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 (Fe(II)Fe(III)$_2$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)-baring 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 H• 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.
A number of studies employing Pd(0) catalysts in various forms have confirmed their exceptional reactivity against a variety of pollutants (Chaplin et al., 2012). These Pd(0) particles have been deployed in a number of different forms, including as native Pd(0) particles, and more commonly on the surface of support materials including carbon, silica, alumina, oxides and metals (Henry, 1998). These supports are often employed due to beneficial effects upon reactivity, where greatly improved reaction rates have been recorded in the presence of secondary “promoter” metals, such as Au (Nutt et al., 2005). However, due to its relatively cheap cost, alumina (Al₂O₃) has gained the most attention as a support for Pd(0) during technological applications (Davie et al., 2008; McNab et al., 2000). Recently, the development of Pd(0) coatings on synthetic magnetite resulted in extremely high catalytic activities towards trichloroethylene (TCE), without the need for costly promoter metals (Hildebrand et al., 2009). The aforementioned, microbially engineered catalyst (Pd-BnM), exhibited equal or superior activity during the Heck reaction, versus colloidal Pd nanoparticles, likely due to reduced agglomeration (Coker et al., 2010). Nanoparticles synthesized via this route have been demonstrated to be effective for the treatment of other common water contaminants; Cr(VI) (Crean et al., 2012; Watts et al., 2015) and azo dyes (Coker et al., 2014).

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

\[
\begin{align*}
C_6H_5NO_2 + 2e^- + 2H^+ &\rightarrow C_6H_5NO + H_2O \quad \text{(eq. 1)} \\
C_6H_5NO + 2e^- + 2H^+ &\rightarrow C_6H_5NHOH \quad \text{(eq. 2)} \\
C_6H_5NHOH + 2e^- + 2H^+ &\rightarrow C_6H_5NH_2 + H_2O \quad \text{(eq. 3)}
\end{align*}
\]
To further compare the reactivity of the un-functionalized Fe(II) mediated reductant, BnM, compared to the catalytic, Pd-BnM, they were both also tested towards dechlorination of the relatively more recalcitrant contaminant PCE. PCE is dechlorinated via a series of potential intermediate compounds, via the idealized reactions:

\[
\begin{align*}
\text{C}_2\text{Cl}_4 + 2\text{H}^+ + 2\text{e}^- & \rightarrow \text{C}_2\text{HCl}_3 + \text{HCl} \quad \text{(eq. 4)} \\
\text{C}_2\text{HCl}_3 + 2\text{H}^+ + 2\text{e}^- & \rightarrow \text{C}_2\text{H}_2\text{Cl}_2 + \text{HCl} \quad \text{(eq. 5)} \\
\text{C}_2\text{H}_2\text{Cl}_2 + 2\text{H}^+ + 2\text{e}^- & \rightarrow \text{C}_2\text{H}_3\text{Cl} + \text{HCl} \quad \text{(eq. 6)} \\
\text{C}_2\text{H}_3\text{Cl} + 2\text{H}^+ + 2\text{e}^- & \rightarrow \text{C}_2\text{H}_4 + \text{HCl} \quad \text{(eq. 7)}
\end{align*}
\]

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

2. Experimental section

2.1. Chemicals

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

2.2. Biogenic magnetite synthesis

BnM was prepared from a starting Fe(III) phase of 2-line ferrihydrite, prepared by alkaline hydrolysis, through addition of 10 N NaOH to a 0.66 M Fe(III)Cl$_3$ solution until reaching a pH of 7 (Lovley and Phillips, 1986; Schwertmann and Cornell, 2008). The precipitates were washed six times using 18.2 MΩ water, separating the precipitates by centrifuging at 17 000 g for 20 minutes, and stored under N$_2$ until required. The physical and chemical properties of these biomineralization products have extensively been characterized previously (Byrne et al., 2011).

A culture of *G. sulfurreducens* was prepared according to Lloyd et al. 2003. Briefly, freshwater medium (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 in to 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 antraquinone-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
The BnM experiment was flushed with pressurized \( \text{N}_2 \) gas, while the Pd-BnM was flushed with pressurized \( \text{H}_2 \), giving a pure \( \text{H}_2 \) headspace within the bottles. The bottles were spiked with known concentrations of \( \text{ArNO}_2 \); 150 \( \mu \text{M} \) for the biogenic magnetite and 5 successive spikes of 1000 \( \mu \text{M} \) \( \text{ArNO}_2 \) 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 \( \text{ArNO}_2 \). When spiked with Pd-BnM the experiment was sampled more regularly at intervals of <5 minutes, up to 104 minutes, after spiking with \( \text{ArNO}_2 \). From both experiments, samples were removed by flushing a needle and syringe with pressurized \( \text{N}_2 \) gas, passed through a 0.22 \( \mu \text{m} \) filter. The samples were then 0.22 \( \mu \text{m} \) 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\(^{-1}\) \( \text{CaSO}_4 \cdot 2\text{H}_2\text{O} \), 30 mg L\(^{-1}\) \( \text{MgSO}_4 \), 2 mg L\(^{-1}\) \( \text{KCl} \) and 168 mg L\(^{-1}\) \( \text{NaHCO}_3 \). 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 + \( \text{H}_2 \) gas, Pd-BnM control (no electron donor), BnM addition and a \( \text{H}_2 \) (no Pd-BnM) control. Bottles requiring electron donor for catalysis were degassed with pressurized \( \text{H}_2 \) gas, giving a pure \( \text{H}_2 \) headspace within the bottles. All other controls were degassed using pressurized \( \text{N}_2 \) 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 \( \text{N}_2 \) 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\( ^\circ \)C ± 2\( ^\circ \)C, on a roller shaker, in the dark. All control experiments were conducted in triplicate while the 2.4 wt % Pd-BnM + \( \text{H}_2 \)
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 µL) 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 µm filtered N₂ gas, introducing 200 µL 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, 5µm; 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, ≥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 ($H_d$) for PCE ($H_d = 0.60$) calculated from (Gossett, 1987) and ethane ($H_d = 20.4$) (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):

$$- \frac{1}{C_{pd}} \frac{dC_{PCE}}{dt} = k_{obs} C_{PCE}$$  \hspace{1cm} (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[PCE] vs $t$. The $k_{obs}$ are normalized by the concentration of Pd in the experiment to give $k_{Pd} \text{ ln } g_{Pd}^{-1} \text{ min}^{-1}$. 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:

$$A_{pd} = \frac{1}{C_{pd} t_{1/2}} = \frac{\ln(C_{t2}/C_{t1})}{\ln C_{pd}(t_{2}-t_{1})}$$  \hspace{1cm} (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, \( C_t \) and \( C_{t2} \) are their corresponding PCE concentrations.

3. Results and discussion

3.1. ArNO\(_2\) 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 ArNO\(_2\) as a chemical probe, with BnM added at the equivalent of 9.4 mM Fe(II). Both the disappearance of ArNO\(_2\) and the formation of the reduction product, ArNH\(_2\), were determined as a function of time (see Fig. 1), hypothesized to react via equations 1-3. The BnM rapidly reduced ArNO\(_2\), completely removing it over the initial 10 minutes of incubation. This was concurrent with the production of ArNH\(_2\) at a slower rate than ArNO\(_2\) was removed, evident from the sum of ArNO\(_2\) and ArNH\(_2\) concentrations (Fig. 1), which shows an initial decrease, possibly indicating reaction via nitroso- and hydroxylamino intermediates. Over the 35 minute time course the 150 µM ArNO\(_2\) was completely reduced to ArNH\(_2\). Taking into account that to reduce 1 M ArNO\(_2\) 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 ArNO\(_2\). Although this assumes the availability of all the structural Fe(II) in the magnetite, it easily accounts for the complete removal of the ArNO\(_2\).

3.2. ArNO\(_2\) 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 ArNO\(_2\) reduction with H\(_2\) 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 µM ArNO\(_2\) (Fig. 2). In total 5000 µM ArNO\(_2\), 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 nontoxic 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.
The $k_{Pd}$ and the $A_{Pd}$ values, calculated from the linear regression of ln[PCE] (Fig. 4), of 334±18 and 492±25 L g$_{Pd}^{-1}$ min$^{-1}$ respectively (Table 1), allow direct comparison of dechlorination rates to literature values. A review by (Chaplin et al., 2012) presented a compilation of the reaction rate constants observed for dechlorination of all chlorinated alkenes by Pd(0) showing rates in the order of 10s to 10000 L g$_{Pd}^{-1}$ min$^{-1}$ for a wide range of substrates including PCE. The values presented here fall within this range of values; the wide range of reported values is probably due to the wide variety of experimental conditions employed (and the wide spectrum of substrates), therefore, it is worth comparing directly with similar studies focusing on PCE. Specifically the PCE dechlorination $k_{catalyst}$ noted using a Pd(0)-Al$_2$O$_3$ catalyst, have been reported to be in the order of 53 L g$_{Pd}^{-1}$ min$^{-1}$, normalized to Pd content, where Pd(0) loading was at 1% (Lowry and Reinhard, 1999). This value is far lower than the 334 L g$_{Pd}^{-1}$ min$^{-1}$ $k_{Pd}$ rate observed in this study, possibly due to the nano-scale of the Pd-BnM employed, compared to the micron scale Pd(0) on Al$_2$O$_3$.

Fewer studies have been carried out on the fully chlorinated PCE, compared to the less (tri) chlorinated TCE, however, as the fully chlorinated compound (PCE) dechlorinates via TCE, it was chosen so as to be relevant to a wider scope of chlorinated compounds. Despite PCE being reported as a more readily reduced than TCE (Elsner and Hofstetter, 2011), a previous study has shown that the TCE is dechlorinated slightly faster than PCE by Pd(0) catalysts (Lowry and Reinhard, 1999). An extremely active catalyst was identified for the dechlorination of TCE, in the form of Pd(0)-Au, recording a $k_{Pd}$ dependent upon Pd(0) %, ranging from 173 - 943 L g$_{Pd}^{-1}$ min$^{-1}$, for 33 and 1.9 wt% Pd(0) respectively (Nutt et al., 2005). This study also found conventional unsupported Pd(0) to have a $k_{Pd}$ of 62 L g$_{Pd}^{-1}$ min$^{-1}$ and a Pd(0)-Al$_2$O$_3$ catalyst to have a $k_{Pd}$ of 12 L g$_{Pd}^{-1}$ min$^{-1}$. In a later study, also with a Pd(0)-Au catalyst, a higher $k_{Pd}$ of 1956 L g$_{Pd}^{-1}$ min$^{-1}$ was achieved for a Pd loading of 12.7 wt % upon Au, equivalent to between 50 and 75% surface Pd coverage of the 4 nm Au particle (Nutt et al., 2006). The high reactivity of the Pd(0)-Au catalyst was inferred to be a result of the promoting effect of Au due to electronic or geometric controls.

The specific catalyst activity ($A_{Pd}$) value presented here (Table 1) is below that reported previously for an unsupported Pd(0) nanoparticle and a Pd-on-Au catalyst; exhibiting $A_{Pd}$ values of 2100 ± 300 L g$_{Pd}^{-1}$ min$^{-1}$ and 2150 ± 480 L g$_{Pd}^{-1}$ 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 $520 \pm 180 \text{ L g}_{\text{Pd}}^{-1} \text{ min}^{-1}$ was obtained, comparable to the value presented here for 2.4 wt % Pd(0) (492 ± 25 L g$_{\text{Pd}}^{-1}$ min$^{-1}$) loading towards PCE. An inverse relationship with Pd content and specific reaction rate was noted, with the highest rate of $6100 \pm 180 \text{ L g}_{\text{Pd}}^{-1} \text{ min}^{-1}$ 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>
<thead>
<tr>
<th>Catalyst</th>
<th>Pd g L$^{-1}$</th>
<th>$K_{\text{obs}}$ (min$^{-1}$)</th>
<th>$R^2$</th>
<th>$K_{Pd}^a$ (L g$_{\text{Pd}}^{-1}$ min$^{-1}$)</th>
<th>$A_{Pd}^b$ (L g$_{\text{Pd}}^{-1}$ min$^{-1}$)</th>
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<tbody>
<tr>
<td>Pd-BnM</td>
<td>7.94 x 10$^{-4}$</td>
<td>0.265 ± 0.014</td>
<td>0.97</td>
<td>334 ± 18</td>
<td>492 ± 25</td>
</tr>
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</table>

$^a$ 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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