“Everything must be made as simple as possible, but not simpler.”

Albert Einstein

Promoters:

Prof. dr. ir. Nico Boon
Department of Biochemical and Microbial Technology, Faculty of Bioscience Engineering, Ghent University, Gent, Belgium

Prof. dr. ir. Willy Verstraete
Department of Biochemical and Microbial Technology, Faculty of Bioscience Engineering, Ghent University, Gent, Belgium

Members of the examination committee:

Prof. dr. Rikke Louise Meyer
Interdisciplinary Nanoscience Center, Department of Bioscience, Aarhus University, Aarhus, Denmark

Prof. dr. Geoffrey Gadd
Molecular Microbiology Division, College of Life Sciences, University of Dundee, Dundee, United Kingdo

Prof. dr. ir. Tom Van Gerven
Department of Chemical Engineering, Faculty of Engineering Science, Catholic University of Leuven, Leuven, Belgium

Prof. dr. ir. Arne Verliefde
Department of Applied Analytical and Physical Chemistry, Faculty of Bioscience Engineering, Ghent University, Belgium

Prof. dr. ir. Gijs Du Laing
Department of Applied Analytical and Physical Chemistry, Faculty of Bioscience Engineering, Ghent University, Belgium

Prof. dr. ir. Christian Stevens
Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Belgium

Dean:

Prof. dr. ir. Guido Van Huylenbroeck

Rector:

Prof. dr. Paul Van Cauwenberghe

OPTIMIZATIONS, LIMITATIONS AND INNOVATIONS OF BIOSUPPORTED PALLADIUM NANOCATALYSTS

ir. Simon De Corte

Thesis submitted in fulfillment of the requirements for the degree of Doctor (Ph.D.) in Applied Biological Sciences

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Optimalisaties, beperkingen en innovaties van Pd nanokatalysatoren op biologische dragers

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Notation index

2,4-DPO	2,4-difluorophenyl-piperidin-4-yl-oxime
2-APA	2-anilinophenylacetate
2-(2-Cl)-APA	2-(2-chloro)-anilinophenylacetate
AAS	Atomic absorption spectroscopy
ANOVA	Analysis of variance
AOP	Advanced oxidation process
AOX	Adsorbable organic halogens
APTES	3-aminopropyltriethoxysiloxane
CAS	Conventional activated sludge
CDW	Cell dry weight
CFC	Chlorofluorocarbon
DMSO	Dimethylsulfoxide
DNAPL	Dense non-aqueous phase liquid
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetra-acetic acid
EDX	Energy dispersive X-ray spectroscopy
EN	Electronegativity
EtOH	Ethanol
EU	European Union
FID	Flame ionization detector
FWHM	Full width at half maximum
GC	Gas chromatography
GHG	Greenhouse gas
HPLC	High performance liquid chromatography
ICM	Iodinated contrast media
ICP-OES	Inductively coupled plasma – optical emission spectroscopy
LC	Liquid chromatography

LC_{50}	Lethal concentration for 50 % of the test population
LCD	Liquid crystal display
LC-MS^n	Liquid chromatography coupled to multiple mass spectrometry
LOQ	Limit of quantification
LSD	Least significant difference
LTQ	Linear trap quadrupole
MS	Mass spectrometry
NHS	N-hydroxysuccinimide
OD	Optical density
PBDE	Polybromodifenylethers
PCB	Polychlorinated bifenyl
PCE	Perchloroethylene
PEI	Polyethyleneimine
PFC	Perfluorinated compound
PGM	Platinum group metals
PI	Propidium iodide
REE	Rare earth element
RI	Refractive index
SG	SYBR Green I
SPR	Surface plasmon resonance
STEM	Scanning transmission electron microscopy
TBAB	Tetrabutylammoniumbromide
TEM	Transmission electron microscopy
TCE	Trichloroethylene
UV	Ultraviolet
WWTP	Wastewater treatment plant
XRD	X-ray diffraction
µXRD	Micro X-ray diffraction

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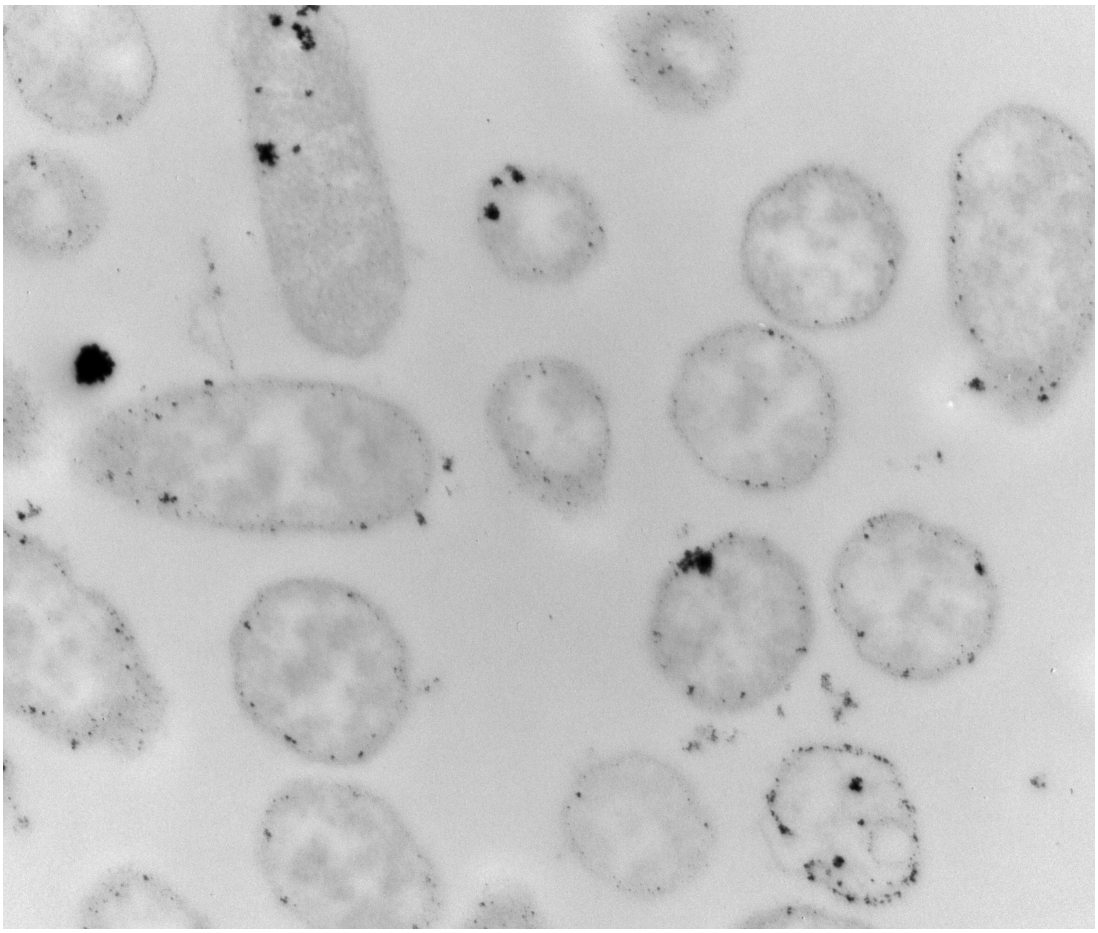
Part I

Introduction



Chapter 1
Introduction

1 Palladium: a precious catalyst

The chemical element palladium (Pd) was discovered in 1803 by the English chemist and physicist William Hyde Wollaston. It is a transition element with atomic number 46 and an atomic mass of 106.42. Its common oxidation states are 0, +1, +2 and +4. Together with rhodium (Rh), ruthenium (Ru), osmium (Os), iridium (Ir) and platinum (Pt), it forms the platinum group elements (PGM). Together with gold (Au) and silver (Ag), the PGM are considered as 'precious metals'. The worlds largest producers of Pd are Russia (44%) and South Africa (40%). Pd finds its applications in:

- Electronics: Pd and Pd-Ag electrodes are used in multilayer ceramic capacitors;
- Jewelry: Pd is used to make white gold alloys;
- Dentistry: dental crowns are made of alloys containing Pd;
- Catalysis: the most important application.

Pd is used as a catalyst in both oxidative and reductive reactions. About half of the world's Pd is used for three-way converters in vehicles. These catalytic beds oxidize hydrocarbons to CO_2 and reduce nitrogenous compounds to N_2. An important characteristic of Pd and probably the main reason for its popularity as a catalyst is the interaction with hydrogen (H). Pd can dissociatively adsorb H_2, causing a homolytical dissociation into H-radicals, which are stored within the free spaces of the Pd crystal lattice. This characteristic has made heterogeneous Pd catalysts (the Pd catalyst is in a solid phase on a carrier material, whereas the reagents are in a dissolved or gaseous phase) as one of the most important catalysts for several reaction types such as hydrogenation, dehydrogenation, hydrodehalogenation. In addition, Pd is also used for

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the oxidation of alcohols and for C-C coupling reactions in organic chemistry, such as the in Suzuki-, Heck-, Negishi-coupling reactions. The latter authors won the Nobel Prize for Chemistry in 2010. For an overview of Pd-catalyzed reactions, see Tsuji (1995).

More recently, the use of Pd for environmental purposes and water treatment has also been investigated, for example for the dehalogenation of chlorinated contaminants such as TCE, PCE, PBDEs and halogenated pharmaceuticals, the reduction of oxyanions such as nitrate and perchlorate and the N-N hydrogenolysis of azo dyes (for a review on Pd in water treatment, see Chaplin et al. (2012)).

2 Palladium nanoparticles

2.1 Nano vs. bulk

These days, heterogeneous Pd catalysts are more and more used under the form of nanoparticles instead of bulk material. Nanomaterials are defined by the European Commission as materials between 1 and 100 nm in size in at least one dimension. The (re)activity of nanoparticles is bigger than bulk materials because of the increased specific surface area (i.e. the surface area per unit of volume). Moreover, nanoparticles can show completely different characteristics compared to their bulk counterparts, such as different optical (Huang et al., 1997), magnetic (Huang et al., 1999) and electronic characteristics (Lue et al., 1995). This is mainly due to:

- Surface effects: nanoparticles contain proportionally more surface atoms than bulk atoms, resulting in a higher average binding energy per atom;
- Quantum size effects: by altering the size of a particle, the energy gap between the valence and the conduction band can change, resulting in varying optical and electrical properties.

An example of the difference between bulk and nanoparticles, which is very relevant for this work, is the fact that Au nanoparticles can show catalytic activity, whereas bulk Au is inert (see 7.2).

2.2 Conventional production methods

Conventional production methods of nanomaterials can be classified as either physical or chemical methods. Physical methods mainly include ‘top-down methods’: the size of bulk materials is decreased by means of mechanical crushing, pulverization or electrochemical destruction. This results in a powder with a relatively wide particle size distribution. Chemical production methods are mostly ‘bottom-up methods’: atoms are

formed by reduction of metal ions. These atoms aggregate together to form nanoparticles. Conventional reductants are H_2, citrate, $NaBH_4$ and $LiAlH_4$. A suspension of nanoparticles requires addition of a stabilizer (e.g. poylvinylpyrrolidone) to avoid aggregation of the particles into micron-size aggregates. Also capping agents are often used, they prevent growth of the particles. For catalytic purposes, Pd nanoparticles are very often impregnated on a carrier material. This makes the nanocatalysts more easy to retain in the medium and prevents release into wastestreams and the environment. The most frequently applied carriers for Pd nanoparticles are Al_2O_3, SiO_2 and activated carbon. It is clear that a lot of chemicals are involved in the chemical production process of Pd nanoparticles. These substances can be scarce, aggressive, toxic or expensive. In the last decades, a more sustainable production method for Pd nanoparticles, requiring less energy and chemicals, has extensively been explored. It exploits the bioreductive deposition of metals by bacteria. The product obtained by this method is further referred to as 'bio-Pd'.

3 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 1-1). The first reported species with Pd-reducing capacities was the sulphate-reducing bacterium *Desulfovibrio* desulfuricans (Lloyd et al., 1998). After incubation under anaerobic conditions, Pd(II) was added to the culture together with H_2 or formate as electron donor. Subsequently, nanoscale deposits of Pd(0) at the cell surface were observed ('bio-Pd'). When hydrogenases were inhibited with Cu, this reduction did not occur. This strongly indicated that a hydrogenase (possibly in combination with cytochrome c_3) 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 et al. (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 cytoplasmatic membrane. The authors suggested that the enzyme probably supplied the electrons for the reduction process and served as a nucleation site for particle growth.

Table 1-1: Overview of the 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
Desulfovibrio desulfuricans	G -	Anaerobe	Sulphate reducing, metal reducing	Lloyd et al., 1998; Yong et al., 2002
Desulfovibrio vulgaris	G -	Anaerobe	Sulphate reducing	Baxter-Plant et al., 2003; Humphries et al., 2005
Desulfovibrio fructosivorans	G -	Anaerobe	Sulphate reducing	Mikheenko et al., 2008
Shewanella oneidensis	G -	Facultative anaerobe	Metal reducing	De Windt et al., 2005
Paracoccus denitrificans	G -	Facultative anaerobe	Nitrate reducing	Bunge et al., 2010
Pseudomonas putida	G -	Aerobe		Bunge et al., 2010
Cupriavidus necator	G -	Facultative aerobe	Resistance to heavy metals	Bunge et al., 2010
Cupriavidus metallidurans	G -	Facultative aerobe	Resistance to heavy metals	Gauthier et al., 2010
Rhodobacter sphaeroides	G -	Facultative aerobe	Photosynthetic, metal resistant	Redwood et al., 2008
Bacillus sphaericus	G +	Aerobe		Creamer et al., 2007
Plectonema boryanum	G -	Aerobe	Cyanobacterium	Lengke et al., 2007
Calothrix	G -	Aerobe	N-fixing Cyanobacterium	Brayner et al., 2007
Anabaena	G -	Aerobe	N-fixing Cyanobacterium	Brayner et al., 2007
Clostridium pasterianum	G +	Anaerobe	Metal reducing, H_2 producing through fermentation	Chidambaram et al., 2010
Citrobacter braakii	G -	Facultative anaerobe	H_2 producing through fermentation	Hennebel et al., 2011c
Clostridium butyricum	G +	Anaerobe	H_2 producing through fermentation	Hennebel et al., 2011c
Bacteroides vulgatus	G -	Anaerobe	Arsenic reducing, H_2 producing through fermentation	Hennebel et al., 2011c
Klebsiella pneumoniae	G -	Facultative anaerobe	H_2 producing through fermentation	Hennebel et al., 2011c
Escherichia coli	G -	Facultative anaerobe	H_2 producing through fermentation	Deplanche et al., 2010; Hennebel et al., 2011c
Enterococcus faecium	G +	Facultative anaerobe	H_2 producing through fermentation	Hennebel et al., 2011c

Another bacterial species that has been extensively studied in this context is the iron reducing bacterium *Shewanella oneidensis* (Figure 1-1A). 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 (H_2, formate, lactate, pyruvate, ethanol) with H_2 and formate being the most efficient donors (De Windt et al., 2005). Interestingly, the presence of O_2 did not significantly inhibit the reduction process. A similar reduction mechanism as for *D. desulfuricans*, based on the involvement of hydrogenases and cytochrome c_3 was proposed. In addition, the authors suggested a role for the formate dehydrogenase enzyme in the reduction process.

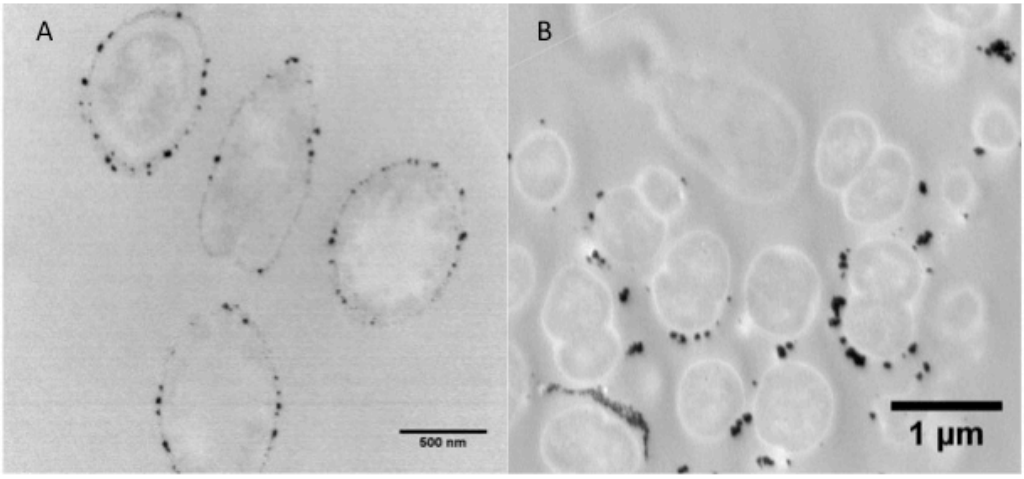


Figure 1-1: A: Nanoparticles of Pd(0) in and on the outer cell parts of *Shewanella oneidensis*. B: Nanoparticles of Pd(0) precipitated by the fermentative bacterium *Citrobacter braakii*.

Bunge et al. (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) nanoparticles were formed both in the periplasm and on the cell surface of *Paracoccus denitrificans*, *Pseudomonas putida* and *Cupriavidus necator* after 14 hours incubation with formate. Pd(0) was also formed with autoclaved 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) nanoparticles out of Pd(II) (Brayner et al., 2007; Lengke et al., 2007). Deposits of Pd(0) were found in the medium, on the cell surface and intracellularly. Brayner et al. (2007) suggested the nitrogenase enzyme to be responsible for the reduction.

A new approach was to produce Pd(0) through fermentative bacteria (Chidambaram et al., 2010; Hennebel et al., 2011c). Several fermentative species produce H_2 during fermentation processes and can subsequently reduce Pd(II) to Pd(0) (Table 1-1). 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 Figure 1-1B. This fermentatively produced H_2 (referred to as 'biohydrogen') can subsequently be used as a reductant for Pd(II) reduction. Addition of Pd(II) to a fermenting culture of *C. 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 et al. (2010) attributed the reduction of Pd(II) by *E. coli* to three types of hydrogenases.

D. 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 cyanobacteria. 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 et al. (2010) and Sobjerg et al. (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 H_2. 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.

4 Use of bio-Pd as a catalyst

4.1 Bio-Pd catalyzed removal of environmental contaminants

Bio-Pd was successfully applied to remove a wide range of environmental contaminants (Table 1-2). 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, …

Table 1-2: 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	*D. desulfuricans* *D. vulgaris* *E. coli* *C. pasteurianum*	Humphries et al., 2005; Mabbett et al., 2006 Chidambaram et al., 2010
ClO_4^-	Reduction	Groundwater and drinking water	*S. oneidensis*	De Windt et al., 2006
Polychlorobifenyls (PCBs)	Dechlorination (1-10 Cl)	Air, water, soil, sediments	*D. desulfuricans* *D. vulgaris* *S. oneidensis*	Baxter-Plant et al., 2003 De Windt et al., 2005
Chlorophenols	Dechlorination (1 Cl)	Industrial wastewaters, groundwater, soil, sediments	*D. desulfuricans* *D. vulgaris*	Baxter-Plant et al., 2003
Lindane	Dechlorination (6 Cl)	Soil and groundwater	*S. oneidensis*	Mertens et al., 2007
Trichloroethylene (TCE)	Dechlorination (3 Cl)	Groundwater	*S. oneidensis*	Hennebel et al., 2009b
Polybrominated diphenyl ethers (PBDE)	Debromination (1-10 Br)	Indoor air and dust	*D. desulfuricans*	Harrad et al., 2007; Deplanche et al., 2009
Iodinated Contrast Media (ICM)	Deiodination (3 I)	Wastewaters and surface waters	*S. oneidensis* *C. braakii*	Hennebel et al., 2010; Forrez et al., 2011b Hennebel et al., 2011c

In all cases, a hydrogen donor (H_2 or formate) was used as the reductive agent to charge the bio-Pd catalyst (Figure 1-2A). 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 species are used which produce biohydrogen (Figure 1-2B) (Chidambaram et al., 2010; Hennebel et al., 2011c).

Bio-Pd catalysts showed a higher catalytic activity than black Pd powder for the dechlorination of PCBs (De Windt et al., 2005), however this is not a fair comparison since this Pd powder does not consist of nanoparticles. Hennebel et al. (2010) mentioned that a nano Pd/Al_2O_3 catalyst was a factor 2,5 to 30 more active than bio-Pd for the deiodination of diatrizoate. No big differences in terms of selectivity were

observed for bio-Pd and chemical Pd catalysts applied for the degradation of environmental contaminants.

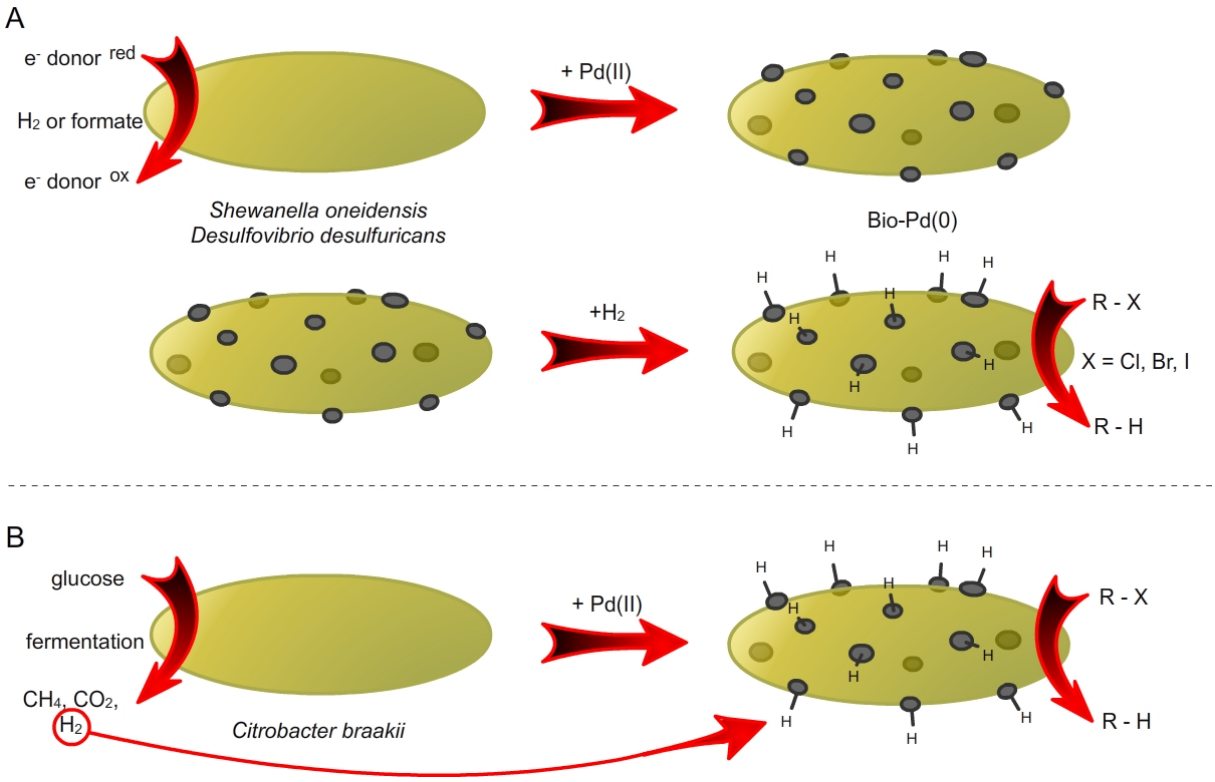


Figure 1-2: A: Two-step dehalogenation of halogenated substances with a bio-Pd catalyst and an external hydrogen donor. B: One-step dehalogenation of halogenated substances with a bio-Pd catalyst produced by fermentative species.

4.2 Bio-Pd as a catalyst in synthetic organic chemistry

Bio-Pd (precipitated by *Desulfovibrio 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-methlisoquinoline, differing from the previous product by only one methyl group, could not be hydrogenated at all. In general, when used in different solvents, bio-Pd gave more consistent results in terms of selectivity compared to Pd/Al_2O_3 (Bennett et al., 2010), indicating that the bacterial carrier is less sensible to changes in reaction condtions than the Al_2O_3 carrier. This is probably due to the location of the particles in the outer layers of the cell matrix, where they have less contact with the reaction

medium, compared to the Al_2O_3 carrier, or to the different surface properties of the carriers. The applicability of bio-Pd catalysts for coupling reactions in synthetic organic chemistry was reported by Sobjerg et al. (2009). Two bacteria (*Pseudomonas putida* and *Cupriavidus necator*) were used to precipitate Pd(0). Both forms of bio-Pd could successfully catalyze the Suzuki-Miyaura coupling of an aryl halide with phenylboronic acid and the Mizoroki-Heck coupling of an aryl halide with n-butylacrylate. Gauthier et al (2010) apply bio-Pd recovered from a metal-containing leachate by 2 different *Cupriavidus* species for the Mizoroki-Heck coupling.

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

5.1 Bio-Pd for in situ groundwater remediation

Chidambaram et al. (2010) showed that Pd(II) could be reduced by a biofilm of *Clostridium 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 µXANES. In a microcosm with viable cells without added Pd(II), no Cr(III) was detected.

5.2 Use of bio-Pd in aqueous suspensions

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 (

Figure 1-3A) 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., 2011b). 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 (

Figure 1-3B). A plate membrane reactor filled with bio-Pd (produced by *S. oneidensis*) was developed by Hennebel et al. (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 H_2 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}).

5.3 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 et al. (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

Figure 1-3C. The encapsulated bio-Pd had at least a six times lower dechlorination rate for TCE to ethane compared to 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 to 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 et al. (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 H_2 was dosed in a controlled way at the other side of the membrane. A scheme of this setup is shown in

Figure 1-3D. The aim of this set-up was to dose H_2 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 hours at pH 10.

Next to encapsulation in polymeric matrices, Hennebel et al. (2009c) coated bio-Pd on zeolites. This coated bio-Pd was 5 times less active for the dechlorination of TCE compared to a bio-Pd suspension. This coating on porous zeolites is illustrated in

Figure 1-3E. Another coating surface is graphite (Hennebel et al., 2011a). This graphite can be further used as a cathode of a bio-electrochemical system. 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 by lowering the activation energy for the anode half-reaction. The maximum power output of a bio-Pd containg cell was 90 % of the power of a cell containing a chemical Pd catalyst.

Another study showed that a biofilm of *Serratia,* which was grown on polyurethane foam, 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).

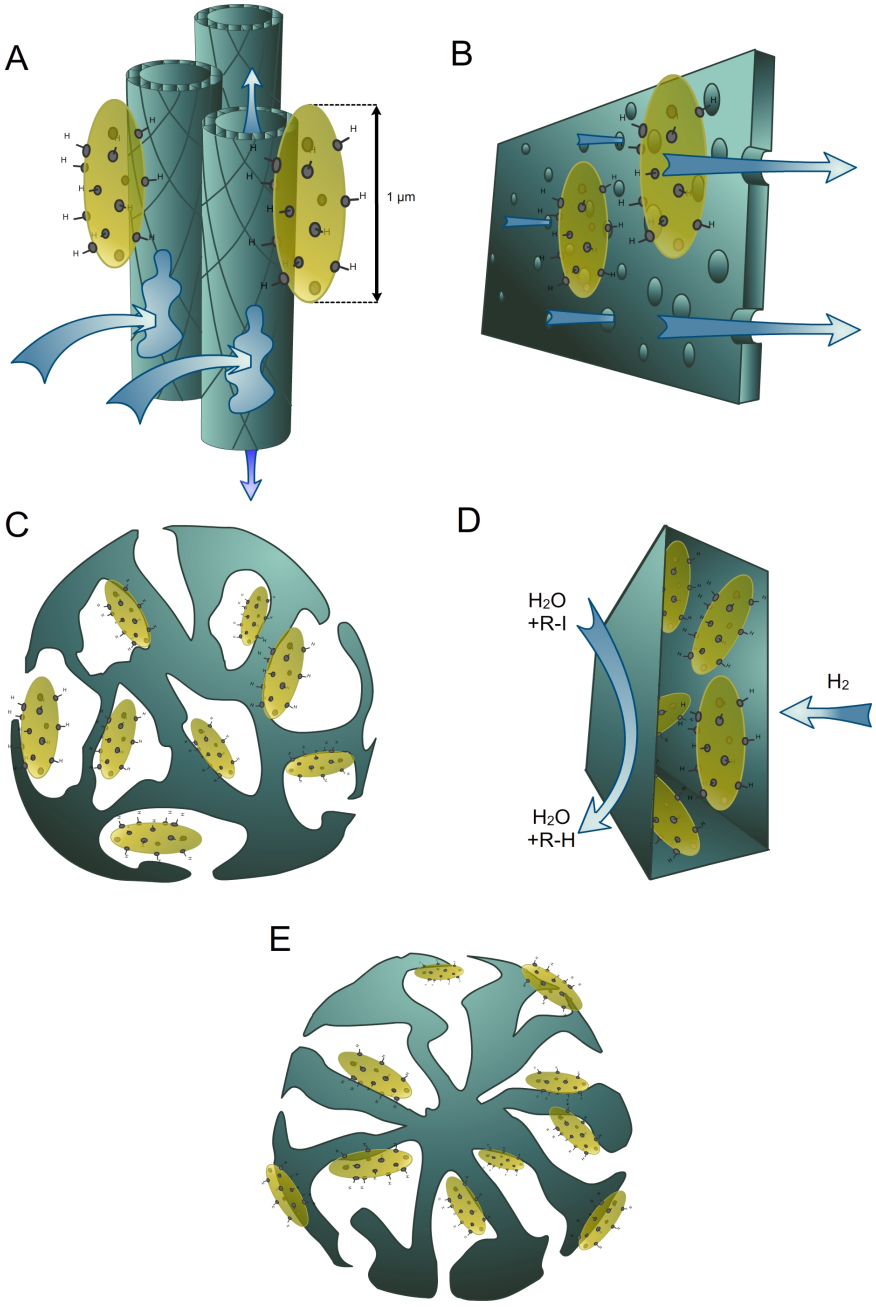


Figure 1-3: Retention mechanisms of bio-Pd in reactor set-ups (not to scale). A: Retention by hollow fibre membranes. B: Retention by plate membranes. C: Encapsulation in alginate beads. D: Encapsulation in polymeric membranes. E: Coating on zeolites.

6 Possible limitations of bio-Pd catalysts

6.1 Lower activity compared to chemically produced Pd catalysts

Bio-Pd catalysts show a higher activity compared to commercial bulk Pd powder (almost complete degradation of 1 mg L^{-1} PCB 21 after 50 minutes using 500 mg L^{-1} bio-Pd and H_2, and only 65 % degradation using 500 mg L^{-1} commercial Pd powder (De Windt et al., 2005)). However, in order to make a fair comparison, the activity of bio-Pd nanocatalysts should be compared to chemically produced Pd nanoparticles. The catalytic activity of Pd nanoparticles on Al_2O_3 largely exceeded the activity of bio-Pd for the deiodination of diatrizoate (first order rate constants of 56.9 ± 5.9 L g_{Pd}^{-1} h^{-1} for bio-Pd (Hennebel et al., 2010) and a factor 50 higher for Pd/Al_2O_3 (Knitt et al., 2008)) and the dechlorination of TCE (1.38 ± 0.22 L g_{Pd}^{-1} min^{-1} for bio-Pd (Hennebel et al., 2009b) vs. 12.2 L g_{Pd}^{-1} min^{-1} for Pd/Al_2O_3 (Nutt et al., 2005)). This could be due to the fact that the bio-Pd particles are partly embedded in the bacterial cell wall and in the periplasmic space and therefore have less catalytic surface available compared to Pd nanoparticles on more conventional carriers.

6.2 Catalyst poisoning by sulfur

Another limitation for the use of Pd catalysts 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 et al., 1999; Alfonso et al., 2003). Poisoning by sulfide occurs already at very low sulfide concentrations: a 20 % activity decrease was observed for the reduction of nitrate at sulfide concentration of 1.42 mg S g^{-1} Pd, whereas a complete inhibition of the activity was observed at 13 mg S g^{-1} Pd (Chaplin et al., 2007). 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).

Another source of sulfur is the bacterial carrier itself. Bacteria typically contain on average between 0.30 and 0.56 fg S $cell^{-1}$, but values up to 13 fg S $cell^{-1}$ have been reported for growing E. coli cells, C/S ratios between 12 and 60 have been reported (Fagerbakke et al., 1996). Due to aggressive conditions or thermal decomposition, the bacterial carrier can be destroyed, sulfur can be released and poison the surface of the catalyst. Therefore, it is important that the bacterial carrier is kept intact and that release of sulfur is prevented. It is advisable that bacteria, which are grown for bio-Pd production are cultivated with as few sulfur as possible in the culture media. Nevertheless, sulfur is an indispensable constituent of some amino acids (cysteine, methionine) and will always be present in bacterial cells. For these reasons, sulfur-free sustainable carrier materials should also be considered as an alternative for bacterial cells.

6.3 High temperature and pressure applications

In advanced synthetic organic chemistry and petrochemistry, homogeneous and heterogeneous Pd catalysts are very frequently used for hydrogenation and dehydrogenation reactions. 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 in the synthesized product. Therefore, bio-Pd could be an interesting heterogeneous Pd catalyst. However, these reactions are often performed at elevated temperatures and pressures. Under these circumstances, the bacterial cells will disintegrate. By doing so, nanoparticles can be released from the carrier and can end up in the final product (see also 6.4). Also aggregation of nanoparticles into micron-size aggregates can occur. Moreover, sulfur can be released and will consequently poison the catalyst (see also 6.3).

6.4 Unknown stability

In order to be a competitive alternative for conventional heterogenous Pd catalysts, the stability of bio-Pd needs to be studied. Three phenomena can occur when preserving bio-Pd for longer periods of time: (i) leaching of the nanoparticles 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 nanoparticles 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 catalyzed Suzuki-Miyaura coupling reactions, also the presence of additives (base, tetrabutylammoniumbromide (TBAB)) and substrates (aryl halides, phenyl boronic acid) play a crucial role.

7 Bimetallic catalysts

7.1 Pd in combination with promoting elements

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. 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 et al., 2001). However, in this case, Fe(0) is most often oxidized to Fe(II). This generates electrons, which can be used for the reduction of H^+ to H_2, which can then serve as a hydrogen donor for the Pd. Since one of the constituents of the bimetal is consumed during reaction, the definition of a 'catalyst' is not really fulfilled anymore.

Most other doping elements are not consumed during the reaction. The exact role of the promoting element is often unclear: it can either improve the geometry of the catalyst or facilitate the transfer of electrons. These bimetallic catalysts can have different structures, such as alloy, core-shell or cluster-in-cluster structures (Figure 1-4). The choice for a certain structure is dependent on the reaction and the desired promoting effect (enabling new reaction pathways, increased reaction rate or increased selectivity). It can be varied by changing the production process of the bimetallic catalyst.

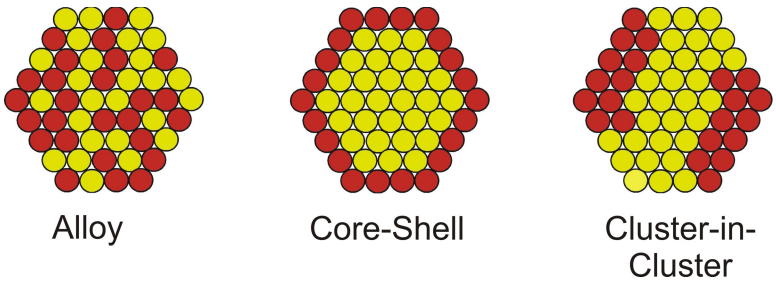


Figure 1-4: Possible structures of bimetallic catalysts: alloy, core-shell or cluster-in-cluster (from: http://www.usask.ca)

A wide variety of elements have been described as promoting elements for Pd catalysts in both oxidative and reductive reactions, some recent examples are: Pt (Valdez et al., 2013), In (Barbosa et al., 2013), Sn (Barbosa et al., 2013), Re (Liu et al., 2013), Ag (Lin et al., 2012), Ni (Sun et al., 2012), Cu (Obraztsova et al., 2012) and Ir (Morfin et al., 2012). One of the most widely described elements as a doping agent for Pd catalysts is Au.

7.2 Pd/Au catalysts

Bulk Au is considered to be inert as a catalyst. Au nanoparticles on the other hand exhibit catalytic activity, for example for the oxidation of CO, the nucleophilic addition to alkenes and the synthesis of H_2O_2 (for a review, see Hashmi et al., 2006)

The Pd/Au catalyst is a very popular bimetallic combination for a whole range of purposes. Some examples are: the oxidative hydrogenation of tetralin (Murugadoss et al., 2012), the Ullmann coupling (Dhital et al., 2012), the oxidation of benzyl alcohol (Dhital et al., 2012) and the hydrogenation of aldehydes (Yang et al., 2012). Gao et al. (2012) attributed the promotional effect of Au to both geometric and electronic effects. The geometric effect was called the ensemble effect, and was caused by the presence of more isolated Pd clusters within the crystal due to the insertion of the Au entities. The electronic effect was called the ligand effect and consisted of a rearrangement of the electronic structure of Pd, causing a weaker attachment of reactants and products to the catalyst surface, increasing sorption and desorption rates and thus increasing reaction rates.

In the hydrodehalogenation reactions, the Pd/Au catalyst is the only described Pd-based bimetallic combination (next to the Pd/Fe catalyst, which consumes Fe, and is not a true catalyst (Ghauch et al., 2010)). Bonarowska et al. (2001a) and (2001b) developed Pd/Au alloys on carbon and silica for the hydrodechlorination of CF_2Cl_2 at 180 °C. These catalysts could increase the selectivity towards CF_2H_2 to 95 %, compared to a selectivity of 40 % obtained by a monometallic Pd catalyst. Nutt et al. (2005) designed bimetallic catalysts with a core of Au and a shell of Pd. In a first phase, 20 nm Au-Pd core-shell nanocatalysts were developed which showed a hydrodechlorination rate of TCE of 943 L g_{Pd}^{-1} min^{-1}, an increase by a factor 10 compared to the monometallic Pd catalysts. With a slightly different preparation procedure using other reductants, new Pd/Au catalysts were designed in a next phase (Nutt et al., 2006). 4 nm particles consisting of an Au core covered with a Pd layer were designed and their activity was again evaluated for the hydrodechlorination of TCE. A maximum activity of 1950 L g_{Pd}^{-1} min^{-1} was obtained at a

Pd surface coverage of 13%. Water with 15 mg L^{-1} TCE was successfully treated with a flow through column containing these particles in a polymeric resin (Wong et al., 2009). The additional investment cost for Au was largely compensated by the tremendous increase in catalytic activity.

One of the aims of this work is to synthesize a biosupported Pd/Au catalyst and to compare its activity for the removal of halogenated micropollutants in wastewaters and in organic C-C coupling reactions with the activity of monometallic bio-Pd catalysts.

8 Halogenated pollutants as target contaminants

8.1 Occurrence and risks

Due to the increasing welfare and life quality, and the continuous progress in synthetic chemistry, the consumption and production of chemicals such as drugs, personal care products, pesticides, solvents and flame retardants has drastically increased. Several of these compounds are halogenated, which means they contain a F, Cl, Br or I atom in their molecular structure. The presence of a carbon-halogen bond makes these molecules very stable, which is often desirable for their application. However, when entering the environment after use or consumption, the stability of the molecules can mean a potential hazard, since these molecules show a very low (bio)degradability.

These compounds can be found in all environmental compartments. PCBs are most often found in sediments, after leakage from electrical equipment. Chlorinated solvents (TCE, PCE, …) form dense non-aqueous phase liquids (DNAPLs) in soils after spills or leakage from dry cleaning activities and are partly dissolved in groundwater. Brominated flame retardants are found in indoor air. Pharmaceuticals are excreted by humans and end up in domestic and hospital wastewaters. They are only very limitedly degraded and sorbed by conventional wastewater treatment plants and, after discharge of the effluent, they end up in surface waters, where they are found in concentrations of pg L^{-1} to µg L^{-1}. Due to dilution effects and transport mechanisms of water and air (the so called ‘grasshopper effect’), these substances are relatively easily transported over longer distances.

Most of these pollutants can be toxic for living organisms. The toxic effects and the concentrations inducing these toxic effects are of course dependent on the compound. Moreover, substances like PCBs and dioxins can bioaccumulate in the food chain. In the case of pharmaceuticals, toxic effects on the aquatic life are in some cases already observed at concentrations at which the contaminants are present. Diclofenac is found in

the effluents of sewage treatment plants in concentrations up to 4 µg L^{-1} (Ternes, 1998). Toxic effects on the hepatic metabolism of the rainbow trout have been observed at 1 µg L^{-1} (Triebskorn et al., 2004).

8.2 Conventional removal techniques

8.2.1 Sorption on activated carbon

Sorption on activated carbon is a very commonly applied technique in for example groundwater sanitation. Groundwater is pumped over a bed of activated carbon and the contaminants are sorbed from the liquid medium to the solid and porous activated carbon. After the carbon is saturated, a new bed is installed and the saturated bed is regenerated. The technique can also be applied in wastewater treatment. The main drawback is that the compound is not degraded at all, but only transferred from a liquid medium to a solid medium, where, in the end, it needs to be processed, for example by incineration.

8.2.2 Advanced oxidation processes

Advanced oxidation processes have in the last decade intensively been studied as a removal technique for micropollutants (for a review, see Ternes et al. (2003)). They consist of the combined application of ozone, H_2O_2 and UV light to degrade organic compounds. Although, a wide variety of contaminants can be degraded by AOPs, others, like the ICM diatrizoate are almost insensitive to AOPs (Ternes et al., 2003). Next to the high cost for implementation, two main drawbacks have been observed:

- There is very little control over the by-products that are formed. Often, only small oxidations and hydroxylations take place, resulting in a whole range of compounds that might even be more toxic than the mother molecule;
- Due to their stability, carbon-halogen bounds are in most cases not broken, resulting in degradation products that can also be persistent.

8.2.3 Biodegradation

Although halogenated compounds are very hard to degrade microbially, some strains, which are able to perform the dehalogenation have been isolated. For example, the strain *Desulfitobacterium dichloroeliminans* is able to dehalogenate 1,2-dichloroethane and dichloropropanes to dechlorinated products due to the presence of a stereoselective dehalogenase enzyme (De Wildeman et al., 2003). However, the process is slow, requiring months-years. Therefore, biodegradation can be a valuable option for groundwater sanitation, where time is often not really a limiting factor. For wastewater

treatment, flow rates are too high and residence times are too short to allow application of biodegradation.

8.3 Opportunities for Bio-Pd and Bio-Pd/Au catalysts

Pd-based catalysts offer the advantage that a real degradation takes place. This degradation can, depending on the concentration of the catalyst, the reagents and the availability of the hydrogen donor, take place within the timeframe of hours to minutes. Moreover, in presence of a hydrogen donor, hydrodehalogenation reactions can occur in a very controlled way, so the formed by-products are relatively easy to predict. Provided that the catalyst is sustainably produced, stable over time and the investment is feasible, Pd-catalyzed dehalogenation of halogenated environmental contaminants can definitely be an alternative for current abatement techniques. De Gusseme et al. (2011) demonstrated that the halogenated pharmaceutical micropollutant diatrizoate became much more biodegradable after a dehalogenation by a bio-Pd catalyst (1 mg L^{-1} diatrizoate was not degraded by nitrifying biomass, whereas 1 mg L^{-1} of the deiodinated product was completely removed within 24 hours).

The use of chemical Pd catalysts for dehalogenation of environmental contaminants has already been demonstrated in batch tests for the removal of PCBs (Korte et al., 1997) chlorophenols (Liu et al., 2001) and the deiodination of diatrizoate (Knitt et al., 2008). A pilot scale reactor with a catalytic bed of Pd was successfully implemented for removal of TCE from a contaminated groundwater in California (Davie et al., 2008). The superior activity of Pd/Au catalysts vs. Pd catalysts was shown for dehalogenation of CFCs (Bonarowska et al., 2001a) and TCE (Nutt et al., 2005). Bio-Pd was shown to dehalogenate a series of halogenated environmental contaminants (see 4.1 and Table 1-2). It is the aim of this work to design a biosupported Pd/Au catalyst and to evaluate its activity for the dehalogenation of environmental contaminants, and compare the activity with monometallic bio-Pd.

Chapter 2

Objectives and outline

The aim of this work was to study some of the possible limitations of bio-Pd catalysts and to try to optimize the catalyst or to look for sustainable alternatives. The outline of this doctoral dissertation is schematized in Figure 2-1.

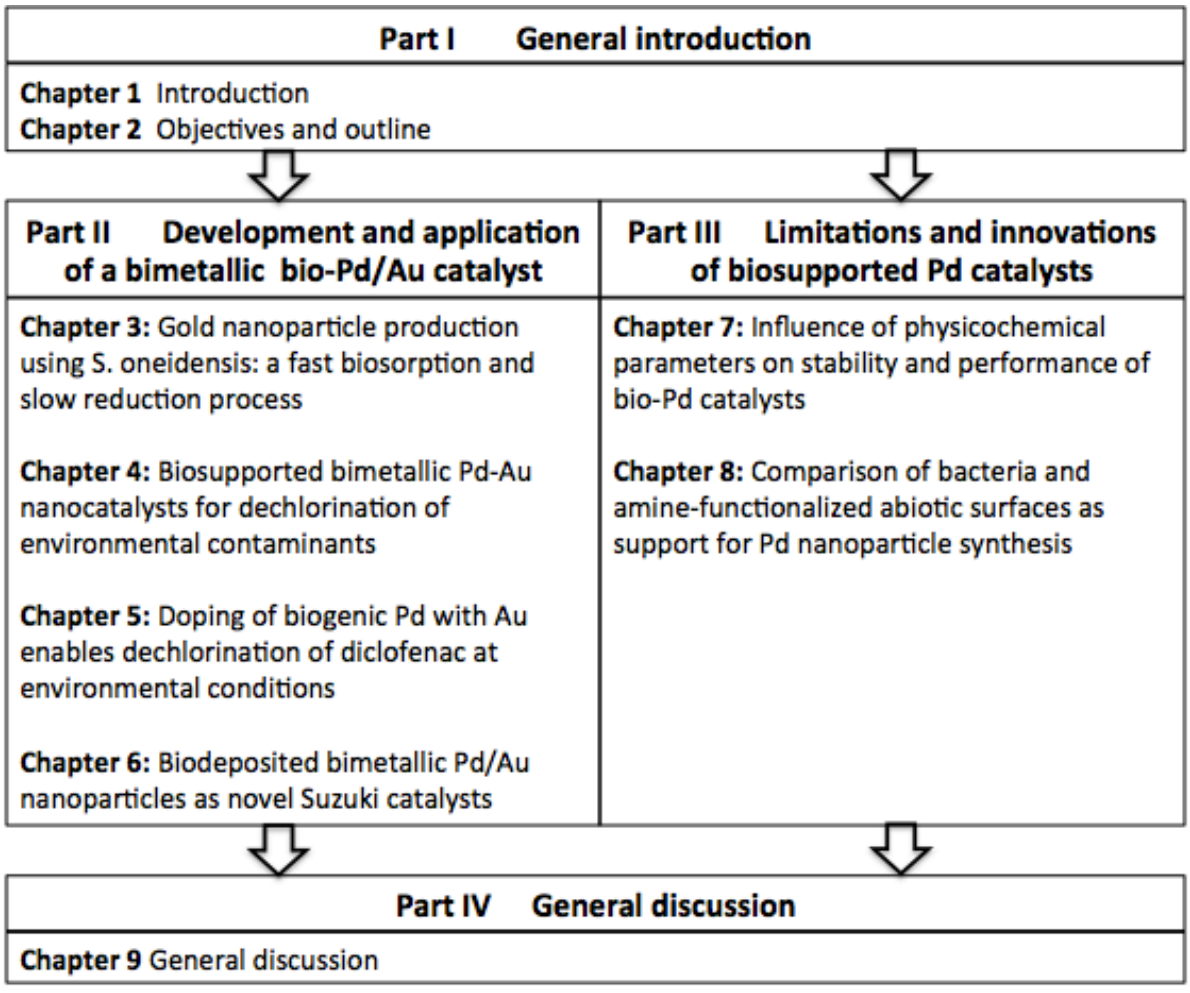


Figure 2-1: Schematized outline of this doctoral dissertation.

1 Optimization of bio-Pd

In **Part II**, a bimetallic Pd/Au catalyst was developed in order to increase the reaction rate of the monometallic bio-Pd catalyst and to enable reactions that cannot be performed using the monometallic bio-Pd. The superior activity of the bimetallic catalyst was demonstrated for the dehalogenation of TCE and diclofenac and for the Suzuki C-C

coupling reaction. The catalyst was also optimized for the dehalogenation of diclofenac and tested in real environmental conditions.

- In **Chapter 3**, the interaction between *S. oneidensis* and Au was studied, as a first step to develop a bimetallic bio-Pd/Au catalyst. The kinetics of both the Au-biosorption and reduction process were studied in detail. The final product ('bio-Au') was studied by transmission electron microscopy (TEM)
- In **Chapter 4**, the biosupported bimetallic bio-Pd/Au was synthesized. Several strategies for its synthesis were evaluated and its superior catalytic activity was demonstrated for the dechlorination of diclofenac and TCE. The catalyst is studied by scanning TEM, EDX and μXRD.
- In **Chapter 5**, the dechlorination of diclofenac by bio-Pd and bio-Pd/Au is studied in detail. The optimal Pd/Au ratio is determined for this reaction and the catalyst is tested for the removal of diclofenac, carbamazepine and diatrizoate at environmental concentrations from the effluent of a hospital wastewater treatment plant.
- In **Chapter 6**, both the activity of the bio-Pd and bio-Pd/Au catalyst were evaluated for the Suzuki C-C coupling reaction of an aryl halide and a boronate. The reaction rates were also compared with a commercial Pd/C catalyst. The effect of different substituents on the reaction rate was tested.

2 Limitations and innovations of bio-Pd

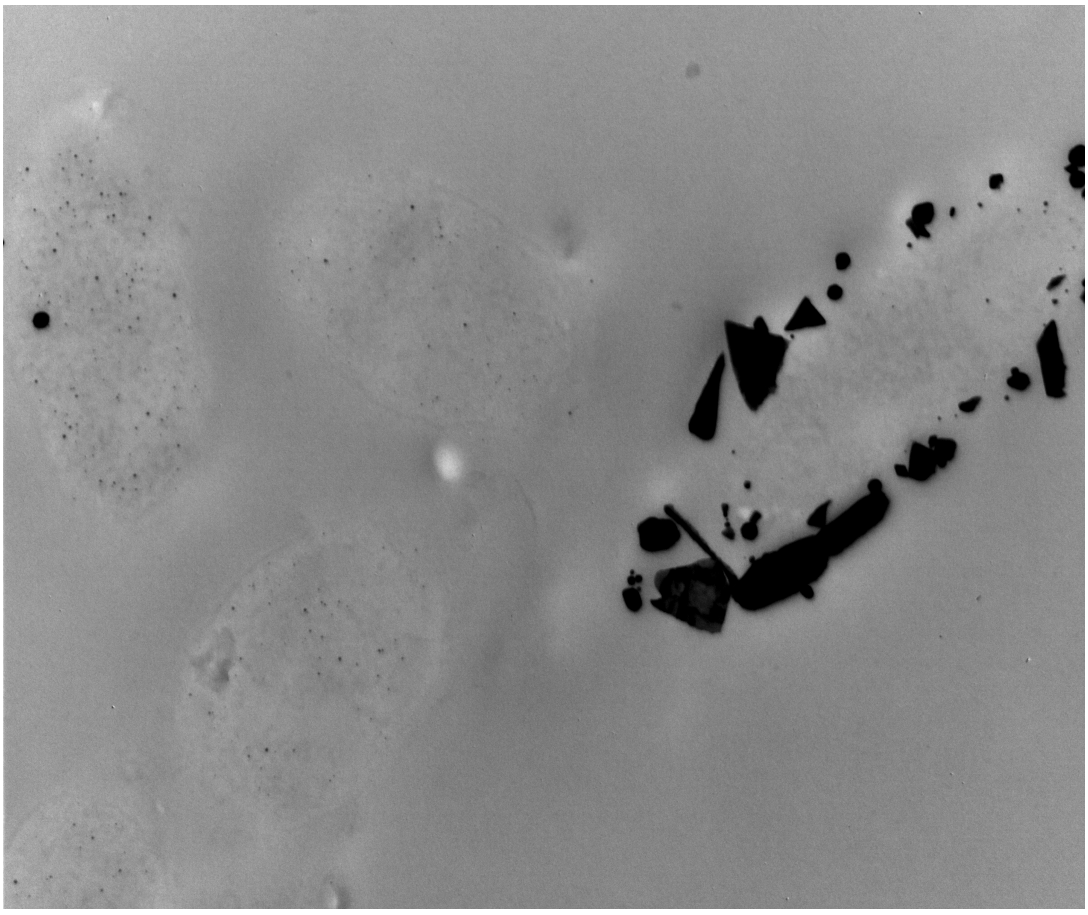
In **Part III**, leaching of Pd was investigated as a possible limitation of bio-Pd. As an innovation, the synthesis of Pd nanocatalysts on silica materials functionalized with amine groups of chemical and biological origin was investigated.

- In **Chapter 7**, the leaching of Pd from the carrier was studied. The leaching was followed as a function of time under different conditions of temperature, medium, pH and atmosphere. The speciation of the leachate was also determined, and the leaching from a *Shewanella oneidensis* and a *Cupriavidus metallidurans* carrier was compared.
- In **Chapter 8**, an alternative for bacteria as carrier was investigated, in order to try to overcome the problem of catalyst poisoning by bacterial sulfur. Silica beads were coated with amine groups, both chemically (APTES and PEI) and with chitosan, a biomaterial containing free amine groups. The obtained catalysts were studied by TEM and the activity was evaluated for the reduction of *p*-nitrophenol.

Part IV (Chapter 9) is the general discussion of the work, discussing potential applications of bio-Pd and bio-Pd/Au in environmental technology, together with some other new possible applications and optimizations. Future perspectives for implementations of biosupported catalysts and the interactions between bacteria and precious metals are discussed.

Part II

Development and application of a bimetallic bio-Pd/Au catalyst



Chapter 3

Gold nanoparticle formation using *Shewanella oneidensis*: a fast biosorption and slow reduction process

Abstract

The metal respiring bacterium *Shewanella oneidensis* has previously been used for reduction of Pd(II) into Pd(0) nanoparticles. This study investigated whether *Shewanella oneidensis* could also perform the reduction of Au(III) to Au(0). The kinetics of both the biosorption and reduction of Au(III) were studied. Biosorption of Au(III) was a fast and efficient process, and depended on the presence of an electron donor, the pH and the medium used. The reduction process however appeared to be a slow process, requiring the presence of an electron donor. As reduction also occurred in heat-killed cells, it is suggested that the reduction is non-enzymatic. At a concentration of 100 mg L^{-1} Au(III), the nanoparticles were mainly smaller than 10 nm and precipitated intracellularly. With H_2 as the electron donor, it was shown that the location of the particles and the size could be steered by changing the concentration of Au(III). After a fast biosorption and slow reduction process, Au(0)-nanoparticles were formed inside the cells or on the cell wall of *Shewanella oneidensis*. In most cases, these particles had interesting properties, like a small size and a narrow size distribution, which can make them interesting for applications, for example in catalysis.

Chapter redrafted after:

S. De Corte, T. Hennebel, S. Verschuere, C. Cuvelier, W. Verstraete, N. Boon. 2011. Gold nanoparticle formation using Shewanella oneidensis: a fast biosorption and slow reduction process. Journal of Chemical Technology and Biotechnology 86(4): 547-553

1 Introduction

Gold nanoparticles find more and more applications in electronics (e.g. as conductors in transistors (Wu et al., 2006), medical diagnostics (cancer detection (Huang et al., 2007)) and catalysis (oxidation of CO, water gas shift reaction, oxidation of alcohols (Hashmi et al., 2006)) because of their unique optical and physicochemical properties (Daniel et al., 2004). Conventional production methods for these gold nanoparticles are based on the use of chemical reductants, stabilizers (which prevent agglomeration of particles) and capping agents (which prevent growth of particles). As an alternative to these chemical synthesis methods, the metal reducing capacities of bacteria can be exploited to produce metallic nanoparticles. Bacterial cells serve as reducing agent as well as carrier matrix for the nanoparticles. In this way the use of toxic and expensive chemicals is prevented, which makes these biological synthesis methods more attractive from an ecological point of view and fit to the concept of green chemistry. Moreover, these methods allow an easy recovery of waste streams into added value products (Hennebel et al., 2009a).

Biosorption of metals by bacteria is a well-known process. Carboxylic groups in the cell wall of *Bacillus subtilis* were found to be the most important sorption sites for metals (Beveridge et al., 1980). Biosorption of Pd by *Desulfovibrio* sp. was optimal at a pH of 3, and decreased with increasing chloride concentrations (de Vargas et al., 2004). Different bacteria that were able to biosorb Au(III) have been described (Tsuruta, 2004). For *Rhodobacter capsulatus* (Feng et al., 2007) and *Pseudomonas maltophilia* (Tsuruta, 2004), the biosorption phenomenon was improved by a factor 5 to 7 at a pH of 3 compared to pH 7.

Bacterial reduction of Au(III) to Au(0)-nanoparticles by bacteria has mainly been described under anaerobic conditions. When Au(III) was added to a concentration of 800 mg Au(III) g^{-1} cell dry weight, the sulfate reducing bacterium *Desulfovibrio desulfuricans* reduced Au(III) to Au(0) in the cytoplasm and in the extracellular matrix (Creamer et al., 2006; Deplanche et al., 2008). Using H_2 as an electron donor, 10 to 50 nm gold nanoparticles were found in the periplasm and on the outer cell surface of *E. coli* (Deplanche et al., 2008), *Pyrobaculum islandicum* and other hyperthermophilic Fe(III)-reducers (Kashefi et al., 2001) and *Shewanella algae* (Konishi et al., 2006). With lactate as electron donor, *Rhodobacter capsulatus* was able to precipitate Au-nanoparticles intracellularly, as well as on the cell membrane and in the growth medium up to levels of 270 mg Au g^{-1} cell culture (Feng et al., 2008). Under aerobic conditions, bacterial reduction of Au(III) to Au(0) is less common. However, bioreduction of $HAuCl_4$ by

cyanobacteria has been reported (Lengke et al., 2006c; Brayner et al., 2007), as well as Au(III) bioreduction by *Bacillus subtilis* (Beveridge et al., 1980). *Rhodopseudomonas capsulata* was able to reduce $HAuCl_4$ extracellularly, with larger sizes at lower pH values (10-20 nm at pH 7 and 50-400 nm at pH 4, He et al., 2007). In general, anaerobic metal-reduction is more efficient and faster than aerobic metal-reduction (Deplanche et al., 2008). However, aerobic metal reduction is also feasible, and can be preferred from a practical point of view. For example, the efficient bioreductive deposition of Pd(0)-nanoparticles on the cell wall and in the periplasmic space of the metal respiring bacterium *Shewanella oneidensis* has been demonstrated under aerobic and anaerobic conditions. Pd(II) was even efficiently reduced when added up to 1000 mg L^{-1} (De Windt et al., 2005). The presence of O_2 did not significantly inhibit bioreduction compared to anaerobic conditions, but the addition of an electron donor, with H_2 and formate the most effective, was required for reduction.

Since it was known that *S. oneidensis* can efficiently produce Pd(0)-nanoparticles (De Windt et al., 2005), the optimal conditions for this process were used as a start point to investigate the reduction of Au(III) into Au(0)-nanoparticles. The aim of this research was to investigate whether gold nanoparticles could be produced under anaerobic and aerobic conditions, using *S. oneidensis,* and which factors influenced their formation. The two main processes driving the nanoparticle formation, i.e. biosorption and reduction were assessed in terms of their kinetics. The metallic character, particle size and size distributions of the precipitates were determined. The synthesis of biosupported Au(0) particles are a first step in the formation of a bimetallic 'bio-Pd/Au' catalyst.

2 Materials and methods

2.1 Bacterial strains and growth conditions

Shewanella oneidensis MR-1 was obtained from the BCCM/LMG Bacterium Collection (Gent, Belgium) under the number LMG 19005. The strain was grown aerobically in Luria-Bertani (LB) medium overnight at 28 °C.

2.2 Batch experiments

S. oneidensis cells were harvested from an overnight LB culture in sterile 50 mL centrifuge tubes (TPP, Switzerland) by centrifuging at 3000 g for 10 min and washed twice with 50 mL distilled water or M9 medium (Sambrook et al., 1989). The washed cells were resuspended in distilled water to a final optical density at 610 nm of 1, corresponding to 50 mg cell dry weight per L. 120 mL Serum bottles were filled with 50

mL cell suspension and capped with inert viton stoppers. Biosorption and reduction assays were performed, either without electron donor, either with formate or H_2. When formate was used as electron donor, the cell suspension was supplemented with 50 mM formate. In the case of H_2 as electron donor, the headspace was replaced with 100% H_2 gas after repeated cycles of overpressure with N_2 and vacuum underpressure. Au(III) was then added as $HAuCl_4.3H_2O$ (Sigma-Aldrich, Germany) to 100 mg L^{-1}. When the pH was adjusted to 7 or 10, this was done with 0.1 M NaOH. The bottles were continuously stirred at 100 rpm. Gold concentrations in solution were determined after separation of the bacteria from the medium using a 0.22 µm PVDF Millipore filter. Subsequently, the gold concentration was measured using an AA-6300 atomic absorption spectroscope (Shimadzu, Kyoto, Japan). All experiments were performed in triplicate.

2.3 Heat treatment

Heat treatment of the bacterial cells was performed by autoclaving the cell suspensions twice. Autoclavation was done for 20 minutes at 120 °C and 1 bar overpressure. Metals, electron donors and other chemicals were added after cooling of the heat-treated cells to room temperature.

2.4 Characterization methods

2.4.1 X-ray diffraction analysis (XRD)

X-ray diffraction analysis of the gold on the cells, dried at 30°C was performed with a Siemens Diffractometer D5000 with BraggBrentano optics (Siemens, Germany). X-rays were generated by a copper X-ray tube with power 1.6 kW (40 kV, 40 mA). Measurements were performed in between 30° and 90° 2-theta with step time of 1.6 s and step size of 0.02 degrees.

2.4.2 UV-Vis spectroscopy

UV-Vis analysis was performed with a Uvikon 932 spectrometer (Kontron Instruments, Switzerland). Wavelength scans were taken in a scan range of 400-700 nm. Data range was 1 nm, and scan speed was 200 nm min^{-1}.

2.4.3 Transmission electron microscopy (TEM)

Samples of bacterial suspensions with Au were allowed to precipitate for 8 hours. Subsequently, the supernatant was removed and the bacteria were fixed in 0.1 M cacodylate buffer containing 4% paraformaldehyde and 5% glutaraldehyde. After postfixation in 1% osmium tetroxide, samples were dehydrated in a series of alcohol and embedded in Epon medium (Aurion, the Netherlands). Ultrathin sections of 60 nm were

contrasted with uranyl acetate and lead nitrate before examination or were examined without contrast by imaging with a Zeiss TEM900 transmission electron microscope (Carl Zeiss, Germany) at 50 kV. Particle size distributions were determined with ImageJ 1.43r freeware. Given the clear contrast between the particles and the background, a binary image could be created by visually adjusting the threshold value. Subsequently, based on the magnification, a table containing each particle and its surface area was obtained. Particle diameters were determined as if the particles were spheres.

3 Results

3.1 Effect of the addition of an electron donor on gold biosorption

Cells of *Shewanella oneidensis* in distilled water were incubated with 100 mg L^{-1} Au(III). Biosorption, including both passive sorption and active uptake processes, of Au(III) from an aqueous solution was a quite fast process (Figure 3-1a). Biosorption rates after 30 minutes of exposure and between 30 minutes and 48 hours of exposure to 100 mg L^{-1} Au(III) are given in Table 3-1. When no electron donor was added, 76.4 ± 8.5 % of the Au(III) was taken up by or sorbed on the bacteria within 30 minutes. After this very fast initial biosorption, the process continued, but at a much slower rate. After 48 hours of exposure, 95.1 ± 0.5 % of the added gold was removed from the solution.

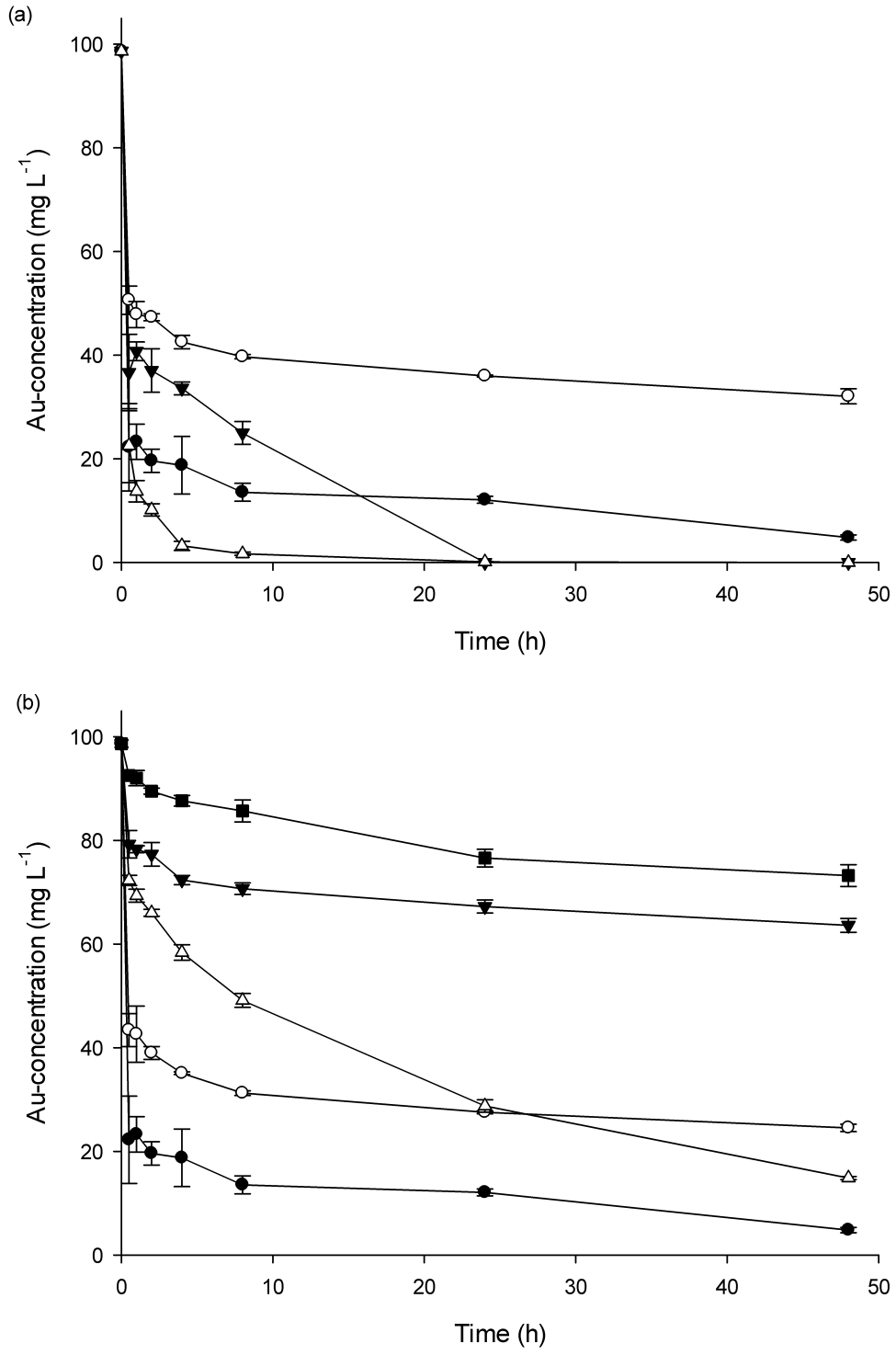


Figure 3-1: Biosorption kinetics of Au(III) by *S. oneidensis*: Au-concentration in solution as a function of exposure time. (a) Effect of heat-killing and addition of an electron donor on Au(III) biosorption: living cells without addition of an electron donor (—●—); heat-killed cells without addition of an electron donor (—○—); living cells with 50 mM formate as electron donor (—▼—); living cells 101,3 kPa H_2 as electron donor (—△—). (b) Effect of medium and pH: pH 3 in H_2O (—●—); pH 7 in H_2O (—○—); pH 10 in H_2O (—▼—); pH 7 in M9 medium (—△—); pH 7 with heat-killed cells in M9 medium (—■—) (n = 3).

In addition, both 101.3 kPa H_2 and 50 mM formate were used as electron donors. When H_2 was supplied as electron donor in the headspace of the flasks, enhanced biosorption was observed. Almost all gold was taken up by or sorbed on the bacteria after 24 hours. With formate, uptake kinetics showed a different pattern: initial uptake was slower but went on after 30 minutes at a higher rate than when no donor was added in the same medium and at the same pH. After 24 hours of exposure, no more gold was found in solution (detection limit: 0,5 mg Au L^{-1}). When Au(III) was added to heat-killed cells, biosorption also took place, but to a lesser extent. After 48 hours, about 67.5 ± 1.5 % of the gold was removed from the solution containing the dead cells.

3.2 Effect of medium and pH on gold biosorption

The kinetics of the uptake of Au at different pH values was followed (Figure 3-1b and Table 3-1). When gold was added to a suspension of bacteria in distilled water as $HAuCl_4.3H_2O$ to a final concentration of 100 mg L^{-1} Au, the pH dropped from 7 to about 3. When the pH of a *S. oneidensis* suspension in water was raised to 7, a significant lower biosorption of Au was seen (75.1 ± 0.7 % biosorbed after 48 hours). This decrease in uptake was even clearer when the pH was increased to 10 (only 35.5 ± 1.3 % biosorbed after 48 hours).

M9 medium contains a buffer (0.07 M phosphate) and was used to maintain a pH of 7 (pH was measured at the beginning and at the end of the experiment). After addition of Au(III) as $HAuCl_4.3H_2O$ to *S. oneidensis* in M9 medium, the pH of the medium remained stable at 7. In M9 medium, the uptake behavior was different from the suspensions in water. The biosorption was slower in the beginning of the experiment. However, at later time points, a faster biosorption rate was observed compared to the bacterial suspensions in distilled water. Moreover, from Figure 3-1a and b, it is clear that the difference in biosorption between heat-killed cells and living cells is larger in M9 medium than in distilled water.

Table 3-1: Biosorption rates after 30 minutes and between 30 minutes and 48 hours of exposure of living and heat-killed cells of *S. oneidensis* in different media to 100 mg L^{-1} Au(III), at different pH and with and without electron donor.

Medium	pH	Electron donor	Heat-killed	Biosorption rate at 30 minutes (mg Au(III) g^{-1} cell dry weight h^{-1})	Biosorption rate between 30 minutes and 48 hours (mg Au(III) g^{-1} cell dry weight h^{-1})
Water	3	-	No	3056 ± 337	7.3 ± 0.2
Water	3	-	Yes	1922 ± 109	7.8 ± 0.6
Water	3	50 mM formate	No	2480 ± 295	31.1 ± 1.9
Water	3	101,3 kPa H_2	No	3044 ± 285	19.1 ± 0.1
Water	7	-	No	2210 ± 127	7.9 ± 0.3
Water	10	-	No	776 ± 105	6.6 ± 0.6
M9	7	-	No	1054 ± 39	24.2 ± 0.1
M9	7	-	Yes	245 ± 39	8.1 ± 0.9

3.3 Reduction of Au(III) to Au(0) in presence of an electron donor

After biosorption of Au(III), the reduction of gold into nanoparticles was examined. Figure 3-2 shows the XRD-spectra of the suspensions of *S. oneidensis* in distilled water when incubated with 100 mg L^{-1} Au(III) for 48 hours, without electron donor and with 101,3 kPa H_2 and 50 mM formate respectively. These confirmed the presence of Au(0) when an electron donor was added. Typical diffraction peaks (at 2θ = 38.19°; 44.39°; 64.58°; 77.55° and 81.72°) for Au(0) were observed. No Au(0)-crystals were present when no electron donor was added (Figure 3-2).

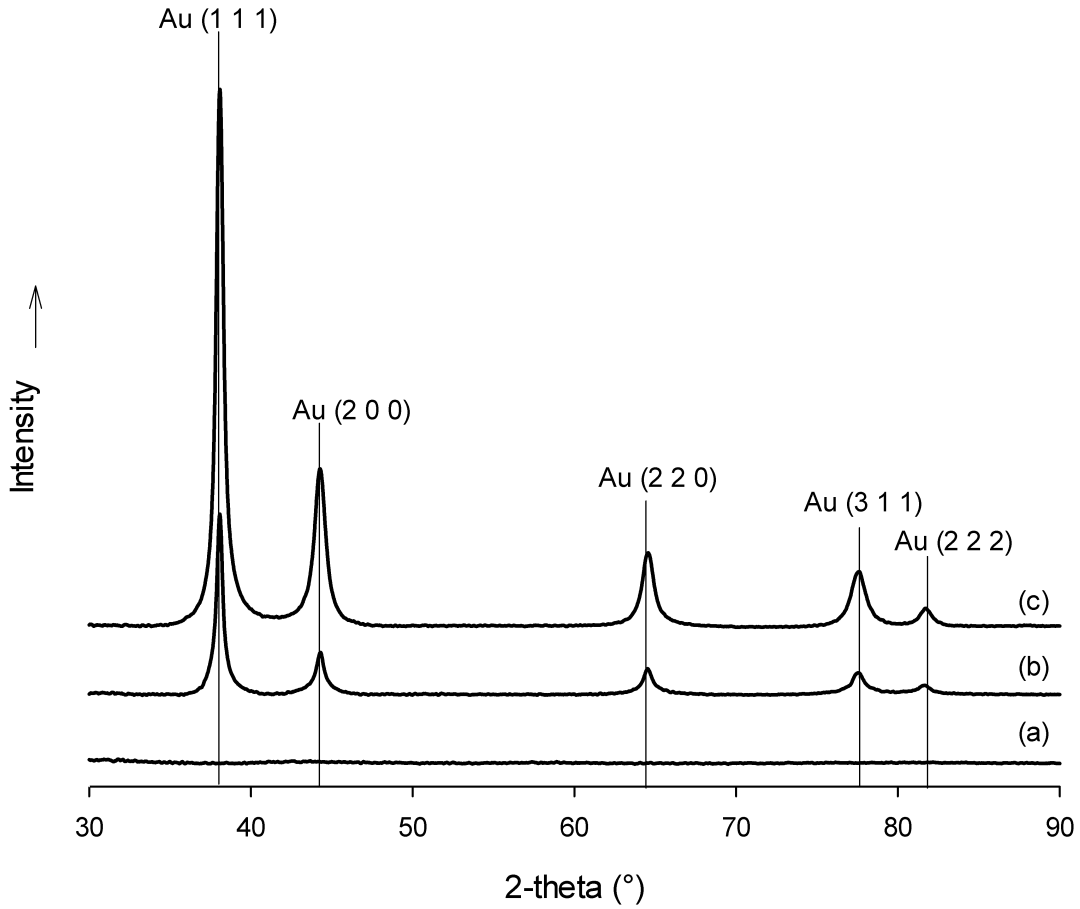


Figure 3-2: XRD analysis of *S. oneidensis* incubated with 100 mg L^{-1} Au(III). (a) with no electron donor added. (b) With 50 mM formate. **(c)** With 101,3 kPa H_2.

When an electron donor was supplied, the color of the suspension changed from pale yellow to purple. The moment of the color change was dependent on the electron donor. The purple color of the suspension was an indication of the formation of Au(0)-nanoparticles (Ruscher et al., 2001). The formation of nanoparticles was monitored in function of time by using UV-Vis spectra of *S. oneidensis* in distilled water with 50 mM formate (Figure 3-3a) or 101,3 kPa H_2 (Figure 3-3b) as electron donor. With formate as electron donor, the color of the solution changed to purple after 8 hours. This was

confirmed by the UV-Vis spectrum (Figure 3-3a), since a surface plasmon resonance (SPR) band around 540 nm was observed, which is characteristic for the presence of Au(0)-nanoparticles (Hanaoka et al., 1998). Because of the presence of the bacteria in the suspension, the SPR band was not always clearly visible. Using H_2 as the electron donor (Figure 3-3b), this SPR-band appeared only after 24 hours. These spectra indicated that, in contrast to biosorption of Au(III) by *S. oneidensis*, reduction of Au(III) is a slow process. It needs to be mentioned that biomass-free controls with Au(III) and 50 mM formate showed a slight color change from pale yellow to pale purple. With H_2 however, biomass-free experiments did not show any color change.

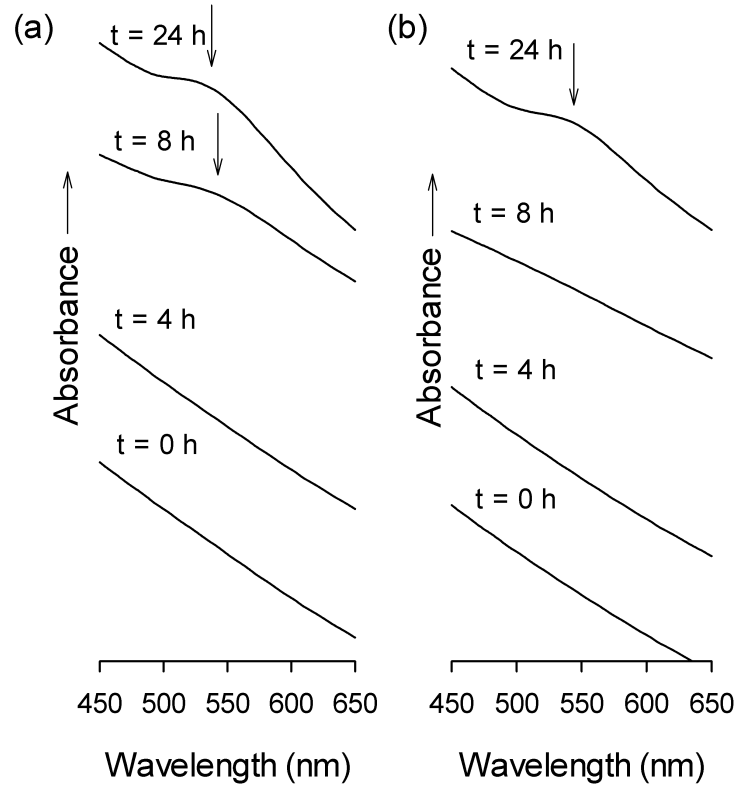


Figure 3-3: UV-Vis spectra of *S. oneidensis*, challenged with 100 mg L^{-1} Au(III) in presence of an electron donor, recorded as a function of time. (a) With 50 mM formate as electron donor. (b) With 101,3 kPa H_2 as electron donor.

3.4 Study of the precipitates by transmission electron microscopy (TEM)

TEM-pictures and particle size distributions of living cells of *S. oneidensis* exposed to 100 mg L^{-1} Au(III) for 48 hours with 101.3 kPa H_2 or 50 mM formate as electron donor are shown in Figure 3-4a and b. From the pictures it is clear that most of the nanoparticles were precipitated intracellularly. Most of the particles had a diameter between 5 and 10 nm. With H_2 and living cells, the mean diameter was 8.4 ± 8.7 nm and

the median diameter was 7.2 nm. The large standard deviation is caused by the presence of the fraction of larger particles (> 30 nm) precipitated on the cell wall (although they represent only 2 % of the total number of particles). With formate and living cells, the mean particle diameter was 6.4 ± 3.2 nm, the median diameter was 5.6 nm. No extracellular precipitates were found. Figure 3-4c shows heat-killed cells of *S. oneidensis* exposed to 100 mg L^{-1} Au(III) in presence of H_2. Also in this case, intracellular nanoparticles were found. These heat-killed cells with H_2 as electron donor showed a mean particle diameter of 6.8 ± 3.8 nm and a median diameter of 5.9 nm.

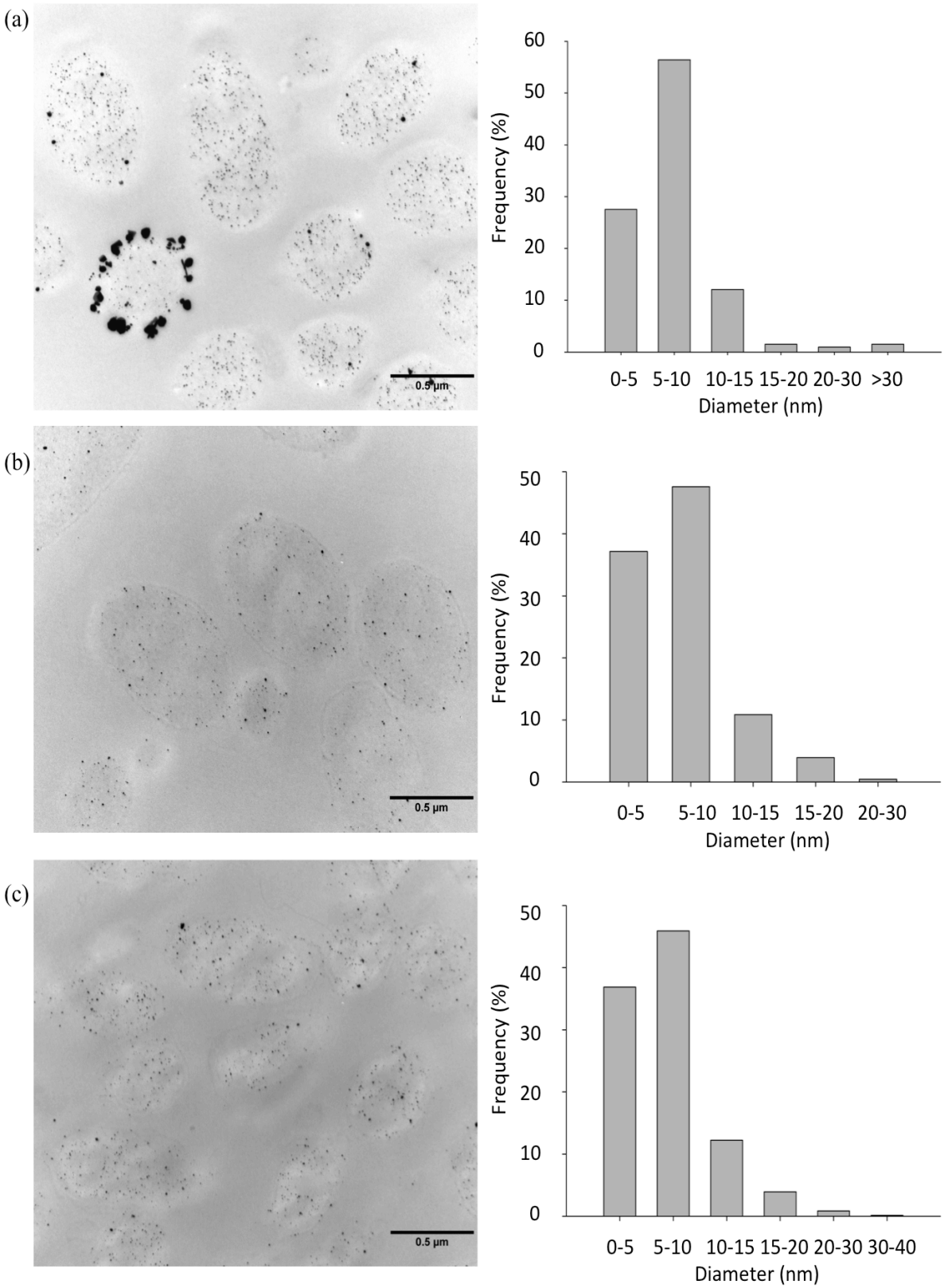


Figure 3-4: TEM-pictures of cells of S. oneidensis exposed to 100 mg L-1 Au(III) and particle size distributions of the Au(0)-nanoparticles. (a) Living cells with 101,3 kPa H2 as electron donor (n = 1154 particles). (b) Living cells with 50 mM formate as electron donor (n = 293 particles). (c) Heat-killed cells with 101,3 kPa H2 as electron donor (n = 621 particles).

With H_2 as electron donor, two types of particles were observed (small intracellular particles and larger particles on the cell wall). It was investigated whether the location of particle deposition and the particle size could be steered by altering concentrations of Au(III). TEM-pictures and particle size distributions of living cells of *S. oneidensis*, exposed to 50 and 200 mg L^{-1} Au(III) and with H_2 as electron donor are shown in Figure 3-5. When the concentration of Au(III) was lowered from 100 to 50 mg L^{-1}, only small intracellular particles (mean diameter 7.3 ± 3.6 nm) and no extracellular deposits were observed (Figure 3-5a). However, when the concentration of Au(III) was increased to 200 mg L^{-1}, mainly extracellular precipitates were observed and not all cells were covered with particles (Figure 3-5b). These precipitates consisted of both small (< 5 nm) and large particles (up to 100 nm and more).

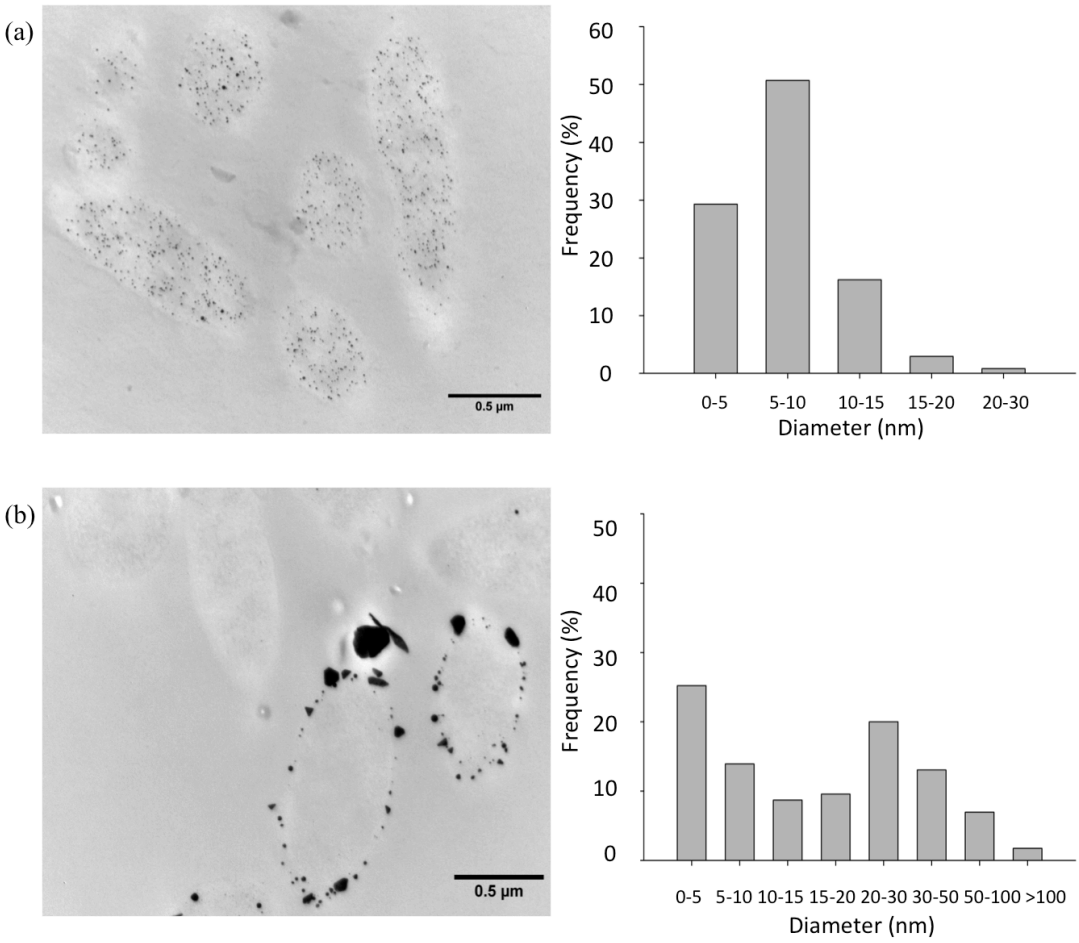


Figure 3-5: TEM-pictures of cells of *S. oneidensis* exposed to Au(III) with H_2 as electron donor and particle size distributions of the Au(0)-nanoparticles. (a) Living cells exposed to 50 mg L^{-1} Au(III) (n = 857 particles). (b) Living cells exposed to 200 mg L^{-1} Au(III) (n = 116 particles).

4 Discussion

This chapter describes the production of Au(0)-nanoparticles by the metal respiring bacterium *Shewanella oneidensis*. The assembly of the metallic Au nanoparticles consisted of two distinctive processes: a fast biosorption process followed by a slow non-enzymatic reduction process. Moreover, reduction was only possible when an external electron donor was present. TEM-pictures revealed mainly intracellular deposits of Au(0)-nanoparticles smaller than 10 nm.

Biosorption consisted of an initial step that occurred very fast (a matter of minutes), after which the process continued for hours at a very slow rate. Biosorption also took place with heat-killed cells of *S. oneidensis*, indicating that Au(III) had disappeared from the solution partly by non-enzymatic sorption processes. Adsorption of Au(III) by heat-killed cells has also been described for *Pseudomonas malthophilia* (Nakajima, 2003). However, when living cells were used, an enhanced Au-removal was seen, so metabolic processes could play a role in the removal of Au(III) from the solution by *S. oneidensis*. A possible reason for the improved biosorption in the presence of H_2 could be the higher metabolic activity of the bacteria in presence of the electron donor. The slower initial biosorption that was observed when formate was used as electron donor could be due to the ionic strength that is increased by adding 50 mM Na-formate. Indeed, the added formate anion is probably able to compete for possible positively charged binding sites with the negatively charged gold-chloride and gold-hydroxide complexes. This effect has been described for other bacteria and other anionic metal complexes (de Vargas et al., 2004; Niu et al., 2000). The faster Au(III) removal rate (factor 4) that was observed at later sample times, compared to the case where no electron donor was added, could be explained by the observation that reduction to Au(0) was already initiated after 8 hours. In this way the uptake equilibrium of Au(III) shifted continuously, creating a driving force for uptake of Au(III). The effect of pH can be explained by the surface charge of the bacteria. When added as $HAuCl_4$, Au is present in water as anionic gold complexes. At a pH of 3, $Au(OH)Cl_3^-$ and $Au(OH)_2Cl_2^-$ are the dominant Au-species, at pH 7, this will be mainly $Au(OH)_4^-$ (Nakajima, 2003). The cell surface of bacteria becomes more negatively charged with increasing pH (Fein et al., 2005), implicating increasing repulsion and decreasing biosorption of anionic gold complexes at pH 7 and pH 10 compared to pH 3. Initial sorption in M9 medium (1054 ± 39 mg Au(III) g^{-1} cell dry weight h^{-1}) happened at a slower rate than in distilled water (3056 ± 337 mg Au(III) g^{-1} cell dry weight h^{-1}). This can be explained by the pH value of 7 on one hand and the higher ionic strength on the other

hand. The higher uptake rate observed at later sample points might be caused by a better survival or higher metabolic activity of the bacteria in the M9 medium than in distilled water. In general, it can be concluded that *S. oneidensis* has a high affinity for biosorption of Au(III). In previous studies with other bacterial species, significantly lower sorption rates up to 70 mg Au(III) g^{-1} cell dry weight h^{-1} have been reported (Nakajima, 2003; Feng et al., 2008). This high affinity for Au(III) makes *S. oneidensis* an attractive species for production of Au(0)-nanoparticles.

In contrast with the fast process of biosorption, reduction of Au(III) to Au(0)-nanoparticles appeared to be a slow process (under aerobic circumstances with formate as well as under anaerobic circumstances with H_2). The moment the color change started was dependent on the type of electron donor used. Using formate as the electron donor, the process started after 8 hours of incubation, with H_2, it took 24 hours. Moreover, XRD analysis showed that an electron donor was indispensable for reduction. This was in correspondence with previous studies describing reduction of Au(III) by *E. coli* and *Desulfovibrio desulfuricans* (Deplanche et al., 2008), Fe(III)-reducing bacteria (Kashefi et al., 2001) and *Shewanella algae* (Konishi et al., 2006). In this study however, reduction took also place with heat-killed cells. Heating will cause denaturing of enzymes. Therefore, it is suggested that reduction of Au(III) is a pure chemical and not an enzymatic process, which is thermodynamically feasible (standard reduction potentials for H^+ to H_2 and $AuCl_4^-$ to Au(0) at pH 7 are – 400 mV and 990 mV respectively). A similar non-enzymatic mechanism has been described for production of Ag(0)-nanoparticles on the cell wall of *Lactobacillus* sp (Sintubin et al., 2009).

TEM-images showed the presence of Au(0)-nanoparticles, mainly in the cytoplasm of the cells at a concentration of 100 mg L^{-1} Au (III). Most of the particles had a size between 5 and 10 nm. Only with H_2 as electron donor, deposits of larger nanoparticles (30-100 nm) on a few cell walls were observed. Even though biomass-free controls with formate showed the presence of Au(0)-nanoparticles in the medium, no metal precipitates were seen in the medium when bacterial cells were present with formate, all particles were found inside the cells. Also in heat-killed cells, intracellular Au(0)-nanoparticles were observed. Although rather unusual for bacteria, intracellular Au(0)-nanoparticles have also been observed in cyanobacteria after exposure to $AuCl_4^-$ (Lengke et al., 2006c,Lengke et al., 2006b) and in sulfate reducing bacteria after exposure to $Au(S_2O_3)_2^{3-}$ (Lengke et al., 2006a). For cyanobacteria, it was shown that an intermediate Au(I)-sulfide complex is involved in the reduction of Au(III)-chloride to intracellular metallic Au(0) (Lengke et al., 2006c). Sources of sulfur in bacteria are amino acids

(cysteine and methionine) and glutathione (Reith et al., 2007, Baruwati et al., 2009). *Sporosarcina ureae* immobilized Au (without precipitation of metallic Au) after exposure to an Au-L-asparagine-complex, the immobilized gold was associated with the low-molecular weight intracellular protein fraction (Southam et al., 2000). In the case of *S. oneidensis*, it could be that Au(III) is reduced to an Au(I)-S complex, which is then further reduced to Au(0) by H_2 or formate. Biomass-free controls of Au(III) with H_2 did not show a color change. A possible mechanism can be that Au(III) must first be bound to or complexed by a bacterial cell component, probably a S-containing molecule, before H_2 can act as a reductant to form metallic Au(0)-nanoparticles. This cytoplasmatic Au-precipitation by *S. oneidensis* differs from the Au-precipitation in the periplasmic space of *S. algae* (Konishi et al., 2006). Au-reduction by *S. algae* is believed to be catalyzed by a periplasmic hydrogenase enzyme. This specific hydrogenase is probably not present in the periplasm of *S. oneidensis*. Different Au-precipitation mechanisms have also been described for different species of the genus *Pyrobaculum* (Kashefi et al., 2001).

The site of particle deposition could be steered by changing the concentration of Au(III). With H_2 as electron donor, both intracellular and extracellular particles were seen at a concentration of 100 mg L^{-1} Au(III). When this concentration was lowered to 50 mg L^{-1}, only small intracellular particles were observed. In contrast, at a concentration of 200 mg L^{-1}, mainly extracellular particles with a diameter up to 100 nm were observed. These different precipitation sites could be caused by the toxicity of Au, which might affect cell wall permeability and explain intracellular or extracellular deposition. However, these observations are in contrast to what has been observed for *S. oneidensis* and Ag (Wang et al., 2010a). When *S. oneidensis* was exposed to 100 µM Ag(I), Ag(0) particles were precipitated intracellularly and the bacteria died. When this concentration was lowered to 10 µM, bacteria survived and were able to keep the Ag(0) out of the cell, and precipitated it on the cell wall (Wang et al., 2010a). So in the case of Ag, intracellular precipitation was seen at higher concentrations and extracellular precipitation at lower concentrations.

5 Conclusions

The observations for reduction of Au by *S. oneidensis* are distinct from those for Pd. Reduction of Pd(II) by *S. oneidensis* was a fast process (within 15 minutes) and Pd(0)-nanoparticles were precipitated on the cell wall and in the periplasmic space (De Windt et al., 2005). The latter may be due to the fact that Pd is less toxic for the bacteria (De Windt et al., 2006). Heat-killed cells in presence of an electron donor showed biosorption of Pd(II), but no reduction to Pd(0) (De Windt et al., 2005). Our study showed that Au(III)

is efficiently sorbed and taken up by *S. oneidensis*. Reduction to Au(0) however is a slow process, which is probably non-enzymatic, after Au(III) has been bound to or complexed by bacterial cell components. Au(0)-nanoparticles were mainly deposited intracellularly at the Au-concentrations used in this study. Their small size (< 10 nm), high specific surface area and uniform size distribution, combined with the fact that particle size and deposition site can be changed by altering the initial gold concentration, can make these bio-supported gold nanoparticles interesting for applications, for example as catalysts in chemical oxidation reactions. Since both Au and Pd can be precipitated as zerovalent nanoparticles by *S. oneidensis*, the synthesis of a bimetallic bio-Pd/Au catalyst is believed to be possible and is investigated in Chapter 4.

6 Acknowledgements

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Chapter 4

Biosupported bimetallic Pd-Au nanocatalysts for dechlorination of environmental contamintants

Abstract

Biologically produced monometallic palladium nanoparticles (bio-Pd) have been shown to catalyze the dehalogenation of environmental contaminants, but fail to efficiently catalyze the degradation of other recalcitrant compounds. This chapter investigates the synthesis of biologically produced bimetallic Pd/Au nanoparticle catalysts. The obtained catalysts were tested for the dechlorination of diclofenac and trichlorethylene. When aqueous Pd(II) and Au(III) ions were both added to concentrations of 50 mg L^{-1} and reduced simultaneously by *Shewanella oneidensis* in the presence of H_2, the resulting cell-associated bimetallic nanoparticles (bio-Pd/Au) were able to dehalogenate 78 % of the initially added diclofenac after 24 hours; whereas no dehalogenation was observed using monometallic bio-Pd or bio-Au. Other catalyst-synthesis strategies did not show improved dehalogenation of TCE and diclofenac compared with bio-Pd. Synchrotron-based XRD, STEM and EDX indicated that the simultaneous reduction of Pd and Au supported on cells of *S. oneidensis* resulted in the formation of a unique bimetallic crystalline structure. This chapter demonstrates that the catalytic activity and functionality of possibly environmentally more benign bio-supported Pd-catalysts can be improved by coprecipitation with Au.

Chapter redrafted after:

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1 Introduction

Pd catalysts have been extensively studied for their potential use as catalysts for hydrodehalogenation in organic synthesis and pollutant degradation (Chapter 1). Pd catalysts doped with other metals to create a bimetallic catalyst have shown enhanced activity, which has been attributed to changes in geometric and electronic properties (Coq et al., 2001). For example, an alloyed Pd/Au catalyst produced by coprecipitation was shown to promote the oxidation of CO (Venezia et al., 2003). A Pd/Au on carbon catalyst, which was produced by reducing Pd and Au sequentially, was able to catalyze the dechlorination of CCl_2F_2, with enhanced selectivity for CH_2F_2 (Bonarowska et al., 2001a). A bimetallic core-shell structure, consisting of an Au-core with a Pd-shell was shown to increase the activity of Pd nanoparticles for the hydrodechlorination of TCE by a factor 10 (Nutt et al., 2005). After optimization of the particle size (4 nm) and degree of Pd coverage (highest activity with 12.7 wt. % Pd) of the Au-nanoparticles, an increase in activity by a factor 34 was observed (Nutt et al., 2006). All of these catalysts were produced using chemical synthesis methods that require the use of expensive substances, such as stabilizers (e.g. polyvinylpyrrolidone) and carrier materials (e.g. Al_2O_3, activated carbon).

It has been shown that Pd nanoparticles can be produced by the metal respiring bacterium *Shewanella oneidensis* in presence of a hydrogen donor. In this context, nanoparticles of Pd supported on the cell wall and periplasmic structures of the bacteria are referred to as bio-Pd (De Windt et al., 2005). Since the bacteria mediate the reduction process and act as a support for the resulting nanoparticles, fewer chemicals are involved in the production process, which makes the process safer and less expensive. Therefore, these biological synthesis methods can be a valuable alternative for chemical methods (Hennebel et al., 2009a). Bio-supported Pd nanoparticles have been shown to catalyze the dehalogenation of a number of important environmental contaminants (Chapter 1), while the degradation of other chlorinated compounds such as 1,2-dichloroethane are not catalyzed by current formulations of Pd catalysts (McNab et al., 2000).

Combining bio-supported Pd nanoparticles with other elements to multimetallic structures could be an option to improve the catalytic activity. Recently, the synthesis of a Pd-on-biomagnetite catalyst was reported (Coker et al., 2010). More specifically, magnetite crystals were produced extracellularly by *Geobacter sulfurreducens* by reduction of Fe(III)-oxyhydroxide. After synthesis of the biomagnetite, Pd(II) was added and reduced chemically on the surface of the biomagnetite crystals. However, biological synthesis of bimetallic structures by simultaneous addition and coprecipitation of 2 metallic elements

has, to our knowledge, not yet been described. Since *S. oneidensis* can both precipitate Pd and Au (De Windt et al., 2005; Chapter 3), we examined if this strain was able to synthesize a bio-supported Pd-Au catalyst.

The aim of this chapter was to synthesize unique bimetallic nano-structures by varying the concentration and sequence of Pd(II) and Au(III) ion additions to viable cultures of *S. oneidensis*. After reduction of the metal ions, the obtained structures were characterized by synchrotron-based X-ray diffraction and electron microscopy. The activities of the different bimetallic catalysts were tested for the dechlorination of diclofenac, a recalcitrant pharmaceutical surface water pollutant, and TCE, a groundwater pollutant. As a benchmark, the degradation rates were compared to monometallic bio-supported Pd and Au catalysts.

2 Materials and methods

2.1 Catalyst preparation

2.1.1 Bacterial strains and growth conditions

S. oneidensis MR-1 was obtained from the BCCM/LMG Bacterium Collection (Gent, Belgium) under the number LMG 19005. The strain was grown aerobically in Luria-Bertani (LB) medium overnight at 28°C. *S. oneidensis* cells were harvested from an overnight LB culture in sterile 50 mL centrifuge tubes (TPP, Switzerland) by centrifuging at 3000 g for 10 min and washed twice with 50 mL distilled water or M9 medium (Sambrook et al., 1989). The cells were resuspended in distilled water to a final optical density at 610 nm of 1, corresponding to 50 mg cell dry weight per L. 120 mL serum bottles were filled with 50 mL cell suspensions.

2.1.2 Metal addition

Monometallic bio-Pd and bio-Au: Bio-Pd (50 mg Pd L^{-1}) was prepared according to De Windt et al. (2005) and bio-Au (50 mg Au L^{-1}) was prepared according to Chapter 3.

Bio-Pd + Bio-Au: 50 mL of a bio-Pd and bio-Au suspension were centrifuged at 3000 g for 10 minutes. The pellets were resuspended in 25 mL of distilled water and mixed to a final volume of 50 mL.

Bio-Au-Pd: Bio-Au was first prepared according to Chapter 3. After reduction of Au, Na_2PdCl_4 was added to 50 mg Pd(II) L^{-1}. Then, the headspace was replaced with H_2 gas after repeated cycles of overpressure with N_2 and vacuum. The resulting suspension was incubated overnight at 28 °C to obtain reduction of Pd.

Bio-Pd-Au: Bio-Pd was prepared as described above. After reduction of Pd, $HAuCl_4.3H_2O$ was added to the suspension to a concentration of 50 mg Au (III) L^{-1} and the headspace was again replaced with 100% H_2 gas after repeated cycles of overpressure with N_2 and vacuum. The resulting suspension was incubated during 48 hours at 28 °C to obtain reduction of Au.

Bio-Au/Pd: $HAuCl_4.3H_2O$ and Na_2PdCl_4 were added simultaneously to a cell suspension in distilled water to a final concentration of 50 mg L^{-1} of both Au(III) and Pd(II). Subsequently, the headspace was replaced with 100% H_2 gas after repeated cycles of overpressure with N_2 and vacuum. The cells were incubated for 48 hours at 28 °C to obtain reduction of Pd and Au.

In all cases, the cells were incubated in deionized water under non-growth conditions and continuously mixed by a shaker at 100 rpm. Catalyst formulations with Pd were incubated for 14 hours and formulations with Au were incubated for 48 hours in order to ensure maximum metal reduction. The preparations of the catalysts together with the precipitation efficiencies are summarized in Table 1. In all cases, the metals were added to a biomass concentration of 50 mg L^{-1}. The metals were in all cases added to a cell suspension with pH 7, after addition of the Au, pH dropped to 3 due to the acid Au-solution. Catalyst preparation was finished 24 hours before starting the experiments. Storage before the experiment was done at 4°C.

2.2 Catalytic removal in liquid microcosms

The pH of the 50 mL suspensions in the 120 mL serum bottles containing the Pd and/or Au catalysts was brought to 7 with a 0.1 M NaOH solution. The headspace was refilled with 1 bar H_2 after repeated cycles of overpressure with N_2 and vacuum.

Diclofenac (Sigma-Aldrich, Germany) was added to a final concentration of 20 mg L^{-1}. TCE (Sigma-Aldrich, Germany) was added to a final concentration of 100 mg L^{-1}. During the experiments, the serum bottles were placed on a shaker with a rotation speed of 100 rpm at room temperature. In the case of diclofenac dechlorination, aqueous samples were withdrawn with a syringe, the bacteria and the catalysts were separated from the reaction medium by filtration over a 0.22 µm Millipore filter. TCE concentration was monitored by taking 1 mL gaseous samples from the headspace of the bottle. Metal-free controls were performed using equal amounts of biomass and pollutant in order to exclude bacterial adsorption and degradation. All experiments were performed in triplicate. Statistical analysis on the data of the experiments was performed using the SPSS 16 software.

2.3 Characterization methods

2.3.1 Benchtop XRD

Bench source X-ray diffraction analysis of the Au and Pd associated with the cells after drying at 30°C was performed with a Siemens Diffractometer D5000 with BraggBrentano optics (Siemens, Germany). X-rays were generated by a copper X-ray tube with power 1.6 kW (40 kV, 40 mA). Measurements were performed between 25° and 90° 2-theta with step time of 1.6 s and step size of 0.02 degrees.

2.3.2 Synchrotron based XRD

X-ray diffractograms were collected at beamline X27A at the National Synchrotron Light Source which is located at Brookhaven National Lab (Upton, NY, USA). The incident beam energy was fixed at 17479 eV using a Si(111) monochromator and the energy was calibrated using the first inflection point of the X-ray absorption spectrum of a zirconium metal foil. Each diffraction pattern was collected for 60 seconds on a CCD detector (Bruker). The diffraction images were background subtracted prior to integrating intensity to produce diffractograms of intensity versus diffraction angle (2θ) (FIT2D V12.012). Subsamples of the bio-catalysts were collected as wet pastes following centrifugation of batch cultures. Samples were kept in polypropylene bags throughout the measurement to preserve full hydration. The detector size and distance to the sample limited the data range so that only the 111 diffraction peaks of the crystalline metal and bimetallic phases present could be observed. Evidence of oxides of palladium and other salts was not observed in any of the samples. The peaks within the diffractograms were fit using a baseline function and the minimum number of Gaussian functions.

2.3.3 Transmission electron microscopy

Samples of bacterial suspensions with Au were allowed to precipitate for 8 hours. Subsequently, the supernatant was removed and the bacteria were fixed in 0.1 M cacodylate buffer containing 4 % paraformaldehyde and 5 % glutaraldehyde. After postfixation in 1 % osmium tetroxide, samples were dehydrated in a series of alcohol and embedded in Epon medium (Aurion, the Netherlands). Ultrathin sections of 60 nm were contrasted with uranyl acetate and lead nitrate before examination or were examined without contrast by imaging with a Zeiss TEM900 transmission electron microscope (Carl Zeiss, Germany) at 50 kV. Particle size distributions were determined with ImageJ 1.43r freeware. Given the clear contrast between the particles and the background, a binary image could be created by visually adjusting the threshold value. Subsequently, based on

the magnification, a table containing each particle and its surface area was obtained. Particle diameters were determined as if the particles were spheres.

Another type of TEM specimens was prepared using whole-mount approach i.e. lacey carbon film on nickel support grid was immersed to diluted bacterial suspension followed by drying on air. Scanning TEM (STEM) mode combined with energy dispersive X-ray spectroscopy (EDX) were applied to study the catalyst particles distribution, morphology and local elemental distribution using a JEM-2200FS/Cs-corrected FEG instrument at 200 kV and probe diameter 1.0 nm.

2.4 Analytical methods

2.4.1 AAS

The bacteria with the nanoparticles were separated from the liquid medium by centrifugation for 10 minutes at 7000 g. The concentrations of Pd and Au in the supernatant were determined using an AA-6300 atomic absorption spectroscope (Shimadzu, Japan). These experiments were performed in triplicate. The detection limit for both Pd and Au was 0.1 mg L^{-1}.

2.4.2 HPLC

Diclofenac was analyzed on a high performance liquid chromatograph (HPLC) consisting of an ASI-100 autosampler, a P580 pump and a STH 585 column oven (Dionex, USA) on an Alltech Genesis C18 column (150 mm x 4.6 mm x 4 µm). Elution was performed isocratically at 25 °C and at a flow rate of 1 mL min^{-1} with 80 % solvent A (100 % methanol) and 20 % solvent B (0.1 % formic acid). Detection was performed with a UV-Vis detector at 203 nm. The limit of quantification (LOQ) of diclofenac was 0.1 mg L^{-1}.

2.4.3 GC-FID

TCE was analyzed by GC (CP-3800, Varian, USA) with a flame ionization detector (FID). The GC conditions were: injection temperature = 50 °C; detector temperature = 250 °C; initial column temperature = 35 °C (hold 2 min), increase to 75 °C at a rate of 5 °C min^{-1}; column pressure = 153 kPa (hold 2 min), increase to 176.5 kPa at a rate of 3 kPa min^{-1}. The column used was a Factor FourTM low bleed capillary column (VF-624 ms, 30 m x 0.25 mm inner diameter, film thickness = 0.25 µm, Varian, USA). The limit of quantification (LOQ) of TCE was 0.1 mg L^{-1}.

3 Results

3.1 Sequential and simultaneous precipitation of Pd and Au

A total of 6 types of bio-supported Pd and/or Au catalysts (bio-Pd, bio-Au and 4 combinations of Pd and Au) were synthesized using the metal respiring bacterium *S. oneidenis* and H_2 gas as the electron donor. In the case of bio-Pd and bio-Au, Pd and Au were added to achieve a total concentration of 50 mg Pd or Au L^{-1}. In the case of formulations that combine Pd and Au, Pd and Au were each added to 50 mg L^{-1} resulting in a total metal concentration of 100 mg L^{-1}. The difference between the four bimetallic combinations of Pd and Au was the different order of adding and reducing Pd and Au. In all cases, more than 90 % of the added Pd and/or Au was associated with the biomass (Table 1). The color change of the solutions to red (for Au(0)) or black (for Pd(0)) indicated the metallic character of Pd and Au in the suspensions. The reduction of Pd(II) and Au(III) ions to the metallic zerovalent state was confirmed with X-ray diffraction, which also showed that measurable quantities of oxides and/or hydroxides did not form in any of the biogenic formulations (Figure 4-1).

Table 4-1: Summary of the different catalyst preparations: concentrations of added Pd and Au, precipitation efficiencies and preparation method.

Catalyst	Pd-concentration (mg L^{-1})	Au-concentration (mg L^{-1})	Preparation	Precipitation efficiency of Pd (%)	Precipitation efficiency of Au (%)
Bio-Pd	50	0	See De Windt et al. (2005)	94.8 ± 0.2	n.a.
Bio-Au	0	50	See Chapter 3	n.a.	98.5 ± 0.3
Bio-Au + Bio-Pd	50	50	Mixture of bio-Pd and bio-Au	94.8 ± 0.2	98.5 ± 0.3
Bio-Pd-Au	50	50	Bio-Pd first prepared, Au(III) added and reduced afterwards	90.6 ± 1.8	96.1 ± 2.0
Bio-Au-Pd	50	50	Bio-Au first prepared, Pd(II) added and reduced afterwards	> 99.8	99.4 ± 0.5
Bio-Pd/Au	50	50	Pd(II) and Au(III) added simultaneously to the cells and then reduced	> 99.8	> 99.8
Heat-killed biomass with Pd/Au	50	50	Pd(II) and Au(III) added simultaneously to heat-killed cells, and then reduced	> 99.8	> 99.8

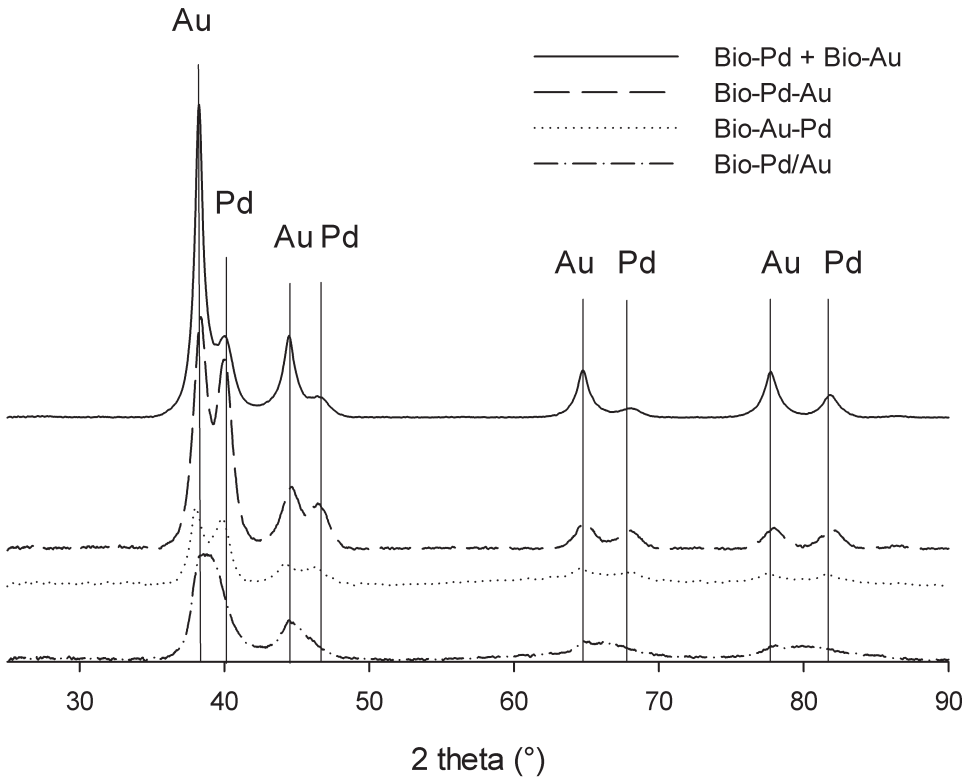

Figure 4-1: Bench source XRD spectra of bio-Pd + bio-Au, bio-Pd-Au, bio-Au-Pd and bio-Pd/Au

3.2 Removal of diclofenac

The different catalysts were subsequently tested for the removal of diclofenac, with H_2 as hydrogen donor at neutral pH. Metal-free controls did not show any removal of diclofenac (data not shown). Pd and Au were in all cases added to achieve a total concentration of 50 mg Pd and/or Au L^{-1}. The concentration of diclofenac was followed as a function of time (Figure 4-2). The monometallic catalysts bio-Pd and bio-Au did not show any catalytic activity for the removal of diclofenac at neutral pH. Also a mixture of the individually produced bio-Pd and bio-Au (referred to as bio-Pd + bio-Au) was ineffective. The catalyst prepared by first reducing Pd(II) to Pd(0) on the cell surface followed by the addition and reduction of Au(III) to Au(0) (referred to as bio-Pd-Au), did not show any activity for the studied reaction either. The preparations where Au was added and reduced prior to the addition and reduction of Pd (referred to as bio-Au-Pd) and the one with Pd and Au added and reduced simultaneously (referred to as bio-Pd/Au) showed significant catalytic activity. Using the bio-Au-Pd catalyst, a diclofenac removal of 36.5 ± 1.8 % after 24 hours was observed (corresponding to a specific removal rate of 0.146 mg diclofenac mg^{-1} Pd d^{-1}). A first order removal rate could be fitted to the curve (R^2 = 0.979), this gave a decay constant of 0.018 ± 0.001 h^{-1}. The bio-Pd/Au preparation catalyzed the removal of 77.8 ± 2.0 % of diclofenac after 24 hours and the curve showed clear first order kinetics (R^2 =

0.975) with a decay constant of 0.078 ± 0.009 h^{-1} (corresponding to a specific removal rate of 0.311 mg diclofenac mg^{-1} Pd d^{-1}). The Pd- and metal-normalized decay values are shown in Table 4-2. The analysis of the degradation products is described in Chapter 5. pH values after reaction showed a maximum decrease of 0.3 pH units.

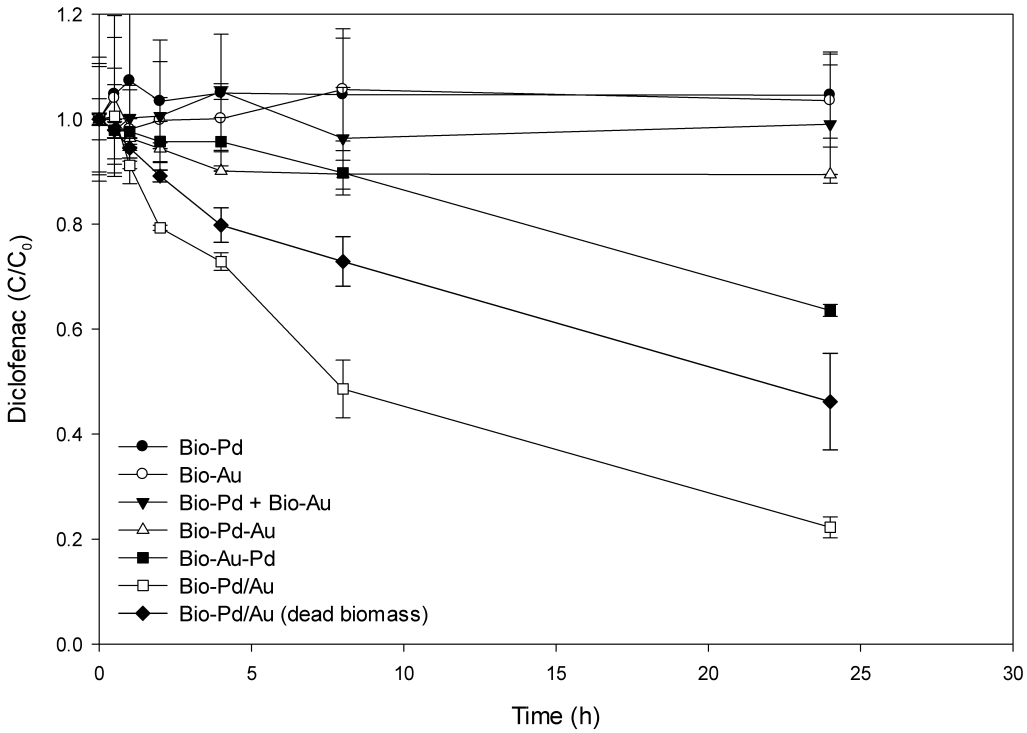

Figure 4-2: Degradation of diclofenac as a function of time using the different bio-supported catalyst formulations and H_2 as hydrogen donor at pH 7 (C_0 = 20 mg L^{-1} and n = 3).

Pd(II) and Au(III) were also added to heat-killed cells of *S. oneidensis*, exposed to an overpressure of H_2 and subsequently tested for the removal of diclofenac. Also in this case, the metals were precipitated for more than 99.8 % (Table 1). This catalyst formulation also showed a first order removal of diclofenac (R^2 = 0.975) with a decay constant of 0.660 ± 0.006 $L.h^{-1}.g_{Pd}^{-1}$.

3.3 Removal of TCE

All six catalyst formulations using living cells were also tested for degradation of TCE with H_2 as the hydrogen donor at neutral pH (pH 7). The concentration of TCE was followed as a function of time (Figure 4-3). No removal was observed in metal-free controls (data not shown). TCE was not degraded using the bio-Au catalyst, but bio-Pd was able to catalyze the removal of TCE. TCE degradation was also observed for bio-Pd-Au, bio-Au-Pd and bio-Pd/Au. A first order removal rate could be fitted to these curves. The decay constants (k-values) for the different catalysts are listed in Table 4-2, together with the metal-normalized decay constants. One-way ANOVA and an LSD post-hoc test showed that bio-Pd/Au showed a significantly faster TCE removal rate relative to the other catalysts, a

factor three more rapid than monometallic bio-Pd. The removal rate of bio-Au-Pd was significantly slower than bio-Pd/Au but significantly faster than the other catalysts. No significant differences in removal rate were found between bio-Pd, bio-Pd + bio-Au and bio-Pd-Au. In all cases, TCE was dechlorinated and hydrogenated with ethane as the only detectable end product. In the case of bio-Pd/Au, with almost complete removal after 40 minutes, 83 ± 8 % of the initially added TCE could be recovered as ethane.

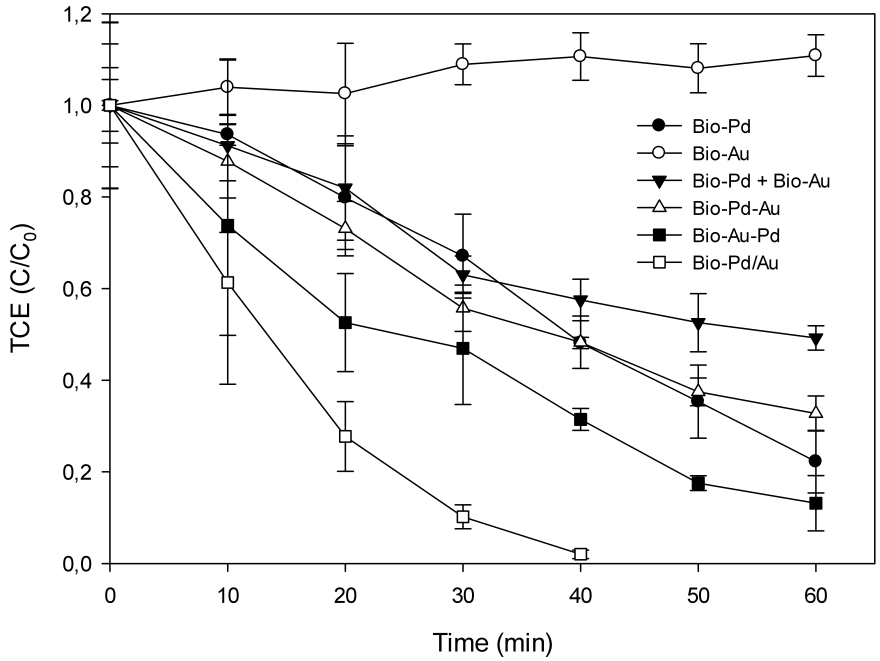

Figure 4-3: Degradation of TCE as a function of time using the different bio-supported catalyst formulations and H_2 as hydrogen donor at pH 7 (C_0 = 100 mg L^{-1} and n = 3).

Table 4-2: First order decay values (k) and metal-normalized decay values (k_{metal}) for the dechlorination of diclofenac and TCE of the different bio-supported catalysts (n = 3).

Contaminant	Catalyst	k_{Pd} ($L.h^{-1}.g_{Pd}^{-1}$)	k_{metal} ($L.h^{-1}.g_{metal}^{-1}$)
Diclofenac	Bio-Au-Pd	0.36 ± 0.03	0.18 ± 0.02
	Bio-Pd/Au	1.55 ± 0.18	0.78 ± 0.09
TCE	Bio-Pd	24.12 ± 3.24	24.12 ± 3.24
	Bio-Pd + Bio-Au	15.72 ± 1.20	7.86 ± 0.60
	Bio-Pd-Au	22.92 ± 1.08	11.46 ± 0.54
	Bio-Au-Pd	36.60 ± 2.40	18.30 ± 1.20
	Bio-Pd/Au	77.64 ± 9.00	38.82 ± 4.50

3.4 Characterization

3.4.1 µXRD

The two most active catalysts (bio-Pd/Au and bio-Au-Pd) were studied in more detail by benchtop XRD, and synchrotron-based µXRD. The benchtop XRD revealed only the presence of metallic Pd and Au, where no peaks of Pd- or Au-oxides or hydroxides could be detected in any of the samples (Figure 4-1). The synchrotron-based µXRD diffractograms shown in Figure 4-4 revealed significant differences among the different catalyst formulations in the region of the 111 Bragg peak. The 111 Bragg peaks of bio-Pd and bio-Au were located at 2θ values of 17.36° and 17.52°, respectively. These 2θ values were converted into d-spacing using Bragg's law in order to more directly compare the distance between 111 planes of atoms and identify unique crystalline domains within the different catalysts. For bio-Pd and bio-Au, these d-spacings were 2.349 and 2.329 Å, respectively. The diffraction pattern of bio-Pd/Au consisted of two distinct peaks with the main peak at 17.92° and the less intense peak at 17.47°. The corresponding d-spacings were 2.277 and 2.335 Å, respectively. The smaller d-spacing of the main peak indicated a significant contraction of the lattice relative to bio-Pd and bio-Au, and thus suggests the formation of structurally unique crystalline domains in bio-Pd/Au. The bio-Au-Pd preparation also showed two distinct peaks, however, the main peak located at 17.45° was consistent with monometallic bio-Pd and bio-Au. The minor peak at 18.11°

corresponded to a d-spacing of 2.253 Å, indicating the presence of a minor crystalline phase with a similar lattice contraction to the dominant phase observed in bio-Pd/Au.

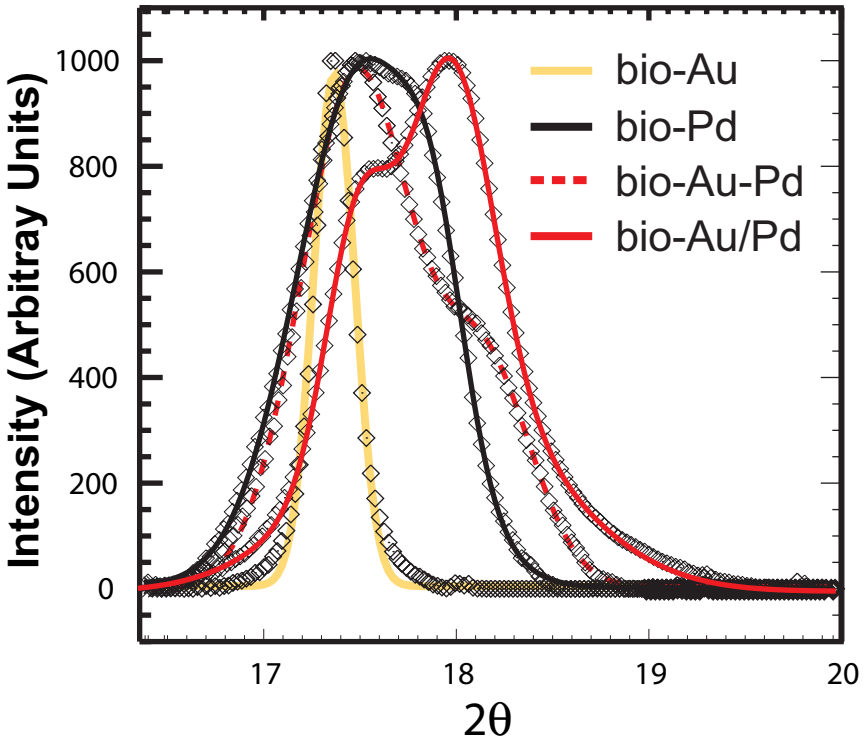

Figure 4-4: μXRD diffractogram of the 111 peak of bio-Pd, bio-Au, bio-Au-Pd and bioPd/Au.

3.4.2 TEM and STEM

The morphology, aggregation and particle diameter of the bio-Au-Pd and bio-Pd/Au catalyst were characterized using TEM and STEM. A thin section of bio-Au-Pd together with the particle size distribution is shown in Figure 4-5a and c. Small nanoparticles (1-10 nm) could be found both intracellularly and on outer cell parts. The largest particles (up to 50 nm) were located on the outer parts of the cells. The thin sections of bio-Pd/Au (Figure 4-5b) showed that most of the particles can be found in the outer parts of the bacterial cells. The particles can be small (< 5 nm), but also larger aggregates (up to 100 nm) were observed. From the particle size distributions, it is clear that bio-Pd/Au consisted of larger particles than bio-Au-Pd . The average diameters were 11.04 ± 13.65 nm and 6.88 ± 5.16 nm for bio-Pd/Au and bio-Au-Pd respectively. These aggregates of the most active catalyst preparation (bio-Pd/Au) were further studied by STEM. An EDX spectrum taken at one point within the aggregate, shown in Figure 4-6a, provided evidence for the presence of both Pd and Au within the same zone of the aggregate (Figure 4-6b). A scan for the presence of Pd and Au was performed along the horizontal line shown in Figure 4-6c. The relative amounts of Au and Pd are shown by the curves in the lower part of Figure 4-6c. These curves show that within one aggregate no zones with pure Pd or pure Au were

present, but that they were always detected together. Figure 4-6d shows the resulting EDX spectrum of the line scan from Figure 4-6c and confirms the presence of Au and Pd within the same aggregate.

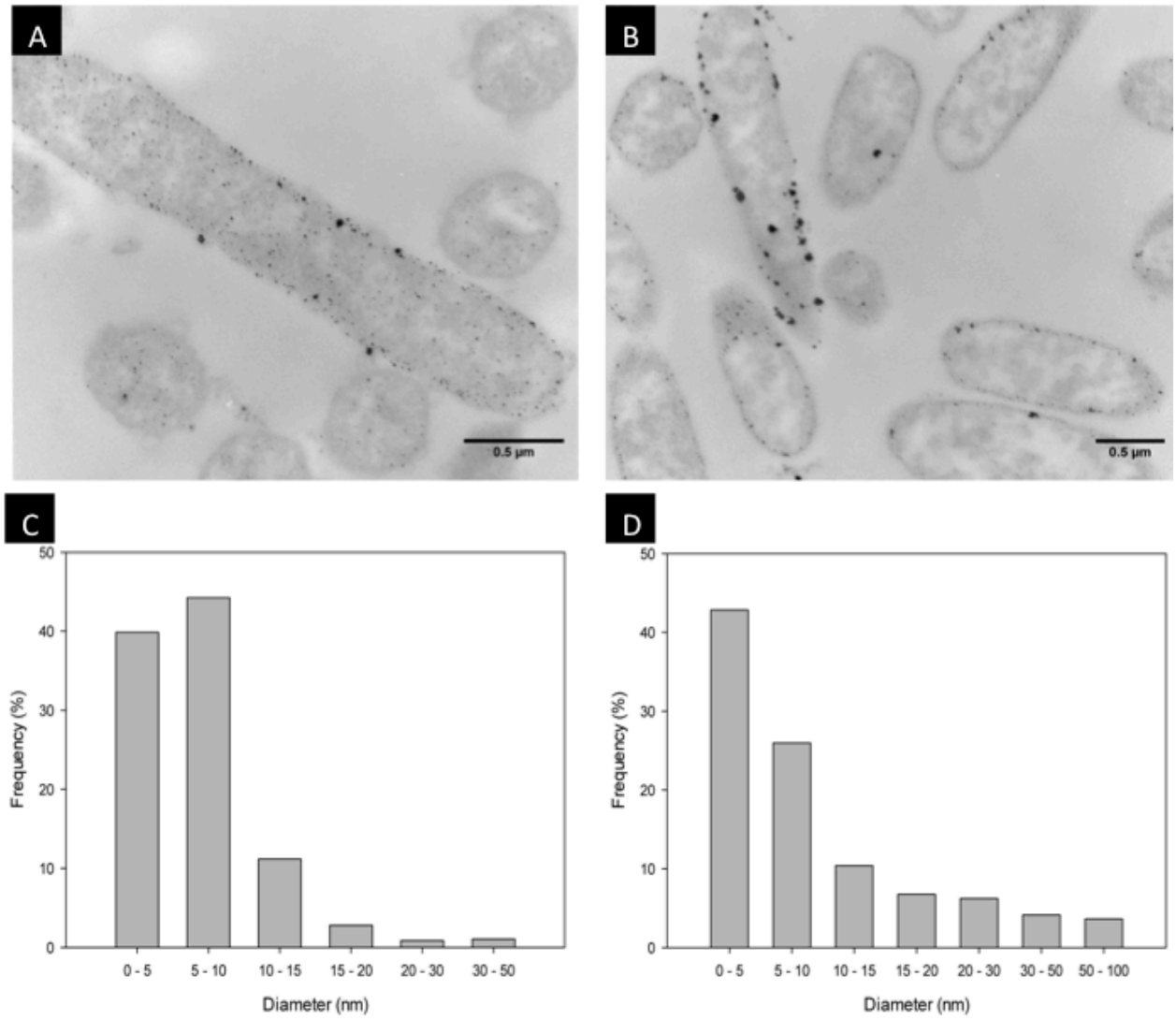

Figure 4-5: TEM bright filed images of (a) Thin section of *S. oneidensis* cells loaded with particles of Pd(0) and Au(0) when Au and Pd were added sequentially (bio-Au-Pd). (b) Thin section of *S. oneidensis* cells loaded with particles of Pd(0) and Au(0) when Au and Pd were added simultaneously (bio-Pd/Au). (c) Particle size distribution of the thin section of bio-Au-Pd. (d) Particle size distribution of the thin section of bio-Pd/Au.

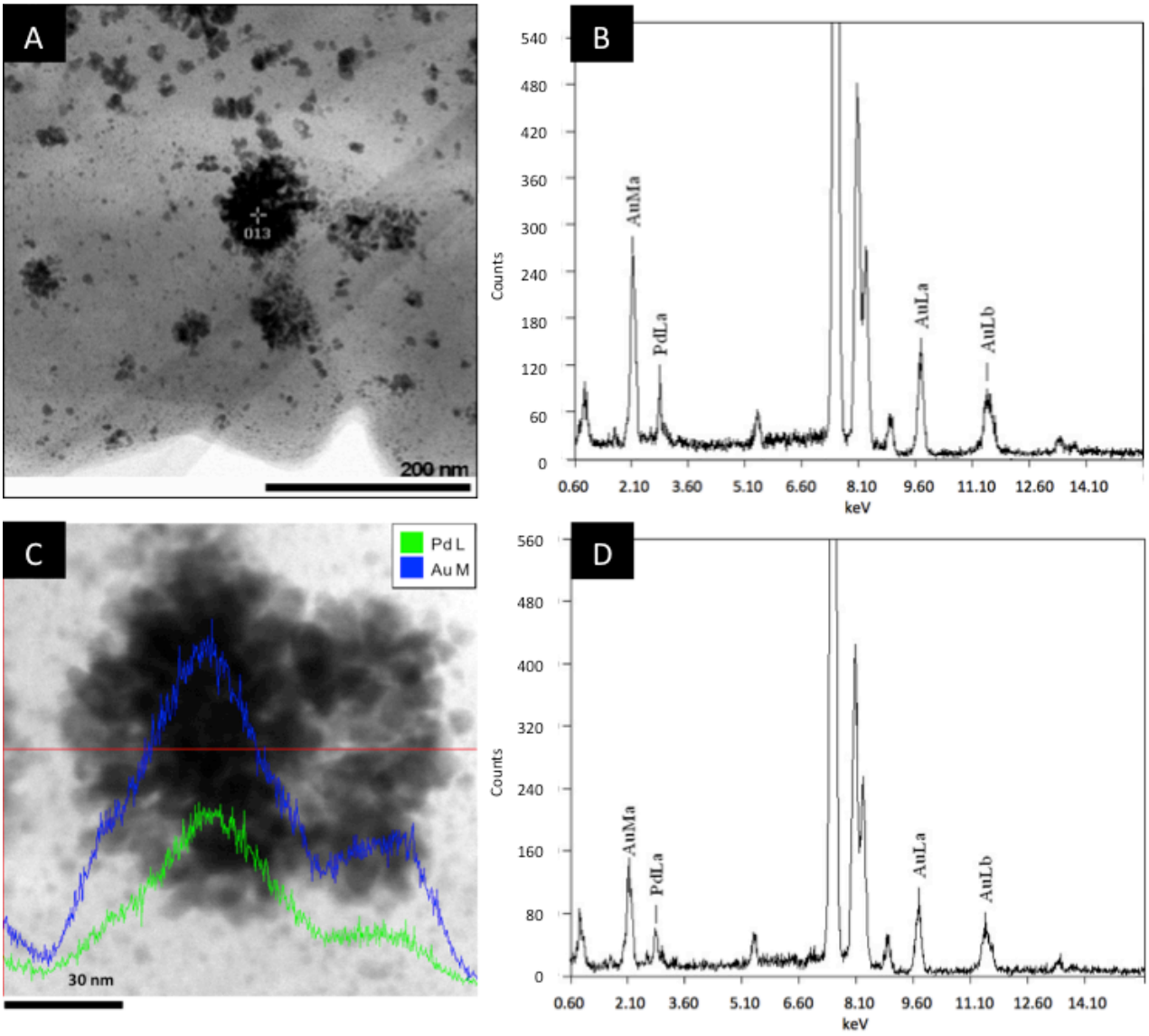

Figure 4-6: STEM bright field images (a) and (c) of a whole–mount bio-Pd/Au aggregate specimen on the cell surface of *S. oneidensis*. (b) EDX spectrum taken from the indicated point '013' on (a). (c) EDX elemental line-scan across a bio-Pd/Au aggregate on the cell surface of *S. oneidensis* and (d) integral line-scan EDX spectrum.

4 Discussion

This study showed that a monometallic Au catalyst supported on the cells of *S. oneidensis* (bio-Au), synthesized according to Chapter 3, was ineffective for the removal of diclofenac and TCE. The bio-supported Pd catalyst (bio-Pd), making use of the same species as De Windt et al. (2005), could catalyze the dechlorination of TCE at a concentration of 50 mg Pd L^{-1}, with H_2 as hydrogen donor and at neutral pH, whereas this was not possible for diclofenac. This is noteworthy since bio-Pd had shown its effectiveness as a catalyst in the dehalogenation of other contaminants (De Windt et al., 2005; Hennebel et al., 2010). In contrast, the catalyst which was obtained when 50 mg Au(III) L^{-1} and 50 mg Pd(II) L^{-1} were

added simultaneously to the bacteria (bio-Pd/Au) in the presence of H_2 as electron donor, successfully catalyzed the removal of diclofenac. Using the bio-supported catalyst with Au added and reduced prior to the addition and reduction of Pd (bio-Au-Pd), dechlorination of diclofenac was observed to a limited extent. A possible explanation for this limited removal relative to the bio-Pd/Au formulation could be the lesser amount of the structurally unique alloyed bimetallic Pd/Au phase observed in the bio-Pd-Au formulation with μXRD. Other sequences of metal addition to *S. oneidensis* were ineffective for the removal of diclofenac. Also in the case of TCE, bio-Pd/Au was the most effective formulation, followed by bio-Au-Pd. The percentage of ethane produced from TCE was similar to the recovery observed previously (Hennebel et al., 2009b). These results indicate that doping of a bio-Pd catalyst with Au, which is inactive as a monometallic catalyst, can improve bio-Pd catalytic activity.

Since it was shown that the catalyst preparation with Pd and Au added simultaneously to heat-killed cells was able to catalyze the dechlorination of diclofenac (but at a slower rate than bio-Pd/Au), it is clear that the formation of a highly active bimetallic structure was not purely enzymatic, but also at least partly occurred via the well established chemical reduction pathway with molecular H_2. However, when using living cells, Pd(II) and Au(III) reduction occur faster than with dead biomass. It appears that both the simultaneous addition and the physiological state of the cells are among the most important factors to obtain a highly active bimetallic catalyst

A combined chemical and biological reduction mechanism was also proposed for bio-Au (Chapter 3) and bio-Pd produced by fermentative species (Hennebel et al., 2011c). In the case of bio-Pd formation by *E. coli*, heat-killed cells showed partial reduction of Pd(II), implying a partly chemical mechanism (Deplanche et al., 2010). In the latter cases in which chemical reduction was involved, larger irregular Pd aggregates were observed, which was in contrast to the smaller mono-dispersed particles resulting from enzymatic bacterial activity.

Chemically produced bimetallic particles have already shown their applicability for dechlorination of diclofenac. Ghauch et al. (2010) investigated the ability of several metals to promote the catalytic activity of Fe particles for the dechlorination of diclofenac under oxic and anoxic conditions. In both cases, Pd was the most effective promoting metal and showed a significantly faster dechlorination than the monometallic Fe catalyst. Although rates are difficult to compare due to the different experimental conditions, the Pd-Fe catalysts showed rates of about a factor 10 higher than the ones presented in this study.

Removal percentages of 80 % were achieved after 20 minutes reaction time, after which degradation stopped. The mode of action of this Pd-Fe catalyst is based on the oxidation of Fe(0) to Fe(II). Dechlorination of diclofenac by a Pd/Au catalyst was not yet reported. The application of Pd/Au bimetallic catalysts for dechlorination of TCE has been described extensively (Nutt et al., 2005; Nutt et al., 2006; Wong et al., 2009). However, these studies make use of particles consisting of an Au core with a Pd shell. After optimization of Au-particle size and Pd-surface coverage, particles catalyzed the dechlorination of TCE with rate constants which are up to a factor 200 higher than for bio-Pd/Au as presented in this study (Nutt et al., 2006). For bio-supported catalysts, attachment to the bacterial biomass might imply a loss of catalyst activity. Moreover, it is possible that the core-shell structure is much more active for the studied reactions than the alloyed bimetallic structures obtained in this study. This study demonstrated that a catalyst with similar activity could not be obtained by first precipitating Au(0) on the cell walls, followed by the precipitation of Pd(0). Similar differences between bio-supported and chemically produced Pd nanocatalysts were also valid for diatrizoate removal with bio-Pd and Pd/Al_2O_3 (Knitt et al., 2008; Hennebel et al., 2010).

The two most active catalysts, bio-Au-Pd and bio-Pd/Au, were characterized more thoroughly by μXRD and TEM. The bio-Pd/Au catalyst showed a clear shift of the 111 peak to the right compared to the monometallic bio-Pd and bio-Au catalysts, implying a smaller d-spacing and thus a contraction of the crystal lattice. This change in lattice structure can be indicative for the formation of a unique bimetallic structure of Pd and Au. A lattice contraction of bimetallic crystals compared to monometallic particles has been demonstrated for crystals of Pt/Cu (Stassi et al., 2006) and Pd/Co (Suo et al., 2007). The improved activity of bio-Pd/Au compared to the other catalyst formulations might be explained by this contraction. This implies that activity of biologically formed catalysts can be steered by selecting different metals, adjusting metal ratios and changing the moment and sequence of metal addition to the bacteria. The spectrum of the bio-Au-Pd catalyst showed a main peak situated at a diffraction angle similar to the one of bio-Pd and bio-Au. A smaller peak, situated at 18.107°, probably indicating a contraction of the lattice, but to a lesser extent, was also present. The reason for this lattice contraction might be that some Au(III), which was added prior to Pd(II), was adsorbed on the bacterial cells without being reduced. This is probable since Au(III) is adsorbed very fast by *S. oneidensis* (76.1 ± 7.1 % was sorbed within 30 minutes), but reduced very slowly (reduction started after 24 hours) (Chapter 3).The unreduced Au(III) can be co-reduced with Pd(II) (similar as for bio-Pd/Au). This fits with the difference that was observed in catalytic activity: bio-Pd/Au

showed a higher activity than bio-Au-Pd. The latter was more active than the monometallic bio-Pd. Since these spectra do not provide information on the spatial positioning of Pd and Au within the crystals, a microscopic study by STEM coupled with EDX was performed. TEM images of the most catalytically active form (bio-Pd/Au) revealed that aggregates of nanoparticles were precipitated mainly on the outer parts of the cells, similar to bio-Pd (De Windt et al., 2005). Nanoparticles are mainly used for their very high specific surface area. In this case however, surface area is not the main activity determining parameter, since bio-Pd/Au contained larger particles than bio-Au-Pd but showed higher removal rates. Degradation rates can be normalized to the specific surface area. In the case of diclofenac removal this results in a normalized first order decay value of 0.144 $nm^3.nm^{-2}.h^{-1}$ for bio-Pd/Au and 0.041 $nm^3.nm^{-2}.h^{-1}$ for bio-Au-Pd , with the specific surface areas calculated based on the average diameter and supposing the particles are spherical. In the case of TCE, these normalized decay constants are 7.20 $nm^3.nm^{-2}.h^{-1}$ for bio-Pd/Au and 4.17 $nm^3.nm^{-2}.h^{-1}$ for bio-Au-Pd.

The promotional effect of Au on Pd catalysis is most likely due to a beneficial geometric effect of Au on the positioning of Pd within the crystal. The presence of Au causes a contraction of the lattice resulting in a structure which can enable the contact between the two reacting substances (in this case diclofenac or TCE and hydrogen). This phenomenon has been reported for other Pd/Au-catalyzed reactions before: Chen et al. (2005) reported that the introduction of Au(0) to a monometallic Pd(0) crystal resulted in the optimal intermolecular distance for the acetoxylation of ethylene to vinylacetate. The increased activity of Pd/Au catalysts relative to monometallic Pd catalysts has also been attributed to electronic effects, where the authors proposed that Au withdraws electron density from Pd, and thereby, increases the interaction potential of Pd with the reactants (Knecht et al., 2008). However, other techniques are needed to confirm beneficial electronic effects of the addition of Au(0) in the current study. Gao et al. (2012) mentioned both geometric effects (more isolated reactive Pd sites due to the insertion of Au in the crystal) and electronic effects (electronic rearrangements, facilitating sorption and desorption of reagents and products).

5 Conclusions

This study showed that the catalytic activity of a biologically produced Pd nanocatalyst, which had already proved its applicability in wide range of environmental applications, can be extended by alloying with Au(0). An aspect of optimization will be the determination the optimal Pd/Au ratio, preferably with a minimal amount of Au, since Au is more expensive

than Pd and will thus mainly determine the cost of the synthesis. The optimal ratio of Pd/Au will likely depend on the pollutant that needs to be dehalogenated. Also other metals that can promote the catalytic activity of Pd should be investigated in this context. Further research for diclofenac and TCE dehalogenation should aim at minimizing reaction times, which can be done by altering reaction parameters such as pH and by changing the concentration of the catalyst. A disproportional increase of catalytic activity with increasing catalyst concentration had been observed in previous studies using a bio-Pd catalyst (Hennebel et al., 2009b). Catalysis by bio-Pd/Au opens perspectives for degradation of extremely recalcitrant pollutants such as fluorinated compounds. Although major advances in the catalytic activity of biogenic catalysts will be required in order to be competitive with chemically engineered bimetallic nanoparticles, this work does provide insights into potential bio-inspired modifications to abiotic bimetallic catalysts.

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Chapter 5
Doping of biogenic Pd catalysts with Au enables dechlorination of diclofenac at environmental conditions

Abstract

Bio-Pd nanoparticles can be used as a catalyst in, for example, dehalogenation reactions. However, some halogenated compounds are not efficiently degraded using a bio-Pd catalyst. The activity of bio-Pd can be improved by doping with Au(0) ('bio-Pd/Au'). In contrast with bio-Pd, bio-Pd/Au could perform the removal of the model pharmaceutical compound diclofenac from an aqueous medium in batch experiments at neutral pH and with H_2 as the hydrogen donor. Dehalogenation was for both catalysts the only observed reaction. For bio-Pd/Au, a disproportional increase of catalytic activity was observed with increasing Pd-content of the catalyst. In contrast, when varying the Au-content of the catalyst, a Pd/Au mass ratio of 50/1 showed the highest catalytic activity. The removal of 6.40 µg L^{-1} diclofenac from a wastewater treatment plant effluent using bio-Pd was not possible even after prolonged reaction time. However, by using the most active bio-Pd/Au catalyst, 44% of the initially present diclofenac could be removed after 24 hours. This chapter shows that doping of bio-Pd nanoparticles with Au(0) can be a promising approach for the reductive treatment of wastewaters containing halogenated contaminants.

Chapter redrafted after:

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1 Introduction

The increasing use of pharmaceuticals combined with the fact that they are easily excreted with urine has resulted in their ubiquitous prevalence as trace pollutants in wastewaters. Their low concentrations (µg L^{-1}) and their complex molecular structure often result in a very limited removal by conventional wastewater treatment systems (Ternes, 1998). Even at these low concentration levels, these compounds can exhibit toxicity to aquatic organisms (Jones et al., 2001; Crane et al., 2006). A significant fraction of pharmaceuticals contains one or more halogen atoms in their molecular structure. An example is the anti-inflammatory drug diclofenac (2-[(2,6-dichlorophenyl)amino]phenylacetate), the model compound used in this study (Schwaiger et al., 2004; Triebskorn et al., 2004). Because of its low biodegradability (Joss et al., 2006), diclofenac has been detected in surface and drinking waters at µg and ng L^{-1} levels, respectively (Ternes, 1998; Heberer, 2002). During the past decade, mostly advanced oxidation processes (AOPs), such as ozonation have been applied to degrade these compounds in an oxidative way. Although these techniques are able to efficiently remove pharmaceuticals from wastewaters (Huber et al., 2005), they can result in a wide variety of mutagenic and toxic transformation products and byproducts (Guzzella et al., 2002; Schmidt et al., 2008). Moreover, some compounds such as the iodinated contrast medium diatrizoate are insensitive to ozonation (Ternes et al., 2003). None of these techniques are able to cleave the carbon-halogen bound, and they can thus not solve the problem of wastewaters containing halogenated compounds. Other techniques such as sorption on activated carbon or retention by membranes may alternatively be applied to avoid the presence of halogenated compounds in effluents, but they do not break the carbon-halogen bound either and thus do not solve the toxicity issue of the concentrated residual fraction. Dechlorination could be obtained by using organochlorine respiring bacteria, but this is a very slow process (De Wildeman et al., 2003). Also gamma irradiation has been described as a dechlorinating treatment (Zona et al., 1999). However, its application in wastewater treatment is very unlikely.

A possibly more effective treatment for halogenated substances at relatively mild conditions, with good selectivity and yields, is reductive hydrodehalogenation catalyzed by Pd in the presence of a hydrogen donor (e.g. H_2). The deiodination of the iodinated contrast media diatrizoate and iopromide by a chemically produced Pd/Al_2O_3 catalyst or biogenic Pd nanoparticles has been reported before (Knitt et al., 2008; Hennebel et al., 2010; Forrez et al., 2011a). The biogenic Pd nanoparticles (also called bio-Pd) are

produced on the cell wall of *Shewanella oneidensis* and have already demonstrated their applicability for the dehalogenation of a variety of environmental contaminants, such as polychlorobifenyls (De Windt et al., 2005), lindane (Mertens et al., 2007) and trichloroethylene (Hennebel et al., 2009b). Since the bacterial cells act both as the reducing agent and as the carrier material of the nanoparticles, the use of possibly toxic, expensive or scarce chemicals as reducing agent, stabilizer or carrier is prevented. The bacterial matrix also allows a better contact between the pollutant and the metal catalyst. Moreover, bacteria can be applied to recover metals from waste streams and produce an added value product in one step (Hennebel et al., 2009a). The main factors determining the feasibility of large scale production and application of biogenic metal nanocatalysts will most probably be the costs for the growth of the bacteria and the price of the noble metal salts.

In Chapter 4 it was shown that diclofenac reduction catalyzed by 50 mg bio-Pd L^{-1} and with externally added H_2 was not possible at neutral pH. However, it was demonstrated that a bimetallic catalyst, obtained by coprecipitation of Pd and Au by *Shewanella oneidensis* (bio-Pd/Au), was able to catalyze the removal of diclofenac at neutral pH with externally added H_2. When Pd and Au were added and precipitated sequentially, there was no or very poor activity for this reaction. The previous chapter focused on the synthesis and characterization of the bimetallic bio-Pd/Au catalyst. In contrast, the goal of this chapter was to apply the bimetallic coprecipitated bio-Pd/Au for the dechlorination of the micropollutant diclofenac. The specific aims were: (i) to investigate the optimal conditions in terms of pH for the removal of diclofenac by bio-Pd and bio-Pd/Au, (ii) to identify the transformation products, (iii) to investigate the effect of different Pd and Au levels of the bio-Pd/Au catalyst and (iv) to study the removal of diclofenac in a real wastewater treatment plant effluent at environmental concentrations.

2 Materials and methods

2.1 Catalyst preparation

Bio-Pd (50 mg Pd L^{-1}) was produced as described by De Windt et al. (2005) and bio-Pd/Au was produced as described in Chapter 4. Metal precipitation efficiencies are also reported in Chapter 4. Catalyst preparation was finished 24 hours before starting the experiments. Storage before the experiment was done at 4°C.

2.2 Catalytic removal in liquid microcosms

Assays for the catalytic removal of diclofenac were performed as described in Chapter 4.

2.3 Hospital wastewater treatment plant effluent

The hospital wastewater treatment plant (WWTP) studied in this research is exclusively treating hospital wastewater. The first treatment step is the mechanical removal of solids by means of a grid screen (ϕ: 40 mm). Subsequently, the wastewater is treated by a conventional activated sludge system (CAS) in 2 aeration tanks. Eventually, the aerated water is treated in a secondary clarifier, allowing the CAS flocks to settle and return (partially) to the aeration tanks. The final effluent is discharged to a nearby surface water. The composition of the wastewater is given in Table 5-1. The hospital WWTP effluent used in the experiments was sampled at 2 pm on April 20th, 2011.

Table 5-1: Characteristics of the hospital WWTP effluent used in this study.

Parameter	**Mean value (± standard deviation)** (mg L^{-1})
pH	7.43
Chemical oxygen demand (COD)	105.61
Total suspended solids (TSS)	(2.29 ± 0.02) * 10^3
Volatile suspended solids (VSS)	(0.30 ± 0.01) * 10^3
Kjeldahl-N	9.83
NH_4^+-N	0.64 ± 0.11
NO_2^--N	0.00 ± 0.00
NO_3^--N	28.85 ± 0.02
PO_4^{3-}-P	1.11 ± 0.02
SO_4^{2-}-S	30.50 ± 0.04

2.4 Analytical methods

Diclofenac and its metabolites were monitored and identified using liquid chromatography coupled to multiple mass spectrometry (LC-MS^n). Chromatography was

carried out on a Thermo Finnigan Surveyor LC system (San Jose, CA, USA) comprising of a quaternary pump and an autosampler, equipped with a Nucleodur C_{18} ISIS column (250 mm x 4.0 mm x 5 µm) obtained from Macherey-Nagel (Bethlehem, PA, USA). Elution was performed at a flow rate of 300 µL/min using a mobile phase of 50% A (0.01 % formic acid) and 50% B (methanol) for 2 minutes, increasing to 100% B in 20 minutes, holding for 10 minutes. before allowing the column to re-equilibrate for 8 minutes. Analytes were detected with an LTQ ion trap mass spectrometer (Thermo Finnigan, San Jose, CA, USA) in the MS^2 or MS^3 positive ion mode using an Atmospheric Pressure Chemical Ionisation interface. A vaporizer temperature of 400°C, a capillary temperature of 270°C, a sheath gas flow of 40 units and an auxiliary gas flow of 5 units were used. Alternating scans were used to isolate $[M+H]^+$ ions at mass 278 for diclofenac, 244 for 2-(2-chloroanilino)phenylacetate and 210 for 2-anilinophenylacetate. The precursor isolation width was set to 2 Da, the activation Q to 0.25 and the collision energy to 25 %.

For the detection of diclofenac, diatrizoate and carbamazapine in the hospital WWTP effluent, LC-MS/MS was performed using the same HPLC system and software as described above, equipped with a Nucleodur C_{18} Pyramid column (100 mm x 2.1 mm, 1.8 µm, Machery-Nagel, PA, USA). The column temperature was set at 35 °C. Analytes were eluted at a flow rate of 0.3 mL min^{-1} using a gradient starting from 98 % A (0.08 % formic acid) and 2 % B (0.08 % formic acid in acetonitrile) for 0.8 min, increasing to 65 % B in 0.5 min, keeping at 65 % B for 0.7 min, increasing to 100 % B in 1 min and keeping at 100 % B for another 2 min, before returning to the initial conditions during 1.9 min. The injection volume was 10 µL. The MS parameters were: a vaporizer temperature of 30 °C, a capillary temperature of 270 °C, a sheath gas flow of 25 units, an ion sweep gas flow of 2 units and an auxiliary gas flow of 5 units. The spray voltage polarity was 3500 V. Argon pressure in the collision cell (Q2) was set at 1.5 mTorr and the mass resolution at the first (Q1) and third (Q3) quadrupole were set at 0.7 Da at full width at half maximum (FWHM).

3 Results and discussion

3.1 Diclofenac removal catalyzed by bio-Pd and bio-Pd/Au

Bio-Pd was ineffective to remove diclofenac at neutral or alkaline pH. However at pH 5 and 6, a clear first order removal of diclofenac was observed (Figure 5-1A). The removal was improved at lower pH (the first order decay constant at pH 5 (0.129 ± 0.020 h^{-1}) is a factor 5 higher than the one at pH 6 (0.027 ± 0.020 h^{-1})). At pH values below the pKa of 4.0, no diclofenac could be detected anymore due to the precipitation of the protonated

form of diclofenac (Llinas et al., 2007). A control experiment using biomass of *Shewanella oneidensis* without Pd at pH 5 and a control with bio-Pd but without H_2 at pH 5 did not show any removal (data not shown). A pH-dependent removal using bio-Pd was also observed before for the iodinated contrast medium diatrizoate (Hennebel et al., 2010). However, in the case of diatrizoate, no pH values were reported at which no removal took place. A possible reason for this improved activity at lower pH is the fact that diclofenac becomes more protonated at lower pH (pKa = 4.0). Hence, there is less repulsion by the negatively charged bacterial cells, which are the carriers of the Pd-nanoparticles.

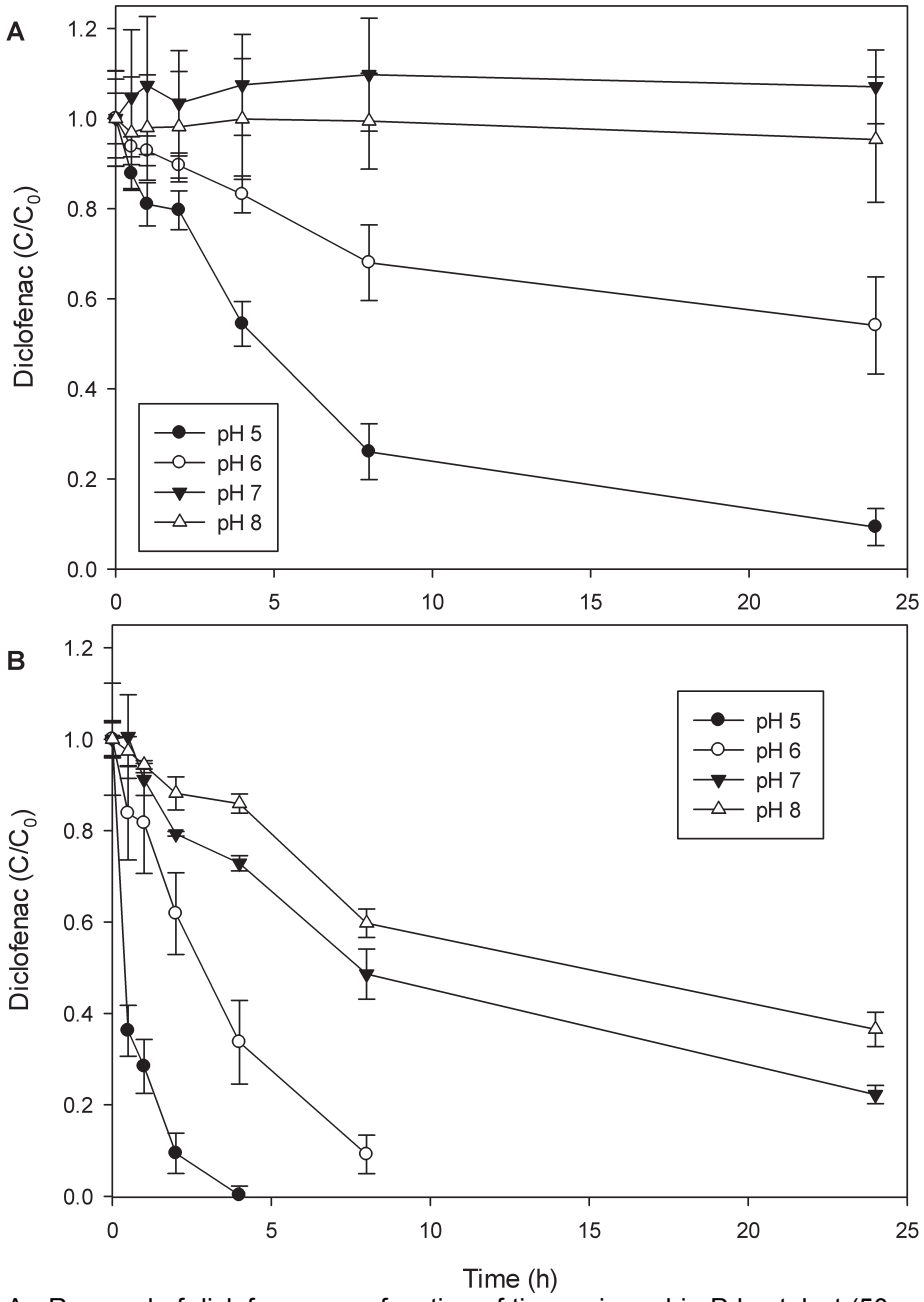

Figure 5-1: A: Removal of diclofenac as a function of time using a bio-Pd catalyst (50 mg Pd L^{-1}) with H_2 as hydrogen donor at pH 5, 6, 7 and 8 (C_0 = 20 mg diclofenac L^{-1}). B: Removal of diclofenac as a function time using a bio-Pd/Au catalyst (50 mg Pd L^{-1} and 50 mg Au L^{-1}) with H_2 as hydrogen donor at pH 5, 6, 7 and 8 (C_0 = 20 mg diclofenac L^{-1}).

Table 5-2: First order decay constants (k-values) of the removal of 20 mg diclofenac L^{-1} using monometallic bio-Pd (50 mg Pd L^{-1}) and bimetallic bio-Pd/Au (50 mg Pd L^{-1} and 50 mg Au L^{-1}) catalysts with H_2 as hydrogen donor at variable pH.

Pd-concentration (mg L^{-1})	**Au-concentration** (mg L^{-1})	**pH**	**k** (h^{-1})
50	0	5	0.129 ± 0.020
50	0	6	0.027 ± 0.004
50	0	7	-
50	0	8	-
50	50	5	1.544 ± 0.257
50	50	6	0.264 ± 0.021
50	50	7	0.078 ± 0.009
50	50	8	0.047 ± 0.005

(- : no removal observed)

In contrast to bio-Pd, the coprecipitated bimetallic bio-Pd/Au allowed removal of diclofenac at neutral and alkaline pH (Figure 5-1B). In addition, a similar trend as for the monometallic bio-Pd, namely an improved removal at lower pH, was observed. These removal curves all showed first order kinetics, the rate constants are listed in Table 5-2. The decay constant at pH 5 was a factor 30 higher than the one at pH 8. At a fixed pH, bio-Pd/Au showed for all pH values from 5 to 8 an improved removal compared to bio-Pd. After reaction, pH remained more or less constant, a maximum decrease of 0.3 pH units was observed in experiments at neutral pH. Doping of Pd catalysts with Au(0) has already been described extensively (Bonarowska et al., 2001a; Nutt et al., 2005; Nutt et al., 2006). These bimetallic catalysts can have several structures, such as alloys (Bonarowska et al., 2001a) or core-shell structures (Nutt et al., 2005; Nutt et al., 2006). However, the exact catalytic mechanisms of these bimetallic systems remain unrevealed. In the previous chapter, we showed that bimetallic alloys of Pd and Au could be produced by the metal respiring bacterium *Shewanella oneidensis.* Simultaneous addition of Au(III) and Pd(II) to the bacterial cells was required in presence of an electron

donor (e.g. H_2). The catalyst obtained by coprecipitation of Pd and Au had an improved catalytic activity for the degradation of the halogenated water pollutants diclofenac and trichlorethylene (TCE) compared to the monometallic bio-Pd. Bio-Au itself was inactive as catalyst. The catalyst obtained by sequential precipitation of Au and Pd did not show a significant improvement of the activity compared to bio-Pd. In contrast to other formulations, the coprecipitated bio-Pd/Au showed a unique bimetallic alloy structure, as was demonstrated by synchrotron-based techniques and (scanning) transmission electron microscopy. The present study furthermore showed that bio-Pd/Au can be a powerful catalyst for dehalogenation of wastewater pollutants, since it can dechlorinate diclofenac at relevant pH values at which wastewater treatment systems are mostly operated. Since these techniques are proposed as effluent polishing technologies, it is advisable that pH values are not varied anymore at this stage of the treatment, making bio-Pd/Au an attractive catalyst for this purpose. The use of chemically produced bimetallic catalysts for removal of diclofenac has been reported before by Ghauch et al. (2010). These authors plated the surface of Fe(0) particles with other elements (Pd, Ni, Cu, Ir, Co and Sn). Both under oxic and anoxic conditions, the Pd/Fe catalyst was the most effective combination. This Pd/Fe catalyst was the only combination that showed higher removal rates than the ones presented in this study. Under oxic conditions, oxidative (e.g. hydroxylated) reaction products were found; under anoxic conditions, reductive (e.g. dechlorinated) transformation products were detected. However, in that case, Fe(0) was not a true 'catalyst', since it was used during the reaction. Indeed, Fe(0) was oxidized to Fe(II), which generated the hydrogen donor H_2 required for catalysis. A periodic renewal of fresh Fe(0) to provide H_2 to the Pd catalyst will thus be required for these Pd/Fe catalysts, in contrast to (bio)-Pd/Au catalysts.

3.2 Transformation products and mass balance

The transformation products after catalytic removal of diclofenac using bio-Pd and bio-Pd/Au in presence of H_2 were determined by means of LC-MS/MS. Figure 5-2A and 5-2B show the chromatograms of the diclofenac removal at pH 5 using H_2 and bio-Pd/Au and bio-Pd after 4 hours reaction time. Three peaks could be identified. Figure 5-2C, D and E show the mass spectra of the peaks with retention time 24.9, 24.4 and 22.4 minutes respectively. The molecular mass of diclofenac is 296. The mass corresponding to the product which eluted at 24.9 minutes was 278 (Figure 5-2C). This mass difference has been reported in literature and is due to the loss of a H_2O fragment during ionization of the molecule (Galmier et al., 2005). The difference in mass between the main peaks shown in Figure 5-2C and D is 34, corresponding to the replacement of a chlorine atom

by a hydrogen atom. The same observation is valid for Figure 5-2D and E. These results show that diclofenac is subjected to a sequential hydrodechlorination. These were the only products that could be identified in all cases where removal took place. The final transformation product was identified as 2-anilinophenylacetate (further referred to as 2-APA). The intermediate with one Cl in the molecule is 2-(2-chloroanilino)phenylacetate (further referred to as 2-(2-Cl)-APA).

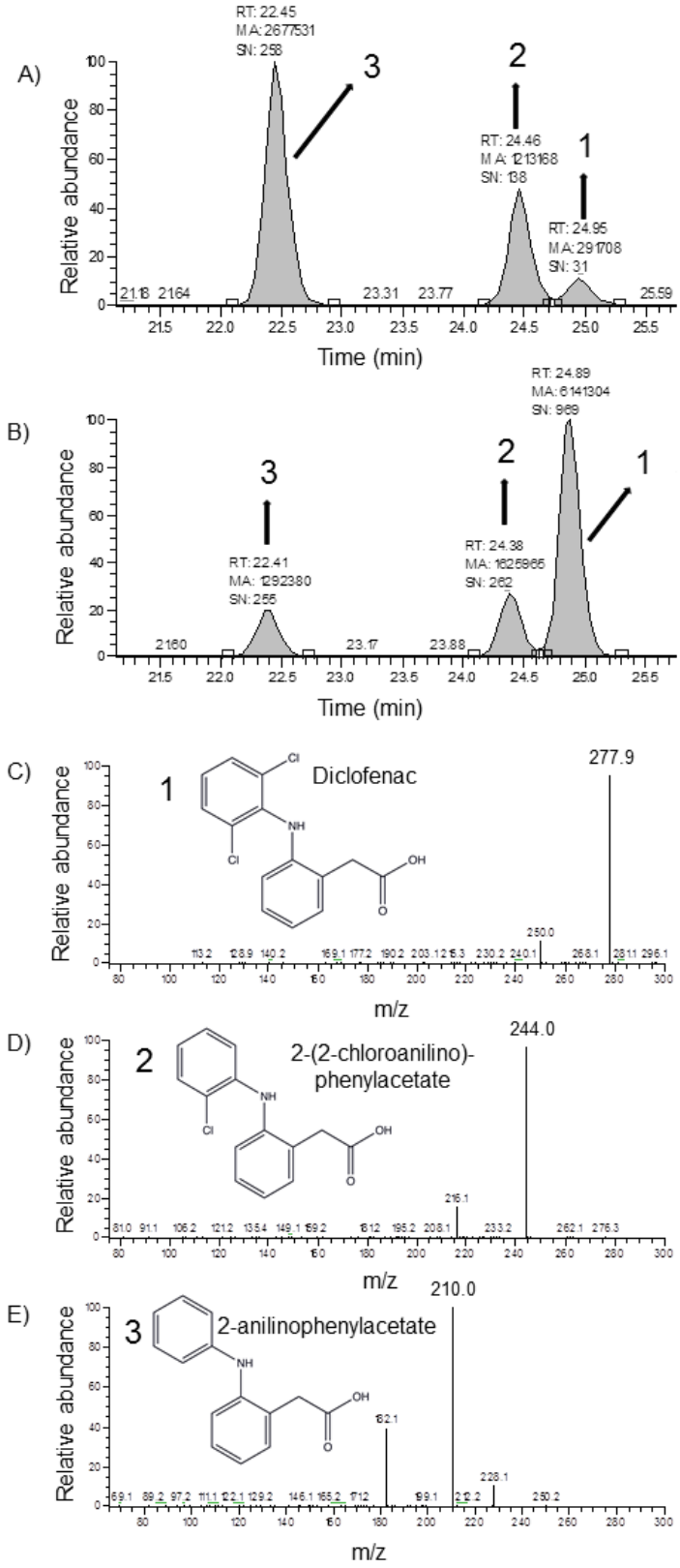

Figure 5-2: LC-MS/MS data of catalytically treated samples after 4 hours treatment at pH 5. (A) LC chromatogram of full MS of sample treated with bio-Pd; (B) LC chromatogram of full MS of sample treated with bio-Pd/Au; (C) MS^2 of peak 1 (diclofenac); (D) MS^2 of peak 2 (2-(2-chloroanilino)phenylacetate); (E) MS^2 of peak 3 (2-anilinophenylacetate).

The kinetics of the formation of the final dechlorinated product 2-APA are given in Figure 5-3. Due to a lack of available reference standards, 2-(2-Cl)-APA could not be quantified. This figure shows that almost all the initially added diclofenac could be recovered as 2-APA after 24 hours reaction time using a bio-Pd catalyst. Using a bio-Pd/Au catalyst, all the initially added diclofenac could be recovered as 2-APA after 8 hours reaction time. It is clear that both the removal of diclofenac and the formation of 2-APA occur at a faster rate when using a bimetallic bio-Pd/Au catalyst compared to the monometallic bio-Pd at a fixed pH (e.g. at pH 5). The diclofenac molecule apparently undergoes a sequential dechlorination. No other reaction products could be detected, so any further reductive reaction (e.g. reduction of the carboxylgroup to an aldehyde) can be excluded. This is in accordance with previous studies using bio-Pd catalysts for removal of halogenated pollutants.

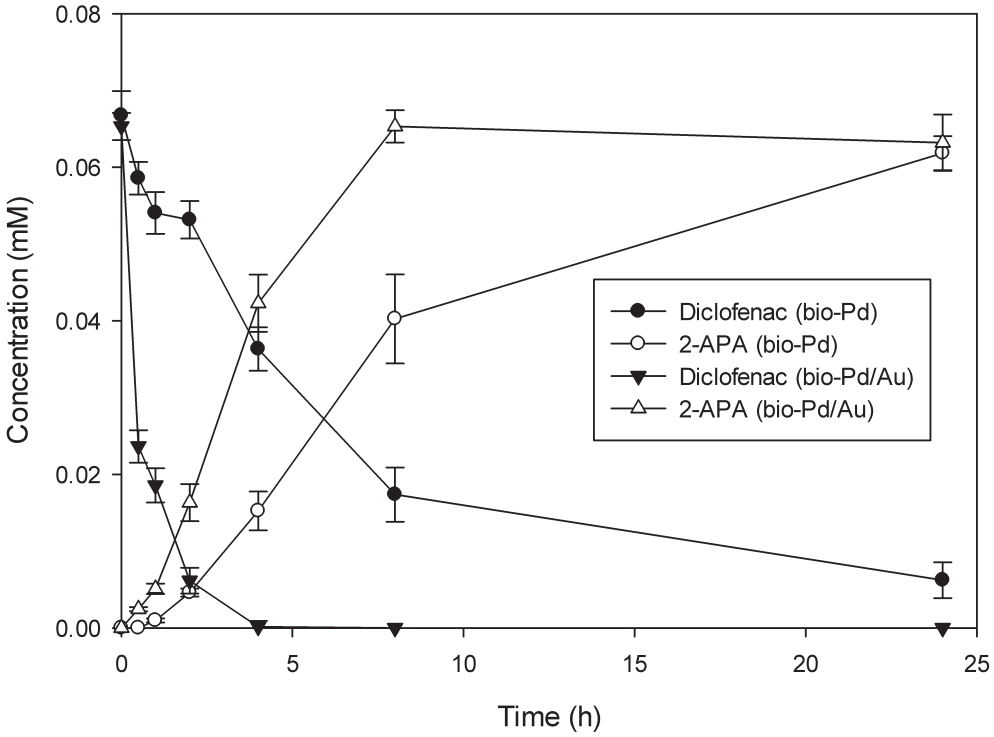

Figure 5-3: Removal kinetics of diclofenac and formation kinetics of 2-anilinophenylacetate (2-APA) using a bio-Pd/Au catalyst (50 mg Pd L^{-1} and 50 mg Au L^{-1}) at pH 5 and with H_2 as hydrogen donor.

Experiments with nitrifying biomass already demonstrated that the dechlorinated 2-APA (88.8 % removal after 10 days) is more biodegradable than diclofenac itself (23.3 % removal after 10 days) (De Gusseme et al., 2012). Oxidative and cometabolic biological processes, for example by nitrifying cultures, have already shown to be a valuable option to further degrade (oxidize) the product obtained after reductive dehalogenation (De Gusseme et al., 2009).

3.3. Catalysis by bimetallic bio-Pd/Au: influence of Pd and Au content

In order to examine the influence of the Pd/Au ratio during precipitation, the concentration of Pd or Au during the synthesis of the catalyst was varied, while the concentration of the other metal was kept constant. The evolution of the first order decay constant for the removal of diclofenac at pH 7 with H_2 is shown as a function of Pd or Au concentration in Figure 5-4. The normalized values of these constants to the Pd-concentration (k_{Pd}) and the total metal concentrations (k_{metal}) are shown in Table 5-3. These results show that an increase in Pd-content resulted in an increase of the removal rate. Also the Pd-normalized decay constants increased with increasing Pd concentration (k_{Pd} at 100 mg Pd L^{-1} is a factor 2 higher than k_{Pd} at 50 mg Pd L^{-1}, which is slightly higher than k_{Pd} at 10 mg Pd L^{-1}). This disproportional increase in catalytic activity was also observed for the deiodination of diatrizoate by monometallic bio-Pd produced by *Shewanella oneidensis* (Hennebel et al., 2010) and *Citrobacter braakii* (Hennebel et al., 2011c). This is in contrast with the observations of Nutt et al. (2006), who demonstrated that there was an optimal Pd surface coverage for their Pd-Au bimetallic core-shell particles, used for the dechlorination of trichloroethylene. Beyond this optimal ratio, a further increase of the amount of Pd led to a decrease in catalytic activity.

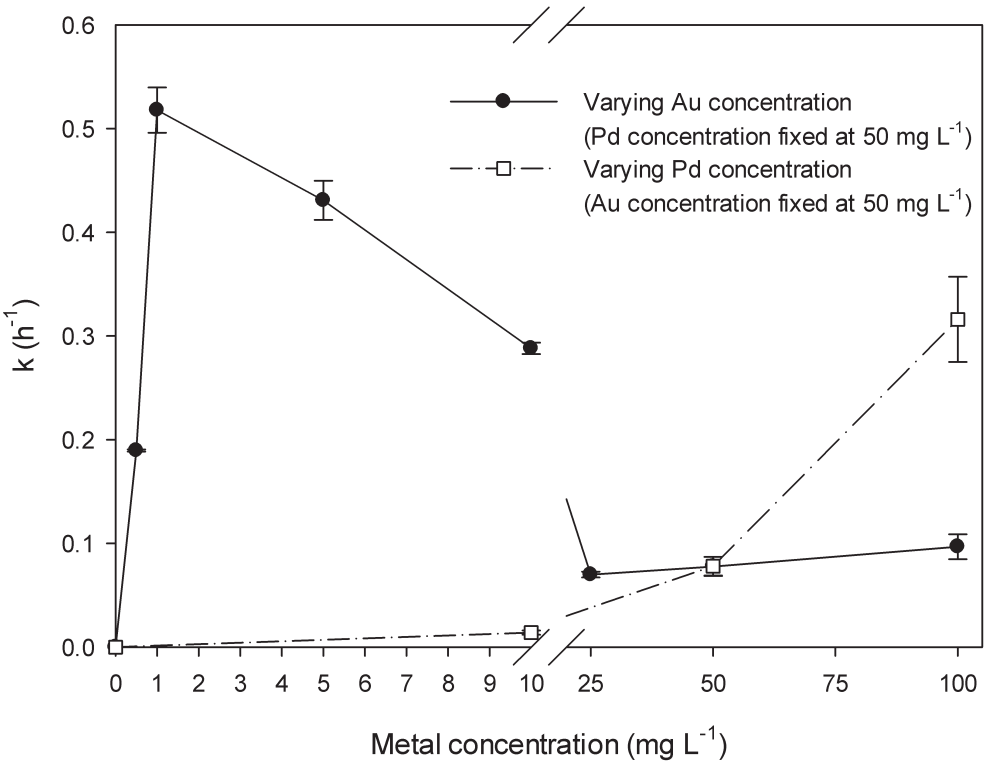

Figure 5-4: Evolution of the first order decay constant (k-value) for the removal of diclofenac at pH 7 using a bio-Pd/Au catalyst as a function of the Au and Pd concentration used for synthesis of the catalyst. The concentration of the other metal was kept constant at 50 mg L^{-1} (C_0 = 20 mg diclofenac L^{-1}).

Table 5-3: Pd-normalized (k_{Pd}-values) and metal-normalized decay constants (k_{metal}-values) for the removal of diclofenac using bio-Pd/Au catalyst with varying Pd-concentration at pH 7 and with H_2 as hydrogen donor. The Au-concentration for the synthesis of the catalyst was kept constant at 50 mg L^{-1}. Decay constants were normalized to the Pd concentration used.

Pd-concentration (mg L^{-1})	**Au-concentration** (mg L^{-1})	**k_{Pd}** (L g^{-1} Pd h^{-1})	**k_{metal}** (L g^{-1} metal h^{-1})
10	50	1.35 ± 0.17	0.23 ± 0.03
50	50	1.55 ± 0.18	0.78 ± 0.09
100	50	3.16 ± 0.42	2.11 ± 0.27
50	0,5	3.79 ± 0.02	3.75 ± 0.02
50	1	10.30 ± 0.40	10.20 ± 0.40
50	5	8.62 ± 0.38	7.83 ± 0.34
50	10	5.76 ± 0.11	4.80 ± 0.09
50	25	1.40 ± 0.06	0.93 ± 0.04
50	100	1.94 ± 0.24	0.65 ± 0.08

Similar as for Pd, the concentration of Au in the different catalysts was varied. In contrast to the observations for a varying Pd-concentration, there was no increasing reaction rate with increasing Au-concentration, but the reaction rate as a function of the Au-concentration showed a clear optimum. The optimal Pd/Au ratio for diclofenac removal at pH 7 was around 50/1 (expressed as mass units). There was a significant drop in activity between Au concentrations of 1 and 25 mg L^{-1}. Between 25 and 100 mg Au L^{-1}, no significant changes in the removal rate were observed. The main objective of varying the Au-concentration during synthesis was to minimize the amount of Au needed, since this is the main cost-determining factor for the synthesis of the catalyst. It is possible that doping of Pd(0) with a small amount of Au(0) improves the transfer of electrons, and hence, the catalytic activity. This effect then partly disappears when too much Au(0) is added, as was also shown for Pd/Au catalysts for the synthesis of H_2O_2 (Hutchings et al., 2010). Doping with 2 mass % of Au(0) will bring about a price increase of the order of 4

percent (based on Pd and Au prices of € 19 000 kg^{-1} € 39 000 kg^{-1} respectively). But this should be largely compensated by the gain in catalytic activity.

3.3 Diclofenac removal from a hospital WWTP effluent

In order to test the catalytic activity in an environmental matrix with relevant pollutant concentrations, a dechlorination assay was performed using the effluent from a hospital WWTP. When bio-Pd and bio-Pd/Au (50/1) were used for the treatment of hospital WWTP effluent, similar trends as in batch tests with spiked distilled water were observed. The presence of four pharmaceutical compounds was monitored as a function of time (Figure 6). Sulfamethoxazole was present at a concentration of 2.21 µg L^{-1} and could not be removed with bio-Pd (50 mg Pd L^{-1}) and bio-Pd/Au (50 mg Pd L^{-1}/1 mg Au L^{-1}) (results not shown). A concentration of 97.83 µg L^{-1} diatrizoate was present and was removed for 100 % within 1 hour using bio-Pd and within 30 minutes using bio-Pd/Au. Carbamazepine could be detected at a level of 1.02 µg L^{-1} and was removed for 20.5 ± 0.1 % after 24 hours using bio-Pd. Using bio-Pd/Au, it could be removed for 100 % after 4 hours. Diclofenac was found to be present at a concentration of 6.40 µg L^{-1}. The pH of this effluent was 7.4. The treatment with bio-Pd was ineffective for removal of diclofenac. Using bio-Pd/Au, 43.8 ± 5.5 % of the initially present diclofenac could be removed. An increasing concentration of 2-APA as a function of time was detected (data not shown). However, compared to the test with bio-Pd/Au and spiked distilled water, the removal from WWTP effluent was rather limited.

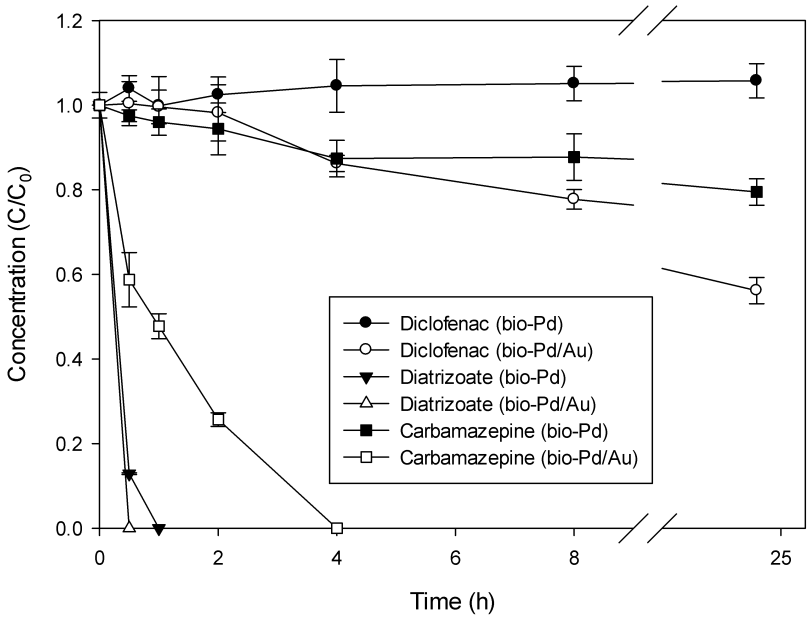

Figure 5-5: Removal of diclofenac (C_0 = 6.40 µg L^{-1}), diatrizoate (C_0 = 97.83 µg L^{-1}) and carbamazepine (C_0 = 1.02 µg L^{-1}) from hospital wastewater treatment plant effluent using bio-Pd (50 mg Pd L^{-1}) and bio-Pd/Au (50 mg Pd L^{-1}/1 mg Au L^{-1}; mass/mass ratio) with H_2 as hydrogen donor.

Possible reasons for this limited removal are the low concentration of diclofenac and the presence of other compounds in the effluent, which might poison the surface of the catalyst. Especially the presence of iodocompounds can decrease the activity of Pd-based catalysts, since they compete with chlorinated compounds for the catalytically active sites (Mackenzie et al., 2006). Diatrizoate is an iodinated contrast medium, which was present in this effluent sample at a concentration of 97.8 µg L^{-1} and thus a possible competitor for the catalytic sites. The higher affinity of bio-Pd for iodocompounds compared to chlorinated compounds has been observed before (De Gusseme et al., 2011). In order to be a valuable technique for effluent treatment, these reaction rates need to be increased (for example by other doping elements, optimal nanoparticle size, pretreatment of the effluent, …). Also the high concentrations of sulfate in the water (30.5 mg SO_4^{2-}-S L^{-1}) can possibly inhibit the activity of the catalyst, since they can be reduced to sulfides, which are known poisoning agents for Pd catalysts (Angeles-Wedler et al., 2009)

4 Conclusions

This study demonstrated the applicability of bio-Pd/Au, of which the unique bimetallic structure had already been demonstrated previously, for the treatment of halogenated micropollutants in the effluents of WWTPs. More specifically, it was shown that diclofenac could be removed from water using a bio-supported bimetallic coprecipitated Pd/Au catalyst at neutral pH. Dechlorination was in all cases where removal was observed the only observed reaction mechanism. The bio-Pd/Au catalyzed dehalogenation can thus be a powerful tool to decrease the load of halogenated micropollutants in wastewaters. The removal rate of diclofenac with bio-Pd/Au increased disproportionally with increasing Pd concentration. Varying the Au-concentration at constant Pd-concentration resulted in optimal catalytic activity at a mass ratio for Pd/Au of 50/1. Bio-Pd/Au was, in contrast to bio-Pd, capable of treating a diclofenac containing WWTP effluent. Dechlorination of halogenated micropollutants using Pd or Pd/Au nanocatalysts can be a valuable alternative to current abatement techniques, which have little control over the by-products that are formed and are not able to cleave the carbon-halogen bound. The remaining structure after dechlorination is more biodegradable than diclofenac.

5 Acknowledgements

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Chapter 6
Biodeposited bimetallic Pd/Au nanoparticles as novel Suzuki catalysts

Abstract

The use of two nanoparticulate palladium based catalysts in the Suzuki reaction was demonstrated. One monometallic (Pd) and one bimetallic (Pd/Au) catalyst were prepared by the environmentally benign method of bioreductive precipitation by *S. oneidensis*. Both catalysts successfully mediated the Suzuki coupling of an arylhalide with a boronate. However, the Au doped catalyst was shown to deliver more reproducible results with a broader reaction scope. Especially with electron donating substituents and with different arylhalides, the bimetallic catalyst showed higher conversions. The obtained reaction rates were not yet competitive with a commercial Pd/C catalyst.

Chapter redrafted after:

T.S.A. Heugebaert, S. De Corte*, T. Sabbe, T. Hennebel, W. Verstraete, N. Boon, C. Stevens. 2012. Biodeposited Pd/Au bimetallic nanoparticles as a novel Suzuki catalyst. Tetrahedron Letters 53 (11): 1410-1412 (*: in equal contribution)*

1 Introduction

Palladium (Pd) is able to catalyze a wide variety of chemical reactions and therefore it is one of the most widely used metal catalysts (Tsuji, 1995). It can be applied both in homogeneous and heterogeneous phases. Heterogeneous catalysts, however, allow a more easy separation from the reaction medium. Due to a higher available catalytic surface, heterogeneous catalysts are more and more used in the form of nanoparticles. However, these small-sized products require more complex production methods, which often imply the use of toxic and expensive chemicals. Therefore, environmentally benign production methods of Pd nanocatalysts are very desirable. A possible option is to exploit the metal-reducing capacities of micro-organisms such as *Shewanella oneidensis* (Hennebel et al., 2009a). It has been shown to be able to precipitate Pd (De Windt et al., 2005) and Au (Chapter 3) in nanoparticulate form on its cell wall structures. The catalytic properties of this biologically precipitated Pd by *S. oneidensis* ('bio-Pd') have already been repeatedly demonstrated (Chapter 1). Furthermore, it was shown in Chapter 4 that co-precipitation of Pd(0) with Au(0) by *S. oneidensis* (bio-Pd/Au) created unique bimetallic structures which display a considerable contraction of the crystal lattice when compared to the monometallic catalysts. These bimetallic nanoparticles showed a greatly improved catalytic activity in dehalogenation reactions compared to the monometallic bio-Pd catalyst.

Dehalogenations of environmental contaminants catalyzed by biologically synthesized Pd catalysts are the best studied reactions for this type of biogenic catalysts. However, more recently, the application of these biogenic metal nanocatalysts in synthetic organic chemistry, e.g. in carbon-carbon coupling reactions, has become the subject of several studies (Sobjerg et al., 2009; Gauthier et al., 2010). The aim of this study was to test the applicability of bio-Pd produced by *S. oneidensis* in the Suzuki coupling reaction and more importantly, to see if bio-Pd/Au showed an advantage in terms of reactivity and/or selectivity for these coupling reactions compared to monometallic bio-Pd.

In this study, two catalyst types were used: bio-Pd, and an Au doped bio-Pd, obtained by co-precipitation of Pd and Au, denoted bio-Pd/Au. Chapter 4 showed that doping of bio-Pd with 2 wt. % Au showed the highest activity for the dechlorination of diclofenac and was therefore chosen as the reference bimetallic catalyst in this study.

2 Materials and methods

2.1 Catalyst preparation

Bio-Pd (50 mg Pd L^{-1}) was produced as described by De Windt et al. (2005), bio-Au (50 mg Au L^{-1}) was produced as described in Chapter 3 and bio-Pd/Au (50/1 mass ratio) was produced as described in Chapter 5. Catalyst preparation was finished 24 hours before starting the experiments. Storage before the experiment was done at 4°C.

2.2 Suzuki cross-coupling reactions

Suzuki cross-coupling reactions were performed as follows: the prepared catalyst solution (50 mL) containing 0.0235 mmol palladium (bio-Pd or Pd/C (10 % Pd, Sigma-Aldrich, Germany)), 0.0235 mmol Pd and 0.25 μmol Au (bio-Pd/Au), or 0.0127 mmol Au (bio-Au) was centrifuged at 4100g and the cells were resuspended in approximately 25 ml of a 2/1 mixture EtOH/water. The flask was equipped with an air condenser and placed under inert atmosphere (N_2). 1.1 mmol of boronic acid, 3 mmol of K_2CO_3 and 1 mmol of aryl halide were added and the resulting solution was heated to 70°C and stirred for a period of 24h. Reaction mixtures were analysed by means of HPLC, conversions were determined by means of integration of UV signals at 235 nm. UV integrations were corrected for the respective response factors, determined from the commercially available compounds (Sigma-Aldrich, Germany and Acros, Belgium). All standard curves contained 4 concentration measurements ranging from 0 to 100 mg L^{-1} and displayed a correlation coefficient above 0.99. Product identities were shown by their retention time on HPLC and by their ESI-MS ionisation pattern after GC analysis.

3 Results and Discussion

3.1 Coupling of iodobenzene and phenylboronic acid

In order to determine the reactivity profile of both catalysts, iodobenzene and phenylboronic acid were used as model substrates. The reactions were run at 70°C in a 2/1 mixture of EtOH/H_2O, using three equivalents of K_2CO_3 as a base and 2.35 molar percentage of palladium. As a reference, Pd/C and a biodeposited gold catalyst, denoted bio-Au were used. 1.27 molar percentage of gold was used for reaction run with the bio-Au catalyst.

As can be seen from Figure 6-1, both catalysts mediated the Suzuki coupling to completion within 24 hours under the chosen conditions. The reaction rate however was increased considerably by gold doping. This effect could be caused by structural

modifications of the Pd surface, since pure Au nanoparticles do not catalyze the Suzuki coupling. Furthermore, as reactions were repeated, it became clear that the reproducibility of results obtained with the Pd catalyst was rather poor. Despite intensive efforts to standardize the production process of the catalyst, one batch would give good results, while the next would give no conversion at all. This was possibly due to the poisoning of the catalyst surface by bacterial sulfur. Results obtained with the Pd/Au catalyst were highly reproducible and similar conversion percentages were observed with every single batch of catalyst produced. It has indeed been shown that the presence of Au salts during palladium reduction not only improves the metal conversion yield due to geometric and electronic effects (as discussed in Chapter 5), but can possibly also sequester sulfur containing compounds from the Pd surfaces, inhibiting the formation of Pd_4S. Gold doping in this way acts as a detoxification agent (Menegazzo et al., 2008).

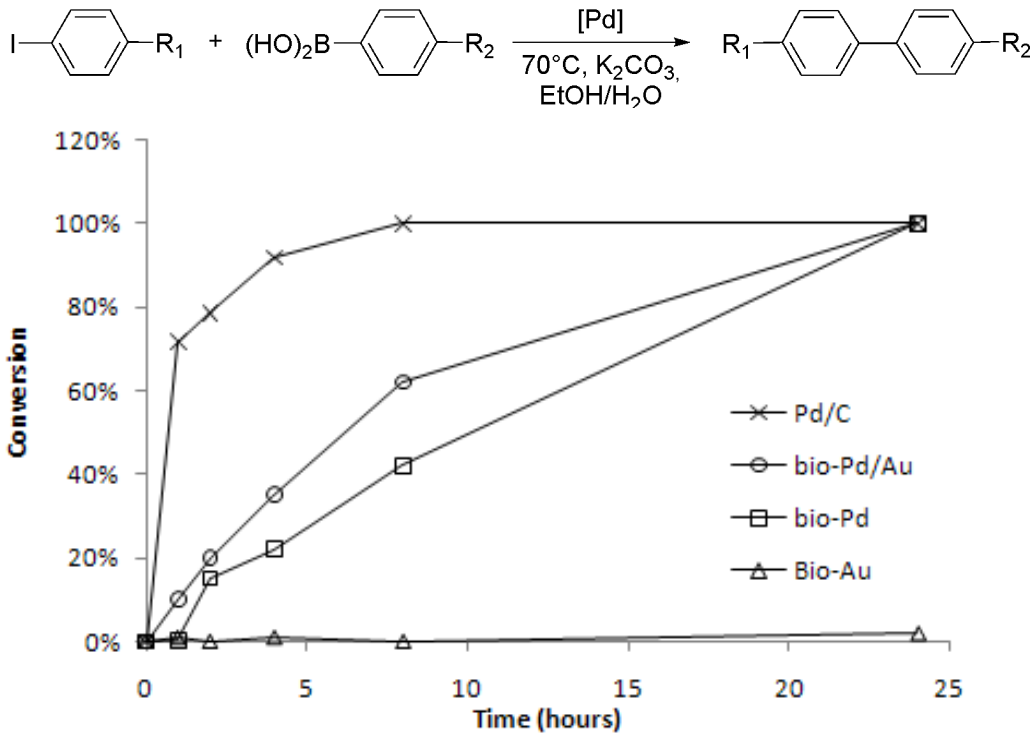


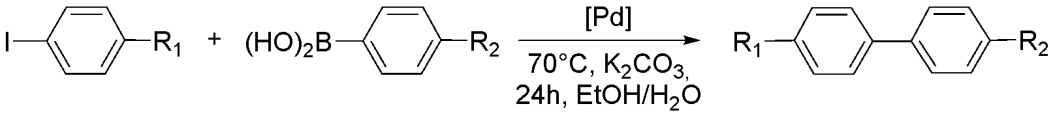
Figure 6-1: Comparison of the conversion rate of the model substrates using Pd/C, bio-Pd, bio-Pd/Au and bio-Au as a catalyst

3.2 Screening of different substrates

These improvements in catalyst efficiency were reflected in the results obtained from the screening of different substrates (Table 6-1). Reactions using the palladium/gold alloyed catalyst continued to completion, whereas some substrates were not completely converted within the 24 hours time frame by the pure palladium catalyst.

Regarding the reaction of substituted aryliodides with phenylboronic acid (entries 1 to 7), it could be clearly seen that the bio-Pd catalyst struggled early on when electron donating substituents were present (entries 4 and 5). The Pd/Au catalyst had a broader scope, however, strong electron donating groups were not tolerated by either one (entries 2 and 3). As for the boronic acids, both catalysts tolerated electron donating and electron withdrawing substituents. Only the very electron poor fluorophenylboronic acid (entry 11) showed a lower conversion when using the non-doped bio-Pd catalyst. When using very electron rich boronates, however, (entries 8 and 14) homo-coupling of the boronate could be observed with the bio-Pd/Au catalyst, causing a significant drop in the reaction yield. This type of conversion was not observed with the Pd catalyst, and as such, bio-Pd may be the better choice in these cases. As can be seen from entries 12 to 15, ortho substitution was well tolerated on both the arylic iodide and the boronic acids, for both catalysts. Only the ortho iodoaniline showed poor reactivity. This was due to the strong electron donating capacity, rather than to steric or ligating effects (compare entries 2 and 12) (Patil et al., 2009).

Table 6-1: Scope study of the Suzuki coupling of different aryliodides and arylboronic acids using both bio-Pd and bio-Pd/Au as a palladium source



			Conversion (%)[a]	
Entry	**R_1**	**R_2**	**Bio-Pd[b]**	**Bio-Pd/Au[c]**
1	H	H	98	100
2	NH_2	H	1	5
3	OMe	H	3	7
4	Ph	H	0	88
5	Me	H	4	100
6	$COCH_3$	H	87	95
7	CN	H	100	100
8	H	OMe	93	77[d]
9	H	Me	90	94[d]
10	H	CN	98	100
11	H	F	58	100
12	o-NH_2	H	0	6
13	o-F	H	100	100
14	H	o-NH_2	98	36[d]
15	H	o-F	94	100

[a]Determined by HPLC [b]Average of two successful trials (see discussion on reproducibility) [c]Average of two trials [d]Conversion was complete, but product yield is lowered by the formation of homo-coupling side products

3.3 Coupling of phenylboronic acid and arylic halides

When moving on to more challenging arylic halides (Figure 6-2), the usual reactivity profile of I>Br>>Cl was observed. The most important observed difference was the significant conversion of bromobenzene by bio-Pd/Au, which was not the case for bio-Pd. In case of iodobenzene and chlorbenzene, much smaller differences were observed. This substantial difference in reactivity shows that not only reproducibility is higher, but the catalyst activity is improved by Au doping. Due to the bimetallic structure the oxidative insertion, the rate determining step, may be facilitated giving the catalyst a broader scope.

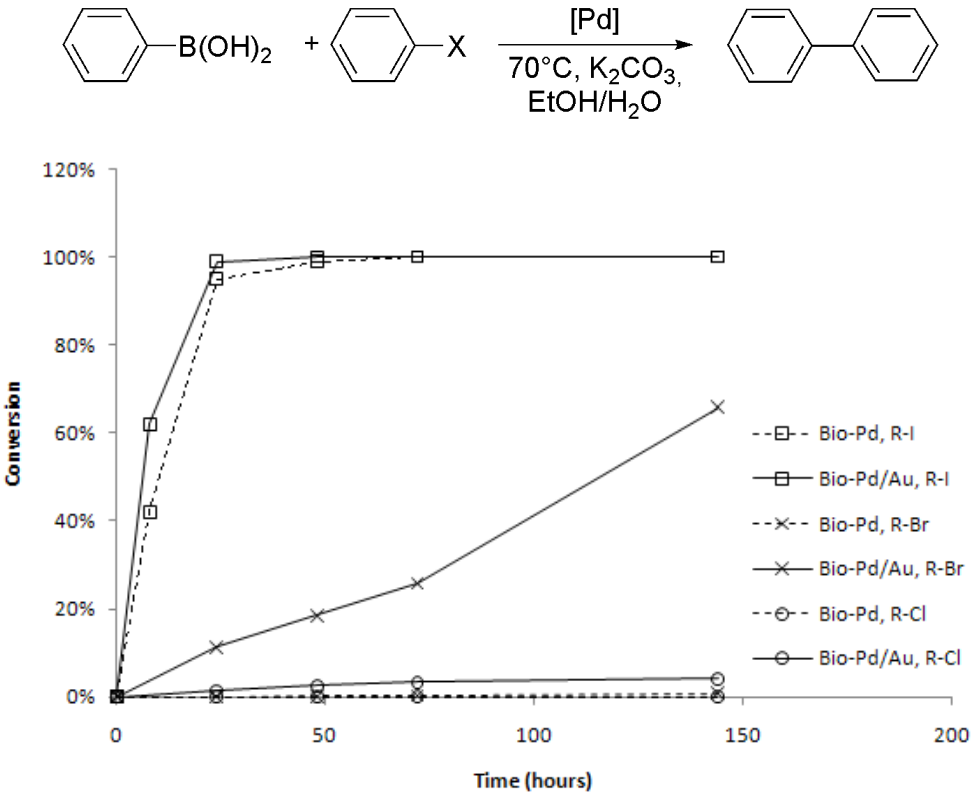


Figure 6-2: Comparison of conversion rates of different phenyl halides (X=I, Br, Cl) using the bio-Pd and bio-Pd/Au catalyst

4 Conclusions

In conclusion, two types of biogenic nanocatalysts containing respectively Pd and Pd/Au, produced by the environmentally benign process of bioreductive precipitation were described, using *Shewanella oneidensis* as the carrier bacterium. Their use as catalysts for the Suzuki reaction was evaluated, showing the benefits of bimetallic catalysts. Both reproducibility and reaction scope were significantly improved by the introduction of 2 wt. % gold within the palladium nanoparticle. Biosupported catalysts can have several

advantages over chemically produced catalysts. They can for example be prepared more sustainably. The bacterial cells serve both as reducing and as stabilizing agent for the nanoparticles, implicating a lower need of chemicals during the production process of the nanoparticles. Moreover, the bacterial cell is a support material with an extremely high specific surface area, which is relatively simple to produce. The bacterial carrier can also have a high affinity for the reacting substances and thus allowing a better contact between the reagents and the catalyst. Nevertheless, the long term stability of biosupported nanocatalysts needs to be further investigated. Especially the leaching of nanoparticles to the reaction medium and to the environment needs to be prevented since the adverse effects of nanoparticles are still of great uncertainty (see Chapter 7). Furthermore, it needs to be mentioned that the highest reaction rates using Pd/Au catalysts have been obtained using bimetallic core-shell structures, consisting of an Au core with a Pd shell (Nutt et al., 2006). It has so far not yet been possible to design a similar core-shell structure on a bacterial cell wall (Chapter 4, Hosseinkhani et al., 2012). However, it is likely that the production process of biosupported bimetallic Pd/Au catalysts can be further optimized, which will make these catalysts more competitive with chemically produced catalysts.

5 Acknowledgements

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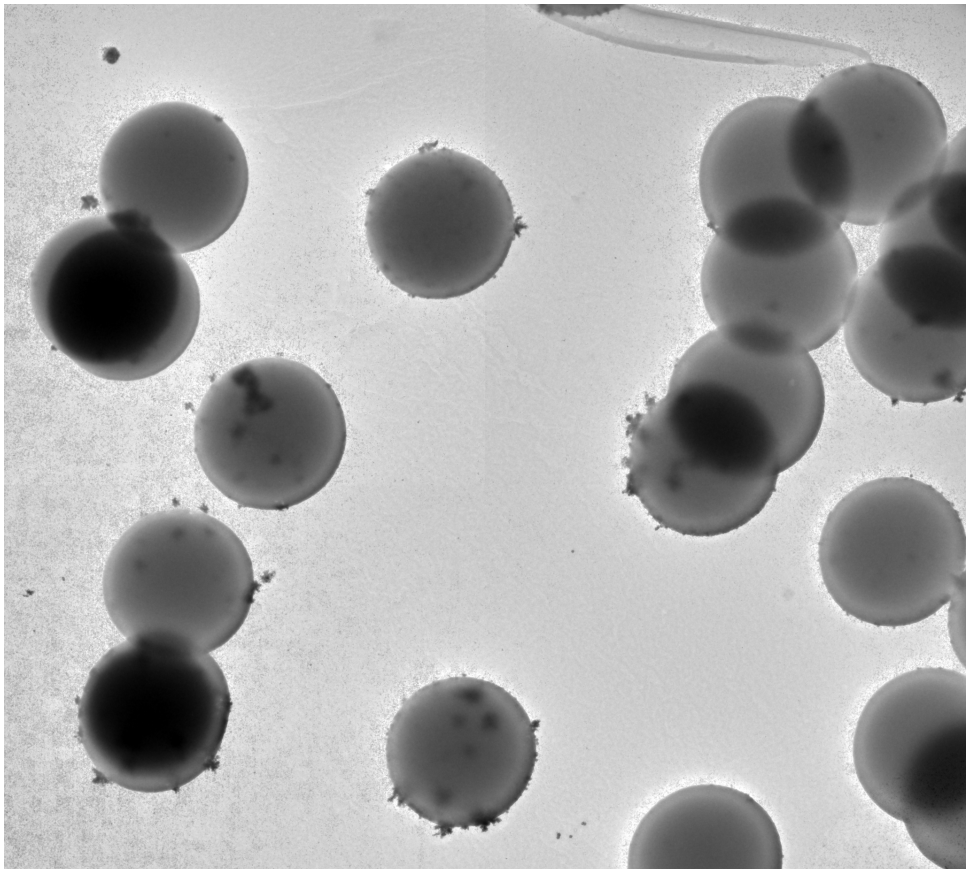
Part III

Limitations and innovations of biosupported Pd catalysts



Chapter 7
Influence of physicochemical parameters on stability and performance of bio-Pd catalysts

Abstract

Bio-Pd catalysts use bacteria as a producer and carrier of Pd nanoparticles. Leaching of Pd from the carrier results in an economical loss and an environmental risk. In this study we therefore investigate the influence of different parameters on the leaching process: temperature (4 - 21 °C), medium (H_2O or mineral medium), pH (1 – 12), atmosphere (air, O_2, N_2, H_2) and bacterial carrier. We show leaching is important for biosupported Pd: 18 % of the Pd was released at 21°C in water after 100 days. Leaching was minimal at lower temperatures, low pH and in an isotonic medium. Up to 5 times more leaching was observed under anaerobic (H_2 or N_2 atmosphere) than under aerobic conditions (O_2 or air atmosphere). The producer/carrier *Cupriavidus metallidurans* leached up to 5 % zerovalent Pd compared with 20% for *Shewanella oneidensis*. No loss of catalytic activity due to leaching was observed when the leachate remained in the reaction medium. This chapter shows for the first time that release of Pd nanoparticles from the bacterial carrier of bio-Pd can be significant (up to 18% when stored at room temperature in distilled water) particularly under anaerobic conditions, at high temperature or high pH.

Chapter redrafted after:

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1 Introduction

The long-term stability of the catalyst is an important condition of Pd nanoparticles in order for them to be applicable at a large scale. A catalyst is, by definition, not consumed during a chemical reaction and can therefore theoretically be considered as a one-time investment cost. In reality, catalysts lose their activity as a function of time. This inhibition can be due to fouling of the catalyst, for example by sulfides (Angeles-Wedler et al., 2009), dissolved organic matter (Chaplin et al., 2006), or harsh reaction conditions resulting in disintegration of the carrier materials (Keane, 2011). Another possibility is the leaching of Pd, which can, in the case of bio-Pd, occur through three mechanisms: (i) oxidation of metallic Pd to dissolved ionic Pd, (ii) detachment of the nanoparticle from the bacterial carrier and (iii) disintegration of the bacterial carrier with subsequent release of Pd. Pd can end up in the final product or in the treated effluent as a consequence of leaching. The loss of catalyst becomes an important cost as the catalyst needs to be periodically renewed; the price of Pd has increased from € 6000 ton^{-1} in 2009 to € 20 000 ton^{-1} in 2012 due to the limited availability. This strongly encourages the careful use of Pd to keep losses to a minimum. Moreover, strict legislation exists for heavy metal contamination in organic chemicals and environmental areas such as soil, groundwater and surface water (for example a maximum of 0.5 to 15 mg L^{-1}, depending on the industrial sector) total metal concentration for wastewater discharged to surface water is imposed in Flanders, Belgium (Vlarem-II, 1995). Several cases of accumulation of heavy metals in soils and groundwater have been reported, resulting in an increased risk of bioaccumulation in the food chain with short and long term impacts (for recent reviews, see Peralta-Videa et al. (2009) and Singh et al. (2011)). By extension, with the increased use of nanoparticles there is a growing concern about their presence in the environment (Chatterjee, 2008). Certain nanoparticles are easily taken up in tissues of living organisms both actively and passively, possibly resulting in bioaccumulation. Perhaps the greatest concern is the contamination of drinking water or agricultural products whereby nanoparticles will enter the human food chain. Few studies have been conducted on the toxicity of Pd nanoparticles: Hildebrand et al. (2010) showed only a minor effect of the exposure of Pd/magnetite nanoparticles on human cell lines, whereas Speranza et al. (2010) demonstrated that the toxicity of Pd nanoparticles (LC_{50}: 1.0 ± 0.3 mg L^{-1}) on kiwifruit pollen was exceeded by the toxicity of $PdCl_2$ (LC_{50}: 8.0 ± 3.2 mg L^{-1}). Since the leaching of Pd in aqueous medium is investigated in this study, especially the toxicity towards aquatic organisms is important. No data were found on the aquatic ecotoxicity of Pd(0) nanoparticles and bulk Pd(0) was found. As a precautionary

principle, the release of Pd nanoparticles into the environment and food chain must be avoided. The LC_{50} values (after 96 hours) of Pd(II) (as $PdCl_2$) to the freshwater worm *Tubifex tubifex* and the freshwater fish *Oryzias latipes* are 0.09 and 4.2 mg Pd L^{-1} respectively (WHO, 2002).

Bio-Pd is mostly applied at room temperature and at neutral pH, particularly for applications in environmental technology. It was demonstrated that the catalytic activity can be largely influenced by varying the pH (Hennebel et al., 2010), with valid applications under aerobic and anaerobic conditions. Recently, the production of bio-Pd has moved beyond *S. oneidensis* to other species such as the metal resistant strain *Cupriavidus metallidurans* (Gauthier et al., 2010; Yong et al., 2010). The storage of bio-Pd is carried out both at room temperature or in a cooled environment at 4°C. The influence of these different parameters - temperature, pH, an-/aerobic, carrier - on the stability of bio-Pd and the effect of long term storage on the performance of the catalyst have so far not been investigated.

This study aimed at quantifying the leaching of Pd from biosupported Pd nanoparticles as a function of time under different conditions of temperature, medium, pH and atmosphere, with different carrier organisms. The speciation of Pd was also studied to determine which type of leaching was dominant. Finally, the evolution of the catalytic activity of bio-Pd as a function of storage time was evaluated for the dechlorination of TCE.

2 Materials and methods

2.1 Preparation of bio-Pd

Bio-Pd (50 mg Pd L^{-1}) was produced as described by De Windt et al. (2005). Unless indicated differently, bio-Pd was prepared in M9 medium, and finally washed and resuspended in distilled water.

2.2 Leaching of Pd from bio-Pd

Batches of bio-Pd were stored at the indicated temperature at pH 7 with an air headspace, unless indicated otherwise. For pH variation experiments, pH was adjusted using 0.1 M HCl or 0.1 M NaOH solutions. For headspace variation experiments, the bottles were flushed for 20 minutes with N_2, H_2 or O_2 and finally the headspace was filled with 1 bar overpressure of the respective gas.

Periodically, samples of the different bio-Pd suspensions (10 mL) were centrifuged (7 min., 8041 g) to separate bacteria in the pellet, from the supernatant, which, based on Stokes' law for sedimentation, contained nanoparticles with diameter smaller than 100 nm. All analyses performed on supernatants refer to centrifugations at 8041 g. Finally, Pd in the supernatant was determined using an AA-6300 atomic absorption spectrophotometer (Shimadzu, Kyoto, Japan).

2.3 Flow cytometry

S. oneidensis cells were grown as described above and samples were incubated at different pH values for 24 hours. Two fluorescent dyes, SYBR® Green I (SG) and Propidium Iodide (PI), were used as a live/dead staining (Wang et al., 2010b). The staining solution was prepared as follows: PI (20 mM in dimethyl sulphoxide (DMSO), from the LIVE/DEAD BacLight Kit, Invitrogen) was diluted 50 times and SYBR® Green I (10 000 times concentrate in DMSO, Invitrogen) was diluted 100 times in 0.22 µm-filtered DMSO. The incubated samples were centrifuged at 4100 g for 7 min. and resuspended in 0.22 µm filtered bottled mineral water in order to eliminate the pH effect on the staining solutions (Wang et al., 2010b). Samples were subsequently stained with 10 µL mL^{-1} staining solution and 10 µL mL^{-1} EDTA (pH 8, 500 mM) for outer membrane permeabilization. Prior to flow cytometric analysis, the stained samples were incubated for 5 min. in the dark at 37°C. Flow cytometry was performed using a CyAn™ ADP LX flow cytometer as described by Boon et al. (2006).

2.4 Speciation analysis

Pd(II) was analyzed according to the method of Ishida (1969). Chromazurol S (1 mM, Sigma-Aldrich, Belgium) was dissolved in distilled water. A buffer solution was prepared by mixing an equal amount of 1 M hexamethylenetetramine and 1 M perchloric acid.

Samples were collected from the supernatants (5 mL) and 0.3 mL of the buffer solution and 1.2 mL of the chromazurol solution were added and further diluted with distilled water to a total volume of 10 mL. After 1 hour reaction time, absorbance was measured at 596 nm using a Biochrom WPA Lightwave II Spectrophotometer. A linear calibration curve was obtained by dissolving 1 to 7 mg Pd(II) L^{-1} (as Na_2PdCl_4, Sigma-Aldrich, Belgium) in distilled water.

2.5 Transmission electron microscopy

A sample of the supernatant (10 µL) of a batch of bio-Pd stored in water at 21°C was dried on a Formvar/Carbon coated nickel TEM grid (Polysciences Inc., Germany).

Images were generated with a Zeiss TEM900 transmission electron microscope (Carl Zeiss, Germany).

2.6 Dehalogenation of trichloroethylene (TCE)

Serum bottles (120 mL), previously flushed with N_2 (0.6 bar) and vacuum repeatedly, were filled with 50 mL bio-Pd suspension. 1 bar of H_2 was then injected into the headspace of the bottles. Finally, the suspensions were spiked with TCE from a stock solution of 20 g L^{-1} in methanol to a final concentration of 100 mg L^{-1}. Periodically, 1 mL samples were withdrawn from the headspace using a needle and syringe and analyzed by GC-FID.

GC conditions were as follows: injection temperature = 200 °C; detector temperature = 50 °C; initial column temperature = 35 °C (held 2 minutes), increase to 75 °C at a rate of 5 °C min^{-1}; column pressure = 153 kPa (held 2 minutes), increase to 176.5 kPa at a rate of 3 kPa min^{-1}. The column used was a Factor Four low bleed capillary column (VF-624 ms, 30 m x 0.25 mm inner diameter, film thickness = 0.25 µm, Varian, Belgium). The detection limit was 0.1 mg TCE.L^{-1}.

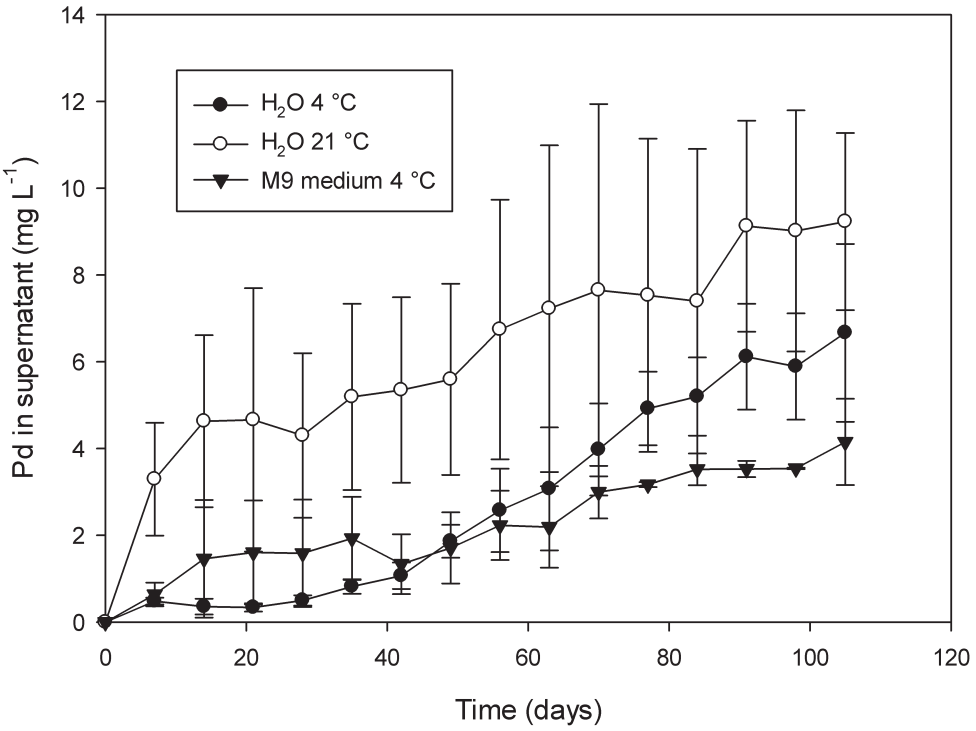
3 Results and discussion

3.1 Effect of temperature on leaching

Three batches of bio-Pd (50 mg Pd L^{-1}) were produced with *S. oneidensis*, washed and resuspended in distilled water then stored at 4°C, where leaching was measured weekly. The leaching of bio-Pd can be clearly observed in Figure 7-1. Initial leaching was rather low and constant (< 1 mg L^{-1}). After 42 days, a linear increase in the leaching was observed. After 15 weeks, 18 % of the reduced Pd was found as in the supernatant following centrifugation. It can thus be concluded that leaching must be an important effect in the application of biosupported nanocatalysts.

Three other batches of bio-Pd in distilled water were stored at 21°C. These batches showed a higher leaching (although not always significantly higher) and a higher variability of Pd concentration in the supernatant than the batches at 4 °C at all time points (Figure 7-1). It is generally known that bacterial cells are more easily lyzed at 21 °C than at 4 °C. The activity of the bacterial community present in the water may also cause a disintegration of the bacterial nanoparticle carrier and therefore release the nanoparticles, probably bound to cell wall fragments. Most applications involving Pd catalysts in organic chemistry are performed at elevated temperatures and would most

likely result in high leaching of a bio-Pd catalyst. The results of our work indicate that the



use of bio-Pd for this type of application is not recommended.

Figure 7-1: Pd concentration in the supernatant after centrifugation (8041 g for 7 minutes) of bio-Pd (50 mg Pd L^{-1}) on *S. oneidensis* stored at 4 °C in distilled water, at 21 °C in distilled water and at 4°C in M9 medium (n = 3).

3.2 Effect of medium on leaching

Three batches were stored at 4°C in mineral M9 medium. Initially, Pd leaching from the bacteria was similar to the leaching in distilled water (Figure 7-1). However, from day 77 onwards, leaching was significantly lower than the batches in distilled water, which showed a slower rate of leaching from day 42 onwards. A possible explanation for this decreased leaching is low osmotic stress for bacteria in M9 medium, whereas distilled water is a hypotonic medium for the cells causing lysis of the cells. Moreover, the presence of bivalent cations such as Ca^{2+} and Mg^{2+} increases the stability of proteins, leading to a higher structural stability of the bacterial cells and subsequently a lower release of Pd (Ugwu et al., 2004).

3.3 Speciation of the leachate

Pd(II) was measured spectrophotometrically in the different supernatants using chromazurol S, however Pd(II) could not be detected in any of the samples. All Pd leached from the bacterial carrier as zerovalent Pd(0) nanoparticles and no reoxidation to

Pd(II) occurred under the circumstances tested. This is in contrast to the previously observed leaching of ionic Ag^+ from biogenic Ag(0) nanoparticles on the cell surface of *Lactobacillus fermentum* (Sintubin et al., 2011). Organic cell fragments are likely to be associated with these free nanoparticles and therefore may be consumed by other (micro)organisms. In this way, the risk of Pd accumulation in the ecosystem and food chain could be increased. The protein fraction associated with these nanoparticles could be determined by the method of Lenz et al. (2011) for biogenic Se nanoparticles. Using gel electrophoresis and LC-MS, they found that peptides with reactive thiol groups, hydrogenases and oxidoreductases were associated with Se nanoparticles produced by *Sulfurospirillum barnesii*.

A TEM picture of the supernatant of a batch stored at 21°C in water is shown in Figure 7-2. Free nanoparticles up to 100 nm can clearly be observed. It should be possible to keep all the catalyst in the reaction medium by means of nanofiltration membranes. However, in practice cheaper techniques will be preferred and Pd nanoparticles will end up in synthesized products or treated effluents. Thorough toxicity studies are required to determine the exact effects of leached zerovalent Pd nanoparticles.

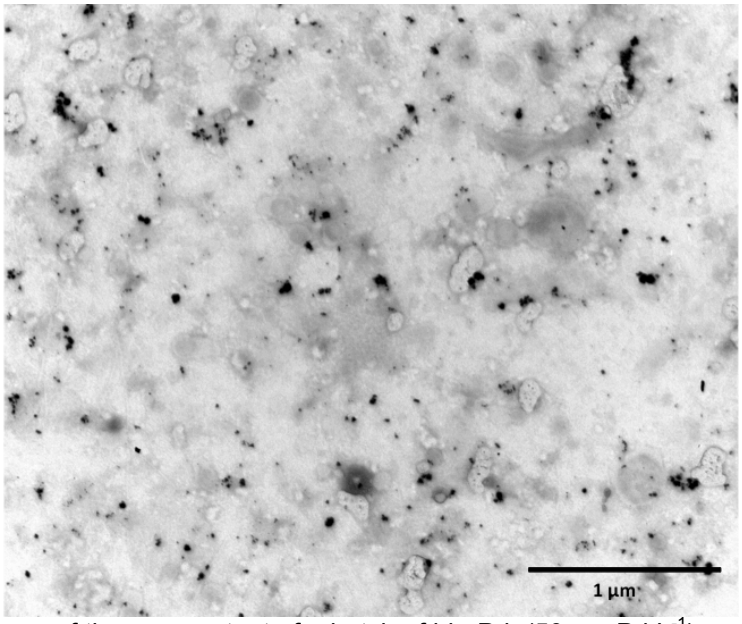
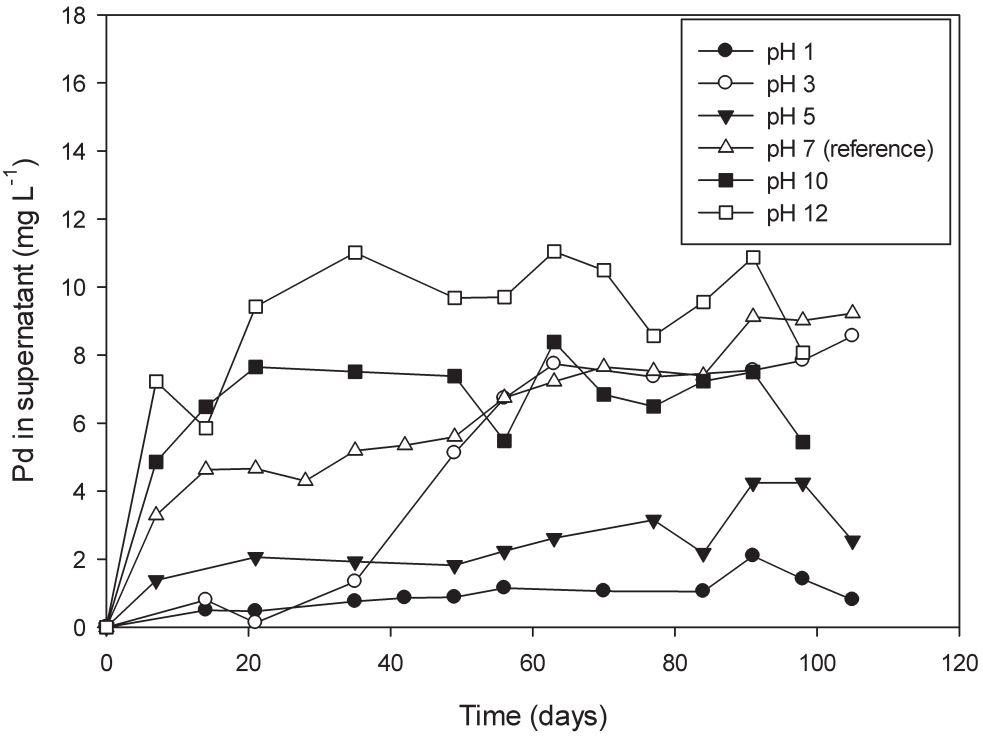


Figure 7-2: TEM image of the supernatant of a batch of bio-Pd (50 mg Pd L^{-1}) on *S. oneidensis* stored at 21°C in distilled water at pH 7 after centrifugation (8041 g for 7 minutes).

3.4 Effect of pH on leaching

Bio-Pd in water or M9 medium presented neutral pH. After preparation at neutral pH, distilled water was adjusted at different pH (1, 3, 5, 10, 12) after production at neutral pH and stored at 21 °C to maximize leaching. Leaching from bio-Pd was followed as a function of time (Figure 7-3). Very high initial leaching was observed at an alkaline pH

(up to 14 % after 1 week), which has been reported before by De Gusseme et al. (2011), who used bio-Pd at the cathode of a microbial electrolysis cell for the deiodination of the contrast medium diatrizoate. After the high initial leaching at elevated pH, no further increase in released Pd was observed from day 21 onwards. A high initial leaching of Pd from bio-Pd followed by a long period of lower leaching was observed when bio-Pd was



applied in a membrane bioreactor for the removal of the groundwater contaminant trichloroethylene, which was operated at neutral pH (Hennebel et al., 2009b). This high initial leaching could be due to the loss of a fraction of Pd nanoparticles that are weakly adsorbed to bacteria, while the rest of the particles were firmly attached.

Figure 7-3: Pd concentration in the supernatant after centrifugation (8041 g for 7 minutes) of bio-Pd (50 mg Pd L-1) on S. oneidensis stored at 21 °C in distilled water at pH 1,3,5,7,10 and 12.

The initial leaching in batches at acidic pH was lower by comparison and stayed low (up to 2 mg L^{-1} or 4 % of the initially added Pd), with the exception the batch at pH 3, where an increase of 1.5 to 5 mg L^{-1} of leached Pd was observed between day 35 and 49, which further increased to 8 mg L^{-1} after 100 days. The pH of the most acidic and alkaline batches (pH 1 and 12) remained more or less constant after 15 weeks, whereas the pH of the other batches had evolved to more neutral pH (between 6 and 8), possibly due to the unbuffered medium. Different phenomena such as bacterial survival, enzyme activity and excretion of cytoplasmatic products could play a role here. Recently, it has been shown that carboxyl and amine groups are crucial adsorption sites for Pd on

bacterial cells (Rotaru et al., 2012). Variations in pH change protonation equilibria of these functional groups may result in the release of Pd.

In order to determine the cell wall and membrane integrity at these different pH values, cells were stained by SYBR Green I and PI and green and red fluorescence was measured by flow cytometry. Green fluorescence was plotted against red fluorescence (Figure 7-4). At pH 1, pH 3 and pH 5 (Figure 7-4A, B and C respectively), both PI and SG entered most cells, which is an indication for cells with (heavily) damaged and permeable membranes. However, this did not affect leaching of Pd. At pH 7 and 10 (Figure 7-4D and E), cells permeable for only SG or both SG and PI can be found. This is a common situation when cells have both intact and damaged membranes, indicating living and damaged bacteria. At pH 12 (Figure 7-4F), neither PI nor SG could enter the cells, an unusual situation for bacterial cells that indicates a significant change in membrane structure and a possible cause for the increased leaching at alkaline pH.

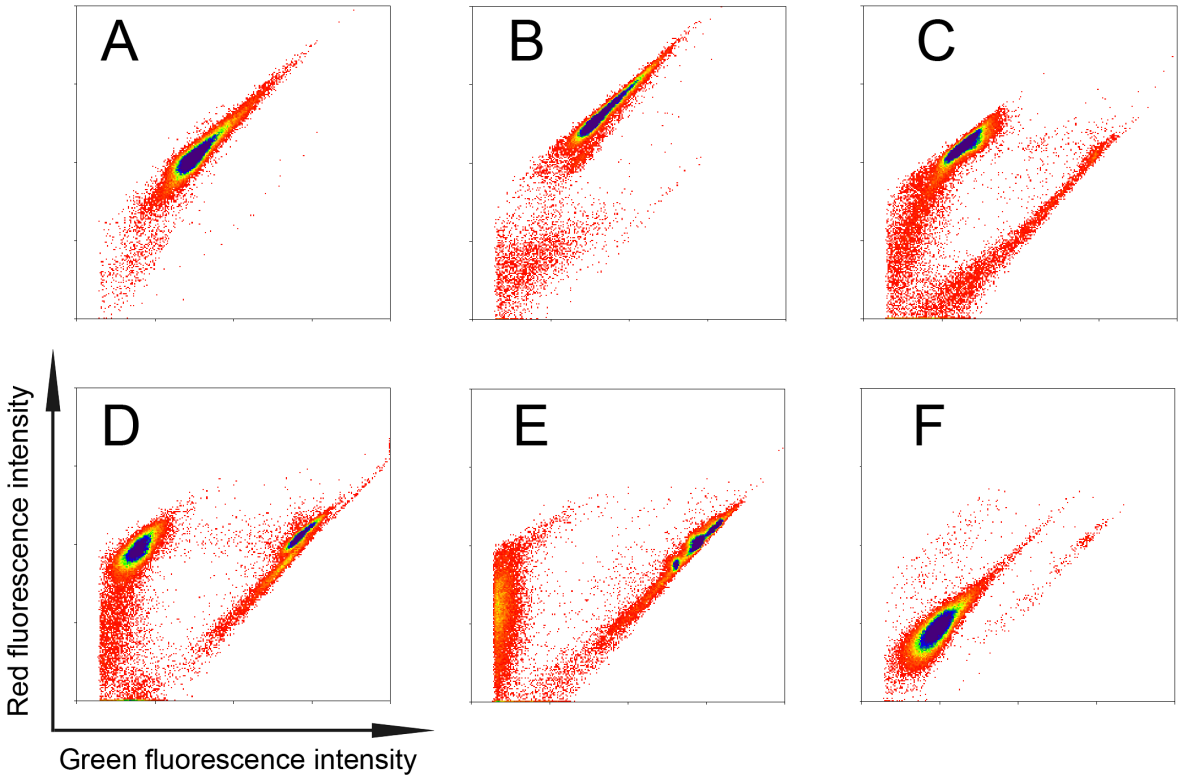


Figure 7-4: Flow cytometry graphs showing the effect of incubation at different pH on the membrane integrity. Incubation at: (A) pH 1, (B) pH 3, (C) pH 5, (D) pH 7, (E) pH 10, (F) pH 12. Bacteria were stained with SYBR Green I and propidium iodide and green fluorescence intensity (x-axis) was plotted against red fluorescence intensity (y-axis).

In practice, bio-Pd is therefore preferably used at a low pH to avoid the need for a recovery step of released Pd from treated effluents or synthesized products. pH was

shown to be critical in the degradation of pollutants when using bio-Pd catalysts. These pH effects could partly be explained by the pKa of the contaminant (e.g. diatrizoate (Hennebel et al., 2010) or diclofenac (Chapter 5)). If bio-Pd is to be applied as an effluent polishing technique for wastewater treatment plants, the pH of the water, which is to be treated, will be more or less neutral. However, if the wastestreams containing contaminants are treated with bio-Pd before any other treatment, the pH can be acidic or alkaline. In the context of wastewater treatment, acidic streams are preferred to alkaline and neutral streams.

3.5 Effect of atmosphere on leaching

The leaching of Pd from bacteria was followed as a function of time for three types of atmospheres (N_2, H_2 and O_2) (Figure 7-5). The highest leaching was observed with H_2 and N_2 in the headspace of the bottles: 52 % (in case of H_2) and 49 % (in case of N_2) of the initially added Pd was leached after 105 days. Most of the Pd had already leached after 28 days. In contrast, the leaching under pressurized (1 bar) O_2 did not differ drastically from the leaching in air at atmospheric pressure.

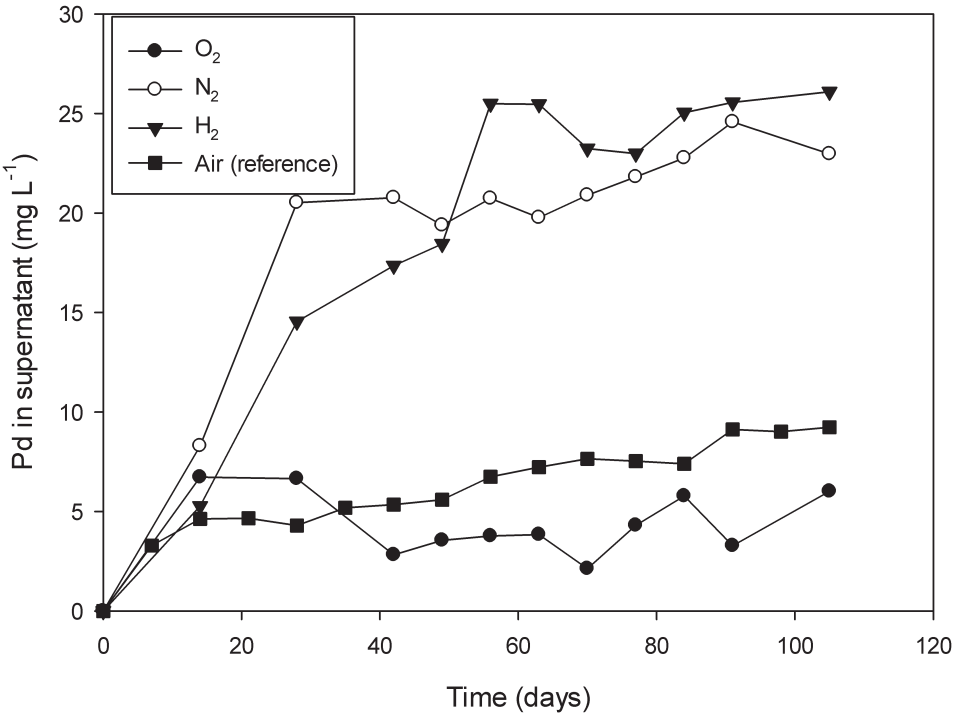


Figure 7-5: Pd concentration in the supernatant after centrifugation (8041 g for 7 minutes) of bio-Pd (50 mg Pd L^{-1}) on *S. oneidensis* stored at 21°C in distilled water under an atmosphere of 1 bar of pure O_2, N_2 or H_2. The values of bio-Pd on *S. oneidensis* stored under atmospheric conditions are shown as a reference.

Wu et al. (2010) described only a minor inhibition of the growth of *S. oneidensis* after exposure to an overpressure of 35 bar N_2 for 17 hours. The atmosphere dependency of

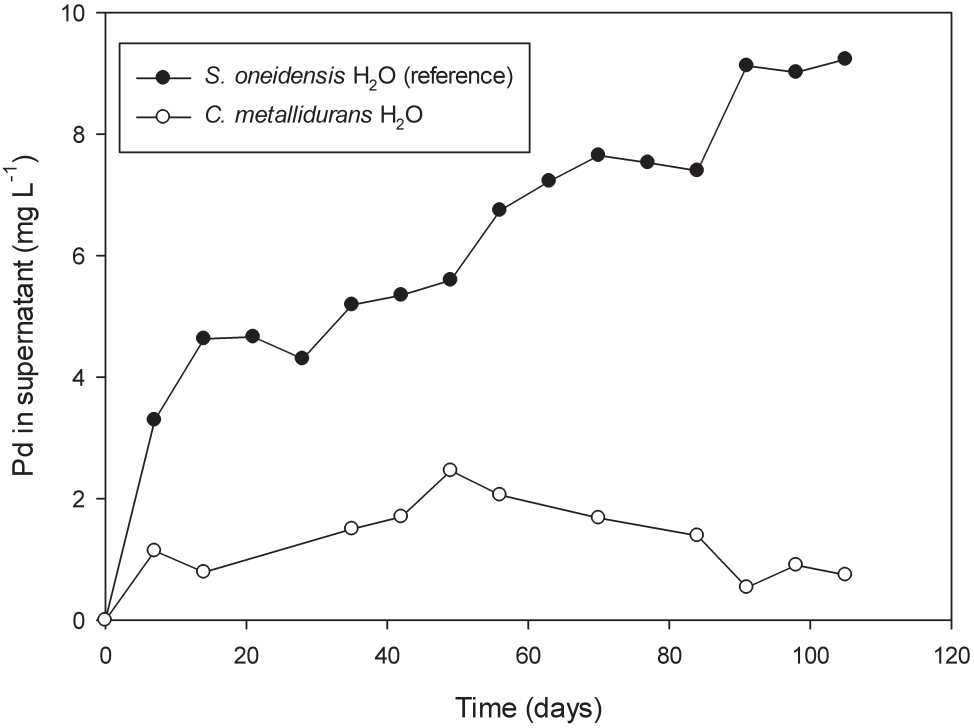
the leaching of Pd from the cells is most likely due to the composition of the gas rather than the overpressure, as these gases interact differently with the bacterial cells. The effect of storage of the cells on membrane integrity under different atmospheres was also investigated using flow cytometry but no significant differences were observed (data not shown). Gases can diffuse through the bacterial cell wall and cause changes to the cell structure or permeability of the cell wall, or interact with the metabolism of the cell. In particular H_2, a known electron donor and reductant for Pd (Lloyd et al., 1998; De Windt et al., 2005), could diffuse easily in the cells. However, little is known about the specific effects of these gasses on *S. oneidensis* cells. Anaerobic conservation of bio-Pd with an overpressure of N_2 or H_2 is not advised since high leaching occurs. For atmospheres of O_2 and N_2, the final pH (6.5 and 7.2 respectively) did not differ drastically from the initial neutral pH after 105 days. However, in the case of H_2, pH of the bio-Pd suspension increased to 9.6, which could partly explain the increased leaching.

Most batch tests investigating the degradation of environmental contaminants have been performed under anaerobic conditions with a H_2 atmosphere (1 bar) (Hennebel et al., 2009b; Hennebel et al., 2010; Chapter 5). From this study, it is clear that leaching was very high under these circumstances. In order to minimize leaching, large scale applications with bio-Pd under anaerobic conditions with overpressure of N_2 or H_2 should preferably be avoided. This could be problematic since H_2 was shown to be the most efficient hydrogen donor for hydrodechlorination reactions (De Windt et al., 2005; Hennebel et al., 2009b). From this point of view, other hydrogen donors that can be applied under aerobic conditions, such as formate or lactate, may be preferred (De Windt et al., 2005).

3.6 Effect of bacterial species on leaching

In the next phase, the influence of the carrier organism on the stability of bio-Pd was investigated. In order to avoid changing the growth conditions, *Cupriavidus metallidurans* was selected as an appropriate alternative to *S. oneidensis*, given that bio-Pd can be produced with the same protocol for both bacteria. *C. metallidurans* is a bacterial species known for its metal resistance, for example against Se, U (Avoscan et al., 2007) Ag (Ledrich et al., 2005) and Cu (Sendra et al., 2006). Recently, *C. metallidurans* has also been applied in the bioproduction of Pd nanocatalysts (Gauthier et al., 2010; Yong et al., 2010). Leaching of Pd from the carrier was followed as function of time in distilled water at pH 7 (Figure 7-6). A maximum leaching of 2 mg Pd L^{-1} (4 % of the initially added Pd) was observed after 49 days, significantly less than with *S. oneidensis* (5.59 mg L^{-1}; 11.18 % after 49 days). From an ecological point of view, *C. metallidurans* can be preferred

over *S. oneidensis* as producer and carrier of Pd nanoparticles. However, the carrier can have a significant effect on the catalytic activity of the biocatalyst. For example, Pd on *S. oneidensis* can catalyze the dehalogenation of TCE within 30 minutes (see 3.6 and Hennebel et al. (2009b)), whereas no removal of TCE was observed when using *C. metallidurans*. In contrast, Pd on *C. metallidurans* was able to catalyze the deiodination



of diatrizoate (first order decay value of 8.9 ± 1.5 h^{-1} L g^{-1} Pd) but at a slower rate than Pd on *S. oneidensis* (first order decay value of 15.9 ± 1.8 h^{-1} L g^{-1} Pd (Hennebel et al., 2010)). It is thus important to consider both stability and catalytic activity in order to determine the choice of bacterial species.

Figure 7-6: Pd concentration in the supernatant after centrifugation (8041 g for 7 minutes) of bio-Pd (50 mg Pd L^{-1}) on *C. metallidurans* stored at 21 °C in distilled water at pH 7. The values of bio-Pd on *S. oneidensis* stored at 21°C in distilled water are shown as a reference.

3.7 Effect of leaching on catalytic activity

The effect of leaching on catalytic activity was tested as follows: batches of bio-Pd in distilled water and M9 medium at 4 °C and in water at 21 °C were used as catalysts for the dehalogenation of TCE (Figure 7-7A, B and C respectively), similar to Hennebel et al. (2009b). Dehalogenation assays were performed immediately after catalyst synthesis and after 15 weeks of conservation under the specified conditions. In all cases ethane was the final degradation product. As a result of long term storage of bio-Pd it can be observed from degradation kinetics that leaching did not have a profound effect on the

catalytic activity, given that the leached Pd remained in the reaction medium. Although a portion of the Pd nanoparticles was not attached to cells, this did not result in an increase of the catalytic activity. Cell disruptions could result in attachment of debris on the catalyst surface and thereby decrease catalytic activity, but this appears not to be the case either. Therefore, bio-Pd can be taken from a larger batch, stored for a longer period of time and used for a batch experiment without the loss of catalytic activity. However, the leaching does have its implications for applications in continuous systems, since part of the catalyst will be lost in the effluent.

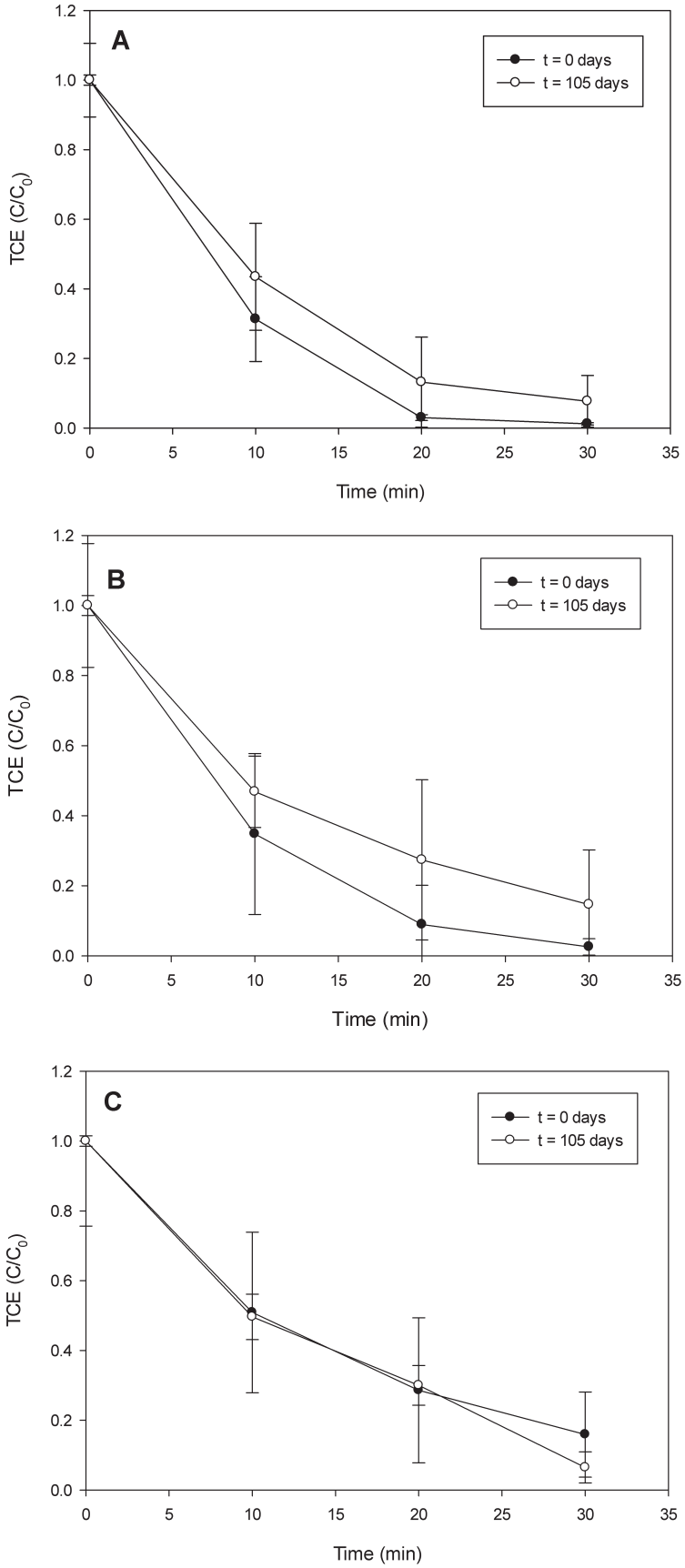


Figure 7-7: TCE degradation (C_0 = 100 mg L^{-1}) using bio-Pd on *S. oneidensis* (50 mg Pd L^{-1}) just after production of the batch (t = 0 days) and after 105 days of storage of the batch at 4 °C in distilled water (A), at 4 °C in M9 medium (B) and at 21 °C in distilled water (C).

These observations are somewhat in contrast to studies where a high catalytic activity at high pH has been reported, which could be attributed to increased leaching at this pH (Hennebel et al., 2010). It is possible that the leachates caused by long term storage and the leachates caused by increased pH differ significantly and have different effects on the catalytic activity and should be investigated further. It is very likely that when Pd is lost in the treated effluent of a reactor, the catalytic activity of the reactor suspension will decrease, since for bio-Pd catalysts disproportional decreases of reaction rates with decreasing Pd concentrations have been reported (Hennebel et al., 2010; Hennebel et al., 2011c).

4 Conclusions

This study demonstrated that leaching of Pd nanoparticles is an important phenomenon when working with biosupported Pd nanoparticles. Leaching appears to be mainly determined by temperature, pH and medium: the lowest leaching was observed in batches stored at low temperature, low pH and in a mineral medium. Leaching was very high (up to 25% after 35 days) at alkaline pH. Also anaerobic storage is to be avoided as significantly higher leaching was observed in anaerobic conditions than under atmospheric conditions. All Pd was found to be leached as zerovalent Pd nanoparticles and no oxidation to Pd(II) occurred. *C. metallidurans* is determined to be a more appropriate organism for production of biosupported Pd nanoparticles than *S. oneidensis* in order to avoid leaching, however the choice of the carrier will be application dependent, as it significantly influences catalytic activity. Leaching caused by long term storage does not affect the catalytic activity if the leached particles remain in the reaction medium. Further research should compare leaching of Pd nanoparticles from different support materials and under different conditions and study how the leached Pd behaves in synthesized products and environmental compartments. Furthermore, the (microbial) recovery of the leached Pd should be the subject of future research.

The results of this study clearly show the importance of Pd leaching from biosupported Pd nanocatalysts and present the conditions in which the leaching can be minimized. An opportunity possibly exists for leached Pd to be recovered on the bacterial cells, for example by working at low Pd : cell dry weight ratios and adding or maintaining live bio-Pd compatible bacteria.

5 Acknowledgements

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Chapter 8
Comparison of bacterial cells and amine functionalized abiotic surfaces as support for Pd nanoparticle synthesis

Abstract

An increasing demand for Pd nanocatalysts has motivated the search for sustainable production methods. An innovative approach uses bacterial cells as support material for synthesizing Pd nanoparticles. Nevertheless, drawbacks of microbially supported Pd catalysts are the low activity compared to conventional Pd nanocatalysts, the limited stability of the catalysts and the possible poisoning of the catalyst surface by sulfur from bacterial proteins. In this study, we explored the possibility of replacing bacteria with amine-functionalized materials, and compared different functionalizations. Pd nanoparticles formed on the support materials were visualized by TEM, and their activity was evaluated for the reduction of *p*-nitrophenol. Surfaces functionalized with 3-aminopropyltriethoxysilane and chitosan are interesting alternatives to bacteria, as the catalytic activity of Pd particles formed on these surfaces was higher than for bio-Pd on *S. oneidensis* cells. Increasing the concentration of *S. oneidensis* cells beyond a certain threshold lead to deactivation of the Pd catalyst. This was not observed for the sulfur-free carriers, implying that such amine-rich materials can provide an excellent support for environmentally friendly synthesis of surface-immobilized Pd nanoparticles.

Chapter redrafted after:

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1 Introduction

Microbially supported synthesis of Pd nanoparticles is considered a sustainable alternative to conventional methods, as the bacterial support material is renewable and does not require many processing steps, in contrast to other support materials like Al_2O_3 and activated carbon (Hennebel et al., 2009a). Fewer chemicals are involved in the production processes as the bacteria also act as a stabilizing agent, and addition of stabilizers (to avoid coalescence of particles) and capping agents (to avoid growth of the particles) is therefore redundant (Ingham et al., 2011). Moreover, bacteria have a high specific surface area and can be applied for recovery of several precious metals from scrap leachate waste streams (Yong et al., 2010). The use of bacteria to recover and regenerate active catalysts from waste provides a closed cycle for heavy metals, as the bio-catalysts can be recycled again after use. The particle size can have great influence on the properties Pd catalyst, the particle size of bio-Pd can to some degree be controlled by manipulating the ratio of Pd to biomass during bio-Pd production (De Windt et al., 2006; Sobjerg et al., 2011). Smaller particles are generally believed to be more reactive due to their surface area. However, the optimum particle size of the Pd catalyst is dependent on the application. For example, perchlorate is reduced much faster by 15 nm particles compared to 50 nm particles, whereas the opposite is the case for dechlorination of polychlorobifenyls (De Windt et al., 2006).

Bio-Pd catalysts have successfully been applied in several reactions (Chapter 1). The reduction rate of *p*-nitrophenol using $NaBH_4$ as hydrogen donor was used by Hosseinkhani et al. (2012) to assess the catalytic activity of monometallic bio-Pd and bimetallic bio-Pd/Au supported on cells of *Cupriavidus metallidurans*. The bimetallic bio-Pd/Au catalyst showed about twice as much activity as the monometallic bio-Pd catalyst (Hosseinkhani et al., 2012). Due to the easy spectrophotometric detection of this reaction, we chose to use *p*-nitrophenol reduction as a model reaction to compare the properties our Pd catalysts in this study.

Bio-Pd catalysts do have some drawbacks. For some applications, they have lower catalytic activity than commercially available Pd nanocatalysts (Sobjerg et al., 2009; Hennebel et al., 2010). They show significant leaching of Pd from the bacterial carrier over time (Chapter 7). Moreover, they risk irreversible poisoning by sulfur from bacterial proteins. This is particularly problematic at high cell dry weight (CDW) to Pd ratios, which are required to obtain the smallest and most monodisperse nanoparticles (Bunge et al., 2010; Sobjerg et al., 2011). A recent study established that active enzymes are not

required to facilitate the synthesis of Pd nanoparticles on the cell surface of bacteria (Rotaru et al., 2012). Instead, the coordination of Pd(II) with amine groups seemed critical for the reductive synthesis of the particles. Motivated by this observation, we explored the possibility of using abiotic materials functionalized with amine groups and amine-rich biomaterials as support material for Pd nanoparticles. The aim of the study was to identify a method for amine-functionalization, which can be applied to a support material of choice, and which facilitates the synthesis of stable, catalytically active Pd nanoparticles without the risk of sulfur poisoning.

To enable direct comparison with bacterial cells, we used spherical silica beads of 1 µm diameter as the abiotic support material. The beads were easily suspended, and the immobilised Pd catalyst could thus be handled in suspension or separated from suspension by centrifugation. Three different strategies were chosen for amine-functionalization of the beads: (1) 3-aminopropyltriethoxysiloxane (APTES), resulting in a functionalization of the silica surface with primary amines; (2) polyethylenimine (PEI), resulting in a secondary amine-functionalized surface and (3) chitosan, a sulfur free biopolymer containing free amine groups. Chitosan had already been described as a stabilizer for metal nanoparticles (Le Bras et al., 2011; Vasseur et al., 2011). However the idea of coating a support material with chitosan for synthesis of immobilised Pd nanoparticles is to our knowledge new. Pd nanoparticle formation on the different support materials was visualized by TEM, and the catalytic properties of the particles were evaluated by measuring the reduction rate of *p*-nitrophenol to *p*-aminophenol.

2 Materials and methods

2.1 Preparation of the carriers

2.1.1 Bacterial cells

Shewanella oneidensis MR-1 (LMG 19005) was obtained from the BCCM/LMG Bacterium Collection (Gent, Belgium). The strain was grown aerobically in Luria-Bertani (LB) medium overnight at 28 °C. *S. oneidensis* cells were harvested in sterile 50 mL centrifuge tubes (TPP, Switzerland) by centrifuging at 3000 g for 10 min and washed twice with 50 mL M9 medium (Sambrook et al., 1989). The washed cells were resuspended in distilled water to a final optical density at 600 nm (OD_{600}) of 1, corresponding to 50 mg L^{-1} cell dry weight and a cell surface area of 1.29 x 10^{10} μm^2 mL^{-1}.

2.1.2 Silica beads

Silica beads (1 µm diameter, 50 mg mL^{-1}, Kisker Biotech, Denmark) were first diluted to obtain 1.29 x 10^{10} $µm^2$ surface area mL^{-1} The beads were first cleaned by applying a Piranha treatment: beads were added to a mixture of miliQ water, 25 % NH_4OH and 35 % H_2O_2 in a volume ratio of 4:1:1, and incubated for 5 minutes at 70-80 °C. The beads were then separated from the solution by centrifuging at 5000 g for 1 min and washed twice in miliQ water and twice in acetone before further functionalization. Unmodified beads were cleaned by Piranha treatment and finally resuspended in water.

Functionalization with APTES was achieved by resuspending beads in 10 vol. % 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich, Germany) solution in acetone and incubated for 15 hours. Finally, the beads were sonicated for 1 hour and washed 3 times in miliQ water.

Functionalization with PEI was obtained by resuspending the cleaned beads in 10 vol. % 3-(triethoxysilyl)propylsuccinic anhydride (ABCR GmbH, Germany) solution in acetone and incubated for 15 hours, in order to obtain a coating with free carboxyl groups. The beads were then washed twice in miliQ water and resuspended in a solution containing 0.1 mg mL^{-1} N-hydroxysuccinimide (NHS, Sigma-Aldrich, Germany) and 1 mg mL^{-1} 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Sigma-Aldrich, Germany) and incubated for 30 minutes. Finally, the beads were washed twice with miliQ water and resuspended in a 3 vol. % of polyethyleneimine (PEI, Sigma-Aldrich, Germany) solution in miliQ water and incubated for 15 hours to facilitate covalent coupling to the carboxyl groups on the silica surface. Finally the beads were sonicated for 1 hour and washed 3 times with miliQ water.

The third form of functionalization was obtained with chitosan using Chitofarm chitosan M (Cognis, Germany), which is 100 % deacetylated and has a broad molecular weight of 300-2000 kDa. Cleaned silica beads were resuspended in 2.5 mL MiliQ water and 1.5 mL of the chitosan solution (15 mg mL^{-1} in 200 mM NaAc/HAc- buffer pH 3.5) was slowly added dropwise. After complete chitosan addition, the glass beads were mixed by vortexing and incubated for one hour at room temperature under slight shaking of 100 rpm. Glass beads were separated from the coating solution by centrifugation at 5000 g for 10 min and washed three times with MiliQ water.

To provide the same surface area of support material as bacterial cell suspensions, the silica beads were resuspended in MiliQ water to obtain a final concentration of particles

corresponding to a surface area of 1.29 x 10^{10} μm^2 mL^{-1}. The functionalized beads were stored in water at 4°C until use.

The zeta potential of chitosan coated glass beads was determined to confirm the presence of surface functionalization, as the positive charge of amine groups was expected to have profound effects on the glass particles negative surface charge. Samples (0.5 mL) of functionalized and unmodified glass beads suspended in MiliQ water were analyzed by dynamic light scattering (backscatter 173°) on a Zetasizer Nano ZS (Malvern, UK) in a clear disposable zeta cell. Three measurements were carried out using default settings (automatic duration, voltage and attenuation selection, temperature 25 °C, model Smoluchowski). The equilibration time was set to 0 seconds. As measurement standards, polystyrene latex (absorption: 0.01, RI: 1590) and dispersant water (25 °C, viscosity: 0.8872 cP, RI: 1.330, dielectric constant: 78.5) were chosen.

2.2 Preparation of the catalysts

Bio-Pd was prepared according to De Windt et al. (2005). Briefly, sodium formate was added to 50 mM to a cell suspension in M9 medium of OD_{600} = 1, corresponding to a surface area of 1.29 x 10^{10} μm^2 mL^{-1} (Rotaru et al., 2012). Pd(II) was added from a 15 g L^{-1} stock solution of Na_2PdCl_4 (Sigma Aldrich, Germany) to a final concentration of either 1 mg Pd(II) L^{-1}, 5 mg Pd(II) L^{-1}, or 50 mg Pd(II) L^{-1}. The final amount of Pd per cm^2 surface area of support material was thus 0.0078; 0.039 or 0.39 µg Pd cm^{-2}, respectively. The suspensions were incubated overnight at 28 °C to obtain reduction of Pd on the surface of the cells. Prior studies confirmed that Pd(II) was reduced completely to Pd(0) under these conditions by measuring the remaining Pd(II) in the supernatant of the suspension after removing cells by centrifugation (De Windt et al., 2005; Sobjerg et al., 2009). The bio-Pd was finally washed twice with 50 mL distilled water.

Pd nanoparticle formation on functionalized beads was performed by preparing 5 mL solutions of beads with a surface of 1.29 x 10^{10} μm^2 mL^{-1} in Exetainers, and then adding Pd(II) from a 1 g L^{-1} stock solution of Na_2PdCl_4 (Sigma Aldrich, Germany) to obtain a final Pd concentration of 1; 5 or 50 mg L^{-1}. The final Pd content per cm^2 support material was thus the same as for samples with bacteria. The resulting solution was subsequently flushed with N_2 for 4 minutes and finally flushed with the H_2 for 4 minutes to achieve reduction to Pd(0) (This is a redox reaction with oxidation of H_2 and reduction of Pd(II) to Pd(0), with a conversion of 100 %).

2.3 Transmission electron microscopy

The morphology of the Pd nanoparticles on the functionalized silica beads and bacterial cells was evaluated using a Philips CM20 TEM equipped with a LaB_6 filament operating at 200 kV and a Gatan CCD camera (Gatan Inc., CA, USA). Samples containing bacterial cells were prepared for TEM by fixation in 2.5 % glutaraldehyde for 10 minutes followed by separation of cells by centrifugation (10 minutes, 5000 g) and washing three times in MilliQ water. Finally the cells were resuspended in MilliQ water. Ten µL of bacterial or silica beads suspensions were placed on Formvar-coated copper grids and air-dried before imaging.

2.4 Reduction of p-nitrophenol

Catalytic activity of bio-Pd and Pd on functionalized silica beads was evaluated by the reduction of *p*-nitrophenol to *p*-aminophenol (Hosseinkhani et al., 2012). The reactions were performed in tubes containing identical concentrations of catalyst (0.5 mg L^{-1} Pd). As the suspensions of cell and silica beads contained either 50, 5 or 1 mg L^{-1} Pd, different volumes had to be added to the *p*-nitrophenol assay to obtain the same amount of Pd catalyst. From the suspensions containing 50 mg Pd L^{-1}, 0.1 mL was transferred to a 12 mL Exetainer, and MiliQ water and *p*-nitrophenol was then added to obtain a volume of 10 mL with a final concentration of 230 µM *p*-nitrophenol (pH = 13, pH adjusted with 5 M NaOH) and 0.5 mg L^{-1} Pd. From the suspensions containing 5 mg Pd L^{-1}, 1 mL was transferred to the Exetainer before addition of MiliQ water and *p*-nitrophenol. The suspensions containing 1 mg Pd L^{-1} had to be concentrated 5 times in order to avoid too much dilution of *p*-nitrophenol. Beads or cells were therefore pelleted by centrifugation (5000 g, 10 minutes) and resuspended in 1/5 of the original volume before transferring 1 mL to Exetainers as described above. Pd could potentially be lost during the centrifugation step, and Pd was therefore measured in the supernatant of these samples, using inductively coupled plasma – optical emission spectroscopy (ICP-OES, Spectro Arcos, Germany) at a wavelength of 340.458 nm. Detection limit for Pd was 0.04 µg L^{-1}.

Exetainers with Pd catalyst and *p*-nitrophenol were finally flushed with N_2 for 4 minutes and transferred to a second Exetainer containing 100 mg $NaBH_4$ powder under N_2 atmosphere (ICN Biomedicals, CA, USA). The reaction started immediately when the powder was dissolved. Mixing of the catalyst during the reaction occurred by the formation of gas bubbles after dissolution of $NaBH_4$. The reaction was run at room temperature, and the Exetainer was placed directly in a spectrophotometer (GE

Healthcare, Sweden) where the OD_{400} was recorded every 20 seconds. All analyses were performed on triplicate samples.

3 Results and discussion

3.1 Pd reduction on bacteria and silica beads

Pd(II) was added to 50 mg L^{-1} to *S. oneidensis* cells and to the silica beads functionalized with APTES, PEI and chitosan. Subsequently the reduction was performed. Pd was reduced in all samples and could be observed visually by a change in color from amber to a clear solution with a black precipitate (data not shown). Nanoparticles were formed on the surface of *S. oneidensis* cells and the three different functionalized silica beads (Figure 8-1). Formation of Pd nanoparticles on *S. oneidensis* appeared similar to previous observations (De Windt et al., 2005). The size of the particles ranged from 20 nm to 100 nm, and all particles were associated with cells (Figure 8-1b-c).

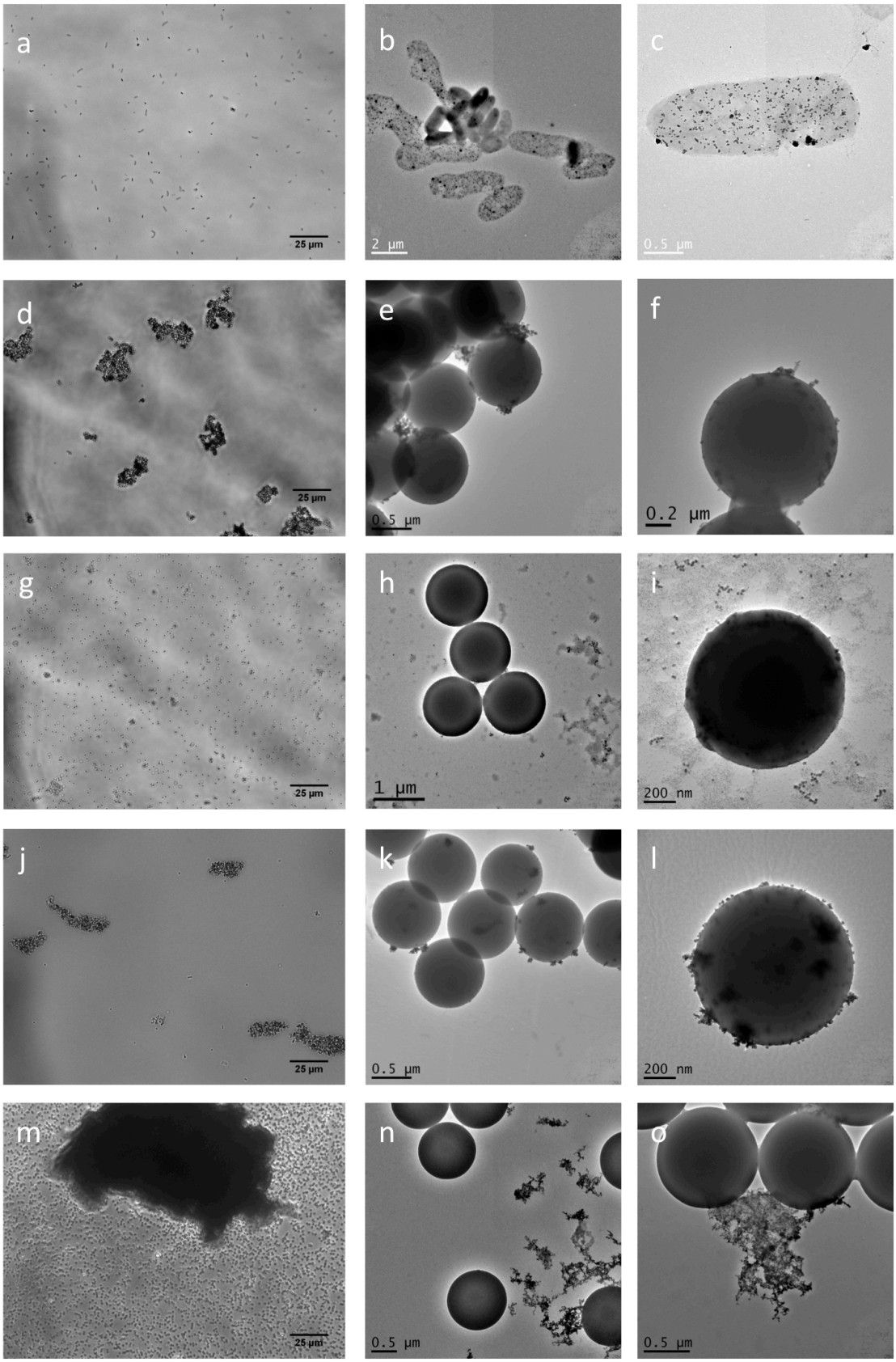

Figure 8-1: Optical (left column) and TEM (center and right column) images of *Shewanella oneidensis* cells (a-b-c) and functionalized silica beads (d to o) after Pd reduction. All samples had a start concentration of 50 mg L^{-1} Pd, i.e. 0.39 µg Pd per cm^2 support material. (a-b-c): bio-Pd on *Shewanella oneidensis*; (d-e-f): APTES functionalized beads; (g-h-i): PEI functionalized beads; (j-k-l): chitosan functionalized beads; (m-n-o): unfunctionalized beads.

Successful functionalization of the beads was confirmed by measuring a significant change in zeta potential following the functionalization procedure. A significant change in the zeta potential was observed for all beads ($p < 0.05$). The unmodified beads had a negative surface charge, whereas beads with APTES were neutral, chitosan slightly positive, and PEI even more positive (Table 1).

Table 8-1: Zeta potentials of the functionalized and unmodified silica beads (n = 3).

Applied coating	**Zeta potential** (mV)
Unmodified silica beads	- 69.19 ± 5.97
APTES	0.04 ± 1.00
PEI	42.23 ± 1.21
Chitosan	26.25 ± 8.01

After Pd(II) reduction, APTES-functionalized beads were covered with 5-20 nm sized Pd nanoparticles, of which many formed aggregates of 30-100 nm (Figure 8-1f). The beads aggregated strongly, which might be explained by the lack of electrostatic repulsion due to their neutral surface charge. Furthermore, Pd has a strong tendency to aggregate in aqueous solution, and the presence of Pd particles on the surface could also contribute to aggregation of the beads.

PEI-functionalized beads were also covered with Pd nanoparticles, but TEM images revealed that most Pd particles were not associated with the beads (Figure 8-1h and i). These "free" Pd particles did not aggregate and had an average size of 9.9 ± 3.0 nm. Pd nanoparticles must be capped with a stabilizing agent to prevent aggregation, and we hypothesized that PEI was partly desorbed from the beads and stabilized the Pd nanoparticles, enabling them to remain in suspension as single particles.

Pd nanoparticles on chitosan-functionalized beads were present as discrete particles of approximately 10 nm in diameter and as larger aggregates up to 100 nm (Figure 8-1j-l). In contrast to the PEI-functionalized beads, all particles appeared to be associated with the beads. The beads tended to aggregate, although not as strongly as observed for APTES-functionalized beads, and aggregates were easily broken by stirring or shaking the suspension (data not shown).

In contrast to the modified beads, no Pd particles were observed on the surface of unmodified silica beads, and Pd was seen as large (hundreds of µm) aggregates (Figure 8-1m-o). Similar aggregates were observed in samples without any support material (data not shown). The absence of Pd particles on the unmodified beads confirms the importance of amine groups in surface-supported synthesis of Pd nanoparticles, as was demonstrated by Rotaru et al. (2012) for bacteria.

3.2 Catalysis of *p*-nitrophenol reduction

The catalytic properties of Pd on silica beads and bacterial cells were subsequently evaluated by using it as catalyst for the reduction of *p*-nitrophenol to *p*-aminophenol. All reactions were performed with a Pd concentration of 0.5 mg L^{-1} to ensure appropriate comparison of samples prepared from different initial Pd concentrations (50, 5 and 1 mg L^{-1}). Thus, the only differences between samples were the type of support material, and the ratio between Pd and the area of support material.

The concentration of *p*-nitrophenol monitored during the reduction assay showed a slightly s-shaped curve, indicating that the reduction rate was low in the beginning, then increased, and finally decreased again as the substrate was depleted (Figure 8-2). Rates of *p*-nitrophenol reduction were calculated from the steepest part of the curve, using at least five successive data points (Figure 8-3).

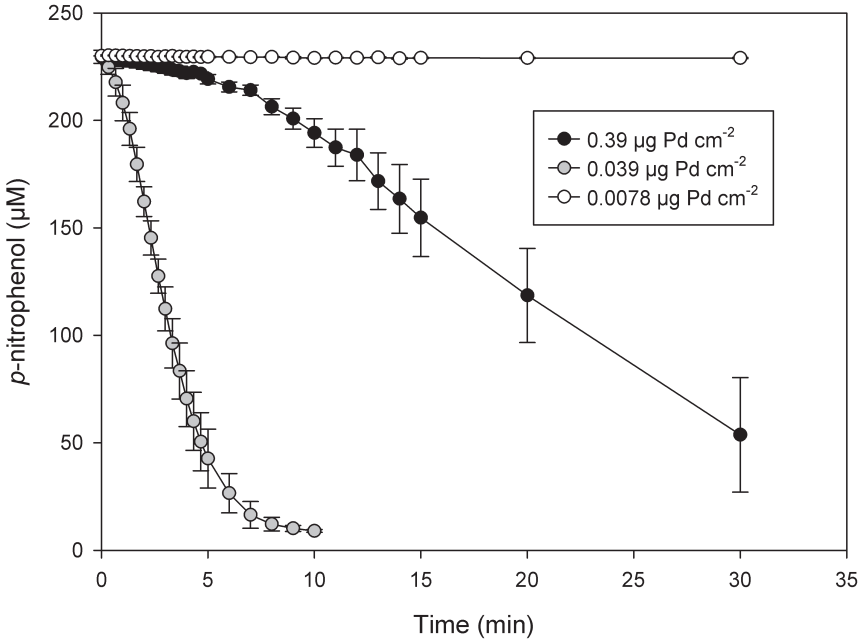

Figure 8-2: Reduction of *p*-nitrophenol catalyzed by Pd nanoparticles immobilised on *S. oneidensis* cells. The concentration of *p*-nitrophenol is shown as a function of time, using Pd catalysts prepared at three different ratios of Pd to surface area of the bacterial cells: 0.39, 0.039, and 0.078 µg Pd cm^{-2} (n = 3).

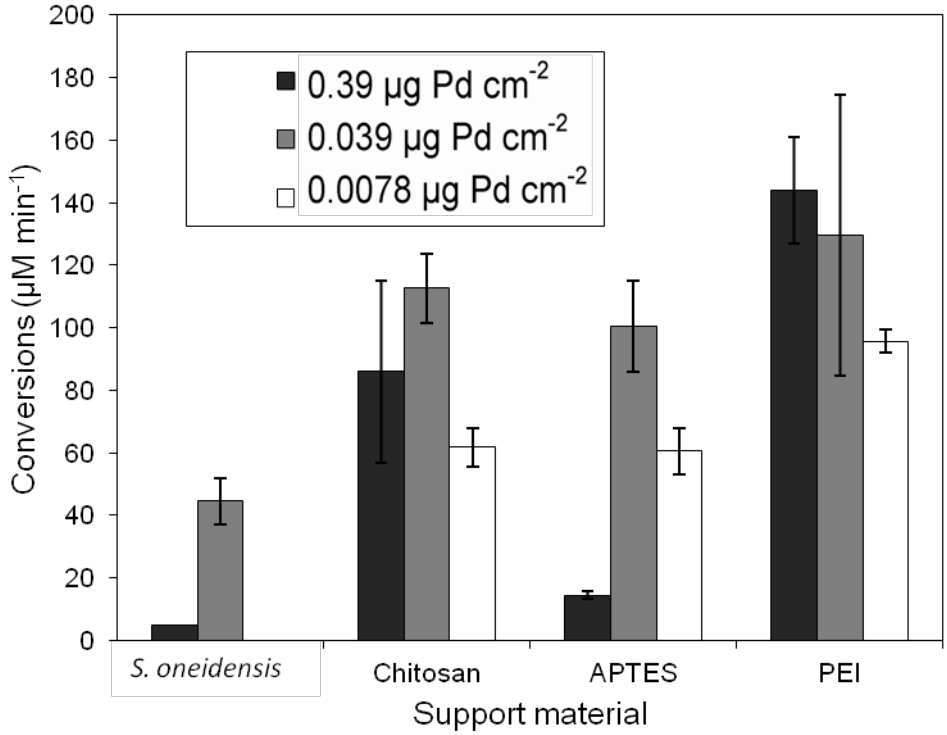

Figure 8-3: Reduction rates of *p*-nitrophenol at a Pd concentration of 0.5 mg L^{-1} using bio-Pd and chitosan, APTES and PEI functionalized silica beads containing Pd. All catalysts were tested at 3 different Pd contents per cm^2 of support surface: 0.39; 0.039 and 0.0078 µg Pd cm^{-2} (n = 3).

Lowering the Pd to surface area ratio from 0.39 to 0.039 µg cm^{-2} lead to a significant increase in the rate of *p*-nitrophenol reduction catalyzed by bio-Pd (Figure 8-2 and Figure 8-3). This effect may be caused by a difference in Pd particle size. We did not determine the particle size distribution, but several studies have demonstrated that synthesis of bio-Pd at lower Pd to cell dry weight ratios leads to smaller Pd particles (De Windt et al., 2006; Bunge et al., 2010). Further decreasing the Pd to surface area ratio 5 fold (to 0.0078 µg cm^{-2}) resulted in complete absence of catalytic activity of the bio-Pd (Figure 8-2 and Figure 8-3). The complete deactivation of the Pd catalyst was probably caused by sulfur poisoning, which was previously observed at similar Pd to surface area ratios of bio-Pd supported on *Cupriavidus metallidurans* and *Staphylococcus sciuri* cells (Bunge et al., 2010). Catalytic assays with Pd to surface area ratios of 0.0078 µg cm^{-2} were prepared slightly differently from the other samples, because the catalyst had to be concentrated by centrifugation before transfer to the *p*-nitrophenol solution. If some Pd nanoparticles were not immobilized on the support material, they could remain in the supernatant and therefore could be lost during the *centrifugation* step. To account for the possible loss of catalyst during sample preparation, we measured the concentration of Pd in the supernatant, which was to be discarded. For bio-Pd, 15% of the initially added Pd was lost during centrifugation (data not shown). This loss could not account for the

complete absence of catalytic activity of the bio-Pd, and sulfur poisoning was the most likely cause for inactivation of the catalyst.

To evade the risk of sulfur poisoning, we explored sulfur-free amine-rich materials as alternatives to bacteria in Pd nanoparticle synthesis. At any given ratio of Pd to surface area, the Pd particles on modified silica beads were superior to bio-Pd as catalysts. This difference could be caused by differences in particle size, aggregation of Pd particles, or by differences in the accessibility of the catalyst. Theoretically, the location of bio-Pd nanoparticles in the periplasmic space of bacterial cells (De Windt et al., 2005) could make them less available for *p*-nitrophenol. However, this hypothesis has not been tested.

Pd nanoparticles synthesized with PEI functionalized beads as support material had the best catalytic properties. No significant difference was observed between samples containing 0.39 and 0.039 µg Pd cm^{-2}, but the *p*-nitrophenol reduction rate was lower in samples prepared with 0.0078 µg Pd cm^{-2} (although the difference to samples prepared with 0.039 µg Pd cm^{-2} was not significant ($p > 0.05$)). The lower reduction rate is probably due to the observed loss of 37% of the Pd during preparation of the 0.0078 µg Pd cm^{-2} sample for the catalytic assay (data not shown). This substantial loss of Pd in the centrifugation step was not surprising, as TEM images had revealed the presence of a large amount of Pd nanoparticles not immobilised to the beads. Although PEI-functionalized beads provided the most active Pd catalyst, the lack of sufficient immobilization does pose important challenges for handling and recycling of the catalyst.

Catalysis of *p*-nitrophenol reduction with Pd particles synthesized on APTES-functionalized beads showed a highly increased activity, when lowering the Pd to surface area ratio tenfold from 0.39 and 0.039 µg Pd cm^{-2} during nanoparticle synthesis (Figure 8-3). Pd particles prepared with 0.39 µg Pd cm^{-2} aggregated strongly (Figure 8-1e and f), whereas this was not the case for particles prepared with 0.039 and 0.0078 µg Pd cm^{-2} (Figure 8-4a and 8-4b). Aggregation of Pd particles strongly affects its surface area, and the lower degree of aggregation can thus explain the improvement in catalytic activity when lowering the Pd to surface area ratio tenfold to 0.039 µg Pd cm^{-2}. However, a further fivefold decrease to 0.0078 µg Pd cm^{-2} did not further improve the properties of the catalyst. On the contrary, the activity nearly halved. This was surprising, as we detected no loss of the Pd catalyst during preparation of these samples, and the lower rate of *p*-nitrophenol reduction must therefore reflect the functional properties of the catalyst.

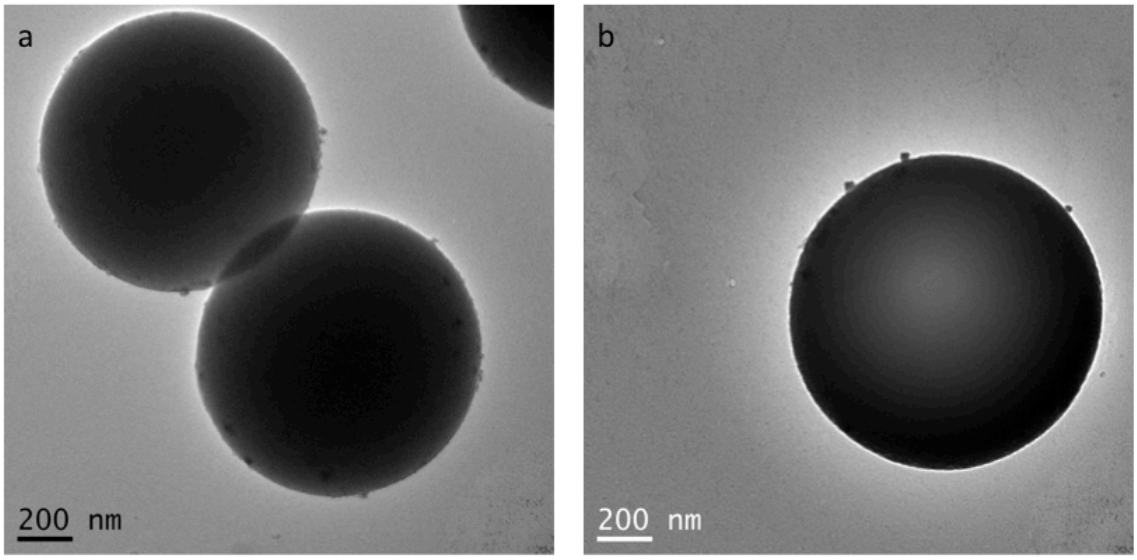

Figure 8-4: APTES functionalized beads with a Pd content of 0.039 µg cm^{-2} (a) and 0.078 µg cm^{-2} (b).

Pd particles synthesized with chitosan-functionalized beads showed a similar trend to those functionalized with APTES, although the catalyst prepared at 0.39 µg Pd cm^{-2} was much more active on chitosan-functionalized beads. For the other samples, the catalytic activity decreased when lowering the Pd to surface area ratio to 0.0078 µg Pd cm^{-2} of APTES-functionalized beads. Whereas plausible explanations for this observed observation could be offered for bio-Pd and PEI-functionalized beads (sulfur cycling and Pd loss during centrifugation), we saw no obvious explanation for the lower catalytic activity of these samples prepared with APTES or chitosan-functionalized beads. It is possible that mass transfer limitation plays a role in samples containing the highest concentration of beads, although sedimentation of the beads was not apparent from visual inspection during the reaction. It would require further investigation to fully address the cause of this observation.

Despite the lower catalytic activity of samples prepared with 0.0078 µg Pd cm^{-2} support material, it should be noted that the even the lowest reduction rates obtained with Pd catalysts prepared with PEI or chitosan functionalized beads were higher than the highest rate obtained with bio-Pd catalysts. Especially seen from the recycling perspective, Pd particles synthesized with chitosan-functionalized beads appeared to be the most of attractive catalyst. The superior catalytic properties of immobilized Pd particles synthesized with chitosan functionalized beads is interesting, as chitosan is a readily available biomaterial obtained by enzymatic deacetylation of chitin – the second most abundant biopolymer in nature. It is a renewable material and can thus considered as a relatively sustainable support material for Pd nanoparticles and is relatively cheap (€

10 – 100 kg^{-1} (Einbu, 2007)). However, additional costs for purification and for the coating process should be taken into account. Chitosan is already used in a wide array of biotechnological applications, for example as slow release fertilizer (Wu et al., 2008), as food preservative (Friedman et al., 2010), as coagulant in wastewater treatment (Fabris et al., 2010) or as drug delivery agent (Hein et al., 2008). We believe it could be an interesting candidate as support material for recovery and regeneration of active Pd catalysts. We only tested the catalytic properties towards one reaction in the present study, and the catalytic activity should be tested for other reactions, such as organic synthesis reactions (Sobjerg et al., 2009) and degradation of halogenated pollutants (Hennebel et al., 2009b; Hennebel et al., 2010). The activity should also be compared with Pd nanoparticles on other conventional support materials (e.g. Al_2O_3, activated carbon).

We used silica beads to provide a support material that was similar in size and shape to bacterial cells, so that experimental conditions were as comparable as possible. However, other materials, such as a mesoporous silica matrix (Keane, 2011) or microfibrous or milled silica glass (Pina-Zapardiel et al., 2011) could easily substitute the beads. Polymers or other materials should also be explored. An important property of support materials, which we did not investigate thoroughly, is the potential leaching of immobilized Pd, which would lead to loss of catalyst during recycling. From analysis of Pd in the supernatant after centrifuging the particles, we conclude that within this short time frame, a substantial amount of Pd was lost from bio-Pd and PEI functionalized silica beads, but not from APTES and chitosan functionalized beads. Hence, APTES and chitosan not only possessed the best catalytic properties, but also look promising with regard to recyclability of the catalyst. However further research is required on the stability of these catalysts. It should be investigated whether phenomena as leaching, sintering and aggregation of the catalysts occur and how they could be prevented.

It is tempting to conclude that bacteria are not interesting as support for Pd nanoparticles. Nevertheless, we believe that bacteria do have value as support material, particularly in applications where living cells produce reductants (e.g. H_2) for continuous regeneration of the Pd catalyst (Hennebel et al., 2011c), or contribute to a conversion process occurring in several steps, where some steps are catalysed enzymatically by the bacteria, and others are catalyzed by the Pd nanoparticles (Foulkes et al., 2011).

4 Conclusions

This study demonstrated that amine-functionalized abiotic materials can be used as support for synthesis of immobilized Pd nanoparticles by reduction from Pd(II) under mild conditions, similarly to what was previously demonstrated for bacterial cells. APTES and chitosan functionalized surfaces were particularly promising, as the catalytic properties of Pd nanoparticles on these surfaces had superior catalytic activity compared to Pd nanoparticles on *S. oneidensis* cells. Furthermore, insignificant amounts of Pd were lost from the surface of these materials during centrifugation of the sample, indicating a high degree of recyclability due to the firm immobilization of Pd nanoparticles on the surface of the support material.

Our results confirm that these amine rich materials coated on silica can be attractive alternatives for bacteria in recovery and regeneration of catalytically active Pd. Nevertheless, the use of bacteria for generation of immobilized catalytically active Pd nanoparticles can have an added value in applications where the catalytic properties of living cells and the Pd catalyst are combined. An example of this concept (glycerol conversion) is further discussed in Chapter 9.

5 Acknowledgements

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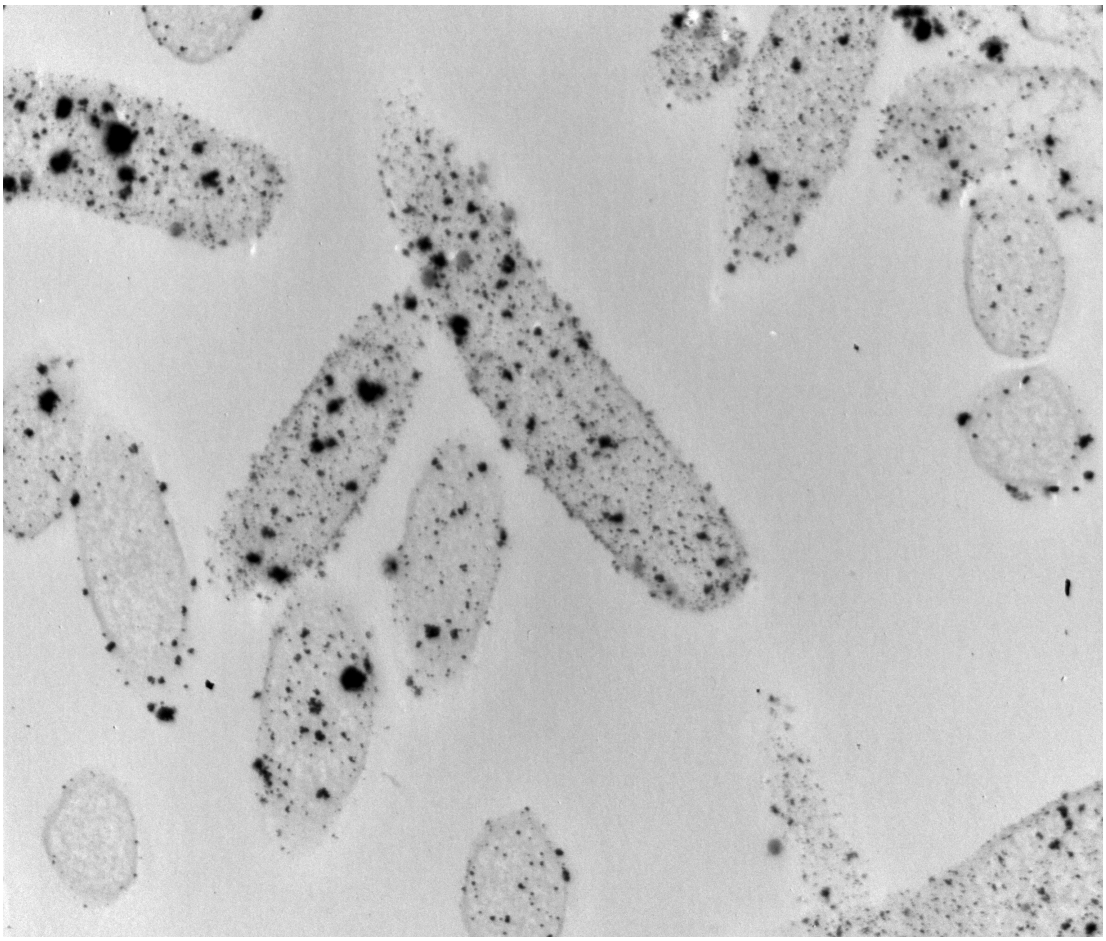
Part IV

General discussion



Chapter 9
General discussion

1 Main outcomes of this work

In this work, some limitations of biosupported Pd nanocatalysts, synthesized on the cell wall of the metal respiring bacterial strain *Shewanella oneidensis*, were put forward and some strategies to overcome these shortcomings were proposed. A first important limitation is the lower catalytic activity compared to conventional heterogenous Pd nanocatalysts (such as Pd/C or Pd/Al_2O_3). In Part II of this work, a bimetallic biosupported bimetallic Pd/Au nanocatalyst was developed. As a first step in this development, the precipitation of Au(0) nanoparticles by *S. oneidensis* was studied in detail in Chapter 1. The reduction of Au(0) was the result of a biosorption combined with a reduction process with very different kinetics. Biosorption was found to be a fast process (within the first minutes of addition of Au(III) to the bacteria), which was influenced by the physiological state of the bacteria (faster sorption with living bacteria), pH (faster sorption at acidic pH), the composition of the medium (faster sorption in water than in mineral medium) and the presence of an electron donor (faster sorption with H_2 than with formate). The reduction process was only initiated after 24 hours and required the presence of an electron donor. At a level of 50 mg Au L^{-1}, small nanoparticles were precipitated mainly intracellular, whereas at 200 mg Au L^{-1}, larger precipitates were formed, located on the cell wall. At 100 mg Au L^{-1}, both phenomena were observed. In Chapter 4, a bimetallic Pd/Au catalyst was synthesized. Different strategies of addition of the metals to the bacteria were tested for the synthesis of this catalyst. The catalytic activity of the different catalyst types was tested for the removal of diclofenac and TCE. Bio-Au was for both compounds inactive as catalyst. It was shown that only the catalyst obtained by simultaneous coprecipitation of Pd and Au showed a substantial improvement of the catalytic activity compared to the monometallic bio-Pd catalyst. Diclofenac could not be removed by a monometallic bio-Pd catalyst at neutral pH, whereas the bimetallic bio-Pd/Au catalyst could perform this removal. The removal rate of TCE by bio-Pd was increased by a factor 7 using bio-Pd/Au. The unique bimetallic nature was demonstrated by synchrotron μXRD, which showed a contraction of the lattice of the bimetallic coprecipitated bio-Pd/Au catalyst compared to the monometallic

bio-Pd catalyst. STEM and EDX images of the bimetallic catalyst showed the presence of aggregates with alloyed Pd and Au. In Chapter 5, the catalytic removal of diclofenac by bio-Pd and bio-Pd/Au was studied in detail. Diclofenac could only be removed by a monometallic bio-Pd catalyst at acidic pH (5-6), whereas the bio-Pd/Au catalyst could perform the removal at all pH values between 5 and 8 (with a better removal at acidic pH). The dechlorinated intermediates were the only degradation products that were present. A maximum dechlorination rate was observed at a Pd/Au mass ratio of 50/1, which demonstrated that only a small amount of Au is needed as a doping agent for Pd catalysts. The monometallic bio-Pd catalyst and the 50/1 bimetallic bio-Pd/Au catalyst were subsequently tested as catalyst for the Suzuki C-C cross coupling reaction in synthetic organic chemistry. The bimetallic catalyst showed better reaction kinetics, more reproducible results and a broader reaction scope than the monometallic bio-Pd catalyst. Nevertheless, the reaction rates were not yet competitive with a conventional Pd/C catalyst.

Part III focused on 2 other possible limitations of bio-Pd catalysts. In Chapter 7, the leaching of Pd from the bacterial carrier was followed as a function of the storage time under different physicochemical conditions. Leaching was found to be an important process, possibly leading to economical losses and environmental risks. Leaching rates increased at increasing temperature, at alkaline pH and under anaerobic conditions. All Pd leached as Pd(0) nanoparticles, no significant reoxidation to Pd(II) occurred. Given that the leached Pd remained in the reaction medium, no loss of catalytic activity was observed for the removal of TCE. In Chapter 8, sulfur-free alternatives for bacteria were investigated in order to prevent catalyst poisoning by biogenic sulfur. Therefore, silica beads with a comparable surface like bacterial cells were functionalized with amine groups, which are important anchoring sites for sorption and reduction of Pd(II). This functionalization was done chemically by PEI and APTES and also by the biomaterial chitosan, a very abundant and relatively inexpensive biopolymer. It was shown that Pd nanoparticles were strongly attached to chitosan and APTES-coated beads. All functionalized beads with Pd particles showed higher catalytic activities than bacteria-supported Pd-catalysts. Moreover, complete inhibition was observed at high carrier/Pd ratios with bacteria, which was not observed with the Pd on the functionalized beads. Especially chitosan was found to be an interesting carrier material for Pd catalysts, due to the stability of the obtained catalyst and the high reaction rates combined with the abundance, price and sustainability of the biopolymer.

2 Biosupported bimetallic catalysts

2.1 Pd/Au catalysts

The Pd/Au catalyst is one of the most widely applied bimetallic catalysts, since the insertion of Au(0) can cause a tremendous increase in both activity and selectivity of monometallic Pd catalysts. Several hypotheses of the promotional effect of Au have been proposed. The most important ones are:

- Geometric effects:
    - A contraction of the crystal lattice allows the reagents to be in a more optimal configuration resulting in increased reaction rates (Chapter 4, Chen et al., 2005);
    - The insertion of Au in the Pd crystal results in the presence of more isolated Pd clusters in the crystal and thus more available catalytic surface area (Knecht et al., 2008; Gao et al., 2012);
- Electronic effects: a rearrangement of the electronic structure takes place due to the withdrawal of electrons by Au (Gao et al., 2012);
- Protective effects: Au acts as a detoxification agent and prevents the Pd catalyst from poisoning by sulfides (Menegazzo et al., 2008).

Only the hypothesis of the lattice contraction could be demonstrated during this work. However, other hypotheses cannot be excluded. The type of promotional effect will most probably also be very dependent on the type of bimetallic structure that is formed. During this work, alloy structures were synthesized (Chapter 4). It is possible that the increase in activity would be even more significant if Au-Pd core-shell structures could be formed, similar to the ones formed by Nutt et al. (2005). These catalysts showed an increase in the activity for TCE removal by a factor 10 and a factor 200 after optimization, whereas the bimetallic catalysts of this work only showed an activity increase by a factor 3. The synthesis of core-shell Au-Pd catalysts supported on bacterial cell walls has so far not been described. Only alloy and similar structures could be synthesized (Chapter 4; Hosseinkhani et al., 2012). The synthesis of core-shell nanoparticles by and on bacterial cells can be an important challenge for future research within this domain.

2.2 Other doping elements

Pd has been used in combination with several other doping elements, which are listed in Chapter 1. Pt and Ni can exhibit catalytic properties themselves and are thus potentially

interesting as doping elements. However, they are probably not easy to reduce on a bacterial cell wall.

The reduction potential of Ni(II) to Ni(0) is -230 mV, which is thermodynamically very unfavorable. To perform this reduction, strong reducing agents are required, which will probably damage the bacterial cell wall. So the production of a biosupported zerovalent Pd/Ni catalyst is unlikely.

Pt is mostly abundant as Pt(IV) (as $PtCl_6^{2-}$) which first needs to be reduced to $PtCl_4^{2-}$ (reduction potential +680 mV), and can then subsequently be reduced to Pt(0) (reduction potential +730 mV). These redox potentials are thermodynamically more favorable than for Ni, but less favorable than the reduction of $AuCl_4^-$ to Au(0) (+990 mV). The reduction of Pt(IV) to Pt(0) has been described previously (Riddin et al., 2009) and could be attributed to the activity of two distinct hydrogenase enzymes. Pt(IV) was removed for 85% after 8 hours and 60% of the initially added Pt could be recovered as Pt(0). It will need to be evaluated to what extent the doping with Pt could increase the activity of bio-Pd catalysts. Moreover, the doping with Au is interesting since a maximum activity was already observed at a minimal doping of only 2 wt. % (Chapter 5). Since Au and Pt have similar prices (39 000 and 40 500 € kg^{-1} respectively), maximum activity with a Pt doping is preferably obtained at similar percentages.

The Pd/Ag catalyst has also been described (Lin et al., 2012), although less than the Pd/Au catalyst. The reduction of Ag^+ to Ag(0) by and on bacteria has extensively been reported (e.g. Sintubin et al., 2009), even for *S. oneidensis* (Wang et al., 2010a). The synthesis of a Pd/Ag catalyst on the cell wall of *S. oneidensis* seems feasible.

Another interesting option could be a Pd/magnetite catalyst, since it would allow an easy separation of the catalyst from the reaction medium, by means of magnets. Recently, the synthesis of a Pd-on-magnetite catalyst on *Geobacter sulfurreducens* was described (Coker et al., 2010). Magnetite (Fe_3O_4) was first synthesized microbially from Fe(III), Pd(II) was reduced to Pd(0) afterwards. This Pd-on-biomagnetite catalyst showed improved reaction rates for the Heck coupling reaction. Moreover, Fe is extremely cheap compared to Au. However, the bacterial synthesis of magnetite seems more delicate (since it is only feasible in a rather narrow redox potential range) than the synthesis of Au(0).

2.3 Economical aspects

The price for the growth of 1 kg biomass dry matter of *S. oneidensis* is estimated at € 1000 (Hennebel et al., 2011b). At a current Pd price of € 19 500 kg^{-1}, the cost of 1 kg bio-Pd (with a Pd/cell dry weight ratio of 1/1) catalyst is roughly estimated € 20 500 kg^{-1} actual Pd mass. Taking into account a current Au price of € 39 000 kg^{-1}, the price for the synthesis of 1 kg bio-Pd catalyst doped with 2% Au, which showed the highest activity for the dechlorination of diclofenac (Chapter 5), can be estimated about € 21 280 kg^{-1} actual Pd mass, meaning an increase of the price of bio-Pd with 3,8%. For most of the applications, this increase in investment cost will be largely compensated by the increase in catalytic activity, which implies less capital investments due to smaller reactor volumes, higher production rates and lower H_2 consumption.

3 Technological perspectives for bio-Pd and bio-Pd/Au

3.1 Treatment of AOX containing wastestreams

Halogenated contaminants are commonly found in both industrial and domestic wastewaters. Although they partially adsorb to the activated sludge, migration through wastewater treatment plants (WWTP) cannot be avoided due to poor biodegradability. Surface waters and other aqueous ecosystems containing varying concentrations of organohalogens do not form exceptions anymore (Ternes, 1999; Ternes et al., 2000). Legislation in the near future will urge plant operators to implement removal techniques for these substances. The European Commission included a list of substances in the European Water Framework Directive, which form a risk for the aquatic environment to such extent that their decrease is a priority. Halogenated substances are most likely to be evaluated under the grouping parameter of 'Adsorbable organic halogens' or AOX by the member states. For the moment, no threshold values have been determined for AOX by the European Commission, only guide values in the order of 10-100 $\mu g\ L^{-1}$. A lot of industries will very likely be affected by normation of AOX emissions, e.g. producers and processers of pesticides, pharmaceuticals, solvents, polymers and cleaning agents. Also hospitals have significant emissions of AOX in their effluents. The currently most applied technique for AOX abatement is sorption on activated carbon. However, this carbon needs to be further treated, once it is saturated, for example by incineration, since the carbon-halogen bound is not broken by sorption. Moreover, advanced oxidation processes (AOPs) do not offer an alternative as the AOX content after treatment remains equal and toxic byproducts are formed (Ternes et al., 2003). There could be

opportunities for bio-Pd and bio-Pd/Au as powerful tools for AOX abatement since it was shown that several micropollutants and pesticides could be dehalogenated using these catalysts (Chapter 1 and Chapter 5). Therefore, catalytic post treatment of WWTP effluents can be suggested. Alternatively, treatment of streams containing high concentrations AOX can be collected separately and treated catalytically before further treatment. Examples could be the collection of urine from hospitals or liquid batches coming from pesticide or medicine production. An important constraint for application of the biosupported catalysts is that the technology will need to deal with high flow rates and low residence times at which the wastewater treatment systems are operated. In Chapter 5, diatrizoate was removed from a hospital WWTP effluent within 60 minutes, whereas diclofenac and carbamazepine were only removed partly within 24 hours. Typical hydraulic residence times in wastewater treatment are in the order of a few hours. In order to avoid too large reactors, removal rates of bio-Pd and bio-Pd/Au should be substantially increased. Increased reaction rates could be obtained with increased catalyst concentrations, which will increase the investment costs. Further research is definitely needed to develop highly reactive sustainably produced (bimetallic) catalyst formulations for the reductive and controlled removal of AOX by hydrodehalogenation.

3.2 Economical aspects

The investment for the synthesis of a bio-Pd/Au catalyst is estimated € 21 280 kg^{-1} actual Pd mass. At a concentration of 50 mg Pd L^{-1}, this means an investment cost of € 1064 m^{-3} reactor volume. Since the catalyst is not consumed during the reaction, this can theoretically be considered as a capital investment cost. However, poisoning by sulfur compounds can occur and leaching of Pd from the carrier is an important phenomenon (Chapter 7), causing significant losses of Pd in the effluent and implying a periodical renewal of the catalyst. The bio-Pd(Au) catalyst is rather to be considered as an operational cost than as a capital investment cost.

Leaching of Pd could probably be decreased by encapsulation of the catalyst. These costs for encapsulation in for example alginate beads were estimated € 300 for 1 m^3 reactor volume containing 100 mg L^{-1} Pd (Hennebel et al., 2009c). Moreover, it needs to be taken into account that reaction rates significantly decrease due to encapsulation (a factor 7 for the dechlorination of TCE (Hennebel et al., 2009c)).

Another important cost which needs to be taken into account is the cost for H_2, which is about € 4 m^{-3} H_2 gas. The amount of H_2 needed is very dependent on the wastewater that is to be treated. One could determine the amount of H_2 that is stoichiometrically

needed and then add an excess of 10 times that amount. Moreover, the use of pure H_2 also implies severe safety constraints. Alternative hydrogen donors such as formate, lactate or ethanol could be used (De Windt et al., 2005), but these show generally a lower activity (Hennebel et al., 2009b; Hennebel et al., 2010).

4 Treatment of other types of contaminants by bio-Pd and bio-Pd/Au

4.1 Fluorinated contaminants

Due to the major difference in electronegativity (EN) between F and C (4.0 vs. 2.5), the C-F bond, is considered as the strongest chemical bond in organic chemistry. The EN difference gives the bond a dipole moment and a partial ionic character. This bond is extremely difficult to cleave, causing organofluorine compounds to be extremely stable. This stability allows a wide range of applications. However, after use, this stability becomes a major drawback, since it causes very high persistence of these compounds in the environment.

4.1.1 Fluorinated pharmaceuticals

Since 1990, the use of the C-F bond in pharmaceuticals has raised enormously, especially on aromatic or heterocyclic structures. Ten of the 30 best sold pharmaceuticals worldwide contain one or more fluorine atoms in their structure (O'hagan, 2010). During production of these compounds, wastestreams containing these pharmaceuticals or production intermediates are generated. These need to be treated. Often, fluorinated compounds are poorly removed and end up in effluents, which are discharged in surface waters. Moreover, just like other pharmaceutical compounds, these fluorinated drugs are excreted after use, enter wastewaters, are poorly removed by conventional treatment plants and can end up in effluents.

In contrast to other C-halogen bonds, the C-F bond shows certain insensitivity to dehalogenation by metal catalysts. However, some studies demonstrating defluorination by Pd-catalysts have been reported. Cellier et al. (2003) described a degradation of fluorbenzene of 51% after 24 hours using 5 mol % Pd/C, with hydrazine as the hydrogen donor.

Preliminary degradation experiments were performed with 2,4-difluorophenyl-piperidin-4-yl-oxime (2,4-DPO), an intermediate of the synthesis of risperidon (structures see Figure

9-1). Risperidon is the active compound of Risperdal, an anti-psychotic drug produced by Janssen Pharmaceutica.

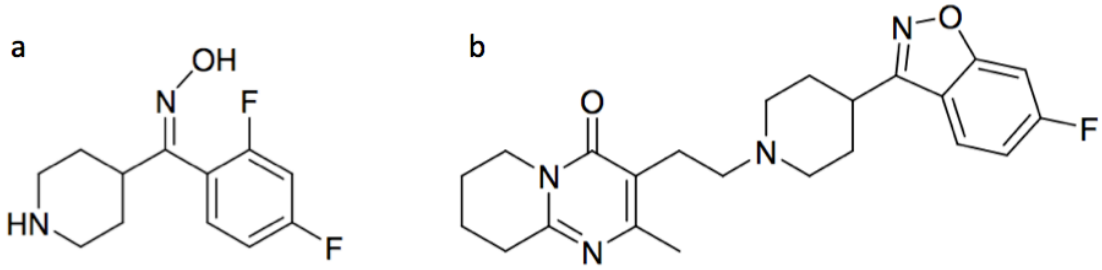


Figure 9-1: Chemical structures of 1,4-DPO (a) and risperidone (b).

Twenty mg L^{-1} 2,4-DPO could only be removed for 15 % using 50 mg L^{-1} bio-Pd and H_2 as hydrogen donor. A complete removal was observed using 50 mg L^{-1} Pd/Al_2O_3. Using a bio-Pd/Au catalyst (50 mg Pd L^{-1} and 1 mg 1 L^{-1}), 66 % removal of 2,4-DPO was observed after 24 hours reaction time (Figure 9-2). This demonstrated again the superior catalytic activity of the Au-doped bio-Pd catalyst compared to the monometallic bio-Pd catalyst. Again, the catalyst with the 50/1 Pd/Au mass ratio showed the highest activity. Both for the chemical Pd/Al_2O_3 catalyst and the bio-Pd/Au catalyst, reaction rates were decreased at acidic pH and increased at alkaline pH.

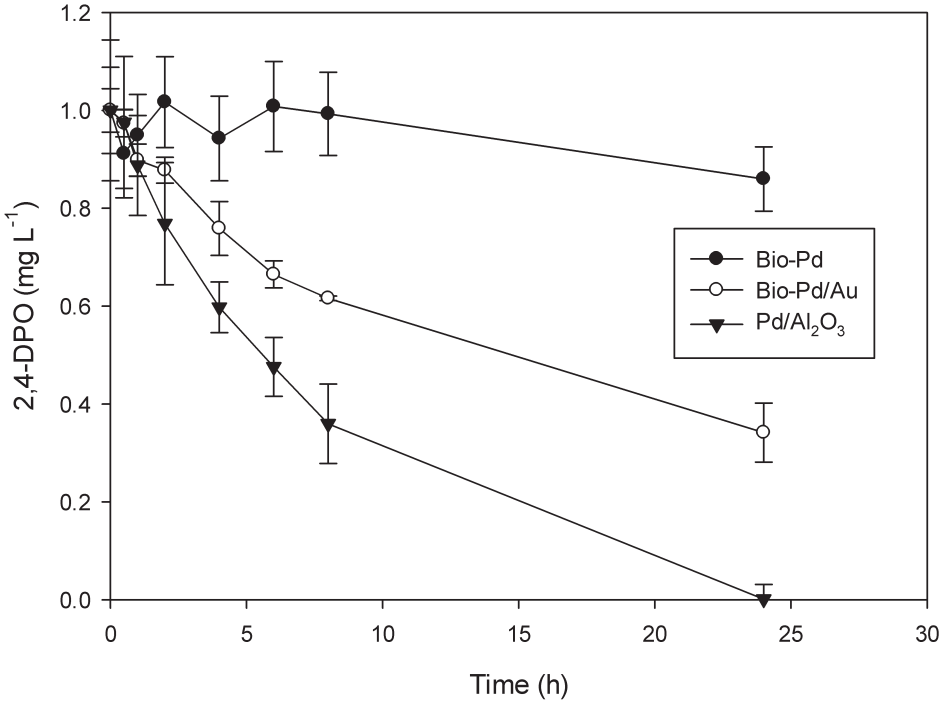


Figure 9-2: Removal of 2,4-DPO using bio-Pd (50 mg Pd L^{-1}), bio-Pd/Au (50 mg Pd L^{-1}, 1 mg Au L^{-1}), Pd/Al_2O_3 (50 mg Pd L^{-1})

The final reaction product was identified as 4-benzylpiperidine (Figure 9-3). Using the chemical Pd catalyst, 56 % of the initially added 2,4-DPO could be recovered as 4-benzylpiperidine, whereas this was only 15 % for the bio-Pd/Au catalyst. The fluoride ion measurements showed a different trend: 54 % of the initially present F could be converted to F^- for the bio-Pd/Au catalyst, whereas this was only 34 % for the chemical Pd catalyst.

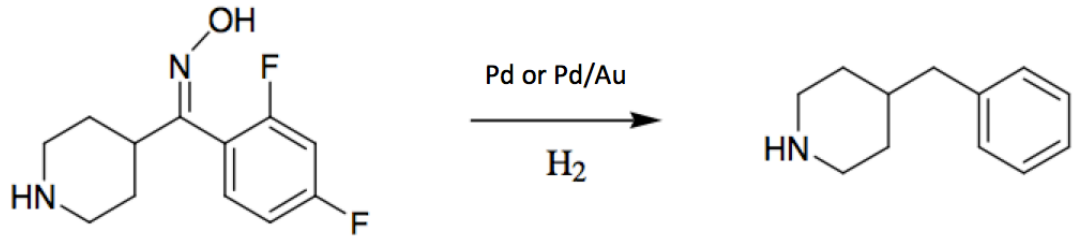


Figure 9-3: Proposed degradation pathway for 2,4-DPO to 4-benzylpiperidine using Pd and Pd/Au catalysts.

These promising results of batch experiments should be upscaled to a reactor technology. Both the polishing of effluents and the treatment of more concentrated streams should be investigated in this context.

4.1.2 Perfluorinated compounds

Perfluorinated compounds (PFCs) consist of an apolar hydrophobic chain of 4 to 14 C-atoms, completely saturated with F-atoms, and a polar hydrophilic head consisting of an acid or sulfonate group. Since they have both hydrophobic and hydrophilic properties, they can interact with different phases and show surface-active characteristics. They are used as surfactants in coatings, paints and fire-resistant foams. They are also used as mother molecules for the synthesis of Teflon. These compounds are highly persistent and toxic and these substances are gradually being banned (Renner, 2006). These compounds are found as micropollutants in all environmental contaminants (including groundwater, surface water and drinking water) and also in food, since they tend to bioaccumulate. The most widely applied PFCs are perfluoroctanoic acid (PFOA) and perfluoroctanesulfonic acid (PFOS) (Figure 9-4). These compounds are highly resistant against biodegradation (Liou et al., 2010), therefore, catalytic defluorination by Pd-based catalysts is worth investigating.

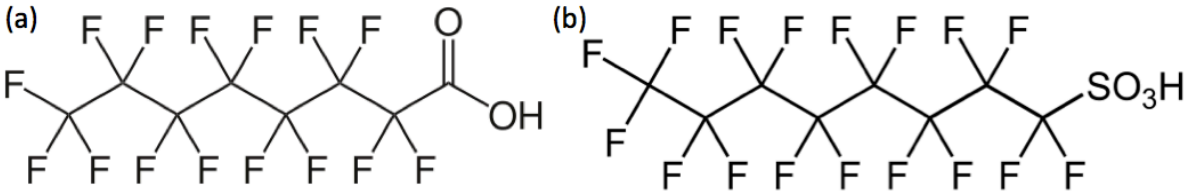


Figure 9-4: Chemical structures of PFOA (a) and PFOS (b).

4.2 Air contaminants

4.2.1 Volatile halogenated compounds

Several halogenated compounds are used in households daily. Some of these compounds can be volatile and release toxic vapors to indoor air, resulting in a decreased indoor air quality. These vapors can be toxic and contribute to the so-called 'sick-building syndrome'. Examples of such compounds are chloroform, used in bleaching agents, para-dichlorobenzene, used in moth balls and deodorants and brominated flame retardants (polybrominated difenyl ethers, PBDEs, Figure 9-5), used in furniture, textile and electronics. The debromination of PBDEs dissolved in water by bio-Pd on *D. desulfuricans* has been demonstrated before (Harrad et al., 2007). Their removal from indoor air has not yet been demonstrated.

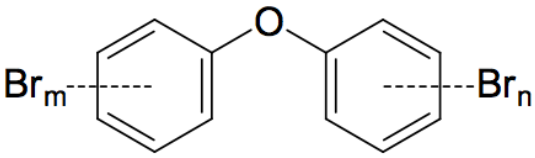


Figure 9-5: Chemical structure of brominated flame retardants (PBDEs).

In order to remove these compounds from indoor air by dehalogenation, a filter apparatus should be developed, which actively pumps the contaminated air over a catalytic bed or surface containing a bio-Pd based catalyst. An important challenge in this context will be the delivery of the hydrogen donor to the catalyst, since the use of H_2 will, due to safety reasons, probably not be feasible for indoor applications, other hydrogen donors should be used. However, these are generally dissolved in water (formate, lactate, ethanol). It should also be investigated whether these compounds can get in contact with the catalyst in a gaseous phase or whether they will need to be dissolved in a liquid first, which might cause important mass transfer limitations.

4.2.2 N_2O

N_2O or laughing gas is an important air contaminant since it is a severe greenhouse gas (GHG) and contributes significantly to global warming. It has a 310 times larger GHG potential than CO_2. Its presence in the atmosphere originates from several processes in the nitrogen cycle, mainly incomplete nitrification and denitrification (Lassey et al., 2007). The removal of N_2O by Pd (McCalman et al., 2012) and also Pd/Au catalysts (Wei et al., 2007) has been demonstrated.

However, a catalytic technology for the removal of N_2O will be extremely difficult to implement since emissions of N_2O are very diffuse. A more concentrated source could be the off-gasses of nitrification and denitrification tanks of wastewater treatment plants.

5 Alternative sustainable carriers for Pd nanocatalysts

5.1 Chitosan

In Chapter 8, amine-coated silica beads were shown to be valuable options as alternatives for bacteria as carriers for Pd nanoparticles. The importance of amine groups for the sorption of Pd to the bacterial surface has been demonstrated before (Rotaru et al., 2012). These free amine groups could be obtained from chemical coating materials such as APTES or PEI or from the biopolymer chitosan. The chemical coating with APTES was shown to be successful and could deliver highly active Pd nanocatalysts. However, the coating process is relatively complex, involves several toxic molecules and cannot really be considered sustainable. The coating with chitosan (Figure 9-6) seems a more sustainable alternative, since the coating involves less chemicals and chitosan is very abundant and relatively cheap (see Chapter 8). Moreover, the obtained Pd-on-chitosan catalysts were shown to be very active (especially compared to the bacteria-supported bio-Pd catalysts) and did not show any significant leaching of Pd on the short term (within 2-3 weeks).

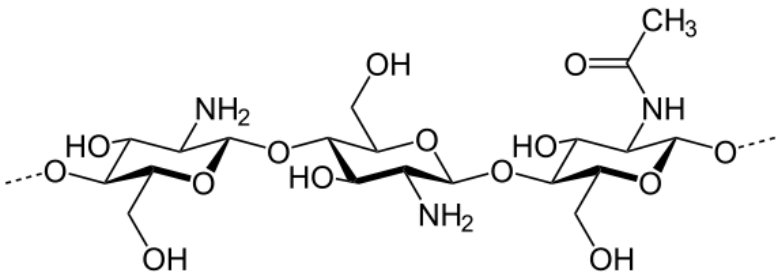


Figure 9-6: Chemical structure of chitosan.

It was shown that chitosan could be successfully coated on silica beads (Chapter 8). It should also be investigated what other materials could be successfully coated with chitosan. By coating materials with chitosan, different filter materials or surfaces with catalytic activity could be designed. The encapsulation of Pd-on-chitosan is possibly also easier than the encapsulation of bacteria-supported bio-Pd (Hennebel et al., 2009c). Also the formation of bimetallic Pd/Au on chitosan needs to be investigated.

5.2 Alginate

Bacteria can grow as free planktonic cells in a growth medium, but also as specific structures such as biofilms and granules. Within these structures, cells are grouped in a matrix of complex biopolymers, also known as extracellular polymeric substances (EPS), which acts as a glue between the different cells in the matrix. A polymer that is often present in these structures is alginate and its derivatives (Figure 9-7). Alginate can possibly be a good carrier for Pd nanoparticles, since it contains free carboxyl groups. The importance of carboxyl groups as anchoring sites for metal biosorption has been demonstrated before (Beveridge et al., 1980). The biosorptive capacities of alginate for metals have also been demonstrated in previous studies (Gotoh et al., 2004; Park et al., 2004).

The growth of bacteria in granules can possibly be advantageous for a better sorption of Pd. In presence of an electron donor, Pd nanoparticles can be formed. The deposition sites of the Pd nanoparticles (inside the granule or on the surface) should be studied first by microscopic techniques. The growth in granules offers also the advantage that the biomass (+ metals) can be easily separated from the reaction medium by sedimentation, so no expensive membranes or encapsulation techniques would be necessary.

The use of biofilms with free carboxyl groups as Pd carriers, could allow the development of different catalytic surfaces or catalytic filter beds in which biofilms with Pd are grown on carrier materials.

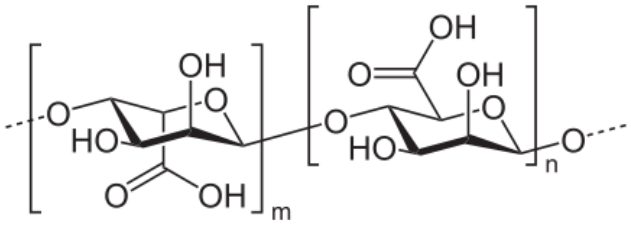


Figure 9-7: Chemical structure of alginate.

6 Added value for bacteria as carriers of Pd nanoparticles

Upon consideration of the different possible limitations of bacteria as carriers for Pd nanoparticles, one could come to the conclusion that bacteria do not have a future as carrier materials for Pd nanoparticles. Nevertheless, one should find an application where bacteria show a clear added value as carriers of the nanoparticles. An added value could be created when bacterial cells remain alive, for example at low metal concentrations (depending on the toxicity of the metal), so they are able to perform metabolic conversions. By doing so, compounds that cannot be converted metabolically can first be transformed by a catalytic reduction or oxidation first and subsequently be metabolized by the bacteria; or vice versa. This concept could be applied in both synthesis and degradation processes. In degradation processes, the goal should be to obtain a complete degradation of contaminants to CO_2 and H_2O or at least to completely harmless degradation products. The final objective of combining metabolic and catalytic processes for synthesis purposes should be to obtain products with a higher added value than the products obtained with separate processes.

An example is the conversion of glycerol. Glycerol, a biorefinery side product, can fermentatively be converted to added value products such as 1,3-propanediol through a complex metabolic pathway by engineered *E. coli* strains. Some conversion steps generate energy, others require energy in the form of reducing equivalents of NADH. When insufficient NADH is supplied, the pathway is stopped at a certain intermediate. Extra reducing equivalents could be delivered by a bio-Pd catalyst loaded with hydrogen. Glycerol could also be converted catalytically by oxidation or reduction first, followed by a metabolic conversion. These processes can take place sequentially in separate cells (Figure 9-8a). However, a final goal should be to combine the metabolic and catalytic conversion in one and the same cell (Figure 9-8b).

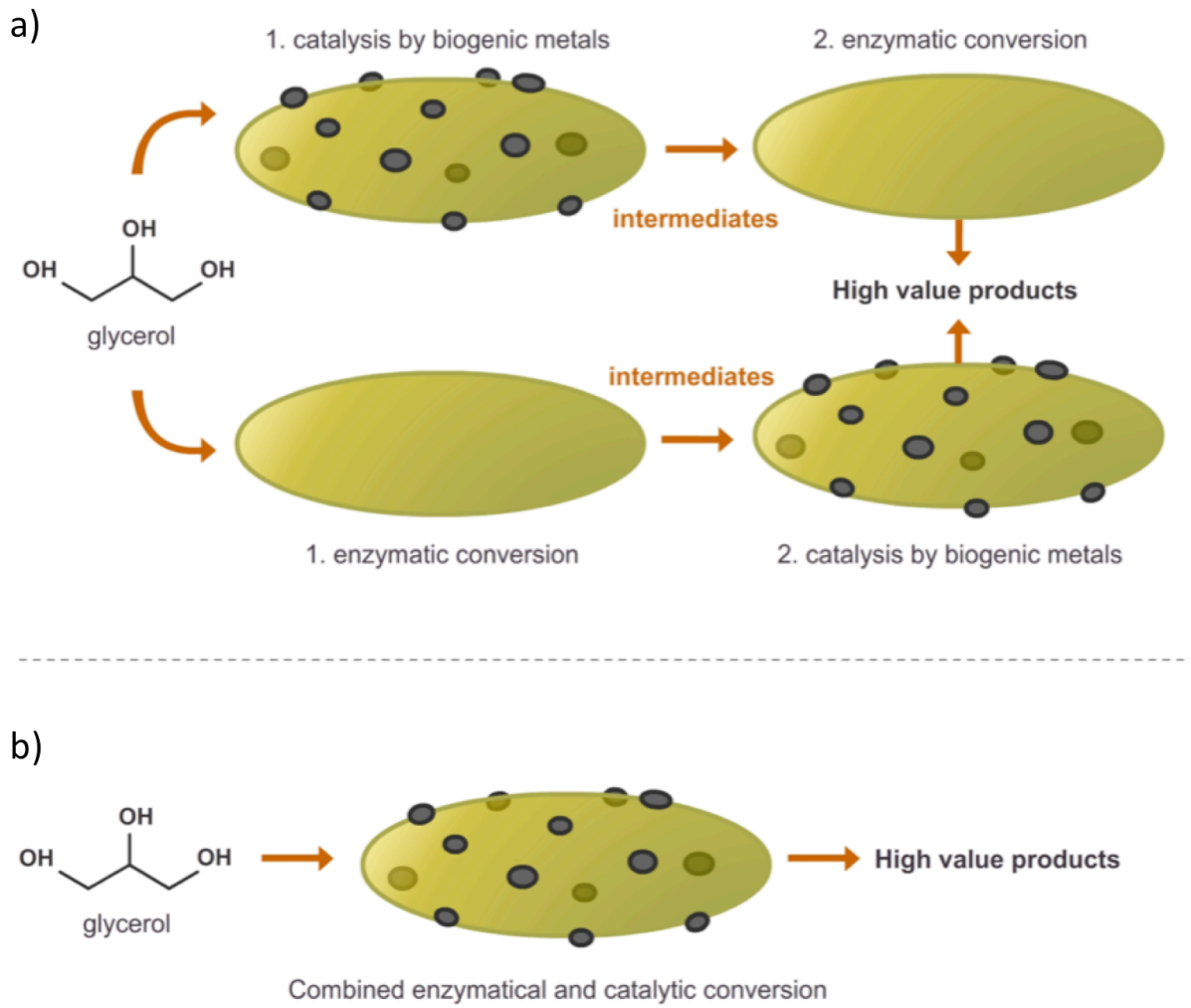


Figure 9-8: (a) Sequential catalytic and metabolic conversion of glycerol, (b) Combined metabolic and catalytic conversion of glycerol by one cell.

7 Biosorption as a metal recovery process

In this work, catalysts were always synthesized starting from a synthetic metal salt. However, the processes metal biosorption and reduction could also be applied to recover metals from wastestreams.

7.1 Critical material recovery

The possible insufficient supply of raw materials (metals and minerals) can possibly hamper the economic development of Europe. In contrast to other continents, Europe is very poor in primary resources and is dependent of import from other continents, where political climates are often instable, causing extremely high resource prices. The geopolitical dependency of primary resources is one of the most important challenges for future generations.

Therefore, the European Union defined a list of 14 critical raw materials in the landmark report 'Critical raw materials for the European Union (2010)'. The criticality of a material based on both the economical importance and the supply risk (Figure 9-9).

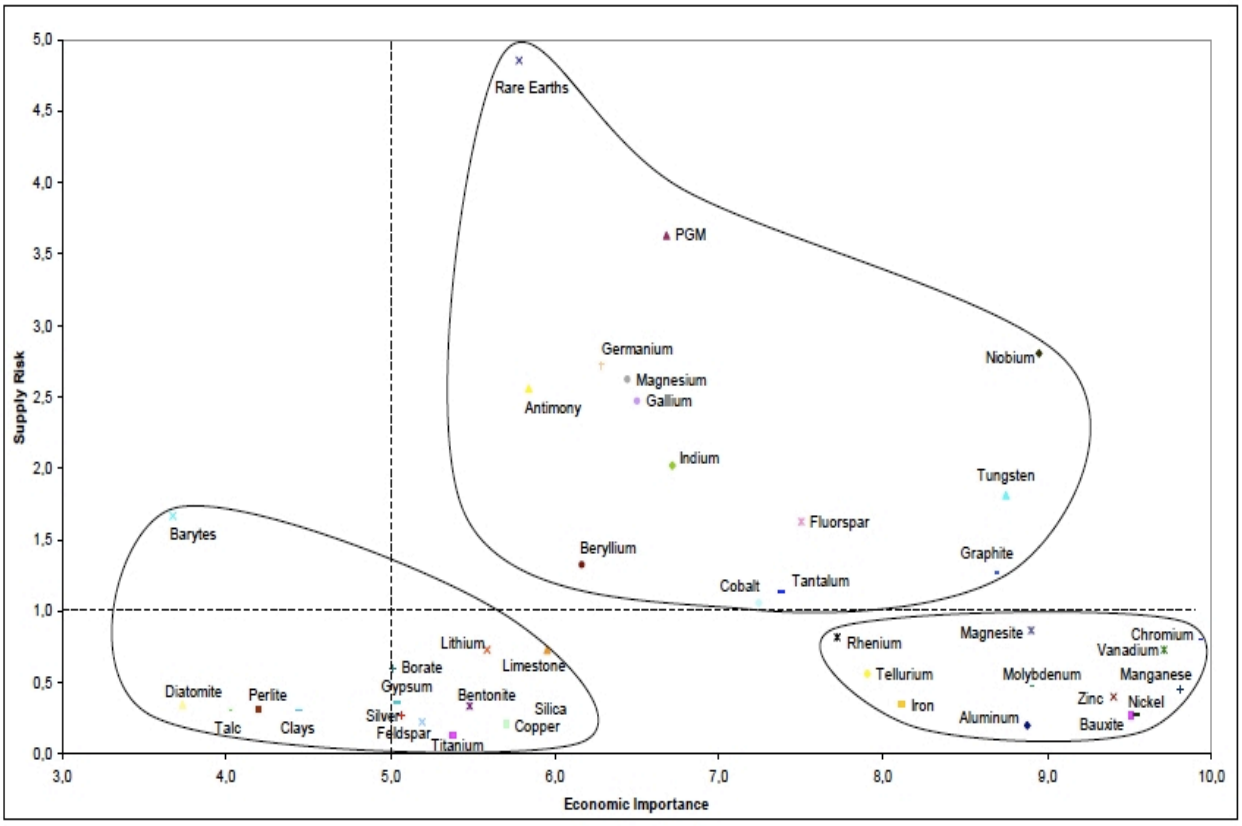


Figure 9-9: Critical materials for the European Union based on their economic importance and supply risk (from the report Critical Raw Materials for the European Union (2010))

Two groups of these 14 materials have a very high supply risk: rare earth elements (REEs) and platinum group metals (PGMs). Especially for PGMs, the instability of their supply is reflected in the price volatility. In the last 5 years, prices of Pd varied from less than € 5000 kg^{-1} in 2008 to more than € 23000 kg^{-1} in 2011 (Figure 9-10).

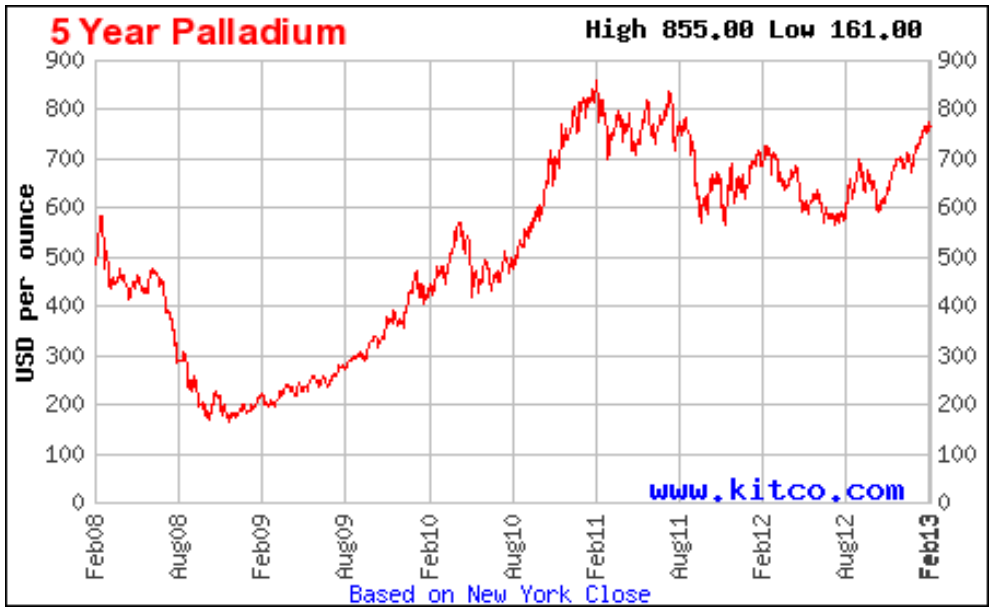


Figure 9-10: Evolution of the Pd price (in US dollar per ounce) between 2008 and 2013 (from www.palladiumprice.org).

A resource efficient economy is a necessary condition to assure technological progress for future generations. This resource efficiency can take place through:

- A sustainable trade and investment policy;
- Increased sustainable primary mining in Europe, including deep-sea mining;
- Substution of critical metals by less critical metals;
- Increased use of secondary raw materials, 'material recovery' (Jones et al., 2011):
    - Direct recycling of pre-consumer manufacturing scrap/residues;
    - Urban mining of post-consumer end-of-life products;
    - Landfill mining of historic and urban wastestreams.

Metal recovery can be important from an economic point of view, since recovered products can serve as resources and valuable materials, which were previously considered as wastes, can be reused. Moreover, it can also have an ecological value, since it prevents metals from entering in the environment and ecosystems, where they can exhibit toxic effects.

7.2 Bacteria for metal recovery

Several bacterial processes are potentially interesting for metal recovery. Metal recovery from solid wastestreams can occur through the process of so-called 'bioleaching'. This process is based on the action of iron and sulfur oxidizing bacteria. It is a slow release process and is industrially applied in South Africa and Chile, mainly for recovery of Fe

and Cu from ores. It is worth investigating whether the principles of bioleaching could also apply for metal containing solid wastestreams such as printed circuit boards, lamp phosphors and phosphogypsum.

More closely related to this work is the recovery of metals from aqueous wastestreams by sorption on bacterial cells and biopolymers. Several industries generate aqueous wastestreams containing critical metals. Examples are wastewaters from metal recycling companies and washing liquids from liquid crystal display (LCD) screens and printed circuit boards. Hospitals also use critical metals, such as Pt in cancerostatic compounds and the REE Gd in radiographic contrast media. Conventional recovery processes make use of hydrometallurgical, pyrometallurgical and/or electrometallurgical techniques. These techniques can be very efficient, but require sometimes a significant input of chemicals and energy. When these metals are present at low concentrations, this can mean a too high energy input per amount of metal recovered. Biosorption processes can be a sustainable alternative for metal recovery from dilute aqueous wastestreams (µg – mg L^{-1}) in cases where other techniques are not feasible anymore.

Biosorption should serve as an upconcentration technique. The resulting product will be sludge loaded with relatively high concentrations of critical metals, which can be processed further by hydro-, pyro- or electrometallurgical techniques. Another possibility is that metal nanoparticles are produced by reduction or oxidation of the metal salts on the cell walls.

Bacteria are suitable sorbents for metals due to their very high specific surface area (> 100 m^2/g), and the presence of different functional groups, of which carboxyl groups (Beveridge et al., 1980) and amine groups (Rotaru et al., 2012) are probably the most important ones. Moreover, bacteria can grow in structures where they produce sorptive biopolymers such as alginates, providing extra functional groups as sites for metal sorption. Granules of bacteria are easy to separate from the reaction medium, which makes them interesting for reactor design, for example in sequential batch reactors (Figure 9-11). Also other biopolymers such as chitosan should be tested as biosorbents.

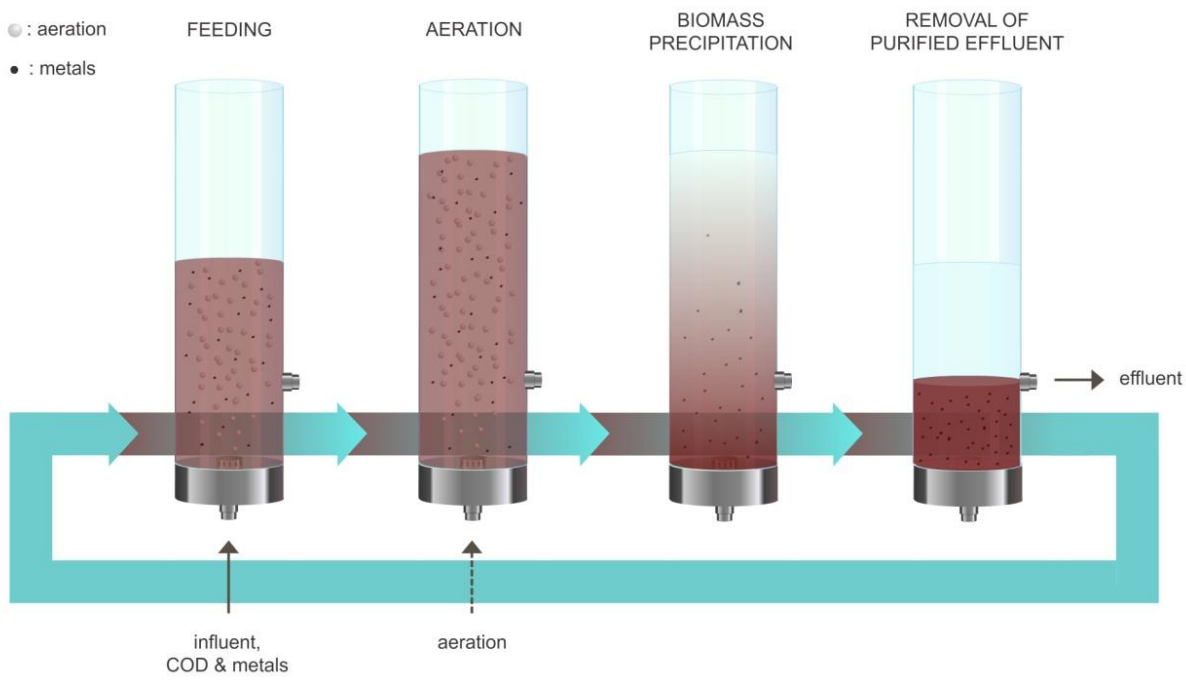


Figure 9-11: Sequential batch reactor for the recovery of critical metals from aqueous wastestreams.

Biosorption processes can be intensified physically, for example by centrifugal contacting. In a centrifugal contactor (for a review: see Vedantam et al. (2006)), the contact between the bacteria and the metal containing wastestream can be optimized in order to minimize contact times and increase the recovery rates. Another potentially interesting technique to intensify biosorption is ultrasound. The use of ultrasound can stimulate microbial growth, but can also stimulate the erosion of solid substrates or suspended particles in water (Sukla et al., 1995; Swamy et al., 2005) (ideas after prof. Tom Van Gerven).

An important challenge for the application of biosorption in metal recovery processes will be the sometimes very aggressive characters of these streams. Wastewaters from metallurgical processes often contain high concentrations (up to several g L^{-1}) of salts and can be extremely acid (pH 1 and lower). The compatibility of the different bacterial strains and cultures with these sometimes harsh conditions should be investigated first. Hospital wastewaters show the disadvantage that these metals are present in very low concentrations (µg L^{-1} and lower), but the composition of the wastewater is probably more compatible with bacteria.

8 Take home messages

During the last decades, bacteria have extensively been investigated as producers and carriers of Pd nanoparticles ('bio-Pd'). In this work some limitations of bio-Pd catalysts were discussed and some possible alternatives were proposed. A first limitation is the limited catalytic activity compared to Pd nanoparticles on conventional supports such as Pd/C or Pd/Al_2O_3. It was shown that doping the bio-Pd catalyst with Au could increase the catalytic activity. Coprecipitation of Pd and Au was required in order to obtain a highly active biosupported bimetallic Pd/Au catalyst. A small doping with a few wt. % of Au can be sufficient to increase the catalytic activity significantly. The increased activity of the bio-Pd/Au catalyst was demonstrated for the dechlorination of TCE and diclofenac and for the Suzuki C-C cross coupling.

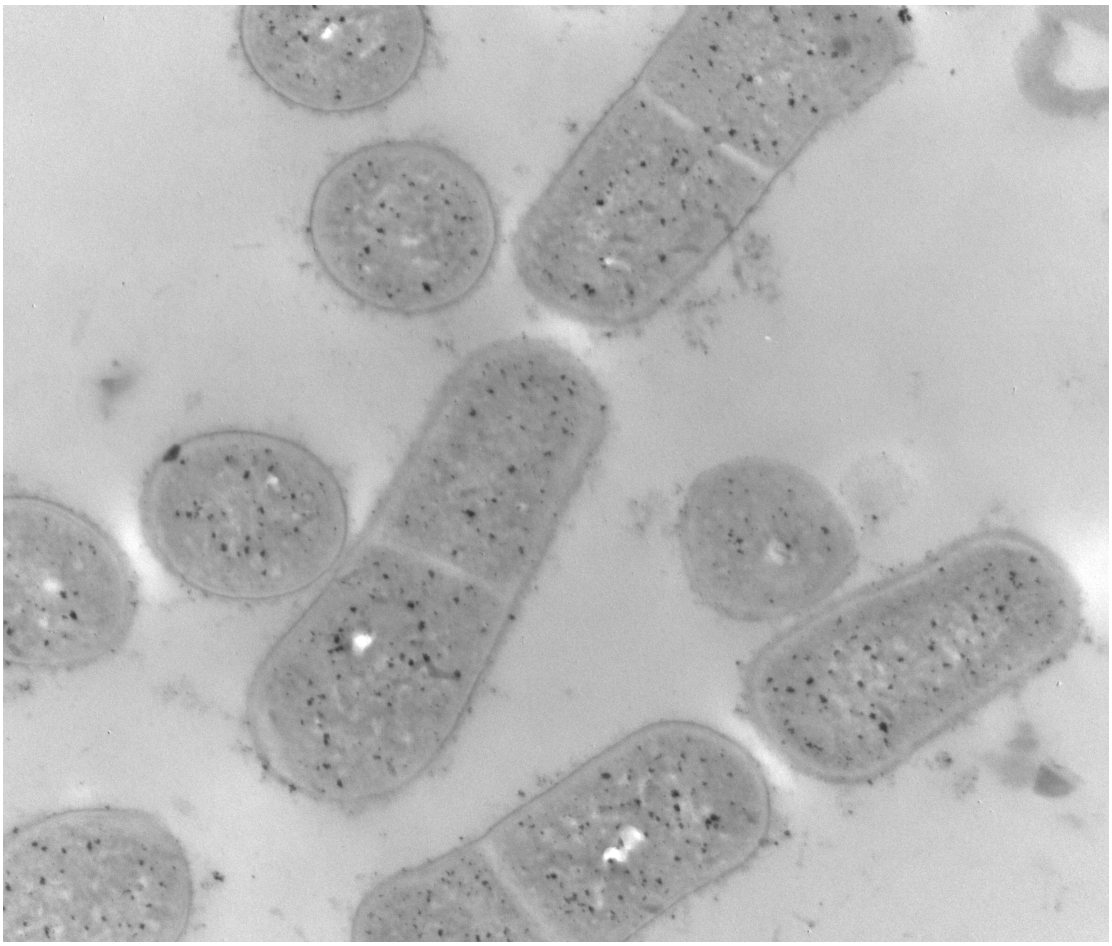
A second limitation is the leaching of Pd from the bacterial carrier. It was shown that leaching can be significant and can occur within a few weeks of storage of the catalyst. To minimize leaching, high temperatures, alkaline pH and anaerobic conditions should be avoided.

In order to avoid poisoning by bacterial sulfur, Pd catalysts can be supported on other biopolymers such as chitosan. The obtained catalysts showed a higher catalytic activity than bio-Pd catalysts and did not show leaching on short term.

Bacteria can have an added value as support, for example when living bacteria covered with metal nanocatalytsts can combine metabolic and catalytic processes. The biosorption of metals by bacteria and biopolymers can potentially be interesting as a metal recovery technique. Especially aqueous wastestreams with low concentrations of critical elements seem interesting for the application of biosorption.

Part V

Appendices



Abstract

Palladium (Pd) has been applied as a catalyst for a wide variety of chemical reactions. Nowadays, Pd catalysts are more and more used under the form of nanoparticles, which have a higher specific surface area (surface area per unit of volume) compared to bulk Pd. The increasing demand for Pd nanocatalysts has stimulated the research for more bio-based production methods. In the last decades, the use of bacterial cells as producers and carriers of nanoparticles has been extensively investigated ('bio-Pd'). Although the applicability of bio-Pd catalysts has been demonstrated for the degradation of environmental contaminants (mainly halogenated contaminants in soils, sediments, groundwater and wastewater) and in synthetic organic chemistry, bio-Pd catalysts show some possible drawbacks and limitations. It was demonstrated that their catalytic activity is lower than the activity of Pd nanoparticles on conventional supports such as Al_2O_3 or activated carbon. The stability of the catalysts has not been thoroughly studied and the catalysts are potentially inhibited by sulfides, which can be of bacterial origin.

It was demonstrated previously that the activity of Pd catalysts can be increased by doping with Au. In a first part of this work, a bio-Pd/Au catalyst was synthesized on the cell wall of *Shewanella oneidensis*. Therefore, the interaction of *S. oneidensis* with Au was investigated first (Chapter 3). It was found that Au(III) sorbed rapidly on the bacterial cells (within minutes). This biosorption process was improved at lower pH and in presence of an electron donor. The reduction process of Au(III) to Au(0) took 8 to 24 hours to initiate and could only take place in presence of an electron donor. The particle size and the deposition site of the Au(0) particles were dependent on the initial Au(III) concentration (small intracellular particles at lower concentrations, bigger particles on the cell wall at higher concentrations). The reduction process of Au on *S. oneidensis* was believed to be mainly abiotic.

In Chapter 4, several strategies were investigated to synthesize a bimetallic bio-Pd/Au catalyst with increased catalytic activity. Only the catalyst obtained by coprecipitation of Pd and Au on the cell walls of *S. oneidensis* could significantly increase reaction rates for the dechlorination of diclofenac, a pharmaceutical micropollutant in wastewaters, and trichlorethylene, a common groundwater contaminant. The unique bimetallic alloy structure of the catalyst, with a contracted lattice compared to the monometallic catalysts, could be demonstrated by synchrotron-based μXRD and electron microscopy

techniques. Several hypotheses can be put forward to explain this effect, mainly geometric (better contact between reagents and more isolated active Pd sites) and electronic effects (electronic rearrangements within the crystal lattice causing better transfer of electrons). Also the protective effect of Au against poisoning by sulfur compounds has been hypothesized. The doping of bio-Pd with Au could be promising. However, several other challenges can be seen in this domain. For example the synthesis of Au-Pd core-shell structures, which were shown to have an extremely high catalytic activity.

The dechlorination of diclofenac by bio-Pd and bio-Pd/Au was studied more in detail in Chapter 5. Diclofenac could be dechlorinated using a bio-Pd catalyst but only at low pH, whereas a bio-Pd/Au catalyst could perform the dechlorination in a broader pH range. A maximum reaction rate was obtained at Pd/Au weight ratio of 50/1 (increase with a factor 7 compared to the Pd/Au 50/50 catalyst). So only a small doping with 2 wt. % Au was sufficient to significantly increase the activity of the bio-Pd catalyst. Whereas the 50/1 bio-Pd/Au catalyst could completely remove 20 mg L^{-1} diclofenac from synthetic water in 24 hours, 6.40 µg L^{-1} could only be removed from the effluent of a hospital wastewater treatment plant for 43 % after 24 hours. This decreased activity was probably due to the presence of sulfur, organic matter and other halogenated compounds. The dehalogenation of halogenated wastewater contaminants over (bio-)Pd and Pd/Au catalysts is promising, since future legislation will evaluate the presence of these compounds (grouped under the parameter adsorbable organic halogens (AOX)) in effluents. However, in wastewater treatment, flow rates are usually high and hydraulic residence times are low. In order to be a competitive technique for effluent polishing of wastewater treatment plants, the reaction rates obtained in this work should be significantly increased.

The difference in catalytic activity between the monometallic bio-Pd and bimetallic bio-Pd/Au catalyst was also tested for synthetic reactions. The bio-Pd/Au catalyst also showed a higher activity than bio-Pd for the Suzuki C-C cross coupling of an aryliodide with a boronate and showed a higher reproducibility than bio-Pd (Chapter 6). Nevertheless, these rates were not yet competitive with a conventional Pd/C catalyst. The higher conversion rates were especially observed with electron donating substituents. Bio-Pd/Au also showed a broader reaction scope than the monometallic bio-Pd when comparing different arylhalides.

In the second part of this work, limitations and innovations of bio-Pd were discussed. In Chapter 7, the stability of bio-Pd catalysts was studied as a function of time. A significant leaching of zerovalent Pd particles from the carrier took place: up to 18 % of the initially added Pd had leached from the carrier after 100 days of storage in water at 21 °C. This leaching decreased when storage was at lower temperature and in a mineral medium. Moreover, an increased leaching was observed at alkaline pH and under anaerobic conditions. Less leaching took place with *Cupriavidus metallidurans* as carrier than with *S. oneidensis*, however, bio-Pd on *S. oneidensis* showed a higher catalytic activity. When the leached Pd was kept in the reaction medium, no decrease of catalytic activity could be observed. Nevertheless, the leaching of Pd from the bacterial carrier can be seen as an important drawback of bio-Pd catalysts, since it can cause economical losses and environmental risks. Another important limitation of bio-Pd catalysts is the poisoning of the catalyst surface with sulfides from bacterial origin, as demonstrated by other studies previously. In Chapter 8, a possible alternative for bacteria as carrier for Pd nanoparticles was developed. Silica particles with a comparable surface area as bacteria were functionalized with amine groups, which were shown to be important functional groups for sorption of Pd(II) and anchoring sites for Pd nanoparticles. This functionalization was performed chemically (APTES provided a functionalization with primary amines, PEI provided a functionalization with secondary amines) and with the biopolymer chitosan. Beads with APTES and chitosan could firmly attach Pd nanoparticles on their surface. These Pd catalysts all showed a higher catalytic activity for the reduction of *p*-nitrophenol to *p*-aminophenol. At low Pd/support material ratios, complete inhibition of catalytic activity could be observed for bacteria supported bio-Pd catalysts. This was not observed with Pd on coated beads. Moreover, these beads did not show significant leaching on short term. Especially chitosan appears to be an interesting alternative support material, since it is a very abundant biopolymer and is relatively cheap. The coating of other surfaces with chitosan and other biopolymers as carriers of Pd nanoparticles should be investigated.

Some further challenges for the above described catalysts in environmental technology can be put forward. The catalytic activity of the different biosupported Pd and Pd/Au catalysts should be tested for degradation of other types of recalcitrant environmental contaminants, such as fluorinated pharmaceuticals and highly persistent perfluorinated surfactants. Also the reductive catalytic treatment of air contaminants should be investigated.

Bacteria can be an interesting support for Pd nanoparticles, but they should have an added value. This could for example be obtained when bacteria are kept alive after the reduction of the metals, so they can perform metabolic conversions. By doing so, metabolic and catalytic conversions can be obtained within one cell. Better degradations of environmental contaminants can thus be obtained and products with higher value could be synthesized. Another important application of the interaction between bacteria and metals is the recovery of critical metals from wastestreams. Especially in Europe, the supply of raw materials, such as Pd, is highly threatened due to the limited presence of ores and geopolitical reasons. Recovery of critical metals can be important both from an economical and environmental perspective. Aqueous wastestreams containing low concentrations (µg – mg L^{-1}) of critical metals seem the most interesting streams to treat biometallurgically, since metals can be concentrated by biosorption on bacterial cells or granules. The interaction between bacterial cells and Pd should be considered not only for the synthesis of catalysts but in the broader perspective of resource recovery from streams, which were previously considered as wastes.

Samenvatting

Palladium (Pd) wordt gebruikt als katalysator voor een hele reeks chemische reacties. Dezer dagen worden Pd katalysatoren meer en meer gebruikt als nanopartikels, deze hebben immers een groter specifiek oppervlak (oppervlakte per volume-eenheid) dan bulk Pd. De groeiende vraag naar Pd nanokatalysatoren heeft het onderzoek naar duurzame productiemethoden gestimuleerd. In de laatste decennia is het gebruik van bacteriële cellen als producenten en dragermateriaal voor Pd ('bio-Pd') uitgebreid onderzocht. Verschillende bacteriële stammen zijn beschreven als producenten van bio-Pd nanopartikels. Hoewel de toepasbaarheid van bio-Pd katalysatoren voor de afbraak van milieucontaminanten (vooral gehalogeneerde contaminanten in bodems, sedimenten, grond- en afvalwater) en in organische synthese uitgebreid werd aangetoond en een aantal reactoren op laboschaal en pilootschaal werden ontwikkeld, tonen bio-Pd katalysatoren enkele mogelijke nadelen en beperkingen. Er werd aangetoond dat hun activiteit lager is dan the activiteit van Pd nanopartikels op conventionele dragermaterialen als Al_2O_3 en actieve kool. The stabiliteit van de katalysatoren werd nog niet grondig onderzocht en de katalysatoren worden mogelijk geïnhibeerd door sulfides, die van bacteriële oorsprong kunnen zijn.

Er werd in eerder onderzoek reeds aangetoond dat de activiteit van Pd katalysatoren verhoogd kan worden door dopering met Au. In een eerste deel van dit werk werd een bimetallische bio-Pd/Au katalysator gesynthetiseerd op de celwand van *Shewanella oneidensis*. Daartoe werd eerst de interactie van *S. oneidensis* met Au onderzocht (Hoofdstuk 3). Er werd vastgesteld dat Au(III) snel werd gesorbeerd op de bacteriële cellen (binnen een tijdsspanne van minuten). Dit biosorptieproces verliep sneller bij lagere pH en in aanwezigheid van een elektrondonor. De reductie van Au(III) tot Au(0) begon pas na 8 tot 24 uur en kon enkel plaatsvinden in aanwezigheid van een elektrondonor. Deeltjesgrootte en de plaats van precipitatie van de Au(0) partikels waren afhankelijk van de initiële Au(III) concentratie (kleine intracellulaire partikels bij lagere concentraties, grotere partikels op de celwand bij hogere concentraties). Het reductieproces van Au op *S. oneidensis* verliep wellicht hoofdzakelijk abiotisch.

In Hoofdstuk 4 werden verschillende strategieën onderzocht om een bimetallische bio-Pd/Au katalysator te synthetiseren met verhoogde katalytische activiteit. Enkel de katalysator die bekomen werd door coprecipitatie van Pd en Au op de celwand van *S.*

oneidensis kon een significante verhoging bewerkstelligen van de reactiesnelheid voor de dehalogenatie van diclofenac, een toxische farmaceutische micropolluent in afvalwaters, en trichloroethyleen, een vaak voorkomende grondwater contaminant. De unieke legeringsstructuur van de katalysator, met een contractie van het kristalrooster vergeleken met de monometallische katalysatoren, kon aangetoond worden met synchrotron-gebaseerde μXRD en elektron-microscopische technieken. Verschillende hypothesen kunnen vooropgesteld worden om dit effect te verklaren, vooral geometrische (beter contact tussen reagentia en meer geïsoleerde actieve Pd sites) en elektronische effecten (verandering van de elektronenstructuur met betere elektronentransfer). Ook het beschermende effect van Au tegen vergiftiging van de katalysator door zwavelcomponenten is als hypothese naar voor geschoven. Het doperen van bio-Pd met Au is veelbelovend. Echter, verschillende andere uitdagingen kunnen in dit domein vooropgesteld worden, bijvoorbeeld de synthese van Au-Pd kern-schil structuren, waarvan de extreme hoge katalytische activiteit reeds aangetoond werd.

De dechlorinatie van diclofenac door bio-Pd en bio-Pd/Au werd meer in detail bestudeerd in Hoofdstuk 5. Diclofenac kon enkel bij een lage pH door een bio-Pd katalysator gedechlorineerd worden, terwijl bio-Pd/Au de dechlorinatie in een breder pH bereik kon bewerkstelligen. Een maximale reactiesnelheid werd bekomen bij een Pd/Au gewichtsverhouding van 50/1 (stijging met een factor 7 vergelijken met de Pd/Au verhouding van 50/50). Dus slechts een beperkte dopering met 2 gewichtsprocent Au was voldoende om te resulteren in een significante verhoging van de activiteit van de bio-Pd katalysator. Terwijl de 50/1 bio-Pd/Au katalysator 20 mg L^{-1} diclofenac volledig kon verwijderen in 24 uur, kon dezelfde katalysator 6.40 μg L^{-1} diclofenac slechts voor 43 % verwijderen uit het effluent van de waterzuiveringsinstallatie van een ziekenhuis. Deze verlaagde activiteit was wellicht te wijten aan de aanwezigheid van zwavel, organisch materiaal en concurrerende componenten. De dehalogenatie van gehalogeneerde afvalwatercontaminanten over (bio-)Pd en Pd/Au katalysatoren is veelbelovend, zeker als toekomstige wetgeving de aanwezigheid van deze stoffen (gegroepeerd onder de parameter AOX) in effluenten zal beperken. Daarbij dient wel vermeld te worden dat in afvalwaterzuivering de debieten hoog zijn en de hydraulische verblijftijden laag (typisch in de grootteorde van een paar uren). Om een competitieve effluentzuiveringstechniek te worden zullen de reactiesnelheden die in dit werk bekomen werden nog significant verhoogd moeten worden.

De bio-Pd/Au katalysator toonde ook een hogere activiteit dan bio-Pd voor de Suzuki C-C cross koppelingsreactie van een aryljodide met een boronaat en vertoonde daarbij ook een hogere reproduceerbaarheid dan bio-Pd (Hoofdstuk 6). Desalniettemin waren deze reactiesnelheden nog niet competitief met een conventionele Pd/C katalysator. De betere omzettingen werden vooral gezien bij elektrongevende substituenten. Bio-Pd/Au vertoonde ook een breder reactiespectrum dan de monometllische bio-Pd katalysator bij vergelijking van verschillende arylhalides.

In het tweede deel van dit werk werden andere beperkingen en innovaties van bio-Pd bestudeerd. In Hoofdstuk 7 werd de stabiliteit van bio-Pd katalysatoren bestudeerd in functie van de bewaringstijd. Een significante uitloging van nulwaardige Pd partikels van de bacteriële carrier vond plaats: tot 18% van het initieel toegevoegde Pd was uitgeloogd van de carrier na 100 dagen bewaring bij 21°C. Deze uitloging kon verlaagd worden door te bewaren bij lagere temperatuur en in een mineraal medium. Een verhoogde uitloging werd vastgesteld bij alkalische pH en onder anaerobe omstandigheden. Minder uitloging vond plaats met *Cupriavidus metallidurans* als drager dan met *S. oneidensis*, maar bio-Pd op *S. oneidensis* vertoonde een hogere katalytische activiteit. Als al het uitgeloogde Pd in het reactiemedium werd gehouden, werd geen daling van de katalytische activiteit waargenomen. Desalniettemin kan de uitloging van Pd van de bacteriële carrier als belangrijk nadeel van bio-Pd katalysatoren beschouwd worden, want dit kan leiden tot economische verliezen en risico's voor het milieu. Een andere belangrijke beperking van bio-Pd katalysatoren is de vergiftiging van het katalysatoroppervlak met sulfiden van bacteriële oorsprong, zoals aangetoond door vroegere studies. In Hoofdstuk 8 werd een mogelijk alternatief voor bacteriën als dragermateriaal voor Pd nanopartikels ontwikkeld. Silica partikels met een gelijkaardig oppervlak als bacteriën werden gefunctionaliseerd met amine groepen, die belangrijke functionele groepen voor Pd(II) bleken te zijn en aanhechtingsplaatsen voor Pd nanopartikels. Deze functionalisatie werd zowel chemisch (APTES zorgde voor functionalisatie met primaire amines, PEI met secundaire amines) als met het biopolymeer chitosan uitgevoerd. Partikels met APTES en chitosan konden Pd nanopartikels stevig op hun oppervlak binden. Deze Pd katalysatoren vertoonden allen een hogere katalytische activiteit dan Pd op bacteriën voor de reductie van p-nitrofenol tot p-aminofenol. Bij lage Pd/dragermateriaal ratio's kon complete inhibitie van de katalytische activiteit vastgesteld worden voor bio-Pd katalysatoren op bacteriën. Deze inhibitie vond niet plaats bij Pd op de gecoate silica partikels. Bovendien werd op korte termijn geen uitloging van Pd vastgesteld. Vooral chitosan lijkt een interessant alternatief dragermateriaal aangezien het een veel voorkomend en goedkoop

biopolymeer is. Het coaten van andere oppervlakken met chitosan en andere biopolymeren als dragermaterialen voor Pd nanopartikels kan verder worden onderzocht.

Er kan gedacht worden over enkele nieuwe uitdagingen voor de hierboven beschreven katalysatoren in de milieutechnologie. De katalytische activiteit van de verschillende Pd en Pd/Au katalysatoren op biomaterialen kan getest worden voor de afbraak van andere types recalcitrante milieucontaminanten zoals gefluoreerde farmaceutica en sterk persistente geperfluoreerde surfactanten. Ook de reductieve katalytische behandeling van luchtcontaminanten zou onderzocht kunnen worden.

Bacteriën kunnen een interessant dragermateriaal zijn voor Pd nanopartikels, maar dan moeten ze wel een toegevoegde waarde bieden. Deze toegevoegde waarde zou kunnen verkregen worden als de bacteriën in leven blijven na de reductie van de metalen zodat ze metabolische omzettingen kunnen uitvoeren. Op die manier kunnen metabolische en katalytische omzettingen door één en dezelfde cel bekomen worden. Zo kan een verdergaande afbraak van milieucontaminanten bekomen worden en kunnen hoogwaardige producten gesynthetiseerd worden. Een andere belangrijke toepassing van de interactie tussen bacteriën en metalen is de terugwinning van metalen uit afvalstromen. Vooral in Europa is de toevoer van grondstoffen zoals Pd sterk bedreigd door de beperkte beschikbaarheid van de ertsen en omwille van geopolitieke redenen. Terugwinning van metalen is belangrijk zowel vanuit een economisch als een ecologisch standpunt. Waterige afvalstromen die lage concentraties (µg – mg L^{-1}) kritische metalen bevatten, lijken de interessantste stromen om biometallurgisch te behandelen omdat de metalen kunnen geconcentreerd worden door biosorptie op bacteriële cellen of granules. De interactie tussen bacteriële cellen en Pd dient dus niet enkel beschouwd te worden voor de synthese van katalysatoren, maar in het bredere perspectief van de terugwinning van metalen uit stromen, die voorheen als afval werden beschouwd.

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Curriculum vitae

Personal details

Full name	Simon De Corte
Date of birth	15/08/1986
Place of birth	Knokke-Heist, Belgium
Nationality	Belgian
Address	Sint-Lievenspoortstraat 287, B-9000 Gent
Phone	+32 472 395580
E-mail	simondecorte@gmail.com

Education

2009 – 2013 **PhD in Applied Biological Sciences**, Laboratory of Microbial Ecology and Technology (LabMET), Ghent University

- *Funding:* Fund for Scientific Research Flanders (FWO-Vlaanderen)
- *PhD thesis:* Optimizations, limitations and innovations of biosupported Pd nanocatalysts
- *Promoters:* Prof. dr. ir. N. Boon and Prof. dr. ir. W. Verstraete

2004 – 2009 **Master in Bioscience Engineering: Environmental Technology**, Ghent University (graduated with great distinction)

- *Master thesis:* Biological Pd nanoparticles in membranes for removal of micropollutants from wastewater
- *Promoters:* Prof. dr. ir. N. Boon and Prof. dr. ir. W. Verstraete
- Erasmus exchange program (Feb – Jun 2008): Polytech Montpellier, France

1998 – 2004 **Science – mathematics (8h)**, Sint-Jozefsinstituut-college Torhout

Professional activities

2013 **Environmental engineer wastewater treatment**, Trevi NV

2009 – 2013 **Scientific collaborator**, LabMET, Ghent University

- Teaching:
  - Coordinator and teacher of courses:
    - Practical exercises ‘Microbial Ecological Processes’

        - Calculation exercises 'Biotechnological processes in environmental sanitation'
    - Tutor of 4 master thesis students in Bioscience Engineering

- Coordinator and researcher on projects commissioned by industry:
    - Janssen Pharmaceutica (Geel, Belgium): B. Mertens
    - Solvay (Brussels, Belgium): F. Desmedt
    - Biorem engineering (Stella Plage, France): W. De Windt
    - Avecom NV (Wondelgem, Belgium): R. Daneels

Jan - Feb 2013 **Internship** at Trevi NV: processing of thick fractions of digestates

Jan - Mar 2012 **Visiting researcher** at Aarhus University, Denmark (Interdisciplinary Nanoscience Centre iNano, Prof. dr. R. Meyer): functionalization of carriers of Pd nanoparticles

Scientific publications

Peer-reviewed publications

T. Hennebel, **S. De Corte,** L. Vanhaecke, K. Vanherck, I. Forrez, B. De Gusseme, P. Verhagen, K. Verbeken, B. Van der Bruggen, I. Vankelecom, N. Boon, W. Verstraete. 2010. Removal of diatrizoate with catalytically active membranes incorporating microbially produced palladium nanoparticles. Water research 44(5): 1498-1506

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Other publications

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S. De Corte, T. Hennebel, S. Verschuere, C. Cuvelier, N. Boon, W. Verstraete. Gold nanoparticle formation using *Shewanella oneidensis*. Environmental applications and implications of nanotechnology at 240th ACS national meeting, Boston, USA, August 22-26 2010. **Oral presentation**

T. Hennebel, S. Van Nevel, **S. De Corte**, J.P. Fitts, D. Van der Lelie, N. Boon, W. Verstraete. Palladium nanoparticle formation by *Citrobacter*: Effective catalysts for diatrizoate removal. Environmental applications and implications of nanotechnology at 240th ACS national meeting, Boston, USA, August 22-26 2010. **Oral presentation (co-author)**

S. De Corte, T. Hennebel, S. Van Nevel, B. De Gusseme, N. Boon, W. Verstraete. Biogenic palladium: a nanocatalyst produced in a green way. 1st international workshop on nanostructured materials for sorption, separation and catalysis, Antwerp, Belgium, October 4-5 2010. **Oral presentation**

S. De Corte, T. Hennebel, S. Van Nevel, B. De Gusseme, N. Boon, W. Verstraete. Biogenic palladium: a nanocatalyst produced in a green way. 1st international workshop on nanostructured materials for sorption, separation and catalysis, Antwerp, Belgium, October 4-5 2010. **Poster presentation**

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W. Verstraete, B. De Gusseme, **S. De Corte**, L. Sintubin, N. Boon, T. Hennebel. Biometals for advanced water treatment. First international conference on sustainable chemistry, Antwerp, Belgium, July 6-8 2011. **Invited oral presentation (co-author)**

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T. Hennebel, **S. De Corte**, N. Boon, W. Verstraete. Biogenic bimetallic nanoparticles: the holy grail in wastewater treatment? Clean water trough bio- and nanotechnology, Lund, Sweden, May 7-9 2012. **Invited oral presentation (co-author)**

W. Verstraete, T. Hennebel, **S. De Corte**, N. Boon. Biogenic metals for applications in environmental technology. 4th International Conference on Engineering for Waste and Biomass Valorization, Porto, Portugal, September 10-13 2012. **Invited oral presentation (co-author)**

S. Maes, **S. De Corte**, T. Hennebel, N. Boon, W. Verstraete. 2013. Recovery of metals using biometallurgy. Third International Slag Valorisation Symposium, Leuven, Belgium, March 19-20 2013. **Invited oral presentation (co-author)**

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