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The effect of biotic and abiotic environmental factors on Pd(II) adsorption and reduction by *Bacillus wiedmannii* MSM

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ABSTRACT

In this paper, we found a bacteria (*Bacillus wiedmannii* MSM) that could not only culture quickly under aerobic condition, but also can biological reduction of Pd (II) under both aerobic and anaerobic conditions. For reducing Pd (II) by *Bacillus wiedmannii* MSM, the best electron donor was sodium formate and the best growth time was 24 h (mid-log growth phase cells). TEM indicated that a lot of palladium nanoparticles (Pd-NPs) were mainly located in the periplasmic space of the live cells. However, the autoclaved cells could not synthesize Pd-NPs, which proved the role of enzyme in the reduction of Pd (II). A few of Pd-NPs were only formed on the surface of Cu^{2+}-treated cells, which proved the main but not the only role of periplasmic hydrogenase in the reduction of Pd (II). XRD and XPS also proved that Pd-NPs could be synthesized by live cells over broad ranges of temperature (20–40 °C) and pH (pH 3.0–7.0). This may be especially useful for in situ reduction and remediation of Pd (II) for both anaerobic and aerobic wastewater.

1. Introduction

The representative, expensive and precious metal, palladium (Pd), has been widely used in catalytic, chemical, photography, electronics, hydrogen storage, refining, dentistry and jewelry etc.(Vlaar et al., 2011). Moreover, the consumption of palladium has outpaced production rates (Sobjerg et al., 2011). Therefore, it is necessary to recover Pd (II) from waste streams and industrial waste (De Windt et al., 2005).

The process of microbial synthesis of Pd-NPs is mild and cheap, without high temperature condition, strong reducing agents (such as sodium borohydrid) and secondary wastes (Jacobsen, 2005). It can reduce the toxicity of Pd (II) through the bacterial reduction of Pd (II). Moreover, microbes can also provide homogeneous sites for palladium nucleation. The biogenic Pd-NPs were generally small and uniformly dispersed, which would be own more catalytic active than that of chemically reduced Pd (0)(De Windt et al., 2006). Therefore, the biogenic Pd-NPs have great potentials of reduction, dehalogenation, hydrogenation, and C–C bond forming reactions et al.(De Corte et al., 2012; Hennebel et al., 2012). Mabbett et al. (2004) used Desulfovibrio desulfuricans-bound Pd(0) to reduce Cr(VI) which demonstrated biomass bound to Pd(0) confers a novel catalytic capability and not seen with Pd base metal or biomass alone. YaTuo et al.(2013) studied *Geobacter sulfurreducens* for the reduction of Pd(II) and production of Pd-NPs capable of reducing Cr(VI). The Pd-NPs that deposited on *Shewanella oneidensis* has also been used in the dechlorination of polychlorobiphenyls (PCBs)(De Windt et al., 2005), lindane (Mertens et al., 2007), trichloroethylene (Hennebel et al., 2009), deiodination of iodinated contrast media (diatrizoate)(Forrez et al., 2011), and reduction of perchlorate (De Windt et al., 2006). In all, the reduction of Pd (II) by bacteria can recover Pd (II), reduce the toxicity of Pd (II) and synthesis high catalytic active Pd (0).

However, the species of reducing Pd (II) were still very limited, mainly focus on *Shewanella oneidensis*, *Desulfovibrio desulfuricans* and *G. sulfurreducens*. Moreover, many bacteria (such as *Desulfovibrio desulfuricans* and *G. sulfurreducens*) was anaerobically culture and reduce Pd (II) under absolute anaerobic condition (Lloyd et al., 1998; Tuo et al., 2013; Yates et al., 2013). The anaerobic operation was complex and the cost was high. Moreover, the bacteria grow slowly under anaerobic conditions. Therefore, so far, the biological reduction of Pd (II) has not yet been applied to practical applications. Therefore, it is necessary and meaningful to enrich more and better aerobic bacterial species for reducing Pd (II) under aerobic condition. In this way, microorganisms can grow rapidly and the process of reducing Pd (II) would be simple and cheap under aerobic condition.

In this paper, we found a bacteria (*Bacillus wiedmannii* MSM) that could not only culture quickly under aerobic condition, but also can biological reduction of Pd (II) under both aerobic and anaerobic conditions. *Bacillus wiedmannii* is a Gram-positive bacterium, which is

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widely distributed in various living environments. The bacteria can produce endophytic spores, which can tolerate a broad range of physical and geochemical conditions in terms of temperature and pH. For reducing Pd (II) by *Bacillus wiedmannii* MSM, the best electron donor was sodium formate and the best growth time was 24 h (mid-log growth phase cells). XRD and XPS characterization indicated that *Bacillus wiedmannii* MSM could reduce Pd (II) over broad ranges of pH (pH 3.0–7.0) and temperature (20–40 °C). The Pd-NPs particle size and location were further assessed by TEM. The enzyme (such as periplasmic hydrogenase) of *Bacillus wiedmannii* MSM played the main but not the only role in the reduction of Pd (II). The reduction of Pd (II) by *Bacillus wiedmannii* MSM was O_2 insensitive, opening the way for recycling and recovery of Pd (II) under aerobic wastewater. To our best knowledge, this was the first study on reduction of Pd (II) by *Bacillus wiedmannii* MSM and may represent an untapped potential for long-term recovery of Pd (II), especially in aerobic wastewater.

2. Experimental section

2.1. Chemicals and bacteria preparation

Na_2PdCl_4 were analytical grade, purchased from Aladdin Industrial Corporation, China. Formic acid, sodium formate, sodium acetate, acetic acid, sodium lactate, lactate, lactic acid and other chemicals were all of analytical grade. *Bacillus wiedmannii* MSM (MG786827) (Supplementary materials S1) was isolated from electronics waste factory, Guangdong, China. *Bacillus wiedmannii* MSM was grown in Luria Broth medium under conditions of aerobic, 30 °C and 150 rpm. The cells were harvested by centrifugation (4000*g*, 10 min) and washed three times with 10 mM PBS buffer and resuspended in 10 mM PBS buffer and stored at 4 °C before use.

2.2. Pd(II) reduction and biosorption

To study the effects of different electron donors, 900 mg/L live cells dry weight and 200 mg/L Pd (II) in the presence of 5 mM different electron donors (formic acid, sodium formate, sodium acetate, acetic acid, sodium lactate, lactate and lactic acid) were transferred to sealed serum bottles under anaerobic condition. Unless otherwise stated, the other conditional experiments (different cell growth period, different temperature (20–60 °C) and different initial pH (1.0–7.0)) were performed with 900 mg/L live cells dry weight and 200 mg/L Pd (II) in the presence of 5 mM sodium formate in glass serum bottles capped with inert Viton stoppers under anaerobic condition. The different oxygen content (aerobic, oxygen limited, anaerobic) and different cells (live cells, autoclaved cells (121 °C for 20 min) and Cu^{2+}-treated cells (0.5 mM Cu^{2+} for 15 min, an inhibitor of periplasmic hydrogenase) were performed with 900 mg/L cells dry weight and 200 mg/L Pd (II) in the presence of 5 mM sodium formate. The controls were no cells in Pd (II) removal experiments initiated by the same procedure as described above. Triplicate bottles were prepared and incubated with shaking at 150 rpm and 30 °C for 12 h.

The residual Pd (II) concentrations were analyzed by flame atomic absorption spectrometry (FAAS, PinAAcle 900T, PerkinElmer, USA). The pH was adjusted with nitric acid and sodium hydroxide and detected by pH meter (PB-10, Shanghai Leici Instrument Inc., China).

2.3. Characterization of Pd-NPs

The biogenic Pd-NPs was examined by X-ray powder diffractometer (Bruker D8 Advance) and X-Ray photoelectron spectroscopy (PHI X-Tool, DE). The XRD spectrum was obtained using Cu Ka radiation (λ = 0.1541 nm) at 40 kV and 40 mA. XRD patterns were recorded from 30° to 90° 2θ with a step size of 0.02°.

The XPS detection were recorded with the Al *Ka* line at 15 kV and 51 W. The binding energies were determined by reference to the C1s component due to carbon being bound only to carbon or hydrogen, set at 284.8 eV.

The Pd-NPs particle size, location and dispersal were further assessed by TEM (TECNAI 10, PHILIPS, NED). The biological slices for TEM analysis were prepared following standard protocols (see supporting information S2).

3. Results and discussion

3.1. The factors affecting the biosorption and reduction of Pd (II)

3.1.1. Effect of different electronic donors

Carbon source is a very important factor affecting the reduction of Pd (II) by microorganism. It can not only provide essential nutrients for microbes, but also provide electron donors for microbial reduction of Pd (II). In the process of the decomposition and metabolism of organic matter, the electron transfer body is produced mainly through a group of Krebs cycles (TCA cycle), but the specific metabolic process is different for different carbon source. Whether carbon source can promote microbial reduction of Pd (II) may be more related to whether it is more suitable for the electron donor reduction of Pd (II)(Gerlach et al., 2011).

For the cells cultured 24 h, compared with no external electron donor, sodium formate, sodium lactate and sodium acetate all support the removal amount of Pd (II) (Fig. 1). And the stimulative effect was sodium formate > sodium lactate > sodium acetate. Theoretically, sodium lactate catabolism would produce more electrons for reduction, while not ruling it out. We suggest that the potentially rate-limiting step in Pd (II) reduction is likely related to the physiological ability of the cells to transfer electrons to Pd (II). However, formic acid, lactic acid and acetic acid did not support the removal amount of Pd (II). It was worth noting that Pd (II) could also be reduced without external electron donors, as the reaction solution was black. These literature show that the color of reaction solution will become black only when Pd (II) were reduced (Lloyd et al., 1998; Lin et al., 2002; Deplanche et al., 2012; Chitam et al., 2016). Formic acid, acetic acid, and lactic acid would decrease the solution pH, then the activity of cells would be reduced and the reduction amount of Pd (II) may be decreased under acid condition. Therefore, the reduction amount of Pd (II) by live cells without electron donors may was greater than that in the presence of formic acid, acetic acid and lactic acid. Moreover, at lower pH, there were electrostatic repulsion between Pd (II) and the positively charged of bacterial surface and the H^+ would also compete with Pd (II). Therefore, the total removal amount of Pd (II) in the presence of formic acid, acetic acid and lactic acid were all lower than that without

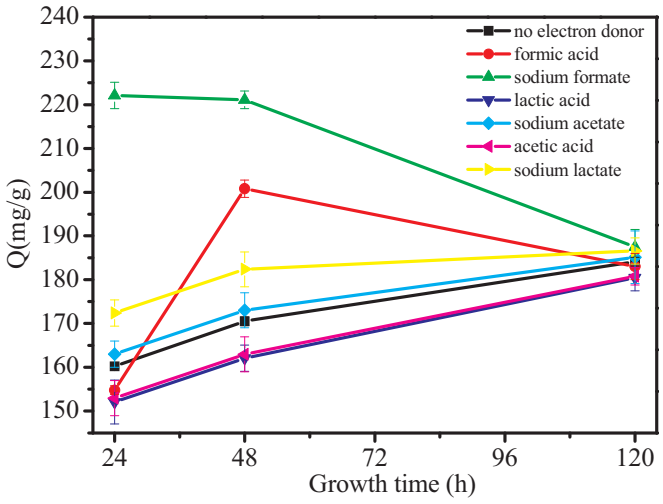

Fig. 1. The removal amount of Pd (II) (mg/g) by cells cultured 24 h, 48 h and 120 h in the presence or absence of different electronic donors.

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