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Biogenic nanopalladium production by self-immobilized granular biomass: Application for contaminant remediation

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ABSTRACT

Microbial granules cultivated in an aerobic bubble column sequencing batch reactor were used for reduction of Pd(II) and formation of biomass associated Pd(0) nanoparticles (Bio-Pd) for reductive transformation of organic and inorganic contaminants. Addition of Pd(II) to microbial granules incubated under fermentative conditions resulted in rapid formation of Bio-Pd. The reduction of soluble Pd(II) to biomass associated Pd(0) was predominantly mediated by H_2 produced through fermentation. X-ray diffraction and scanning electron microscope analysis revealed that the produced Pd nanoparticles were associated with the microbial granules. The catalytic activity of Bio-Pd was determined using p-nitrophenol and Cr(VI) as model compounds. Reductive transformation of *p*-nitrophenol by Bio-Pd was ~20 times higher in comparison to microbial granules without Pd. Complete reduction of up to 0.25 mM of Cr(VI) by Bio-Pd was achieved in 24 h. Bio-Pd synthesis using self-immobilized microbial granules is advantageous and obviates the need for nanoparticle encapsulation or use of barrier membranes for retaining Bio-Pd in practical applications. In short, microbial granules offer a dual purpose system for Bio-Pd production and retention, wherein *in situ* generated H_2 serves as electron donor powering biotransformations.

1. Introduction

Palladium, a precious metal that belongs to platinum group, is one of the most widely used catalysts. Palladium (II) is highly mobile in the aqueous environments and has serious toxic effects on organisms (Kielhorn et al., 2002). Its expansive use in automotive industry and petite existence in nature have spurred interest in the recovery of palladium from wastes for meeting the demand. Recovery of Pd from potential streams such as scrap leachates, which are acidic liquids used to dissolve precious metals from electronic scrap and spent automotive catalysts, is feasible. However, currently employed methods require large investments, labour, and time and generate secondary wastes (De Corte et al., 2012). Microbial reductive precipitation is considered a potential alternative to chemical methods for recovery of soluble Pd (II) in the form of biomass-associated Pd (0) nanoparticles (Bio-Pd). Biological approach combines sustainable synthesis of Pd(0) nanoparticles coupled to efficient recovery. Bio-Pd

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preparation involves adsorption of Pd(II) and reduction of the bio-adsorbed Pd(II) to Pd(0). Applications of Bio-Pd include dehalogenation (Hennebel et al., 2009a, 2009b), reduction (Chidambaram et al., 2010) and hydrogenation (Bennett et al., 2010) reactions. Bio-Pd catalysed dehalogenation of various environmental contaminants such as polychlorobiphenyls (De Windt et al., 2005), chlorophenols (Baxter-Plant et al., 2003), polybromodiphenylethers (Harrad et al., 2007), hexachlorocyclohexane (Mertens et al., 2007) and trichloroethylene (Hennebel et al., 2009a, 2009b) have been reported. Based on the promising applications of Bio-Pd, its integration into full scale wastewater treatment plants has been envisaged for remediation of recalcitrant pollutants (De Corte et al., 2012). In order to retain Bio-Pd in a column format, encapsulation in polymer beads or use of membrane bioreactors have been considered (Hennebel et al., 2009a, 2009b). In the present study, we have investigated granular microbial biomass as a supporting matrix for Bio-Pd preparation, since it may allow retaining Pd in the bioreactor in immobilized form with the granular biomass, making it suitable for practical applications.

Bacterial cultures such as *Desulfovibrio* (Baxter-Plant et al., 2003), *Rhodobacter* (Redwood et al., 2008), *Shewanella oneidensis* (Hennebel et al., 2009b), *Pseudomonas putida*, and *Paracoccus denitrificans* (Bunge et al., 2010), *Clostridium butyricum*, *Citrobacter braakii*, *Klebsiella pneumonia*, *Enterococcus faecium*, *Bacteroides vulgates*, *Escherichia coli* (Hennebel et al., 2011), *Staphylococcus sciuri* and *Cupriavidus necator* (Søbjerg et al., 2011) have been used for Bio-Pd preparation. Bio-Pd demonstrated catalytic activity in the transformation of contaminants, which were otherwise not removed in wastewater treatment plants (De Corte et al., 2012). However, practical applications require that Bio-Pd must be immobilized to a suitable matrix (Hennebel et al., 2009b). The problem may be circumvented by using self-immobilized microbial systems such as biofilms or biogranules. *Serratia* sp. biofilm-associated Pd was prepared and used for the catalytic reduction of Cr(VI) to Cr(III), using formate as the electron donor (Beauregard et al., 2010). To the best of our knowledge, use of biogranules for Bio-Pd preparation has not been attempted earlier. Mixed microbial consortia in the form of aerobic microbial granular biomass (henceforth referred to as microbial granules (MG)) is promising for development of new generation wastewater treatment plants. Granular biomass provides significant improvements in terms of dense and compact biomass, better biomass retention, tolerance to toxic substrates and ability to withstand shock loadings (Morgenroth et al., 1997; Beun et al., 1999; Suja et al., 2012). Aerobic granular biomass has been cultivated for biodegradation of several toxic and recalcitrant compounds through either enrichment (Tay et al., 2004; Nancharaiah et al., 2006) or bioaugmentation (Nancharaiah et al., 2008). The granular structure of the microbial granules was reported to be maintained during incubation under anoxic denitrifying conditions (Nancharaiah and Venugopalan, 2011).

In this study, we used aerobic granular biomass under fermentative conditions for hydrogen-mediated reductive synthesis of Bio-Pd. The synthesized Bio-Pd was characterized using x-ray diffraction (XRD) and scanning electron microscopy (SEM) and the catalytic activity of Bio-Pd was demonstrated by the reduction of oxidized contaminants such as *p*-nitrophenol (PNP) and Cr(VI) using biogenic hydrogen as electron donor.

2. Materials and methods

2.1. Chemicals and palladium stock preparation

All the chemicals were of analytical grade and purchased either from HiMedia, Merck or Sigma. Palladium (II) stock solution was prepared by dissolving sodium tetrachloropalladate (Na_2PdCl_4) in double distilled water. The Pd(II) stock solution was purged with nitrogen gas for 5 min and stored at room temperature.

2.2. Sequencing batch reactor operation and cultivation of microbial granules

Microbial granules were cultivated in a 3 L working volume glass column (diameter 6.2 cm, height 120 cm) operated in sequencing batch reactor (SBR) mode. Details of the SBR setup and operation have been described previously (Nancharaiah et al., 2006). Briefly, the SBR was inoculated with activated sludge (3 g total suspended solids (TSS) L^{-1}) collected from an operating municipal wastewater treatment plant at Kalpakkam, India. The SBR was fed with synthetic wastewater (SWW) consisting of the following (mM): sodium acetate (6.3), $MgSO_4 \cdot 7H_2O$ (0.36), KCl (0.47), NH_4Cl (3.54), K_2HPO_4 (0.42), KH_2PO_4 (0.21), and $CaCl_2 \cdot 2H_2O$ (0.25). Trace elements were provided by adding 1 mL of stock solution (Nancharaiah et al., 2008) to 1 L of SWW. Air was supplied at the bottom of the SBR, at a superficial air velocity of 1.6 cm s^{-1}. The SBR was operated at room temperature (~28 °C) with 66% volume exchange ratio and 6 h cycle period. Addition of SWW at the bottom of the SBR and the withdrawal of the effluent through a port situated at a height of 34 cm from the reactor bottom were performed using peristaltic pumps operated with the help of electronic timers. Microbial granules were collected from the SBR after one month of operation and were used for Bio-Pd preparation.

2.3. Acclimatization of MG to fermentative growth conditions

The microbial granules were incubated in mineral salts medium (MSM) in serum bottles (Nancharaiah and Francis, 2011) for glucose fermentation. The MSM (glucose, 10.0 g; NH_4Cl, 0.5 g; glycerol phosphate, 0.3 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $CaCl_2 \cdot 2H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.005 g; peptone, 0.1 g; yeast extract, 0.1 g; deionized water, 1 L; pH, 6.8) was pre-reduced by boiling for 5 min while purging with nitrogen gas and transferred as 80-mL aliquots into 125 mL serum bottles in an anaerobic glove box (Coy Laboratory Products, USA). The serum bottles were closed with butyl rubber stoppers, sealed with aluminum crimps and autoclaved at 121 °C for 15 min. The autoclaved MSM bottles were inoculated with 5 g (dry weight: 0.174 g) of microbial granules. The serum bottles were incubated at 30 °C without shaking for 48 h. The microbial community of granules before and after fermentative incubation was anlysed using polymerase chain reaction –