

Minireview

Bio-palladium: from metal recovery to catalytic applications

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Summary

While precious metals are available to a very limited extent, there is an increasing demand to use them as catalyst. This is also true for palladium (Pd) catalysts and their sustainable recycling and production are required. Since Pd catalysts exist nowadays mostly under the form of nanoparticles, these particles need to be produced in an environment-friendly way. Biological synthesis of Pd nanoparticles ('bio-Pd') is an innovative method for both metal recovery and nanocatalyst synthesis. This review will discuss the different bio-Pd precipitating microorganisms, the applications of the catalyst (both for environmental purposes and in organic chemistry) and the state of the art of the reactors based on the bio-Pd concept. In addition, some main challenges are discussed, which need to be overcome in order to create a sustainable nanocatalyst. Finally, some outlooks for bio-Pd in environmental technology are presented.

Introduction

In the last decades, there has been a considerable expansion in the use of precious metals in medicine, optical devices, electronics and catalysis (Das, 2010). Among the precious metals, platinum group metals [PGM: e.g. platinum (Pt), rhodium (Rh), ruthenium (Ru) and palladium (Pd)] are widely applied as catalysts. Due to their increasing use, for example in automotive

catalytic converters to reduce harmful gaseous emissions, their availability has become more and more limited (Hoffmann, 1988). Especially Pd has become a very widespread catalyst. Its limited supply has caused extreme price volatility (its price raised from about €7300 per kg in November 2005 to about €19 000 per kg in November 2010). Therefore, recovery of palladium from waste is required. Conventionally, these recycling techniques are hydro- and pyrometallurgical processes. These processes require significant investments, labour and time. Moreover, considerable amounts of chemicals are involved, thus generating a secondary waste stream (Jacobsen, 2005).

There is a trend to switch from the use of bulk material towards nanoparticles (NPs) of palladium, since NPs are more active and thus less catalyst is needed. Conventional production methods of these NPs require the use of a series of toxic and expensive chemical agents (Hennebel *et al.*, 2009a). Strong reductants (e.g. NaBH₄) are required to reduce metal salts into their zerovalent metallic form. This reduction is often performed in solvents. Stabilizers (e.g. polyvinylpyrrolidone) and carrier materials (e.g. Al₂O₃) prevent the particles from aggregation and release into the environment. A promising alternative for chemical synthesis is to exploit the bioreductive deposition of metals by bacteria (Hennebel *et al.*, 2009a). This biotechnological approach can serve both as a sustainable production method of nanomaterials as well as an efficient recovery technique for palladium and other metals.

This review aims at giving a summary of different bacteria that are able to perform the reduction of Pd(II) to Pd(0) and the mechanisms behind these bioreductive depositions. Subsequently, an overview is given of the demonstrated applications using these microbially synthesized Pd nanoparticles ('bio-Pd'), both as metal recovery technique and as nanoscale catalyst for several reduction reactions. Subsequently, the state of the art of the reactor set-ups with bio-Pd is discussed, together with some new challenges, which need to be fulfilled in order to create a stable, sustainable and applicable biocatalyst. Finally, a few outlooks for applications of bio-Pd are discussed.

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Bioreductive deposition of Pd(II) as Pd(0) nanoparticles

Different bacterial species are able to reduce Pd(II) to Pd(0) and each of them has specific properties that make them attractive for metal reduction (Table 1). The first reported species with Pd-reducing capacities was the sulfate-reducing bacterium *Desulfovibrio desulfuricans* (Lloyd *et al.*, 1998). After incubation under anaerobic conditions, Pd(II) was added to the culture together with H₂ or formate as electron donor. Subsequently, nanoscale deposits of Pd(0) at the cell surface were observed. When hydrogenases were inhibited with Cu, this reduction did not occur. This strongly indicated that a hydrogenase (possibly in combination with cytochrome c₃) was responsible for the reduction of Pd(II) to Pd(0). A more detailed study on the involvement of hydrogenases in the reduction of Pd(II) by *Desulfovibrio fructosivorans*, a species with very well-characterized periplasmic enzyme systems, was performed by Mikheenko and colleagues (2008). The role of different hydrogenases in the reduction process of Pd(II) was confirmed, since no depositions in the periplasmic space but only some Pd(0) clusters in mutants lacking these enzymes were observed on the cytoplasmic membrane. The authors suggested that the enzyme probably supplied the electrons for the reduction process and served as a nucleation site for particle growth.

Another bacterial species that has been extensively studied in this context is the iron-reducing bacterium *Shewanella oneidensis* (Fig. 1A). The bioreductive deposition of Pd(0) on the cell wall and in the periplasmic space of *S. oneidensis* has been described in presence of a series of electron donors (H₂, formate, lactate, pyruvate, ethanol) with H₂ and formate being the most efficient donors (De Windt *et al.*, 2005). Interestingly, the presence of O₂ did not significantly inhibit the reduction process. A similar reduction mechanism as for *D. desulfuricans*, based on the involvement of hydrogenases and cytochrome c₃, was proposed. In addition, the authors suggested a role for the formate dehydrogenase enzyme in the reduction process.

Bunge and colleagues (2010) stated that the bacterial reduction of Pd(II) is not restricted to bacteria that reduce metals in a dissimilatory way (*D. desulfuricans* and *S. oneidensis*), but that a broader spectrum of Gram-negative bacteria can sorb and subsequently reduce Pd(II). Pd(0) NPs were formed both in the periplasm and on the cell surface of *Paracoccus denitrificans*, *Pseudomonas putida* and *Cupriavidus necator* after 14 h incubation with formate. Pd(0) was also formed with auto-claved cells, where all hydrogenases had been inactivated and thus excluding the necessity of hydrogenase for the reduction process.

Cyanobacteria are another interesting group of bacteria that are able to form Pd(0) NPs out of Pd(II) (Brayner

Table 1. Overview of de different Pd-reducing species, their Gram staining, oxygen tolerance and properties attractive for metal reduction.

Species or genus	Gram staining	Aerobe/anaerobe	Attractive properties for Pd reduction	Reference
<i>Desulfovibrio desulfuricans</i>	G –	Anaerobe	Sulfate reducing, metal reducing	Lloyd <i>et al.</i> (1998); Yong <i>et al.</i> (2002b)
<i>Desulfovibrio vulgaris</i>	G –	Anaerobe	Sulfate reducing	Baxter-Plant <i>et al.</i> (2003); Humphries and Macaskie (2005)
<i>Desulfovibrio fructosivorans</i>	G –	Anaerobe	Sulfate reducing	Mikheenko <i>et al.</i> (2008)
<i>Shewanella oneidensis</i>	G –	Facultative anaerobe	Metal reducing	De Windt <i>et al.</i> (2005)
<i>Paracoccus denitrificans</i>	G –	Facultative anaerobe	Nitrate reducing	Bunge <i>et al.</i> (2010)
<i>Pseudomonas putida</i>	G –	Aerobe		Bunge <i>et al.</i> (2010)
<i>Cupriavidus necator</i>	G –	Facultative aerobe	Resistance to heavy metals	Bunge <i>et al.</i> (2010)
<i>Cupriavidus metallidurans</i>	G –	Facultative aerobe	Resistance to heavy metals	Gauthier <i>et al.</i> (2010)
<i>Rhodobacter sphaeroides</i>	G –	Facultative aerobe	Photosynthetic, metal resistant	Redwood <i>et al.</i> (2008)
<i>Bacillus sphaericus</i>	G +	Aerobe		Creamer <i>et al.</i> (2007)
<i>Plectonema boryanum</i>	G –	Aerobe	<i>Cyanobacterium</i>	Lengke <i>et al.</i> (2007)
<i>Calothrix</i>	G –	Aerobe	N-fixing <i>Cyanobacterium</i>	Brayner <i>et al.</i> (2007)
<i>Anabaena</i>	G –	Aerobe	N-fixing <i>Cyanobacterium</i>	Brayner <i>et al.</i> (2007)
<i>Clostridium pasterianum</i>	G +	Anaerobe	Metal reducing, H ₂ producing through fermentation	Chidambaram <i>et al.</i> (2010)
<i>Citrobacter braakii</i>	G –	Facultative anaerobe	H ₂ producing through fermentation	Hennebel <i>et al.</i> (2011c)
<i>Clostridium butyricum</i>	G +	Anaerobe	H ₂ producing through fermentation	Hennebel <i>et al.</i> (2011c)
<i>Bacteroides vulgatus</i>	G –	Anaerobe	As reducing, H ₂ producing through fermentation	Hennebel <i>et al.</i> (2011c)
<i>Klebsiella pneumoniae</i>	G –	Facultative anaerobe	H ₂ producing through fermentation	Hennebel <i>et al.</i> (2011c)
<i>Escherichia coli</i>	G –	Facultative anaerobe	H ₂ producing through fermentation	Deplanche <i>et al.</i> (2010); Hennebel <i>et al.</i> (2011c)
<i>Enterococcus faecium</i>	G +	Facultative anaerobe	H ₂ producing through fermentation	Hennebel <i>et al.</i> (2011c)

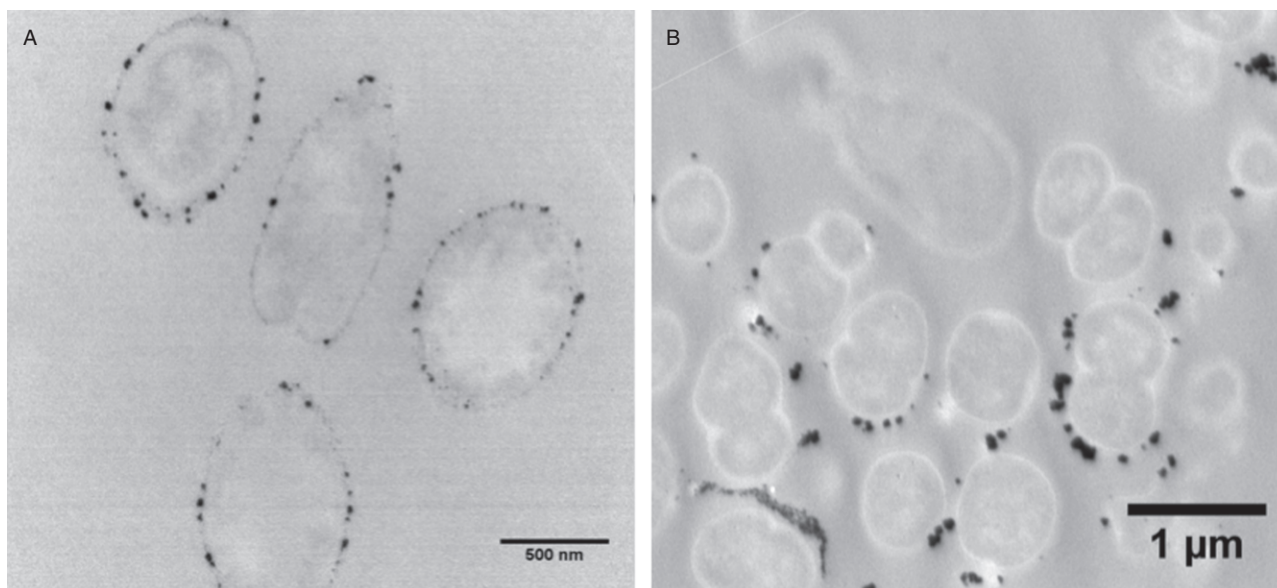


Fig. 1. A. Nanoparticles of Pd(0) in and on the outer cell parts of *Shewanella oneidensis*. B. Nanoparticles of Pd(0) precipitated by the fermentative bacterium *Citrobacter braakii*.

et al., 2007; Lengke *et al.*, 2007). Deposits of Pd(0) were found in the medium, on the cell surface and intracellular. Brayner and colleagues (2007) suggested the nitroreductase enzyme to be responsible for the reduction.

A new approach was to produce Pd(0) by fermentative bacteria (Chidambaram *et al.*, 2010; Hennebel *et al.*, 2011c). Several fermentative species produce H₂ during fermentation processes and can subsequently reduce Pd(II) to Pd(0) (Table 1). These bacteria were cultivated under fermentative conditions in an anaerobic minimal medium in which glucose was supplied as a carbon source before Pd(II) was added. A picture of bio-Pd produced by the fermentative *Citrobacter braakii* is shown in Fig. 1B. This fermentatively produced H₂ (referred to as 'biohydrogen') can subsequently be used as a reductant for Pd(II) reduction. Addition of Pd(II) to a fermenting culture of *Clostridium pasteurianum* resulted in the formation of NPs of Pd(0) on the cell wall and in the cytoplasm of the bacteria (Chidambaram *et al.*, 2010). Moreover, this biohydrogen could further serve as hydrogen donor for the catalytic activity of the Pd(0). A reactive catalyst could thus be obtained in one step. Deplanche and colleagues (2010) attributed the reduction of Pd(II) by *Escherichia coli* to three types of hydrogenases.

Desulfovibrio desulfuricans and *S. oneidensis* are the most studied organisms in the context of the bioreductive synthesis of Pd(0) NPs. The NPs are formed at the outer surface of the bacterial cells (Lloyd *et al.*, 1998; De Windt *et al.*, 2005), which makes them available for applications as catalyst. This is in contrast with for example cyanobac-

teria. Moreover, the particles synthesized by *D. desulfuricans* and *S. oneidensis* are small and show a narrow size distribution (De Windt *et al.*, 2006; Bennett *et al.*, 2010) and the precipitation occurs within minutes, which is not really the case for the particles produced by the Gram-negative species described by Bunge and colleagues (2010) and Sobjerg and colleagues (2009). Still, to increase the catalytic activity of bio-Pd, the size of the nanoparticles should be decreased to 1–10 nm. Application of fermentative bacteria can be promising due to *in situ* production of H₂. However, the particles show poor cell adhesion and a large particle size distribution (Hennebel *et al.*, 2011c). Due to the location on the outer parts of the cells, the narrow size distribution and the fast reduction, bio-Pd produced by *D. desulfuricans* and *S. oneidensis* have shown their applicability in catalysis. Nevertheless, it is possible that the conditions for deposition of Pd(0) by other strains can be optimized so that also other forms of bio-Pd become more interesting for applications as catalyst.

Pd recovery from waste streams

Due to the limited availability of Pd and consequently its price volatility, the recovery and recycling of Pd has become of particular interest. Waste streams from spent automotive catalysts and printed circuit boards contain substantial amounts of Pd and other precious metals. For these waste streams, bacterial recovery of Pd(II) is a promising recovery technique since it is less labour intensive and requires less waste-generating chemicals than

conventional recovery techniques (Jacobsen, 2005). However, significant amounts of Cu(II) [up to 25 weight % and more (Creamer *et al.*, 2006)], a known hydrogenase inhibitor, are present in the leachates of these scraps. For this reason, native biomass of *D. desulfuricans* was not able to remove Pd(II) from a printed board circuit leachate in presence of H₂ (Creamer *et al.*, 2006). To enable the recovery of Pd(II), cells which reduced Pd(II) to Pd(0) in absence of Cu(II) could be used. Indeed, a 'pre-palladized' culture of *D. desulfuricans* was capable of removing Pd(II) from the leachate. The recovery is in that case not enzymatic anymore but based on the autocatalytic growth of Pd(0) clusters on the cells. The same findings were valid for *E. coli* (Mabbett *et al.*, 2006). The observations for *D. desulfuricans* and *E. coli* were in contrast to the observations of Gauthier and colleagues (2010), who showed that this pre-palladization was not required for recovery of Pd from a scrap leachate by *C. necator* and *Cupriavidus metallidurans*.

Several reactor types were built for the purpose of recovering Pd(II) from metal-rich waste streams. An electrobioreactor for Pd recovery was developed by Yong and colleagues (2002a). In this system, H₂ was produced electrochemically and could be delivered efficiently to a biofilm of *D. desulfuricans* on a Pd-Ag membrane electrode, which allowed efficient transfer of H₂. Creamer and colleagues (2006) applied a similar system to bio-recover Au(0), Pd(0) and Cu(II) but this time from real waste scrap leachates. The presence of Cu(II) played a crucial role since it largely influenced the enzymatic reduction of Pd(II) but not of Au(III). This allowed for a stepwise separation of these three metals: Au(III) was first reduced

by native biomass of *D. desulfuricans*, followed by the reduction of Pd(II) by pre-palladized biomass and finally, Cu(II) could be precipitated as hydroxides and sulfates using the off gas of a bacterial culture.

These studies showed that the concept of Pd biorecovery is feasible when a waste stream containing pure Pd(II) is treated. When a mixture of different metals is present, the efficiency of the recovery process can be much lower. Especially Cu(II) appears to have an inhibiting effect on the process. Moreover, the concentrations of the metals in the considered solutions and scraps were quite high (several mg l⁻¹), the efficiency of biorecovery should also be evaluated at lower metal concentrations.

Use of bio-Pd as catalyst

Bio-Pd catalysed removal of environmental contaminants

Bio-Pd was successfully applied to remove a wide range of environmental contaminants (Table 2). The reactions that are described in this context are reduction of inorganic contaminants and dehalogenation of organic molecules. These contaminants result from different industrial processes and from the use of pesticides, pharmaceuticals, solvents etc. In all cases, a hydrogen donor (H₂ or formate) was used as the reductive agent to charge the bio-Pd catalyst (Fig. 2A). Noteworthy is that the particle size and the reactivity can be steered by altering the Pd/cell dry weight ratio (De Windt *et al.*, 2006) and that the external addition of a hydrogen donor can be omitted when fermentatively cultivated

Table 2. Overview of the different environmental contaminants that were successfully degraded with a bio-Pd catalyst, together with the reaction mechanism and the Pd-reducing species used in the study.

Compound	Type of reaction	Polluted environmental compartment	Pd-reducing species	Reference
Cr(VI)	Reduction	Industrial wastewaters	<i>D. desulfuricans</i> <i>D. vulgaris</i> <i>E. coli</i> <i>C. pasteurianum</i> <i>S. oneidensis</i>	Humphries and Macaskie (2005); Mabbett <i>et al.</i> (2006) Chidambaram <i>et al.</i> (2010) De Windt <i>et al.</i> (2006)
ClO ₄ ⁻	Reduction	Groundwater and drinking water	<i>D. desulfuricans</i> <i>D. vulgaris</i> <i>S. oneidensis</i>	Baxter-Plant <i>et al.</i> (2003) De Windt <i>et al.</i> (2005) Baxter-Plant <i>et al.</i> (2003)
Polychlorobiphenyls (PCBs)	Dechlorination (1–10 Cl)	Air, water, soil, sediments	<i>D. desulfuricans</i> <i>D. vulgaris</i> <i>S. oneidensis</i>	De Windt <i>et al.</i> (2005) Baxter-Plant <i>et al.</i> (2003)
Chlorophenols	Dechlorination (1 Cl)		<i>D. desulfuricans</i> <i>D. vulgaris</i>	Baxter-Plant <i>et al.</i> (2003)
Lindane	Dechlorination (6 Cl)	Soil and groundwater	<i>S. oneidensis</i>	Mertens <i>et al.</i> (2007)
Trichloroethylene (TCE)	Dechlorination (3 Cl)	Groundwater	<i>S. oneidensis</i>	Hennebel <i>et al.</i> (2009b)
Polybrominated diphenyl ethers (PBDE)	Debromination (1–10 Br)	Indoor air and dust	<i>D. desulfuricans</i>	Harrad <i>et al.</i> (2007); Deplanche <i>et al.</i> (2009)
Iodinated contrast media (ICM)	Deiodination (3 I)	Wastewaters and surface waters	<i>S. oneidensis</i> <i>C. braakii</i>	Hennebel <i>et al.</i> (2010); Forrez <i>et al.</i> (2011) Hennebel <i>et al.</i> (2011c)

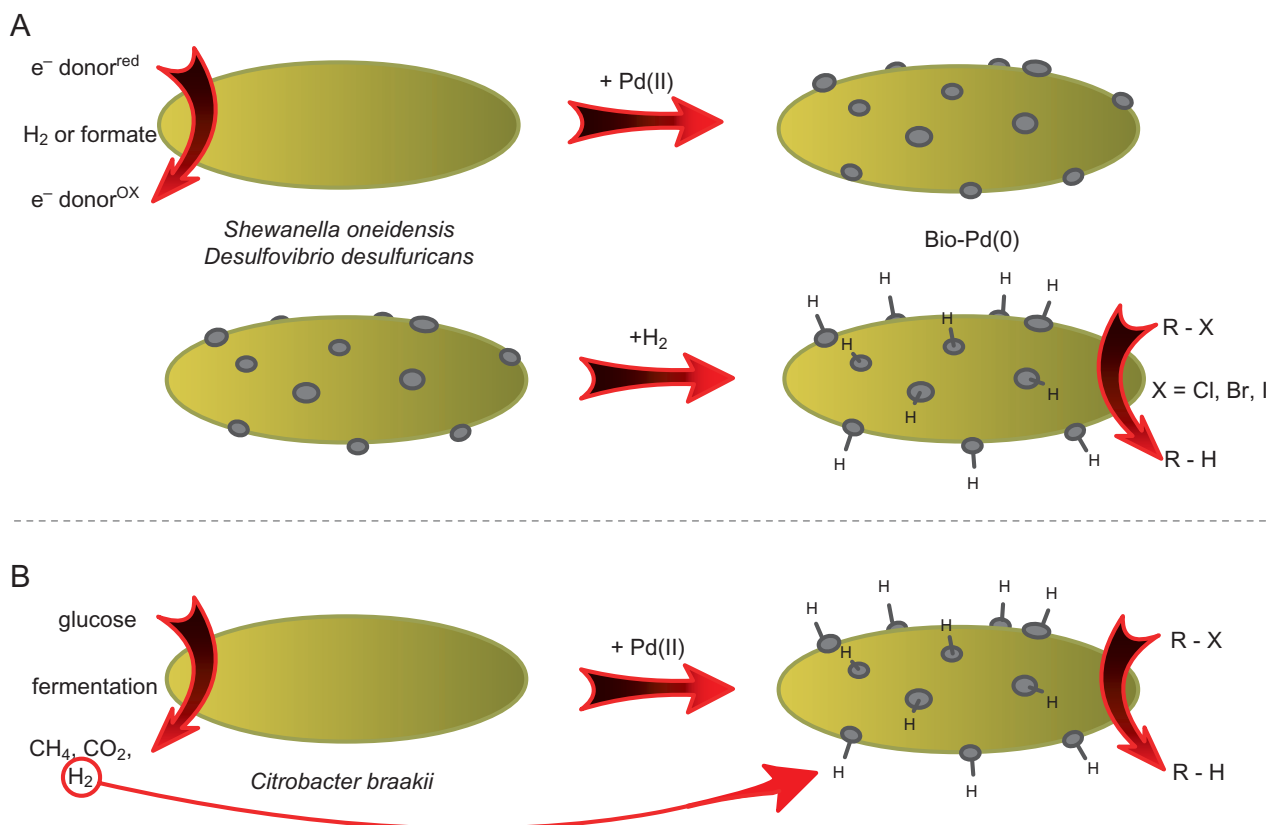


Fig. 2. A. Two-step dehalogenation of halogenated substances with a bio-Pd catalyst and an external hydrogen donor. B. One-step dehalogenation of halogenated substances with a bio-Pd catalyst produced by fermentative species.

species are used which produce biohydrogen (Fig. 2B) (Chidambaram *et al.*, 2010; Hennebel *et al.*, 2011c).

Bio-Pd as a catalyst in organic chemistry

Bio-Pd (precipitated by *D. desulfuricans* and *Bacillus sphaericus*) was applied as catalyst in the hydrogenation of organic molecules, e.g. the hydrogenation of itaconic acid (Creamer *et al.*, 2007), 2-pentyne (Bennett *et al.*, 2010) and a wide variety of other organic molecules (Deplanche *et al.*, 2009). The reaction rates were dependent on the solvent in which the reaction was performed and extremely dependent on the molecule that is to be hydrogenated. Molecules with very high structural similarity showed large differences in hydrogenation efficiency. For example, 3,4-dihydroisoquinoline could be hydrogenated to 1,2,3,4-tetrahydroisoquinoline with a yield of 47%, whereas 3,4-dihydro-1-methylisoquinoline, differing from the previous product by only one methyl group, could not be hydrogenated at all. In general, bio-Pd gave more consistent results in terms of selectivity compared with Pd/Al₂O₃ (Bennett *et al.*, 2010). The applicability of bio-Pd catalysts for coupling reactions in synthetic organic chemistry was

reported by Sobjerg and colleagues (2009). Two bacteria (*P. putida* and *C. necator*) were used to precipitate Pd(0). Both forms of bio-Pd could successfully catalyse the Suzuki-Miyaura coupling of an aryl halide with phenylboronic acid and the Mizoroki-Heck coupling of an aryl halide with n-butylacrylate. Gauthier and colleagues (2010) applied bio-Pd recovered from a metal-containing leachate by two different *Cupriavidus* species for the Mizoroki-Heck coupling.

Reactor set-ups using a bio-Pd catalyst: state of the art

Bio-Pd for *in situ* groundwater remediation

Chidambaram and colleagues (2010) showed that Pd(II) could be reduced by a biofilm of *C. pasteurianum* grown on sand grains. Aquifer microcosms were developed for *in situ* remediation of groundwater contaminated with Cr(VI). In one of these microcosms, Pd(II) was added and subsequently reduced to Pd(0). In this microcosm, Cr(VI) could be reduced to Cr(III), which was confirmed by μ XANES. In a microcosm with viable cells without added Pd(II), no Cr(III) was detected.

Use of bio-Pd in an aqueous suspension

The attachment of the nanoscale Pd(0) catalysts to a microscale bacterial scaffold allowed separation of the catalysts from the reaction medium by relatively simple techniques like microfiltration in membrane reactors. Several membrane systems were used to keep the bio-Pd catalyst in the reaction medium and to prevent leaching of the catalyst in the effluent. The use of hollow fibre membranes for the retention of bio-Pd was demonstrated for the removal of the groundwater contaminant lindane and the removal of iodinated contrast media (ICM) from wastewaters (Mertens *et al.*, 2007; Forrez *et al.*, 2011). Lindane was removed for 83% using 100 mg Pd l⁻¹ and the ICM iohexol, iomeprol, iopromide and diatrizoate were removed for more than 90% using 141 mg Pd l⁻¹. These hollow fibres allow a high membrane area per unit of volume but are very vulnerable to clogging. A more robust alternative are plate membranes (Fig. 3B). A plate membrane reactor filled with bio-Pd (produced by *S. oneidensis*) was developed by Hennebel and colleagues (2009b) for the removal of the chlorinated solvent TCE from groundwater. The highest removal rates (2340 ± 144 mg TCE day⁻¹ g⁻¹ Pd) were obtained using H₂ as electron donor and 100 mg Pd l⁻¹. Almost no Pd leaching to the effluent took place (a maximum effluent concentration of 180 µg Pd l⁻¹).

Retention of bio-Pd by encapsulation and by coating on surfaces

An alternative for the retention of bio-Pd by membranes is the encapsulation in polymeric matrices. Bio-Pd was encapsulated in several materials in order to prevent leaching of Pd in the effluent of the reactors and to facilitate separation and recycling of the catalyst.

Hennebel and colleagues (2009c) incorporated bio-Pd, produced by *S. oneidensis*, in a series of encapsulation materials: polyurethane, polyacrylamide, alginate and silica. Encapsulation in alginate beads is illustrated in Fig. 3C. The encapsulated bio-Pd had at least a six times lower dechlorination rate for TCE to ethane compared with a bio-Pd suspension. Polyurethane cubes were used in a fixed bed reactor. In a flow-through system, the maximum TCE removal rate was 865 ± 151 mg TCE g⁻¹ Pd day⁻¹. This removal rate is significantly lower compared with a bio-Pd suspension in a membrane reactor (Hennebel *et al.*, 2009b).

Polymeric membranes have also been used as encapsulation matrix for bio-Pd. Hennebel and colleagues (2010) made catalytically active membranes by incorporating bio-Pd (produced by *S. oneidensis*) in PVDF (polyvinylidene fluoride) and PSf (polysulfone) membranes. These membranes were prepared by the immersion-precipitation method. The deiodination rates of diatrizoate obtained with bio-Pd membranes were only slightly lower

than the ones with a suspension of bio-Pd. The PVDF membranes containing bio-Pd were used in a membrane contactor set-up. In this contactor, water spiked with diatrizoate was circulated on one side of the membrane and H₂ was dosed in a controlled way at the other side of the membrane. A scheme of this set-up is shown in Fig. 3D. The aim of this set-up was to dose H₂ in a more controlled way and directly to the catalyst with less limitations due to its limited water solubility. In this system diatrizoate was removed for 77% after 48 h at pH 10.

Next to encapsulation in polymeric matrices, Hennebel and colleagues (2009c) coated bio-Pd on zeolites. This coated bio-Pd was five times less active for the dechlorination of TCE compared with a bio-Pd suspension. This coating on porous zeolites is illustrated in Fig. 3E. Another coating surface is graphite (Hennebel *et al.*, 2011a). This graphite can be further used as a cathode of a bio-electrochemical system (see *Efficient delivery of the hydrogen donor*). Also the anode of microbial fuel cells (MFCs) can be coated with bio-Pd, for example coated on a Teflon-coated carbon paper (Ogi *et al.*, 2011). The aim for the use of bio-Pd was to maximize the power output of the cell. The maximum power output of a bio-Pd-containing cell was 90% of the power of a cell containing a chemical Pd catalyst.

In a recent study, a biofilm of *Serratia* was grown on polyurethane foam, which could reduce Pd(II). The obtained Pd(0) was successfully used for reduction of Cr(VI). However, reactors containing this biofilm-Pd showed heterogeneities in reaction rates and dead spots (Beauregard *et al.*, 2010).

Comparison of the different set-ups

The different set-ups, as discussed above, differ in two important aspects: the production of Pd (production *in situ* or pre-production) and the retention of bio-Pd (filtering by membranes, encapsulation in a matrix or coating on a surface). The production *in situ* offers the advantage of a cheaper production requiring no pure cultures, no complex growth media and especially no external hydrogen (when working with H₂-producing species). Still, addition of an external carbon source can be needed. However, when the *in situ* production of bio-Pd is used for groundwater remediation (as described by Chidambaram *et al.*, 2010), the injection of a heavy metal like Pd into the soil might raise questions. The presence of Pd in groundwater should definitely be avoided. Looking at the different retention mechanisms, the size of the bacteria (about 1 µm) makes the retention of bio-Pd by microfiltration membranes very straightforward. Of course, this requires a strong attachment of the nanoparticles to the cell wall in order to avoid leaching of nanoparticles into the effluent. Moreover, the membranes can be sensitive to clogging

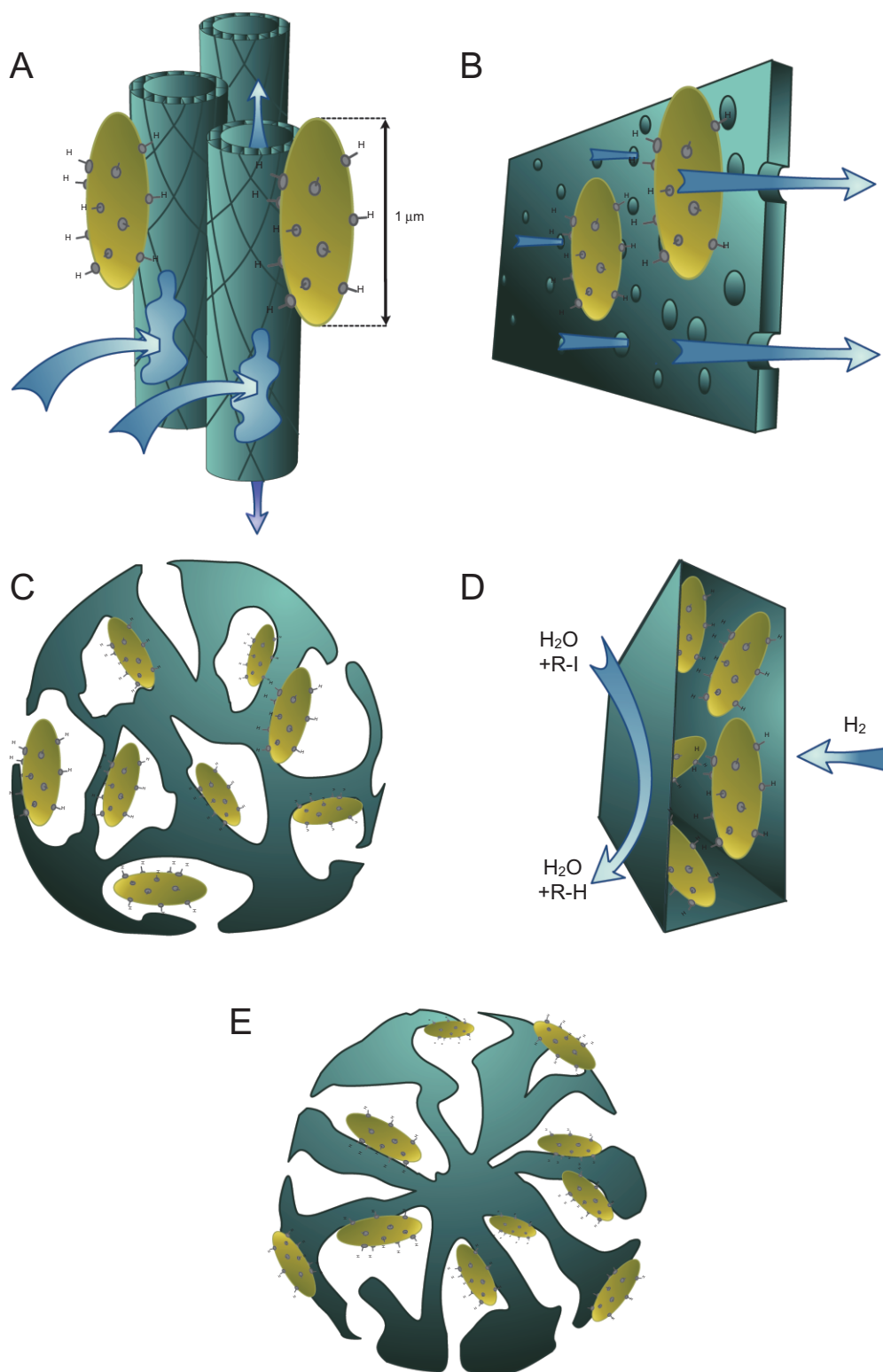


Fig. 3. Retention mechanisms of bio-Pd in reactor set-ups.

- A. Retention by hollow fibre membranes.
- B. Retention by plate membranes.
- C. Encapsulation in alginate beads.
- D. Encapsulation in polymeric membranes.
- E. Coating on zeolites.

and regular backwash can decrease the efficiency of the reactor. Encapsulation could be an option to assure better retention of the nanoparticles in the system. These reactors are insensible to clogging but this encapsulation will cause a decrease of the reaction rates. The challenge is to find a matrix which is porous enough to limit the loss of catalytic activity but still assures the good retention of the nanoparticles inside the matrix. Another challenge is to evaluate the long-term stability of these reactor systems. Conclusively, the optimal reaction configuration will be very dependent on the type of application.

Challenges for the usage of bio-Pd as catalyst

Catalytic application of biorecovered Pd

Ideally, bio-Pd is synthesized from a Pd-containing scrap leachate and subsequently applied as a catalyst in order to obtain a closed cycle of Pd. This was demonstrated by Gauthier and colleagues (2010), who biorecovered Pd from a heavy metal scrap leachate using *C. necator* and *C. metallidurans*. The obtained bio-Pd was subsequently successfully applied as a catalyst for the Mizoroki-Heck coupling of n-butylacrylate with 4-iodoanisole. However, it is probable that the success of both the biorecovery and the catalytic application are very dependent on the composition of the leachate. The scrap can contain compounds which inhibit the biorecovery process and other metals present on the bacteria after the recovery process might decrease the catalytic activity of Pd.

Doping of bio-Pd with other metals to enhance activity

In order to increase reaction rates or to enable new reactions, there is a growing interest in the use of bimetallic catalysts. In the case of Pd, a whole range of elements has been used as promoting element which can improve either the geometry of the catalyst or the transfer of electrons. For example, the combination of chemically produced Pd and Fe is a very well-documented bimetallic catalyst for reduction reactions (Zhang *et al.*, 1998; Crabb and Marshall, 2001). Also other transition metals like Cu, Ni and Ag have been used as promoting element for Pd catalysts (Tzitzios and Georgakilas, 2005; Massard *et al.*, 2007; Kim *et al.*, 2008). The combination of Pd and Au is also widely applied for reductive reactions, for example dechlorination of TCE (Nutt *et al.*, 2005). These bimetallic catalysts can have different structures, such as alloys (Venezia *et al.*, 2003) or core-shell structures (Nutt *et al.*, 2005). Doping of bio-Pd, produced by *S. oneidensis*, with Au(0) is currently under investigation. First results indicate that a bio-Pd/Au catalyst, synthesized by co-precipitation of Pd and Au, can significantly increase the reaction rates of dechlorination reactions (S. De Corte, T. Henebel, J.P. Fitts, T. Sabbe, W. Verstraete and N. Boon, unpubl. results).

Assuring long-term stability of the product

A topic that urgently requires more in-depth studies is the long-term stability of bio-Pd as a product. Three phenomena can occur when preserving bio-Pd for longer periods of time: (i) leaching of the NPs from the biomass, (ii) leaching of Pd(II) from the metallic nanoparticle and (iii) poisoning of the catalyst.

Leaching of Pd nanoparticles can be due to detachment from the cell wall or desintegration of the bacterial biomass. Degradation of the bacterial support will cause release of NPs and thus needs to be avoided. The cell structures need to stay intact for longer periods of time by an appropriate preservation technique. Little is known about the fate of nanoparticles in the environment and potential adverse health effects, but due to the precautionary principle, their release needs to be prevented. The leaching of ions is commonly reported for catalysts based on transition metals (Calvo *et al.*, 2010). Leaching of Pd(II) depends mostly on the type of solvent and the pH of the reaction medium. In the case of chemical synthesis reactions, such as the Pd catalysed Suzuki-Miyaura coupling reactions, also the presence of additives (base, TBAB) and substrates (aryl halides, phenyl boronic acid) play a crucial role.

Another limitation for the use of bio-Pd might imply the poisoning of the catalyst. For example, sulfides are known to have a strong affinity for the Pd metal and may block the active sites of the catalyst via formation of strong Pd-S bonds and layers of sulfide around the Pd clusters (Gravil and Toulhoat, 1999; Alfonso *et al.*, 2003). Since sulfide is a natural water constituent under reducing conditions produced by microbial sulfate reduction, sulfide induced catalyst deactivation is a crucial issue which hinders the full exploitation of the catalyst potential as a treatment technology for groundwater remediation (Angeles-Wedler *et al.*, 2009). A possible approach to prevent sulfide poisoning is the oxidative removal of sulfide prior to any contact with the noble metal (Angeles-Wedler *et al.*, 2008).

High temperature applications in organic chemistry

In advanced synthetic organic chemistry and petrochemistry, homogeneous and heterogeneous Pd catalysts are very frequently used. Heterogeneous catalysts allow an easy separation of the catalyst from the reaction medium. Due to the attachment of the nanoparticles to the bacterial cell wall structures, the use of bio-Pd could prevent the leaching of Pd NPs in the synthesized product. Therefore, bio-Pd could be an interesting heterogeneous Pd catalyst. However, these reactions are often performed at high temperatures. Under these circumstances, the bacterial cells will disintegrate. By doing so, sulfur will be released and will consequently poison the catalyst. Preliminary

tests in dehydrogenation reactions at 250°C indicated a very fast loss of catalytic activity due to sulfur poisoning (S. De Corte, T. Hennebel, W. Verstraete and N. Boon, unpubl. results). This phenomenon will be difficult to overcome because sulfur is an essential constituent of microbial biomass.

Efficient delivery of the hydrogen donor

Several studies showed that multiple hydrogen donors can be used for Pd driven reactions. Indeed, not only hydrogen gas but also formate, formic acid, isopropanol and ethanol can effectively donate reactive hydrogen species for the reduction and dehalogenation of pollutants (Kopinke *et al.*, 2004; De Windt *et al.*, 2005; Hennebel *et al.*, 2009b). In the case of bio-Pd, only H₂, formate and formic acid were effective to charge the nanoparticles and it was demonstrated that H₂ was more effective than formate (Hennebel *et al.*, 2009b). However, a major challenge is the efficient supply of the hydrogen donor to the catalyst. Yet, the external supply of H₂ gas can give rise to large costs and technical difficulties because of the high safety risks for gas storage (Hennebel *et al.*, 2011b). As discussed in *Reactor set-ups using a bio-Pd catalyst: state of the art*, several reactor types were constructed to limit the hydrogen supply, for example by a membrane contactor (Hennebel *et al.*, 2010), or to produce hydrogen gas in a sustainable way, for example by using microbially produced H₂ (Humphries *et al.*, 2007), or by using a H₂-producing species for Pd reduction, e.g. *C. pasteurianum* (Chidambaram *et al.*, 2010).

A promising configuration for the gentle delivery of a hydrogen donor to a catalyst is a microbial electrolysis cell (MEC). In MEC systems, electrons travel through an external circuit to a cathode, where they can be consumed for H₂ formation (Mu *et al.*, 2009). Yet, electrical energy needs to be supplied to the electrical circuit by means of a power source in order to provide the correct potential for H₂ generation (−0.414 V). Hennebel and colleagues (2011a) have demonstrated the opportunities of such a system by the development of a MEC with bio-Pd coated on graphite at the cathode for catalytic dehalogenation of diatrizoate and TCE. Applying −0.8 V with a power source, 93% removal of the contaminant was achieved after 1 h of recirculation at neutral pH and complete removal after 3 h, whereas only 48% removal was observed using the same MEC with non-Pd coated graphite granules. Biocatalysed electrolysis required a quarter (Rozendal *et al.*, 2006) to half (Hennebel *et al.*, 2011a) of the energy needed for conventional water electrolysis (Rasten *et al.*, 2003). The price of hydrogen produced through water electrolysis is strongly dependent on the electricity price (Turner, 2004). As this can be expected for

biocatalysed electrolysis as well, the reduced consumption of electrical energy per unit of hydrogen is a strong advantage of biocatalysed electrolysis.

Outlooks

Design of a cost-effective technology

The economical feasibility is the main determining factor for pilot-scale and full-scale bio-Pd applications. Several costs need to be taken into account. A first cost is attributed to the production of the bio-Pd. The culturing and subsequent handlings of the bacteria can amount to €1000 kg^{−1} cell dry weight. The cost of the palladium salt can be approximately €20 000 kg^{−1} and is dependent on the extremely volatile price of Pd. This cost can be 70% lower if the Pd is recovered from a Pd(II) containing waste stream or leachate (personal communications with Umicore, Belgium). However, some synthetic Pd salt might still be required for pre-palladization of the biomass. When Pd nanoparticles are purely chemically produced, the cost for culturing of the bacteria is omitted, but extra costs are attributed to the use of chemicals: reducing agents (e.g. NaBH₄), stabilizers (e.g. polyvinylpyrrolidone) and carrier materials (mostly activated carbon or Al₂O₃).

In order to prevent leaching of the Pd in reactor effluents, one can choose either for membrane filtration or for encapsulation. The cost for microfiltration membranes can be estimated on about €100 m^{−2}. Hennebel and colleagues (2009c) estimated the cost of encapsulation of bio-Pd in several materials. For a reactor with 100 mg bio-Pd l^{−1}, the price for bio-Pd coated on zeolites was about €75 m^{−3} whereas for polyacrylamide beads this cost amounted to €2250 m^{−3}. Moreover, the loss of activity due to encapsulation needs to be taken into account. Also the cost of the hydrogen donor needs to be included. Since H₂ is mostly produced electrochemically, its cost is very dependent on the energy price. A less expensive alternative for H₂ production is a MEC (see *Efficient delivery of the hydrogen donor*). When fermenting bacteria are used, H₂ can be delivered *in situ* and this cost can be omitted. However, the cost for culturing these strains can be higher due to the continuous need for anaerobization and the continuous dosage of a carbon source.

Application of bio-Pd to treat groundwater, soils and sludges

The largest environmental market for the technologies presented in this work is soil remediation. Indeed, halogenated pollutants are ubiquitous in groundwater and soils. The suggested technologies can be applied as a part of a 'pump-and-treat' strategy for the sanitation of groundwater or as *in situ* remediation technology by supplying the Pd catalyst in the soil. Conventional pump-and-treat methods

involve pumping contaminated water to the surface followed by treatment with for example air stripping and/or adsorption on activated carbon. A successful pump-and-treat-based field-study using chemical Pd coated on egg shells in a fixed bed reactor was performed at Edward Air Force Base (California, USA; Davie *et al.*, 2008). Until now, only one field-study explored the applicability of bio-Pd at larger scale. Groundwater contaminated with a mixture of hexachlorohexanes and chlorobenzenes was treated in a fluidized bed reactor with 20 g of bio-Pd encapsulated in alginate at a flow rate and contact time of 50 l h⁻¹ and 4 h respectively (Hennebel *et al.*, 2011b). Further up-scaling projects will need to examine the feasibility of full-scale bio-Pd treatments. Biocatalytic treatment can economically compete with currently used other pump-and-treat technologies. However, it needs to be shown that bio-Pd offers advantages, such as price or sustainability of the production process, over chemically produced Pd catalysts.

The pump-and-treat technology will not be effective for contaminants which strongly interact with the soil matrix. For example, PCBs tend to adsorb on soil particles due to their hydrophobicity. Also metals are sometimes difficult to pump due to their affinity to soil components. In these cases, the pump-and-treat with bio-Pd will be ineffective. Therefore, an alternative technology was developed consisting of the supply of a Pd(II) salt and a carbon source (e.g. glucose) to a soil. It was shown that Pd NPs were formed by biofilms of an inoculated culture of *C. pasteurianum* on sand grains in aquifer mesocosms (Chidambaram *et al.*, 2010). Subsequently, an almost complete reduction of the toxic Cr(VI) was obtained. However, the inoculation is very likely not required in a remediation programme, as many bacteria that reduce Pd and form Pd NPs will belong to the indigenous microbial community. The advantage of such a technology would be the good contact between catalyst, pollutant and soil matrix. A drawback of this technology might be the low efficiency of Pd reduction and hence the environmental implications and costs related to the unreduced Pd(II). An example of a niche market for this bio-Pd-based technology might be the clean-up of river and lake sludges contaminated with PCBs. One could mix the bio-Pd and a slowly degradable organic carbon source with these sludges at extremely low concentrations approaching the background values of metals. Autochthonous bacteria would degrade the carbon source and form hydrogen gas in these anaerobic environments. The latter could be used as electron donor to charge the bio-Pd. A final and more controlled way of bio-Pd application in soils could be in reactive barriers or wells. It is clear that bioremediation strategies are not yet elaborated in comparison with the pump-and-treat strategy. Many fundamental aspects such as the durability of the dehalogenation process, the sensitivity for poisoning and

the *in situ* hydrogen production need to be addressed in batch and pilot scale experiments. Moreover, technological aspects such as a homogeneous dosing of the catalyst and the electron donor to soils or sludges need to be studied.

Application of bio-Pd for wastewater treatment

A major issue of conventional wastewater treatment is the presence of micropollutants such as pharmaceuticals and pesticides at concentration levels of µg l⁻¹ in the effluents of WWTPs. The use of bio-Pd-based reactors holds promising opportunities for reductive post-treatment of WWTP effluents and hospital wastewaters in order to decrease the environmental load of halogenated micropollutants from these point sources. Catalytic dechlorination and deiodination using biogenic palladium NPs should be further explored for these pharmaceuticals that cannot be treated by other advanced treatment methods. As proof of principle, the removal of ICM was demonstrated (see *Bio-Pd catalysed removal of environmental contaminants*). These ICM are not effectively removed in WWTPs and because of their high degree of substitution of the benzene ring, they are even resistant to ozonation and other advanced oxidation techniques (Ternes *et al.*, 2003). Another recalcitrant compound of recent interest is diclofenac, a non-steroidal anti-inflammatory drug. It is one of the pharmaceuticals most detected in WWTP effluents worldwide (Zhang *et al.*, 2008; Okuda *et al.*, 2009; Sodre *et al.*, 2010). According to the precautionary principle, the environmental input of such persistent and hence long-living, mobile compounds should be limited. Moreover, these contaminants considerably contribute to the absorbable organic halogen (AOX) content in wastewater, especially in hospital wastewaters and effluents from radiographic practices (Putschew *et al.*, 2000; Schitko *et al.*, 2004). The AOX will become an important parameter of wastewater regulation. Consequently, the development of innovative post-treatment methods is of utmost importance in the coming decade.

Application of bio-Pd for drinking water production

In drinking water production, the application of reductive treatment methods should be considered since pharmaceutical residues can be introduced into groundwater through surface water filtration, leakage and groundwater recharge. Because the sorption affinity of pharmaceuticals to sludge and suspended matter is limited, their retention by bank filtration is insufficient, which makes that they were detected in drinking water supplies (Seitz *et al.*, 2006). For example, trace concentrations of diclofenac were detected in Berlin tap water (Heberer, 2002). Therefore, we suggest the implementation of a bio-Pd-based reactor system at the inlet of drinking water facilities that

make use of groundwater resources. Immediately after pumping the water out of the subsoil, the water is anoxic, which makes it possible to maintain a reducing environment for catalytic dehalogenation in the reactor tank. After aeration, the dehalogenated metabolites can be further degraded/metabolized by oxidative metabolic or cometabolic processes (De Gussemé *et al.*, 2009; 2011), for example during sand filtration.

Conclusions

In the past decade, a wide range of microorganisms were reported to be capable of precipitating Pd(0) NPs ('bio-Pd') from solutions containing Pd(II). It was shown that bio-Pd can be a potentially powerful tool as metal recovery technique and in environmental applications such as reduction of inorganic contaminants and dehalogenation of organic pollutants. The microbial scaffold allows a green synthesis of NPs and offers some advantages and perspectives for the development of reactor technologies for metal recovery and soil and water treatment (groundwater, wastewater and drinking water). However, some main issues need to be addressed. For example, the hydrogen donor should be delivered to the catalyst in a sustainable way. In addition, up-scaling of the production process needs to be optimized and the long-term stability of the catalyst needs to be assured. If these conditions are fulfilled, an economically feasible and effective full scale technology can be developed.

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