainsa *et al.* for Ag nanoparticles obtained with *Aspergillus fumigatus* (Bhainsa & D'Souza 2006). Whereas, Sanghi *et al.* assessed Au nanoparticles with AFM method and reported a broader size range of 10-100 nm with *Phynerochaete chrysosporium* (Sanghi et al. 2011). Espinoza-Ortiz *et al.* reported the formation of needle-like tellurium nanoparticles and Se-Te particulate alloys of various morphologies with a broad size range for Te and Se-Te particles being as high as 20-465 nm and 50 - 600 nm respectively due to presumably agglomeration of the particles (Espinoza-Ortiz et al., 2016, 2017). Recently, *P. chrysosporium* was reported for the synthesis of very fine (1.5-2 nm in size) cadmium sulfide (CdS) quantum dots where a special stabilizing role of cysteine amino acid of the fungal proteins was investigated (Chen et al., 2014). Similarly, the binding and stabilizing properties of the fungal proteins were emphasized in the synthesis of Ag NPs formed as the result of *P. chrysosporium* mycelium treatment with silver nitrate solution, though broad range of the particles size was reported (50-200 nm) indicating some aggregation of metallic silver (Vigneshwaran et al., 2006).

Overall, the palladium biomineralization on the surface of *P. chrysosporium* yielded well defined palladium nanoparticles within the range of 10-14 nm which were easily characterized by different physico-chemical methods. It was deduced that only 44 % of the adsorbed palladium was reduced to Pd(0) state whereas the reduction undergoes two steps (i.e. Pd(IV) → Pd(II) → Pd(0)). Both proteins and chitin could be responsible for the adsorption of palladium and the fungal mycelium is believed to provide high surface area. Yet, further studies on the reducing activity of the fungal enzymes are required.

3.3 Catalytic Testing of bio-derived Pd nanoparticles in Heck reaction

A great interest in the recovery of PGMs in general and palladium in particular is due to their high catalytic activity in Suzuki and Heck coupling reactions widely used in pharmaceutical industry. The Heck reaction of stilbene synthesis seen in Fig. 5 was used to study the catalytic activity of the bio-derived palladium nanoparticles in comparison to a traditional 5 % Pd/C catalyst (Sigma-

Aldrich). The initial reaction rates for both catalysts were found to be comparable, 0.052 mmol s⁻¹ for 4.9 % Pd/fungi and 0.048 mmol s⁻¹ for 5.0 % Pd/C. Although the bio-derived catalyst reached somewhat lower final conversion of 95 % in 65 min in comparison to 98 % for the Pd/C catalyst, Pd/fungi demonstrated a similar performance in the studied reaction and even somewhat greater catalytic activity per unit of Pd(0) if taken into account that only 44 % of palladium on the fungus biomass was in the reduced state. Previously, only bio-Pd obtained via bacterial route has been studied in catalytic reactions and Bennett *et al.* demonstrated that bacteria supported Pd maintained the same or superior activity in the Heck coupling of ethyl acrylate and iodobenzene in dimethylformamide and in the hydrogenation of 2-pentyne in comparison to commercial palladium catalysts (Bennett *et al.* 2013; Bennett *et al.* 2010). It is still not clear how bacterial support affects the nanoparticles activity though some stabilization of the nanoparticles via anchoring to the bacteria was suggested (Bennett *et al.* 2013). Overall, bacteria supported bio-Pd demonstrated great potential as catalyst in various chemical reactions in comparison to traditional palladium catalysts, such as Pd/C and Pd/Al₂O₃ (Macaskie *et al.* 2017; Zhu *et al.* 2016; Deplanche *et al.* 2014; Bennett *et al.* 2010). However, high costs for the production of the bacterial bio-Pd (H₂ is required) hampers the implementation of the technology. Fungal route, on the contrary, could become an economically feasible method for the production of catalytically active palladium nanoparticles via recovery of palladium from industrial effluents. In the current report, the same key catalytic properties have been demonstrated for palladium nanoparticles supported by *P. chrysosporium*, such as well defined, small size particles of 10-14 nm, 5-12% loading of the metal and comparable to the commercial catalyst catalytic activity in a Heck reaction. Moreover, the fungal biosynthesis can be further used for the recovery of other PGMs and an example of the synthesis of platinum nanoparticles has been demonstrated with *Fusarium oxysporum* fungus (Syed & Ahmad 2012).

4. Conclusions

The fungal biomineralization of metals reported in literature is mainly devoted to gold and silver whereas, to the best of our knowledge, this is the first report on the synthesis of catalytically active palladium NPs via a simple method of palladium adsorption from an aqueous solution onto fungus *Phanerochaete chrysosporium*. Overall, high surface area of the fungal support and lower production costs make the fungal biomineralization of precious metals more advantageous in comparison to bacterial biomineralization. The demonstrated method in this work opens the way for the development of fungi-based membranes to be used in cleaning industrial effluents from PGMs and at the same time converting precious metals into valuable nanoparticles.

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Tables

Table 1

Combined data presenting a set of various adsorption experiments with different initial concentrations and final loadings of palladium onto *P. chrysosporium* determined by AAS and XPS techniques (STDV based on triplicates).

Metal form	Salt used	C^0_{Pd} , ppm	Pd_{eq} ads, %	Pd load, %wPd/wb (AAS)	Pd load, %wPd/wb (XPS)
Pd (IV)	K_2PdCl_6	48	81.5 ± 1.6	12.8 ± 0.2	12.6 ± 2.0
		24	74.5 ± 1.4	4.9 ± 0.3	6.2 ± 1.0
		12	63.4 ± 1.0	2.2 ± 0.1	7.7 ± 1.5

Figures

Fig. 1. Adsorption of Pd from an aqueous solution of 48 ppm Pd onto the fungus vs. time (left) and adsorption isotherm for Pd and its linearized form (right), (a – control sample with untreated fungus in the medium, b – after addition of Pd^{4+} salt solution, c – after 48 hours of adsorption), (20 ml total volume, 37 °C, 80 rpm, Pd concentration monitored by AAS after addition into the medium with 5 days' culture of *P. chrysosporium*, 12, 24, 48 ppm of the initial palladium concentration was tested).

560

561

562 Fig. 2. Comparative FTIR spectra of the dried *P. chrysosporium* with or without Pd treatment (left)
 563 and an SEM image of the fungal mycelium made of branched filaments with a diameter of 1.3 ± 0.1
 564 μm (right).

565 Fig. 3. The XRD diffraction pattern (left) and XPS spectrum of Pd 3d (right) of the 12.6 %
 566 W_{Pd}/W_{fungal biomass} sample.

567
 568 Fig. 4. The TEM image of the 12.6 % wPd/wfungal biomass sample (left) and the corresponding particle
 569 size distribution (right).

570
 571 Fig. 5. Comparison of the rate of the Heck reaction catalysed by bio-derived palladium
 572 nanoparticles and traditional Pd/C catalyst (schematic of the Heck reaction is included).
 573