NOTES

Adsorption and reduction of palladium (Pd^{2+}) by *Bacillus licheniformis* R08

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Abstract **Preliminary study on the mechanism of Pd^{2+} biosorption by resting cells of *Bacillus licheniformis* R08 biomass has been carried out by means of chemical kinetics and AAS, TEM, XRD and FTIR methods. The results showed that at 30℃ and pH 3.5, when dry R08 biomass powder (800 mg/L) was mixed with Pd^{2+} (100 mg/L) for 45 min, the rate constant *k* of biosorption of Pd^{2+} attained a maximum of 5.97×10^{-2} min^{-1} and the half life period of the reaction reached 12 min. The part of Pd^{2+} adsorbed by R08 biomass was reduced to elemental, cell-bound Pd^0 at the same condition. The cell wall of R08 biomass was the primary location for accumulating Pd^{2+}, and aldoses, i. e. hydrolysate of a part of polysaccharides on the peptidoglycan layer in the acidic medium, serving as the electron donor, *in situ* reduced the Pd^{2+} to Pd^0.**

Keywords: ***Bacillus licheniformis*, biosorption, palladium, non-enzymatically mediated bioreduction, FTIR.**

Precious metal recoveries by biotechnological methods have gained much attention[1—5] for its low cost, easy to operate and potential applications in environmental protection. Brierley et al.[6] utilized granulated, non-living microbial biomass to produce a metal removal gent (MRA) to recover gold, silver, platinum and palladium from wastewater. Mycelial waste of *Streptomyces aureofaciences* procured from the aureomycin fermentation industry is used as biosorbent for Au^{3+}[7]. Relatively few reports[8, 9], however, appear on the mechanism of the interaction of microorganisms with Pd^{2+} or other metal ions or substrate. To probe these processes, the essentials of the biochemical behavior of Pd^{2+} adsorbed and reduced by *Bacillus licheniformis* R08 biomass on their interface were investigated by spectroscopy techniques, so that evidence might be provided for the development of methods for the treatment of contaminated waters, recovery of precious metal ions and improvement of the preparation of highly dispersive supported precious metal catalysts by biotechnological methods.

The bacteria were isolated from the soil of mining areas and R08 biomass was screened out from the different bacterial biomasses for its stronger ability of adsorbing and reducing Pd^{2+} than any others. It was identified as *Bacillus licheniformis* R08. When resting cells of R08 biomass (800 mg/L) were mixed with Pd^{2+} (100 mg/L) for 45 min at 30℃ and pH 3.5, the biosorptive efficiency could attain 93.2% and the biosorptive capacity reached 116.5 mg/g. The mechanism of biosorption and bioreduction of Pd^{2+} was investigated by means of chemical kinetics, atomic adsorption spectrophotometry (AAS), transmission electron microscopy (TEM), X-ray powder diffractometry (XRD) and infrared spectroscopy (FTIR) methods.

1 Experimental

(ⅰ) Biomass culture and dry biomass preparation. The strain *Bacillus licheniformis* R08 biomass was cultivated in accordance with ref. [10], and then harvested by centrifuging and washed three times with deionized water. The resulting biomass was dried at 80℃, ground into powder and stored in a desiccator for use.

(ⅱ) Pd^{2+} biosorption. To each 30 mL of palladium chloride aqueous solution (100 mg/L Pd^{2+}), 24 mg of dry R08 biomass powder was added, the pH was adjusted in accordance with the experimental requirements. After being shaken (130 r/min) in an incubator at 30℃, the mixtures were filtered through 0.22 μm pore-size filter membranes and the filtrates were assayed using atomic adsorption spectrophotometer (WFX-IE2 model, China) to determine the residual Pd^{2+} concentrations. The residual, R08 biomass adsorbing Pd^{2+}, was washed three times with deionized water, dried at 80℃ and ground into powder for use.

(ⅲ) TEM characterization. R08 biomass suspended in palladium chloride aqueous solution was agitated for 6 h at 30℃ and then coated on the copper grid covered with Formvar membrane, dried at room temperature and detected by transmission electron microscope (JEM-100CXⅡ, Japan) at 100 kV.

(ⅳ) XRD spectral characterization. A sample of 200 mg dried R08 biomass powder having adsorbed Pd^{2+} for 6 h was examined by X-ray powder diffractometer (Rigaku Rotaflex D/max-RC model, Japan).

(ⅴ) FTIR spectral characterization. Equal amounts of blank dried powder of R08 biomass and that having adsorbed Pd^{2+} for 6 h were mixed with dry KBr powder (1∶20) respectively, ground thoroughly and pressed into discs (0.2 mm thick) and then examined by infrared spectrometer (Nicolet 740SX model, USA), MCT-B detector. The spectra were recorded at the resolution of 4 cm^{-1} with 32 scans.

2 Results and dicussion

(ⅰ) Pd^{2+} biosorption

(1) Effect of pH on biosorption. Pd^{2+} biosorption can be considered as a simple elementary reaction during the process of R08 biomass adsorption. Since the

concentration of active groups on the cell wall of the biomass is much greater than that of Pd^{2+}, i.e. $[a] \gg [Pd^{2+}]$, the adsorption should thus be a first order reaction. Based on the chemical kinetics of a first order reaction $-\frac{dc}{dt} = kc$, where c is the instantaneous concentration of Pd^{2+} and k is the rate constant, the rate constant k of Pd^{2+} biosorption on R08 biomass and its half life period $t_{1/2}$ at different pH were calculated at 30℃ and pH 3.5, when Pd^{2+} (100 mg/L) was mixed with dry R08 biomass powder (800 mg/L) for 45 min. The results are listed in table 1.

Table 1 Influence of pH on rate of Pd^{2+} biosorption

pH	$k\times10^{-2}$/min^{-1}	$t_{1/2}$ / min
1	1.41	49
2	2.70	26
3	4.34	16
3.5	5.97	12

The k in table 1 displays the speed of Pd^{2+} biosorption, $t_{1/2}$ indicates how much time R08 biomass adsorbing 50% of Pd^{2+} takes. The data show that Pd^{2+} biosorption is a pH-dependent process, in which pH 3.5 is the optimum. When pH < 3.5, most of H^+ competed with Pd^{2+} for the binding sites of the active groups on the cell wall of the microorganism and the rate of Pd^{2+} biosorption decreased with pH falling; when pH $\geqslant$ 4.0, it caused precipitation of palladium hydroxide. The pH of palladium chloride aqueous solution in which R08 biomass suspended dropped from 3.5 to 1.98 after biosorption for 6 h. This showed that R08 biomass released H^+ in the process of Pd^{2+} biosorption.

(2) Effect of time on biosorption. At pH 3.5 and 30℃, the rate constant k of Pd^{2+} biosorption (Pd^{2+}: 200 mg/L, R08 biomass: 800 mg/L) and its half life periods $t_{1/2}$ at different periods of time are listed in table 2.

Table 2 Influence of time on rate of Pd^{2+} biosorption

t / min	$k\times10^{-2}$/min^{-1}	$t_{1/2}$ / min
3	29.0	2.34
8	11.9	5.82
11	9.14	7.58
15	7.09	9.78
30	3.84	18.1
45	2.77	25.0
60	2.08	33.3

Table 2 shows that the rate of Pd^{2+} biosorption decreased rapidly with prolonging absorptive time. Pd^{2+} biosorptive reaction reached near kinetic equilibrium after absorption for 45 min. It indicates that the biosorption of Pd^{2+} on R08 biomass is a rapid process.

(3) Effect of temperature on biosorption. The rate constant k of Pd^{2+} biosorption for 45 min in the solution mentioned above and its half-life periods $t_{1/2}$ at different temperatures are listed in table 3.

Table 3 Influence of temperature on rate of Pd^{2+} biosorption

T / K	$k\times10^{-2}$/min^{-1}	$t_{1/2}$ / min
278	3.17	22
293	3.41	20
303	3.43	20
313	3.47	20
323	3.64	19
333	3.68	19

As shown in table 3, influence of temperature on the rate of Pd^{2+} biosorption by R08 biomass is very small. This indicates that R08 biomass is heat-resisting, and the biosorption needs little activation energy and can take place at normal temperature.

(ii) TEM characterization of Pd^{2+} biosorption. The appearances of Pd^{2+} adsorbed by R08 biomass for 6 h was observed on TEM and it was found that there were gathered black metallic particles that were distributed unevenly on the cell surface (fig. 1). These tiny black particles were detected using electron diffraction by TEM. From the pattern obtained (fig. 2), it is evident that the electron opaque particles on the surface of R08 cell are palladium metallic particles. This result indicates that R08 biomass has the ability of adsorbing and reducing Pd^{2+}. The cell wall biopolymers are the primary location for accumulating metal ions and Pd^{2+} in solution is adsorbed on the surface of R08 biomass through interactions with the chemical functional groups in the cell wall.

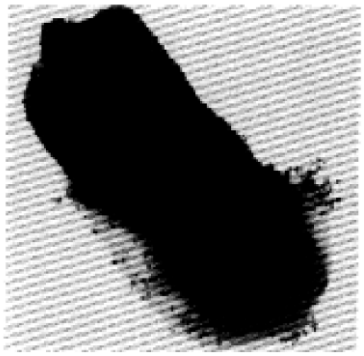

Fig. 1. Electron micrograph of Pd^{2+} adsorbed by R08 biomass for 6 h (× 20000).

(iii) XRD characterization of Pd^{2+} biosorption. The phase of dried Pd-load R08 biomass powder was analysed by X-ray powder diffractometer (fig. 3). Three bands, i.e. 2.245, 1.948 and 1.382 were found. These bands correspond to the diffraction signals of Pd^0 (111), Pd^0 (200) and Pd^0 (220) crystal planes respectively. The result shows that part of Pd^{2+} had been reduced to Pd^0 after contacted with R08 biomass. The mechanism of Pd^{2+} biosorption there-

fore involves an apparent reductive reaction[6, 11]. We have made use of this property of R08 biomass to prepare highly dispersed supported palladium catalyst[10]. Since the resting cells of R08 biomass was used in this work, the reduction was non-enzymatically mediated bioreduction of metal[11].

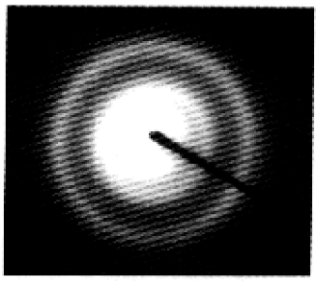

Fig. 2. Electron diffraction pattern of Pd^0 particles on R08 biomass.

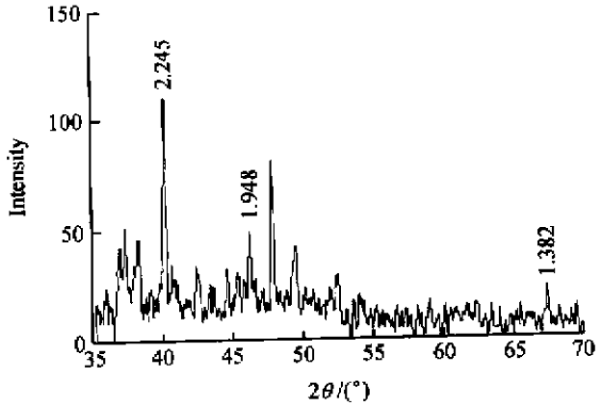

Fig. 3. XRD spectrum of Pd^{2+} adsorbed by R08 biomass.

(iv) FTIR characterization and reductive mechanism of Pd^{2+} biosorption. The cell of R08 biomass is mainly composed of a thick cell wall, about 90% of which are made up of peptidoglycan whose major components are polysaccharides and peptides. Therefore the major IR absorbed bands of R08 biomass are saccharides and proteins, with the absorbed bands of the rest components (about 10%) being covered. Since Pd^{2+} biosorption occurs on the peptidoglycan layer[12,13], the interaction of Pd^{2+} with saccharides and proteins on the cell wall of R08 biomass can be investigated by the FTIR technique.

It has been reported that the binding of palladium to R08 biomass was related to chemical functional groups possessing N or O atoms found on the cell wall of R08 biomass[12–14]. Fig. 4 shows the IR spectra of R08 biomass (1) and Pd^{2+} adsorbed by R08 biomass for 6 h (2). Bands at 1656 cm^{-1} and 1550 cm^{-1} on curve 1 may be assigned respectively to C═O stretching vibration band ($\nu_{C=O}$) and a coupled vibration involving the N—H bending and the C—N stretching modes ($\delta_{N-H} + \nu_{C-N}$) of amido bond of the peptidoglycan layer on the cell wall of R08 biomass[15,16]. It was found that the two bands were reduced to 1649 cm^{-1} and 1536 cm^{-1} respectively after Pd^2 biosorption (fig. 4, curve 2). The results imply that Pd^{2+} may be adsorbed or complexed by O or N atoms of amido bond, which shifted the two bands to lower frequencies.

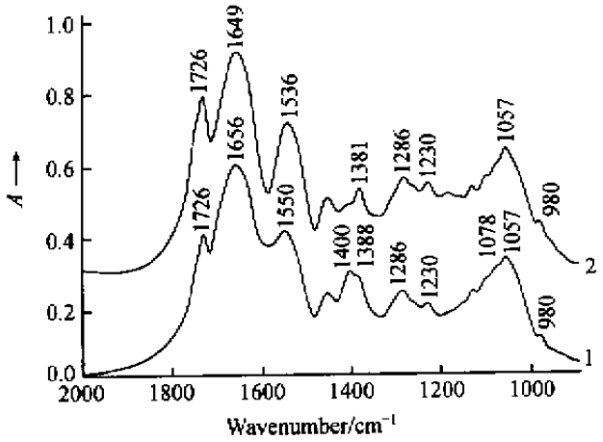

Fig. 4. FTIR spectra of R08 biomass (1) and Pd^{2+} biosorbed by R08 biomass for 6 h (2).

Bands near 1400 cm^{-1} and 1388 cm^{-1} (fig. 4, curve 1) may be due to an ionized carboxyl group (—COO^-) of remnant amino-acid C—O vibration band (ν_{C-O})[15] and the mixed vibration of C—O and C—C band ($\nu_{C-O} + \nu_{C-C}$)[16] respectively. Normally, the ionized carboxyl group C═O stretching vibration band is at about (1580±10) cm^{-1}[15,16], but it is overlapped. After R08 biomass reacted with Pd^{2+}, most of the carboxyl ions (—COO^-) might complex or chelate with Pd^{2+}, which made C═O stretching band move to a higher frequency[15,16] and caused the peak valley between 1656 cm^{-1}—1550 cm^{-1} deepened, ν_{C-O} lowered, band at 1400 cm^{-1} nearly vanished, and band at 1388 cm^{-1} shifted to 1381 cm^{-1} with its intensity weakened (fig. 4, curve 2).

Band at 1078 cm^{-1} (fig. 4, curve 1) may be due to a coupled vibration band ($\delta_{O-H} + \nu_{C-O}$) of hydroxyl group (C—O—H) of saccharides[15]. After Pd^{2+} biosorption, the band at 1078 cm^{-1} disappeared nearly. This is possibly due to the fact that Pd^{2+} had reacted with the oxygen atom of hydroxyl group, which caused the band to shift to a lower frequency.

Both curves 1 and 2 show the absorption bands at 1726 cm^{-1} and 980 cm^{-1} which may be assigned respectively to $\nu_{C=O}$ and δ_{O-H} of non-ionized carboxyl group (—COOH) of remnant amino-acid (fig. 4). The bands on

curve 2 are stronger than those on curve 1. There are two possibilities that account for the increase of intensity of absorption band of carboxyl group. One is the hydrolysis of some peptide chains of the peptidoglycan layer on the cell wall in the acidic medium because the peptide chains break drown to shorter peptides and amino acid should increase the amount of free carboxyl groups which will intensify the corresponding IR bands. The other is the likelihood of the aldehyde group in the aldose being oxidized to carboxyl group. In order to prove the possibility of the hydrolysis of peptide chains, the R08 biomass cultured in the same batch was shaken (130 r/min) in an incubator with deionized water at 30℃ and pH 3.5 for 6 h and its IR spectrum was recorded. It was found that the intensity of $\nu_{C=O}$ (1726 cm^{-1}) absorption band of non-ionized carboxyl group, which is the most sensitive to infrared light, was not enhanced (fig. 5, dotted line). This indicated that the peptide chains had not hydrolyzed. However the broader absorption band between 3373 cm^{-1} and 3425 cm^{-1} and the peak at 1057 cm^{-1} arising from ν_{O-H} and $\delta_{O-H} + \nu_{C-O}$ of saccharides both intensified (dotted line), which indicates that the quantity of free hydroxyl group increased. The result showed that a part of polysaccharides on the peptidoglycan layer had hydrolyzed in the acidic medium. Therefore the intensified IR absorption bands of the carboxyl group (fig. 4, curve 2) did not result from the hydrolysis of the peptide chains but from the redox reaction of palladium ions with the saccharides.

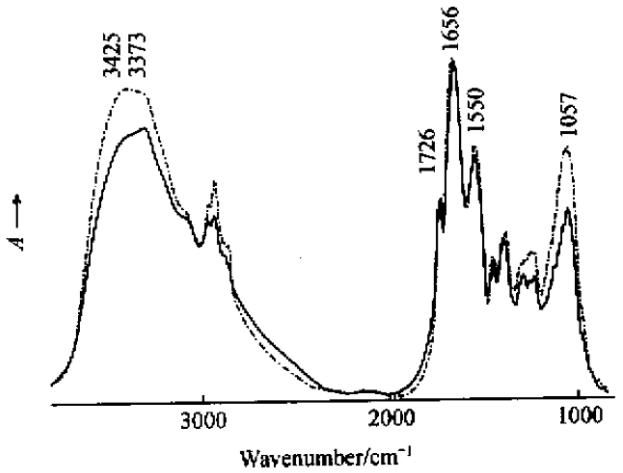

Fig. 5. IR spectra of R08 biomass (solid line) and hydrolyzed R08 biomass (dotted line).

At 30℃ and pH 3.5, polysaccharides on the cell wall of R08 biomass may partially hydrolyze to aldoses which possess the reducting property. Since the standard electrode potential of Pd, E^0 (Pd^{2+}/Pd), is 0.85 V (middle-strong oxidant), when Pd^{2+} is contacted with aldose, the following redox reaction can occur on the cell surface:

$$Pd^{2+} + R—CHO + H_2O \rightarrow R—COOH + Pd^0 + 2H^+$$

The aldoses generally do not have $\nu_{C=O}$ absorption in their IR spectra, so that they do not interfere with the vibration bands of the carboxyl group. The aldehyde groups of monoses were unceasingly oxidized to carboxyl groups, which led to the enhancement of the intensity of carboxyl absorption bands in the IR spectrum clearly (fig. 4, curve 2). This is similar to the process of Au^{3+} being reduced to Au^0 on the cell wall of *Bacillus megatherium* D01 biomass[17].

Lloyd et al.[11] noted that the resting cell of *Desulfovibrio desulfuricans* could reduce soluble Pd^{2+} ions to cell bound Pd^0 with pyruvate, formate, or H_2 as the electron donor. Our results have indicated that the electrons transport in the redox reaction can directly occur to the metallic ions and the cell wall of killed R08 biomass, without any chemical cofactors.

3 Conclusions

The biosorptive interactions of Pd^{2+} with R08 biomass have been characterized by AAS, TEM, XRD and FTIR. The evidence suggests that the rate of Pd^{2+} biosorption on R08 biomass is highly pH-dependent. The biosorption is a rapid and non-temperature-dependent process, in which H^+ is released from the chemical functional groups in the cell wall of R08 biomass. The major location of Pd^{2+} deposition is the peptidoglycan layer on the cell wall of R08 biomass. The net-like structure of peptidoglycan layer forms porosity on the surface, which provides wider space for active groups absorbing, complexing or chelating with Pd^2. The hydroxyl group of saccharides, amido bond of peptides and ionized carboxyl of remnant amino-acids are active groups, in which O and N are coordination atoms and they may provide lone electron pairs for vacant 4d or 5s of Pd^{2+} valence electron orbit to form coordinate bonds and combine quantitatively[13]. Hydrolysate of partial polysaccharides on the peptidoglycan layer—aldoses serving as the electron donating agent, *in situ* reduce the Pd^{2+} to Pd^0.

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