# PALLADIUM NANOPARTICLES PRODUCED BY FERMENTATIVELY GROWN BACTERIA AS CATALYST FOR DIATRIZOATE REMOVAL WITH BIOGENIC HYDROGEN

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## INTRODUCTION

There is a growing interest to use microorganisms for the synthesis of metal nanoparticles that can be applied for catalysis or disinfection. This biological synthesis is a green technology not requiring expensive and harmful compounds, as used for the chemical synthesis of nanoparticles. Biologically produced nanopalladium (bio-Pd) is such a catalyst that was reported to efficiently catalyze the reduction of chromate, perchlorate and nitrate and the dehalogenation of for example PCBs, trichloroethylene (TCE) and lindane (Hennebel *et al.*, 2009). In these reduction and dehalogenation reactions, the Pd nanoparticles are activated by adding hydrogen gas as external electron donor. However, the use of hydrogen gas resulted in large costs and technical difficulties due to safety or supply.
Two model organisms have been primarily used to produce bio-Pd: *Desulfovibrio desulfuricans* and *Shewanella oneidensis* (Hennebel *et al.*, 2009). Recently, the nanoparticle formation process was coupled to the supply of hydrogen using the fermentatively grown *C. pasteurianum* BC1, producing hydrogen (Chidambaram *et al.*, 2010). Pd(0) nanoparticles were formed after the addition of Pd(II) to *C. pasteurianum* and were used to reduce Cr(VI) with bio-hydrogen as electron donor. The goal of this study was to examine the general applicability of this process by testing the Pd nanoparticle formation by two other fermentative bacteria. Additionally, a process consisting of microbial hydrogen formation and Pd nanoparticle catalysis without the need for an external electron donor was studied in dehalogenation reactions in batch tests as well as in a membrane bioreactor (MBR). Diatrizoate was used as model compound. This halogenated compound belongs to the iodinated contrast media (ICM) and can hardly be removed from wastewater due to its recalcitrance (Ternes *et al.*, 2003).

## MATERIAL AND METHODS

### Bacterial growth and synthesis of Pd nanoparticles

*Clostridium butyricum* (LMG 1217) and *Citrobacter braakii* (ATCC 6750) were anaerobically grown overnight in brain heart infusion medium (BHI) at 37°C. Subsequently, 1 mL of the cultures were inoculated in 30 mL medium containing 10 g $L^{-1}$ glucose, 1 g $L^{-1}$ $NH_4Cl$, 0.2 g $L^{-1}$ $MgSO_4.7H_2O$, 0.02 g $L^{-1}$ $CaCl_2.2H_2O$, 0.5 g $L^{-1}$ NaCl, 6 g $L^{-1}$ $Na_2HPO_4$ and 3 g $L^{-1}$ $KH_2PO_4$, and incu-

bated until an $OD_{610nm}$ of 1.6 was obtained. Palladium was added to the culture as Pd(II) solution to a final Pd concentration of 10 or 50 mg $L^{-1}$. To analyze the amount of palladium associated with the microbial biomass, the bio-Pd suspensions were centrifuged at 11000 g for 30 minutes. Subsequently, the organic material in the pellet and the supernatant were completely oxidized by boiling with $HNO_3$ and $H_2O_2$. The palladium concentrations were measured using an atomic absorption spectroscope (AAS). Analysis of hydrogen gas was performed by gas chromatography (GC) with a thermal conductivity detector (TCD). The Pd precipitated on the biomass was speciated by X-ray diffraction (XRD) (Hennebel *et al.*, submitted).

### Biocatalytic activity of bio-Pd towards dehalogenation of diatrizoate

Diatrizoate (Applichem, Darmstadt, Germany) was added to a bio-Pd suspension of 10 and 50 mg L-1 to a final concentration of 20 mg $L^{-1}$. Samples of 1 mL were withdrawn and analyzed by high performance liquid chromatography (HPLC) (Hennebel *et al.*, submitted). To remove diatrizoate in a continuous system, based on bio-Pd nanoparticles biogenic hydrogen gas as electron donor, a MBR was used. The reactor consisted of a 9 L reservoir with 1 plate membrane made of chlorinated polyethylene (Kubota, Japan). The reactor was supplied with 8 L of a 50 mg $L^{-1}$ bio-Pd suspension. The influent contained 0.5 g $L^{-1}$ glucose, 0.1 g $L^{-1}$ $NH_4C1$, 0.05 g $L^{-1}$ NaCl, 0.6 g $L^{-1}$ $Na_2HPO_4$ and 0.3 g $L^{-1}$ $KH_2PO_4$ and was spiked with 20 mg $L^{-1}$ diatrizoate. The system was maintained at a hydraulic retention time of 1 day.

## RESULTS AND DISCUSSION

### Reduction of Pd(II) to Pd(0) by fermentative bacteria

The Pd(II) reduction and Pd(0) nanoparticle formation capacity of *Citrobacter braakii* and *Clostridium butyricum* were evaluated with AAS, XRD and TEM. After the addition of 50 mg $L^{-1}$ Pd(II) to a bacterial culture of $OD_{610nm}$ = 1.6, the valency of the Pd associated with the bacterial cell mass was studied with XRD. The color change of the medium after the addition of the aqueous Pd(II), from pale yellow to black, was indicative for the formation of metallic Pd particles. TEM images showed nanoparticles associated with the bacteria (Figure 1A) while their oxidation state was confirmed to be zerovalent. Subsequently, the amount of Pd associated with the bacterial cell mass was determined using AAS. *C. butyricum* was able to bind 84 ± 3 % of the Pd mass, while in case of *C. braakii,* only 38 ± 4 % of the Pd mass was associated with the biomass. This large difference can be caused by several parameters including a difference in enzymatical reduction activity, cell structure and composition, Pd toxicity resistance, fermentation product type and distribution, and biogenic hydrogen production. Since the hydrogen concentration was expected to largely influence both the Pd reduction and in a later stage the dehalogenation mechanisms, the hydrogen gas production of the bacterial strains during the fermentation of glucose was measured after 24 h of growth. *C. braakii* and *C. butyricum* produced 43.7 ± 5.0 mmol $H_2$ $L^{-1}$ and 28.0 ± 6.3 mmol $H_2$ $L^{-1}$ respectively. The higher production of $H_2$ by *C. braakii* can provide a possible explanation for their low Pd-binding on their cells. Indeed, the Pd(II) will be readily reduced and deposited on every possible nucleation site such as cell debris or exudates. Detailed adsorption experiments, removing the hydrogen to avoid precipitation, are needed to de-

termine if the differences in the amount of biomass associated Pd can also be related to differences in cell structure or to a complex combination of other factors involved.

**Dehalogenation of diatrizoate with biogenic Pd(0)**

In a second stage of the research, the ability of bio-Pd nanoparticles produced by *C. braakii* and *C. butyricum* to dehalogenate diatrizoate was tested. The removal of diatrizoate was shown previously using bio-Pd produced by *S. oneidensis* and applying external hydrogen gas as electron donor (Hennebel *et al.*, 2010). In this study, no external hydrogen gas was added as it was produced during the fermentation reaction (Figure 1B). This is the main advantage of bio-Pd produced by fermenting bacteria compared to bio-Pd produced by *S. oneidensis*. At a concentration of 10 mg $L^{-1}$ bio-Pd, the dehalogenation of diatrizoate followed first order kinetics for both bacteria. The first order rate constant $k_{obs}$ amounted to 0.45 ± 0.03 $h^{-1}$ for *C. braakii* while *C. butyricum* showed almost no activity ($k_{obs}$ = 0.023 ± 0.002 $h^{-1}$). Apparently, there was no relation between the activity and the amount of Pd precipitated on the bacteria as *C. braakii* showed by far the highest activity but had the lowest amount of Pd on its cells. More likely, the observed difference can partly be explained by the varying amount of produced hydrogen gas. Indeed, *C. braakii* showed the highest activity and produced the highest amount of hydrogen gas. An increased amount of hydrogen gas can improve the Pd-catalyzed reaction since hydrogen gas functions as electron donor in the dehalogenation reaction.

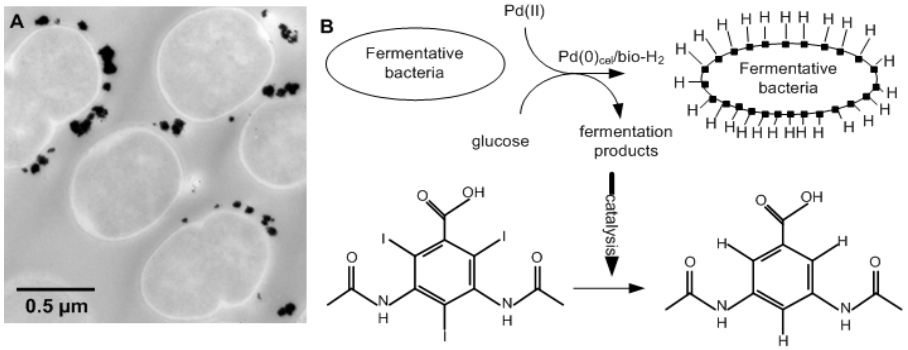


Figure 1: (A) TEM image of Pd nanoparticles on *Citrobacter braakii*. (B) Schematic representation of diatrizoate dehalogenation, catalyzed by bio-Pd particles precipitated on fermentative bacteria.

**Removal of diatrizoate by biogenic Pd(0) produced by *Citrobacter braakii* in an MBR**

An MBR was constructed to continuously supply glucose, ammonium and a phosphate buffer to ensure continuous production of biogenic hydrogen and avoid bacterial die-off due to pH decrease, as seen in batch experiments. The removal of diatrizoate was tested in an MBR, provided with a one-time spike of 50 mg $L^{-1}$ Pd(II) in order to generate and establish bio-Pd before contaminant introduction (20 mg $L^{-1}$ diatrizoate). Nearly complete diatrizoate removal was observed during the first days of MBR operation. Subsequently, a gradual decline in activity was observed and the effluent concentration reached a

maximum of 16.7 mg $L^{-1}$ diatrizoate after 8 days. After day 7, the glucose concentration of the influent was increased from 0.5 g $L^{-1}$ to 2 g $L^{-1}$ to stimulate the biogenic formation of hydrogen gas. This resulted in a temporarily improvement of the contaminant removal. However, the efficiency decreased again from day 12 to day 19 until almost no removal was observed anymore. During the entire experiment, a conversion of 22 mg diatrizoate $mg^{-1}$ Pd was obtained without adding external hydrogen gas. Loss of Pd from the reactor and the covering of Pd nanoparticles by growing bacterial cells could partly explain why complete removal could not be maintained over a longer period. Further research challenges include the need to completely retain the bio-Pd in the reactor while maintaining its catalytic activity. The ultimate goal would be to develop an MBR-type system in which reduced Pd species are produced and continuously regenerated by bio-$H_2$.

## CONCLUSION

This work demonstrates the general applicability of fermentatively grown bacteria to generate catalytic Pd(0) nanoparticles and bio-H2 as electron donor. These biomass-associated nanoparticles can be used to dehalogenate iodinated contrast media without an external source of hydrogen. The suggested mechanism can be applied in MBRs to treat aqueous streams of industrial effluent and as part of pump-and-treat systems.

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