Chemosphere 117 (2014) 462–470

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Synthesis of novel palladium(0) nanocatalysts by microorganisms from heavy-metal-influenced high-alpine sites for dehalogenation of polychlorinated dioxins

Michael Schlüter [a], Thomas Hentzel [a], Christian Suarez [a], Mandy Koch [b], Wilhelm G. Lorenz [b], Leonard Böhm [c], Rolf-Alexander Düring [c], Karin A. Koinig [d], Michael Bunge [a,*]

[a] Institute of Applied Microbiology, Research Centre for BioSystems, Land Use, and Nutrition (IFZ), Justus-Liebig-Universität Giessen, Germany
[b] Institute of Chemistry, Research Group Food and Environmental Chemistry, Martin-Luther-Universität Halle-Wittenberg, Germany
[c] Institute of Soil Science and Soil Conservation, Research Centre for BioSystems, Land Use, and Nutrition (IFZ), Justus-Liebig-Universität Giessen, Germany
[d] Institute of Ecology, Universität Innsbruck, Austria

HIGHLIGHTS

- Heavy-metal tolerant microorganisms were isolated from serpentinite-influenced alpine ponds.
- A high-throughput assay was used for screening of microbial growth dynamics in the presence of Pd(II).
- Pd(0) nanocatalysts were synthesized by using Pd(II)-tolerant *Pseudomonas* species.
- These "bioPd(0)" nanocatalysts were active in the dehalogenation of dioxins.
- Identified dioxin dehalogenation pathways represent a "safe route" via non-lateral intermediates.

ARTICLE INFO

Article history:
Received 16 December 2013
Received in revised form 13 July 2014
Accepted 15 July 2014
Available online 19 September 2014

Handling Editor: O. Hao

Keywords:
Palladium
Nanoparticles
Catalysts
Totalp
Serpentinite
Dehalogenation

ABSTRACT

In a search for new aqueous-phase systems for catalyzing reactions of environmental and industrial importance, we prepared novel biogenerated palladium (Pd) nanocatalysts using a "green" approach based on microorganisms isolated from high-alpine sites naturally impacted by heavy metals. Bacteria and fungi were enriched and isolated from serpentinite-influenced ponds (Totalp region, Parsenn, near Davos, Graubünden, Switzerland). Effects on growth dynamics were monitored using an automated assay in 96-well microtiter plates, which allowed for simultaneous cultivation and on-line analysis of Pd(II)- and Ni(II)-mediated growth inhibition. Microorganisms from Totalp ponds tolerated up to 3 mM Pd(II) and bacterial isolates were selected for cultivation and reductive synthesis of Pd(0) nanocatalysts at microbial interfaces. During reduction of Pd(II) with formate as the electron donor, Pd(0) nanoparticles were formed and deposited in the cell envelope. The Pd(0) catalysts produced in the presence of Pd(II)-tolerant Alpine *Pseudomonas* species were catalytically active in the reductive dehalogenation of model polychlorinated dioxin congeners. This is the first report which shows that Pd(0) synthesized in the presence of microorganisms catalyzes the reductive dechlorination of polychlorinated dibenzo-*p*-dioxins (PCDDs). Because the "bioPd(0)" catalyzed the dechlorination reactions preferably via non-lateral chlorinated intermediates, such a pathway could potentially detoxify PCDDs via a "safe route". It remains to be determined whether the microbial formation of catalytically active metal catalysts (e.g., Zn, Ni, Fe) occurs *in situ* and whether processes involving such catalysts can alter the fate and transport of persistent organic pollutants (POPs) in Alpine habitats.

1. Introduction

Apart from their relevance as anthropogenic contaminants, heavy metals occur naturally in the environment. Surprisingly, Alpine habitats are often impacted by natural sources of heavy metals (Nowack et al., 2001; Thies et al., 2007). For instance, due

* Corresponding author. Address: Institute of Applied Microbiology, Research Centre for BioSystems, Land Use, and Nutrition (IFZ), Justus-Liebig-Universität Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany. Tel.: +49 641 9937354; fax: +49 641 9937359.
E-mail address: michael.bunge@umwelt.uni-giessen.de (M. Bunge).

http://dx.doi.org/10.1016/j.chemosphere.2014.07.030
0045-6535/

to the presence of metamorphic mafic and ultramafic rocks (e.g., serpentinite) and the effects of weathering and pedogenic processes, the abundance of heavy metals such as chromium and nickel is unexpectedly high in some Alpine regions which can even exceed legal limits (Gasser et al., 1995; Bonifacio et al., 2010). In contrast to anthropogenically contaminated areas, microorganisms present at sites with a high natural background of heavy metals have been exposed to these metals on a geological time scale. It is therefore expected that microorganisms in such environments would have developed efficient metal resistance mechanisms and would thus be optimally adapted to the elevated heavy-metal concentrations. Sites with a high natural background of heavy metals, including Alpine serpentinitic areas, might therefore represent an as yet overlooked reservoir of heavy metal-tolerant bacteria that may be useful in geobiotechnological applications, such as bioremediation of industrially contaminated sites or biomining via bio-oxidation or bioleaching of metals (Rawlings, 2002; Haferburg and Kothe, 2007).

Noble-metal nanocatalysts can be synthesized at microbial interfaces (Lloyd, 2003; Gericke and Pinches, 2006; Korbekandi et al., 2009; Narayanan and Sakthivel, 2010; Thakkar et al., 2010; Dhillon et al., 2012). Such biologically produced nanoparticles may exhibit useful catalytic or antimicrobial properties (Baxter-Plant et al., 2003; Baxter-Plant et al., 2004; Mabbett et al., 2004; Humphries and Macaskie, 2005; De Corte et al., 2012; Senior et al., 2012). We previously reported on the formation of Pd(0) nanocatalysts at microbial interfaces and demonstrated their superior catalytic properties in a number of advanced synthetic organic chemistry reactions, including Suzuki–Miyaura and Mizoroki–Heck reactions (Søbjerg et al., 2009; Bunge et al., 2010; Gauthier et al., 2010). The catalytic activity of microbially synthesized Pd(0) (nano)catalysts has been also demonstrated through the transformation of environmentally relevant contaminants, such as the reduction of carcinogenic Cr(VI) to Cr(III) (Mabbett et al., 2004; Humphries and Macaskie, 2005; Humphries et al., 2006; Creamer et al., 2008; Macaskie et al., 2012) and the reductive/hydrodehalogenation of persistent environmental pollutants (Baxter-Plant et al., 2003; De Windt et al., 2005; Mertens et al., 2007; Hennebel et al., 2010; De Corte et al., 2011; De Gusseme et al., 2011; Hennebel et al., 2012; Macaskie et al., 2012).

For sustainable and environmentally benign production of highly active catalysts using bacteria, the key to this process is the reduction of Pd(II) to Pd(0) in the presence of microbial cells. Due to the high cytotoxicity of Pd(II) ions, the production of "bioPalladium" (bioPd) primarily involves separate steps for cultivation, cell harvesting, Pd(II) reduction, and Pd(0) nanoparticle formation. To our knowledge, none of the existing synthesis routes employing microorganisms utilized single-stage processes of simultaneous cell cultivation, Pd(II) reduction, and Pd(0) nanocatalyst formation. However, continuous single-stage processes might be better suited for biotechnological implementation than multi-step processes which consist also of spatially and temporally separated processes for growth and reduction. In this regard, Pd(II)-tolerant microorganisms capable of catalyzing the reduction of Pd(II) to Pd(0) and/or serving as "scaffolds" for the deposition of bioPd nanoparticles would be ideal. Microorganisms meeting these requirements would need to be isolated from sites contaminated with anthropogenic heavy metals or from habitats affected by natural sources of heavy metals. Due to the long-term multi-metal exposure of microorganisms (e.g., exposure to the heavy metals Cr and Ni) in Alpine serpentinite areas and the low specificity of some metal resistance mechanisms (Bruins et al., 2000; Nies, 2003; Harrison et al., 2007), we would expect to find a high prevalence of Pd(II)-tolerant microorganisms useful for single-stage formation of Pd(0) nanocatalysts via Pd(II) reduction.

Covering reactive nanoparticles with lipophilic cell constituents or residues may enhance the degradation of organic environmental contaminants through an increase in reaction rates brought about by synergistic effects resulting from sorptive enrichment of the hydrophobic pollutants in the vicinity of the nanocatalyst particles. Hence, one goal of our study was to test biologically synthesized Pd(0) catalysts against representatives of one of the most relevant classes of POPs: polychlorinated dibenzo-*p*-dioxins (PCDDs). The specific tasks undertaken in our study were as follows: (i) collection of samples from heavy-metal influenced serpentinitic sites in the Alps, (ii) selective cultivation of microbial communities on media spiked with a heavy-metal (Pd(II) or Ni(II)), (iii) successive transfers, enrichment, and isolation of particular strains tolerant of elevated concentrations of Pd(II) or Ni(II), (iv) physiological characterization, as well as identification of the isolates through their small subunit rRNA genes or fungal internal transcribed spacer (ITS) region sequences, (v) synthesis of Pd(0) nanocatalysts in the presence of the selected microorganisms and characterization of the nanocatalysts, and (vi) extraction and testing of the biosynthesized nanocatalysts for the dehalogenation of model dioxin compounds. The potential for *in situ* formation of metal catalysts and the consequences for the fate and transport of POPs will be discussed.

2. Materials and methods

2.1. Sampling, enrichment, and isolation of Pd(II)- and Ni(II)-tolerant microorganisms

Rock-attached biofilms and sediment samples were collected from four different ponds and five sampling points in the Totalp region (approx. 2500 m a.s.l., Parsenn, near Davos, Graubünden, Switzerland; Table 1). Samples were transferred to sterile 50-mL plastic tubes and immediately stored at 2–4 °C prior to the experiments. After 24 h, dilutions (up to 10^{-6}) of homogenized biofilm/sediment samples were used to inoculate primary enrichment cultures on solidified growth medium (nutrient agar, Difco) supplemented with 1 mM Ni(II)-chloride or 0.5 mM sodium tetrachloropalladate(II) (98%, Sigma–Aldrich). After incubation for 5 d at 25 °C, single colonies ($n = 76$) were picked from the primary enrichment cultures. For culture preservation, cells were resuspended in 500 μL of sterile 0.9% (w/v) NaCl solution and mixed with 500 μL of sterile 20% (v/v) glycerine. Colony material was transferred into fresh liquid culture medium supplemented with 0, 1, 2, or 10 mM Pd(II) or Ni(II) salts. Tolerant species were selected for further enrichment, purification, isolation, time-resolved characterization of heavy metal tolerance and identification via sequencing of PCR-amplified partial 16S rDNA fragments or fungal ITS sequences.

Table 1
Origin of isolates.

Sampling site	GPS coordinates	Altitude a.s.l. (m)
P03	N 46°50′16.9″ E 9°48′14.5″	2556
P04a	N 46°50′17.8″ E 9°48′16.3″	2558
P04b	N 46°50′18.2″ E 9°48′16.3″	2558
P05	N 46°50′18.3″ E 9°48′33.5″	2537
P06	N 46°50′17.0″ E 9°48′37.5″	2508