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Highly efficient degradation of organic pollutants using a microbially-synthesized nanocatalyst

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Abstract

Magnetite is a common subsurface mineral, formed biogenically in anaerobic environments. Containing Fe(II), it is reactive towards a variety of common redox sensitive subsurface contaminants. To extend the reactivity of biomagnetite it is possible to add a coating of Pd(0) nanostructures, which is capable of sustained catalytic reactivity. Here we assess the reactivity of biogenic nano-magnetite (BnM), formed by the reduction of Fe(III) oxyhydroxide by *Geobacter sulfurreducens*, to the model organic compounds nitrobenzene (ArNO₂) and tetrachloroethylene (PCE), and compare its performance to biomagnetite functionalized with Pd(0) (Pd-BnM). The BnM and the Pd-BnM were both found to be highly reactive towards ArNO₂, quantitatively transforming it to the reduced product aniline (ArNH₂). When applied to tetrachloroethylene (PCE), the BnM was found to be poorly reactive, while the Pd-BnM rapidly dechlorinated the PCE to the benign product, ethane, at rates comparable to synthetic nano-scale catalysts. The biological synthesis route proposed is highly scalable and offers a green, environmentally benign route for the production of highly reactive nanoparticles for environmental clean-up.

1. Introduction

The mixed-valent iron mineral magnetite ($\text{Fe(II)Fe(III)}_2\text{O}_4$) is widespread in the natural environment (Cornell and Schwertmann, 2003), formed via a diverse range of biotic and abiotic processes, including the bioreduction of poorly crystalline Fe(III) phases by dissimilatory iron reducing bacteria (Cutting et al., 2009; Lovley et al., 1987), the abiotic oxidation of ferrous or zero valent iron (ZVI) (Gu et al., 1999; Olowe et al., 1989) and the bio-oxidation of Fe(II) coupled to denitrification (Kappler and Straub, 2005). Early studies noted that in the absence of aqueous and sorbed Fe(II) species, magnetite is relatively unreactive towards nitroaromatic and chlorinated compounds over timescales of hours (Elsner et al., 2004; Klausen et al., 1995). Other studies, however, have demonstrated the ability of both synthetic and biogenic magnetite to reduce a range of environmentally relevant contaminants such as trichloroethylene (Lee and Batchelor, 2002), carbon tetrachloride (Danielsen and Hayes, 2004), nitrobenzene (Gorski and Scherer, 2009), nitramine explosives such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Gregory et al., 2004), Hg(II) (Wiatrowski et al., 2009), Cr(VI) (Cutting et al., 2010), U(VI) (Missana et al., 2003) and Tc(VII) (Lloyd et al., 2000; McBeth et al., 2011).

Ultimately the reactivity of magnetite (or other solid state reductants) appears to be governed by their ability to store (capacitance) and donate electrons from Fe(II) , with Fe(II)/Fe(III) stoichiometry noted to have a profound control on reactivity (Gorski et al., 2010; Latta et al., 2012). In an attempt to overcome the limitation of a finite capacitance inherent to Fe(II) -bearing magnetite particles, composite catalysts have been fabricated by supporting nano-scale metallic palladium (Pd(0)) clusters on the surfaces of biogenic magnetite nanoparticles (Coker et al., 2010; Crean et al., 2012). These nanoparticles, synthesized using an Fe(III) -reducing bacterium, were functionalized with Pd through a one-step reduction method (Coker et al., 2010). The resulting particles consisted of a biogenic magnetite (BnM) carrier (~20 nm diameter) hosting metallic Pd particles (~5 nm) on its surface (Pd-BnM). When supplied with a suitable electron donor, typically H_2 gas, Pd(0) is able to dissociate and absorb reactive $\text{H}^\bullet$ in to its bulk structure, available for reaction (Conrad et al., 1974; Tierney et al., 2009; Yamauchi et al., 2008). The ability to supply the reaction with fresh electron donor improves the reduction potential and longevity of particle reactivity, in comparison to the finite Fe(II) of magnetite.

52 A number of studies employing Pd(0) catalysts in various forms have confirmed their exceptional
 53 reactivity against a variety of pollutants (Chaplin et al., 2012). These Pd(0) particles have been deployed
 54 in a number of different forms, including as native Pd(0) particles, and more commonly on the surface of
 55 support materials including carbon, silica, alumina, oxides and metals (Henry, 1998). These supports are
 56 often employed due to beneficial effects upon reactivity, where greatly improved reaction rates have
 57 been recorded in the presence of secondary “promoter” metals, such as Au (Nutt et al., 2005). However,
 58 due to its relatively cheap cost, alumina (Al₂O₃) has gained the most attention as a support for Pd(0)
 59 during technological applications (Davie et al., 2008; McNab et al., 2000). Recently, the development of
 60 Pd(0) coatings on synthetic magnetite resulted in extremely high catalytic activities towards
 61 trichloroethylene (TCE), without the need for costly promoter metals (Hildebrand et al., 2009). The
 62 aforementioned, microbially engineered catalyst (Pd-BnM), exhibited equal or superior activity during the
 63 Heck reaction, versus colloidal Pd nanoparticles, likely due to reduced agglomeration (Coker et al.,
 64 2010). Nanoparticles synthesized via this route have been demonstrated to be effective for the treatment
 65 of other common water contaminants; Cr(VI) (Crean et al., 2012; Watts et al., 2015) and azo dyes
 66 (Coker et al., 2014).

67 Here the catalytic potential of biologically synthesized nanomaterials for the treatment of common
 68 organic contaminants is assessed. Nitrobenzene (ArNO₂) and tetrachloroethylene (PCE) are used as
 69 model contaminant compounds, since these occur at a variety of contaminated sites, often as co-
 70 contaminants (Albright, 2011). The ArNO₂ was used to probe the reactivity of BnM, produced by
 71 incubating an Fe(III) starting phase with pre-grown cells of the Fe(III)-reducing bacterium *Geobacter*
 72 *sulfurreducens*. To increase the reactivity and extend its reactive lifetime the BnM was functionalized
 73 with a surface loading of Pd(0) nanoparticles, to create a biosynthesized, magnetically recoverable nano-
 74 scale catalyst (Pd-BnM).. ArNO₂ is a useful probe compound as it is known to undergo reduction to
 75 aniline (ArNH₂), via the following reactions (Scherer et al., 2001):

76

80 To further compare the reactivity of the un-functionalized Fe(II) mediated reductant, BnM, compared to
81 the catalytic, Pd-BnM, they were both also tested towards dechlorination of the relatively more
82 recalcitrant contaminant PCE. PCE is dechlorinated via a series of potential intermediate compounds,
83 via the idealized reactions:

88 The assessment of the reactivity of the particles towards PCE includes a detailed assessment of the
89 rates and end points of degradation catalyzed by BnM and Pd-BnM, and a critical comparison of
90 performance with a range of synthetic analogues.

91

92 **2. Experimental section**

93 **2.1. Chemicals**

94 All chemicals used in this study were of analytical grade or purer, unless otherwise stated, and used as
95 received without further purification prior to use. All water used in the experiments was of a minimum
96 18.2 MΩ quality.

97 **2.2. Biogenic magnetite synthesis**

98 BnM was prepared from a starting Fe(III) phase of 2-line ferrihydrite, prepared by alkaline hydrolysis,
99 through addition of 10 N NaOH to a 0.66 M Fe(III)Cl₃ solution until reaching a pH of 7 (Lovley and
100 Phillips, 1986; Schwertmann and Cornell, 2008). The precipitates were washed six times using 18.2 MΩ
101 water, separating the precipitates by centrifuging at 17 000 g for 20 minutes, and stored under N₂ until
102 required. The physical and chemical properties of these biomineralization products have extensively
103 been characterized previously (Byrne et al., 2011).

104 A culture of *G. sulfurreducens* was prepared according to Lloyd et. al. 2003. Briefly, freshwater medium
105 (Lovley and Phillips, 1988) was prepared, containing 20 mM acetate as the electron donor and 40 mM of

fumarate as the electron acceptor, prepared under an N₂-CO₂ (80:20) atmosphere, adjusted to a pH of 7 using NaOH, determined using a Denver Instrument UB-10 bench top meter and a calibrated P Cole Parmer 5990-45 CCP probe. This medium was decanted into serum bottles and sealed using a rubber bung and aluminum crimps, leaving a headspace of N₂-CO₂ (80:20), prior to autoclaving for sterility. An inoculum of a growing culture of *G. sulfurreducens* was added at a 10% v/v ratio. For incubation with the Fe(III) starting phases, a late log phase culture of *G. sulfurreducens* was harvested by centrifugation (Sigma 6k15), at 4920 g for 20 minutes, and washed using a bicarbonate buffer under N₂-CO₂ (80:20) gas. This concentrated cell suspension was used to inoculate sealed serum bottles containing 10 g L⁻¹ of Fe(III) starting material (at 0.6 mg protein mL⁻¹), 20 mM Na acetate, 30 mM NaHCO₃ and 10 μM anthraquinone-2,6-disulfonate (AQDS) under an atmosphere of N₂-CO₂ (80:20) gas. These inoculated serum bottles were incubated at 30°C in the dark until transformation to magnetite was complete. Following incubation, the resulting magnetite was magnetically separated and washed using 18.2 MΩ water and stored at 4°C in the dark until use. The Fe concentration of the BnM slurry was determined by the acid extraction and ferrozine assay, as detailed in section 2.6.

2.3. Functionalization of biogenic magnetite with Pd(0)

Biogenic magnetite was used to support surface-localized Pd(0), precipitated via the method detailed in (Coker et al., 2010). Briefly, under an N₂ atmosphere an aliquot of the washed BnM slurry was added to a sterile serum bottle and crimp sealed using a rubber bung and aluminum crimps. An N₂ degassed solution of Na₂PdCl₄ was prepared, at a concentration to give a final concentration of Pd on the magnetite as 2.4 wt % Pd. This solution was then added, using an N₂ degassed syringe, to the BnM slurry while shaking prior to agitating on a roller shaker for 12 hours. In order to remove excess Cl⁻ ions the resulting Pd-BnM was washed under an N₂ atmosphere, with 18.2 MΩ water. The resulting slurry was sampled and solubilized using concentrated HCl and sent for ICP-AES analysis to confirm the concentration of Fe and Pd, data not shown.

2.4. Batch ArNO₂ reduction experiments

ArNO₂ reduction by the BnM and the Pd-BnM was tested using batch reaction experiments. These experiments were conducted in 50 mL serum bottles containing 30 mL of a 20 mM MOPS buffer solution (pH 7.0) and a known concentration of BnM or Pd-BnM. The serum bottles were sealed using a rubber

septum and aluminum crimps. The BnM experiment was flushed with pressurized N₂ gas, while the Pd-BnM was flushed with pressurized H₂, giving a pure H₂ headspace within the bottles. The bottles were spiked with known concentrations of ArNO₂; 150 µM for the biogenic magnetite and 5 successive spikes of 1000 µM ArNO₂ for the Pd-BnM experiment. During batch BnM experiments, samples were removed after 1 minute followed by every 5 minutes up to 35 minutes after spiking with ArNO₂. When spiked with Pd-BnM the experiment was sampled more regularly at intervals of <5 minutes, up to 104 minutes, after spiking with ArNO₂. From both experiments, samples were removed by flushing a needle and syringe with pressurized N₂ gas, passed through a 0.22 µm filter. The samples were then 0.22 µm filtered to remove the particle suspension and transferred immediately to a sealed high performance liquid chromatography (HPLC) vial for analysis by HPLC.

2.5. Batch PCE dechlorination experiments

The dechlorination of PCE by Pd-BnM was tested using batch headspace reaction experiments. These experiments were conducted using a buffered synthetic groundwater containing; 30 mg L⁻¹ CaSO₄•2H₂O, 30 mg L⁻¹ MgSO₄, 2 mg L⁻¹ KCl and 168 mg L⁻¹ NaHCO₃. 10 mL sub-samples of this synthetic groundwater were placed in 20 mL glass serum bottles, leaving a 1:1 ratio of solution to headspace, and sealed using PTFE lined rubber septa and aluminum crimps. The following experimental treatments were prepared; Pd-BnM + H₂ gas, Pd-BnM control (no electron donor), BnM addition and a H₂ (no Pd-BnM) control. Bottles requiring electron donor for catalysis were degassed with pressurized H₂ gas, giving a pure H₂ headspace within the bottles. All other controls were degassed using pressurized N₂ gas. A concentrated methanol stock solution of PCE was added to the test solutions to make a final concentration of 50 mg L⁻¹, along with an internal standard (2.5 mg L⁻¹ hexane). The final pH of this solution was 6.8, maintaining this value over the duration of the experiment. The serum bottles were shaken vigorously by hand, and left on the roller shaker to allow the PCE to equilibrate between the liquid and gas phase. Prior to the addition of Pd-BnM, a headspace sample was taken for gas chromatography – flame ionization detector (GC-FID) analysis (see section 2.8). Addition of the Pd-BnM or BnM was carried out using an N₂ degassed syringe to make a final concentration of 0.025 g Fe L⁻¹ for the Pd-BnM, or 1 g Fe L⁻¹ for the BnM control. All experiments were maintained at 20°C ± 2°C, on a roller shaker, in the dark. All control experiments were conducted in triplicate while the 2.4 wt % Pd-BnM + H₂

experiment was conducted 4 times. Each of these experiments was conducted with different individual timepoints, all of which are plotted in the results. Samples were removed at appropriate intervals, to give a good coverage over the experimental time span, while also aiming to minimize time between sampling and injection in to the GC. During sampling, the serum bottle was removed from the roller shaker and a headspace sample (200 μ L) removed. Samples were taken using a lockable gastight syringe (Hamilton SampleLock 1750sl 0.5 mL syringe, Supelco Park, CA,USA), which had previously been degassed with 0.22 μ m filtered N₂ gas, introducing 200 μ L of N₂ in to the serum bottle so as to maintain atmospheric pressure inside the vessel, locking the syringe prior to removal. Once removed the headspace sample was immediately injected in to the GC-FID for analysis.

2.6. Analytical method – Fe(II) determination

The Fe(II) concentration of the BnM was determined by the spectrophotometric ferrozine assay (Stookey, 1970). Initially the magnetite was extracted in hydrofluoric acid and the resulting extract then reacted with the ferrozine solution (1 g L⁻¹ Ferrozine, 11.96 g L⁻¹ HEPES buffer, adjusted to pH 7), according to (Lovley and Phillips, 1986). Analysis was performed on a Jenway 6715 UV/Vis spectrophotometer compared to calibration standards (FeSO₄·7H₂O solution).

2.7. Analytical method –ArNO₂

ArNO₂ batch experiment samples were analyzed by HPLC, performed on a Dionex GP50 gradient pump system using a Supelco LC-18, 5 μ m; 250 x 4.6 mm column, at a flow rate of 1 mL min⁻¹. The analytes were detected using a Dionex UVD 170U 4-channel UV-vis detector by comparison to standards for ArNO₂ and the product ArNH₂.

2.8. Analytical method – PCE

Headspace samples of the PCE batch experiments were analyzed using GC-FID according to methods adapted from (Burris et al., 1996; Lowry and Reinhard, 1999); samples were injected at 50°C (held for 2 minutes) and the oven was programmed to 220°C at 40°C min⁻¹, at which it was held isothermal for 3 minutes. Helium (He) was used as carrier gas at 30 mL min⁻¹, identification was achieved by comparison to a PCE standard and quantification using an internal standard of 2.5 mg L⁻¹ hexane (Sigma-Aldrich CHROMASOLV Plus, for HPLC, $\geq$ 95%) added to the vial prior to analysis. Analysis was performed on an

Agilent 7890A GC fitted with an Agilent J&W GC HP-PLOT/Q column (30 m length and 0.535 mm diameter), equipped with on-column injector (set in splitless mode at 275°C) and an FID (FID temperature was 275°C). The retention time of ethane was recorded from a gas standard. Its concentration was estimated using Henry's Law, with (dimensionless) Henry's constants (H_c) for PCE ($H_c = 0.60$) calculated from (Gossett, 1987) and ethane ($H_c = 20.4$) (Mackay and Shiu, 1981), to determine partitioning between the headspace and solution. This allowed an estimation of the ethane concentration from the GC-FID carbon response.

2.9. Evaluation of reaction kinetics - PCE

To provide a comparison with previously reported kinetic data, for catalytic dechlorination of chlorinated hydrocarbons, the PCE dechlorination reaction was modeled using a simple pseudo-1st order rate law, as employed previously in (Lowry and Reinhard, 1999):

$$-\frac{1}{C_{Pd}} \frac{dC_{PCE}}{dt} = k_{obs} C_{PCE} \quad (\text{eq. 8})$$

where C_{Pd} and C_{PCE} represent the concentration of Pd and PCE respectively, the k_{obs} is the pseudo-1st order reaction rate constant calculated from the linear regression of $\ln[\text{PCE}]$ vs t . The k_{obs} are normalized by the concentration of Pd in the experiment to give k_{Pd} in $\text{L g}_{Pd}^{-1} \text{min}^{-1}$. Due to the presence of a pure H_2 headspace and the saturation of the aqueous phase in respect to H_2 , it is assumed these are present in vast stoichiometric excess and do not impair reaction rates. For further comparison of the rate kinetics, the specific catalyst activity (A_{Pd}) as previously employed by (Hildebrand et al., 2009) was also calculated:

$$A_{Pd} = \frac{1}{C_{Pd} t_{1/2}} = \frac{\ln(C_{t1}/C_{t2})}{\ln C_{Pd}(t_2 - t_1)} \quad (\text{eq. 9})$$

where $t_{1/2}$ represent the half life of the PCE during the reaction, t_1 and t_2 are two arbitrary sampling times, C_{t1} and C_{t2} are their corresponding PCE concentrations.

3. Results and discussion

3.1. ArNO₂ reduction by biogenic magnetite

Biogenic nano-magnetite (BnM) was synthesized from 2-line ferrihydrite powder using whole cells of *G. sulfurreducens* within 2 days of inoculation. The reactivity of the BnM investigated here was assessed using ArNO₂ as a chemical probe, with BnM added at the equivalent of 9.4 mM Fe(II). Both the disappearance of ArNO₂ and the formation of the reduction product, ArNH₂, were determined as a function of time (see Fig. 1), hypothesized to react via equations 1-3. The BnM rapidly reduced ArNO₂, completely removing it over the initial 10 minutes of incubation. This was concurrent with the production of ArNH₂ at a slower rate than ArNO₂ was removed, evident from the sum of ArNO₂ and ArNH₂ concentrations (Fig. 1), which shows an initial decrease, possibly indicating reaction via nitroso- and hydroxylamino intermediates. Over the 35 minute time course the 150 μ M ArNO₂ was completely reduced to ArNH₂. Taking in to account that to reduce 1 M ArNO₂ it would require the oxidation of 6 M Fe(II), the starting concentration of Fe(II) used here is in 21 times stoichiometric excess to the ArNO₂. Although this assumes the availability of all the structural Fe(II) in the magnetite, it easily accounts for the complete removal of the ArNO₂.

3.2. ArNO₂ Reduction by Pd(0) functionalized biogenic magnetite

The intrinsic limitation of the availability of electrons at the mineral-solution interface in the magnetite system makes its application to the reduction of contaminants, in the absence of a method to replenish Fe(II), limited. To address this, the surface of the magnetite was functionalized with Pd(0) and again tested for prolonged ArNO₂ reduction with H₂ gas supplied as the electron donor.

The sustained reactivity of the Pd-BnM is evidenced by the maintenance of the reductive capacity following repeated spiking with 1000 μ M ArNO₂ (Fig. 2). In total 5000 μ M ArNO₂, was removed without loss of activity, equivalent to the stoichiometric generation of 30 mM of electron reduction equivalents.

This represents a far greater removal than is potentially possible by the Fe(II)-mediated BnM systems (without the Pd coating), due to the replenishment of catalytic reactivity in the presence of the electron donor H₂. The hydrogenation of ArNO₂, by a Pd(0) catalyst, has been described previously by several authors (Gelder et al., 2002; Sangeetha et al., 2009), and is the typical method employed for the production of ArNH₂ in industrial processes (Wisniak and Klein, 1984). The sustained reactivity presented here, under ambient conditions, would indicate that the employment of Pd(0) supported on BnM is an efficient reduction mechanism for the treatment of ArNO₂.

3.3. PCE reduction

In addition to the hydrogenation of ArNO₂, the BnM and the Pd-BnM were used to treat the more recalcitrant PCE. Dechlorination of PCE by magnetite has been observed previously (Lee and Batchelor, 2002), however, this was found to proceed slowly over 100 days of reaction. In these experiments, a buffer reflective of realistic groundwater composition was used to give a closer approximation to contaminated land and water scenarios. In line with this, even in a great stoichiometric excess of magnetite (1 g Fe L⁻¹), little dechlorination over the short timescale of this experiment was noted (Fig. 3(a)). By contrast, upon functionalization with Pd(0), dechlorination proceeded rapidly to the hydrogenated end product ethane (Fig. 3(a)). It should be noted that a minor decrease in the mass balance (PCE + ethane) is noted within the first 5 minutes of the reaction, indicating the presence of degradation intermediates, described in equations 4-7. However, with minimal detection of partially chlorinated products or the unsaturated hydrocarbon, ethylene, it is unclear what is responsible for this loss in mass. This mass loss is not likely the result of adsorption, where for Pd-BnM in the absence of H₂ (Supporting information Fig. S1), no PCE removal occurred. The good overall selectivity towards non-toxic ethane production is desirable, where no evidence for significant by-product accumulation was noted, and wholly consistent with previous studies employing a variety of Pd(0) catalysts; Pd(0)-Al₂O₃ (Lowry and Reinhard, 1999), Pd(0)-Au (Nutt et al., 2006; Nutt et al., 2005) and unsupported Pd(0) (Heck et al., 2009). In addition, no PCE removal was observed in the presence of H₂ but the absence of the Pd-BnM (Supporting information Fig. S1), evidence that the catalytic Pd(0) is required for dehalogenation of the PCE.

268 The k_{Pd} and the A_{Pd} values, calculated from the linear regression of $\ln[PCE]$ (Fig. 4), of 334 ± 18 and
269 $492 \pm 25 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$ respectively (Table 1), allow direct comparison of dechlorination rates to literature
270 values. A review by (Chaplin et al., 2012) presented a compilation of the reaction rate constants
271 observed for dechlorination of all chlorinated alkenes by Pd(0) showing rates in the order of 10s to 10000
272 $\text{L g}_{Pd}^{-1} \text{ min}^{-1}$ for a wide range of substrates including PCE. The values presented here fall within this
273 range of values; the wide range of reported values is probably due to the wide variety of experimental
274 conditions employed (and the wide spectrum of substrates), therefore, it is worth comparing directly with
275 similar studies focusing on PCE. Specifically the PCE dechlorination $k_{catalyst}$ noted using a Pd(0)-Al₂O₃
276 catalyst, have been reported to be in the order of $53 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$, normalized to Pd content, where Pd(0)
277 loading was at 1%) (Lowry and Reinhard, 1999). This value is far lower than the $334 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$ k_{Pd} rate
278 observed in this study, possibly due to the nano-scale of the Pd-BnM employed, compared to the micron
279 scale Pd(0) on Al₂O₃.

280 Fewer studies have been carried out on the fully chlorinated PCE, compared to the less (tri) chlorinated
281 TCE, however, as the fully chlorinated compound (PCE) dechlorinates via TCE, it was chosen so as to
282 be relevant to a wider scope of chlorinated compounds. Despite PCE being reported as a more readily
283 reduced than TCE (Elsner and Hofstetter, 2011), a previous study has shown that the TCE is
284 dechlorinated slightly faster than PCE by Pd(0) catalysts (Lowry and Reinhard, 1999). An extremely
285 active catalyst was identified for the dechlorination of TCE, in the form of Pd(0)-Au, recording a k_{Pd}
286 dependent upon Pd(0) %, ranging from $173 - 943 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$, for 33 and 1.9 wt% Pd(0) respectively
287 (Nutt et al., 2005). This study also found conventional unsupported Pd(0) to have a k_{Pd} of $62 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$
288 and a Pd(0)-Al₂O₃ catalyst to have a k_{Pd} of $12 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$. In a later study, also with a Pd(0)-Au catalyst,
289 a higher k_{Pd} of $1956 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$ was achieved for a Pd loading of 12.7 wt % upon Au, equivalent to
290 between 50 and 75% surface Pd coverage of the 4 nm Au particle (Nutt et al., 2006). The high reactivity
291 of the Pd(0)-Au catalyst was inferred to be a result of the promoting effect of Au due to electronic or
292 geometric controls.

293 The specific catalyst activity (A_{Pd}) value presented here (Table 1) is below that reported previously for an
294 unsupported Pd(0) nanoparticle and a Pd-on-Au catalyst; exhibiting A_{Pd} values of $2100 \pm 300 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$
295 ^{1} and $2150 \pm 480 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$ respectively (Hildebrand et al., 2009). This study also compared a

synthetic magnetite supported Pd(0) heterostructure towards TCE dechlorination with varying wt % Pd. For wt % Pd(0) values similar to those used by (Hildebrand et al., 2009) of 5 wt % loading on magnetite, a A_{Pd} of $520 \pm 180 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$ was obtained, comparable to the value presented here for 2.4 wt % Pd(0) ($492 \pm 25 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$) loading towards PCE. An inverse relationship with Pd content and specific reaction rate was noted, with the highest rate of $6100 \pm 180 \text{ L g}_{Pd}^{-1} \text{ min}^{-1}$ observed at a Pd loading of 0.15 wt % Pd on magnetite. This greater reactivity of the lower Pd(0) loading in (Hildebrand et al., 2009) was hypothesized to be a result of increased dispersion of smaller Pd(0) particles increasing exposed surface Pd(0) atoms for reaction. The high reactivity of the synthetic Pd(0) on magnetite and the biogenic magnetites presented here would seem to provide a highly reactive alternative to the costly promoter metal Au, with the dual advantage of providing a magnetically recoverable support.

Table 1. Calculated psuedo-1st order reaction rate constants and specific catalyst activity for dechlorination of PCE by Pd-BnM

Catalyst	Pd g L ⁻¹	K_{obs} (min ⁻¹)	R^2	K_{Pd}^a (L g _{Pd} ⁻¹ min ⁻¹)	A_{Pd}^b (L g _{Pd} ⁻¹ min ⁻¹)
Pd-BnM	7.94×10^{-4}	0.265 ± 0.014	0.97	334 ± 18	492 ± 25

^a 1st order rate constant normalized to g⁻¹ Pd (see section 2.9)

^b specific catalyst activity (see section 2.9)

3.4 Implications for bio-nanoremediation

There is a growing realization that the metabolic processes of microorganisms can be harnessed for the production of functional nanomaterials, with a wide variety of applications, including contaminant remediation (Hennebel et al., 2009; Lloyd et al., 2010). These materials are proving to have comparable or better functional properties than synthetic alternatives (Coker et al., 2010), leading to several studies focusing on their reactivity towards contaminant remediation (De Corte et al., 2012; Mabbett and Macaskie, 2002; Watts et al., 2015). The present study supports the growing realization of the comparable reactivity of biogenic nanoparticles to synthetic alternatives. It also demonstrates the improvements gained through functionalization of nanoparticles, a process which can be achieved

through a one-step reaction with BnM (Coker et al., 2010). The possibilities of modifying the biogenic particles is also a key focus (Byrne et al., 2011; De Windt et al., 2006; Redwood et al., 2008; Sobjerg et al., 2011) and its impact on reactivity remains to be fully explored. The challenges of applying this technology at field scale also need to be addressed, with work being done to scale up their bioproduction (Byrne et al., 2015), and development of novel *in situ* deployment technologies (Chidambaram et al., 2010).

4. Conclusions

In summary both the un-functionalized BnM and the Pd-BnM catalyst (the latter supplied with H₂), exhibited high reactivity towards ArNO₂ in batch systems. Both were able to fully transform ArNO₂ to the reduced product ArNH₂, mediated by Fe(II) in the magnetite, and reducing power replenished in the Pd-BnM treatment by the H₂ electron donor. Indeed, the Pd-BnM was able to efficiently reduce high quantities of ArNO₂, 5000 µM in total, without showing any loss in reactivity. Moving towards treatment of the more recalcitrant organic contaminant, PCE, the BnM was un-reactive on the short timescales of the experiment employed here. In contrast, the Pd-BnM was highly reactive, resulting in the fast degradation to the benign product ethane. The modeled reaction rates are extremely fast and are comparable to those previously reported in the literature for 5% Pd(0) on synthetic magnetite towards reaction with TCE. The Pd-BnM employed here, therefore represents a more reactive nanoparticle treatment than the Fe(II) based BnM alone and is better suited to treatment of more recalcitrant contaminants. However, the BnM still exhibited good reactivity towards the less recalcitrant test compound ArNO₂. Biotechnological routes for BnM and Pd-BnM offer scalable, environmentally benign alternatives to the synthetic production processes currently available for highly reactive nanoparticles, resulting in the tunable synthesis of bionanoparticles that are well suited to the treatment of a wide range of organic and inorganic contaminants. Current research focuses on their synthesis from waste materials and subsequent deployment in a range of contaminated land scenarios.

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