Deployment of nZVI to soil for polychlorinated biphenyl remediation: impacts on soil microbial communities

Liz Shaw
Outline

• Background
  – PCBs: use of ZVI and biodegradation for remediation

• Experimental Data
  – impacts of nZVI on microbial functions over short and long timescales

• Summary of nZVI impacts on microbes

• Wider questions
  – Environmental health and safety considerations
  – Identify challenges for the development, commercialisation and application of nanotechnology
Polychlorinated biphenyls (PCBs)

- A class of chloro-organic chemicals with between 1 to 10 chlorine atoms attached to a biphenyl backbone
- 209 congeners
- Widespread historical use as dielectric fluids but production is now banned
- Toxic, bioaccumulative, persistent
- Soil pollutants as a result of historical and continued releases

![Chemical structure of PCBs](attachment:image.png)

Aroclor 1242
Nano- (and micro-) scale ZVI/ bimetallic ZVI particles are active in PCB dechlorination

<table>
<thead>
<tr>
<th>Reference</th>
<th>Test conditions</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowry &amp; Johnson (2004) <em>Environ. Sci. Technol.</em> 38, 5208.</td>
<td>ZVI (30-50 nm) in methanol/water with PCB congeners 22′, 34′, 234, 22′35′, 22′45′, 33′44′</td>
<td><img src="image" alt="Graph showing mass of PCB congeners over time" /></td>
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<tr>
<td>He et al. (2009) <em>J. Hazard. Mat.</em> 164, 126.</td>
<td>Pd/ZVI (1-2 μm) in soil with congener 22′455′</td>
<td>Production of 22′4-trichlorobiphenyl</td>
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ZVI treatment may not always result in complete dechlorination but could produce lower-chlorinated congeners from more highly chlorinated parent PCBs.
Depending on the extent and pattern of chlorination, microorganisms may dehalogenate or completely mineralize PCBs

- Under anaerobic conditions, bacteria may slowly reductively dehalogenate higher chlorinated PCB congeners
- Under aerobic conditions, bacteria may mineralize lower (<3 chlorines) chlorinated PCB congeners
A two-step PCB remediation process combining nZVI and bioremediation?

1. Use of nZVI to dechlorinate higher chlorinated PCBs
2. Use of bioremediation to remove the resulting lower-chlorinated products
   • Step 2 could involve monitored natural attenuation, bioaugmentation, biostimulation, rhizoremediation

OVERALL AIM
• **How compatible is step 2 with step 1?**
• **What is the impact of nZVI on soil microbial communities that are responsible for:**
  • Chloroaromatic degradation
  • Plant nutrient acquisition (nutrient mineralization and symbioses)

*http://www.nerc.ac.uk/research/programmes/nanoscience/background.asp*
Short and long timescales

Time (d)

0

• Addition of aroclor 1242 (50 mg kg\(^{-1}\))
• Addition of ZVI (10 mg kg\(^{-1}\))

28

• Soil redox
• Soil microbial activity (global and chloroaromatic degraders)

112

• Plant growth
• Plant N fixing symbioses
• Soil microbial activity (chloroaromatic degraders)

Expt 1 (n & mZVI)

Expt 2 (nPd/ZVI)
ZVI particles

TEM digital images of nano and micro-particles. The image was obtained using a Phillips CM20 TEM operated at 200 kV. For sample preparation, a droplet of the slurry was placed on a copper grid with a lacy carbon film and allowed to dry in air. nZVI and nPd/ZVI were produced by ball-milling (Golder Associates) and mZVI was obtained from BASF.

Polyacrylic acid (PAA) (MW = 1,200 g / mol) was used as a dispersant at a concentration of 5 % wt / wt with the ZVI.
Short and long timescales

Time (d)

0
28
112

- Addition of aroclor 1242 (50 mg kg\(^{-1}\))
- Addition of ZVI (10 mg kg\(^{-1}\))

Revegetation

- Soil redox
  - Soil microbial activity (global and chloroaromatic degraders)

Expt 1 (n & mZVI)

- Plant growth
- Plant N fixing symbioses
- Soil microbial activity (chloroaromatic degraders)

Expt 2 (nPd/ZVI)
Effects on soil redox potential are consistent with Fe corrosion chemistry

- Addition of nZVI created an immediate and significant reducing environment
- Redox potential thereafter recovered at a rate related to the age of the nZVI particles
- Effects produced by mZVI were less pronounced

\[ 2\text{Fe}^0 + 4\text{H}^+ + \text{O}_2 = 2\text{Fe}^{2+} + 2\text{H}_2\text{O} \]

\[ \text{Fe}^0 + 2\text{H}_2\text{O} = \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^- \]
Short and long timescales

Time (d)

0

- Addition of aroclor 1242 (50 mg kg\(^{-1}\))
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- Revegetation
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Expt 1 (n & mZVI)

Expt 2 (nPd/ZVI)
nZVI impacts on dehydrogenase activity

- **Dehydrogenase assay** - estimates the activity of the membrane bound electron transport chain and therefore measures the activities of intact microbial cells in the decomposition of organic matter.

- In the assay, the substrate (INT) acts as a terminal electron acceptor in microbial respiration and is reduced to INTF.

- There was no abiotic reduction of INT to INTF.

- nZVI stimulated dehydrogenase activity.
ZVI impacts on FDA hydrolysis activity

- **Fluorescein diacetate hydrolysis (FDA) assay** - estimates the global hydrolytic activity of soil enzymes (intra- and extracellular) in the depolymerisation reactions involved in organic matter breakdown

- Neither nanoscale nor microscale ZVI had any significant (p>0.05) effect on soil microbial global hydrolytic activity
Short and long timescales

Time (d)

0

- Addition of aroclor 1242 (50 mg kg$^{-1}$)
- Addition of ZVI (10 mg kg$^{-1}$)
- Revegetation

28

- Soil redox
- Soil microbial activity (global and chloroaromatic degraders)

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- Plant growth
- Plant N fixing symbioses
- Soil microbial activity (chloroaromatic degraders)

Expt 1 (n & mZVI)

Expt 2 (nPd/ZVI)
nZVI short-term impacts on chloroaromatic degrader number and activity

- nZVI initially depressed numbers of chloroaromatic degrading microbes, but numbers recovered over the same timescale as the recovery in soil redox potential
nZVI short-term impacts on chloroaromatic degrader number and activity

Degrader activity was assessed through a mineralization assay

\[ Y = \frac{a}{1 + 10^{\log(EC_{50}) t}} \]

‘Hillslope’ parameter used to examine treatment and time effects on mineralization kinetics
nZVI short-term impacts on chloroaromatic degrader number and activity

- ZVI significantly decreased the rate of chloroaromatic mineralization in comparison to the PAA control at all time points.
- Although degrader numbers appeared to recover with time, their chloroaromatic degradation activity was inhibited.
  - Direct or indirect effect of nZVI on expression of catabolic pathway?
Short and long timescales

- Addition of aroclor 1242 (50 mg kg\(^{-1}\))
- Addition of ZVI (10 mg kg\(^{-1}\))

Time (d)

- 0
- 28
- 112

Revegetation

- Soil redox
- Soil microbial activity (global and chloroaromatic degraders)

Expt 1 (n & mZVI)

- Soil microbial activity (chloroaromatic degraders)
- Plant growth
- Plant N fixing symbioses

Expt 2 (nPd/ZVI)
Pd/nZVI long-term impacts on chloroaromatic degrader number and activity

- Pd/nZVI had no effect on the number or activity of chloroaromatic degradative microorganisms in the developing clover rhizosphere
Short and long timescales

- Addition of aroclor 1242 (50 mg kg\(^{-1}\))
- Addition of ZVI (10 mg kg\(^{-1}\))

Time (d)

0

- Soil redox
- Soil microbial activity (global and chloroaromatic degraders)

28

- Soil microbial activity (chloroaromatic degraders)
  - Plant growth
  - Plant N fixing symbioses

112

- Revegetation

Expt 1 (n & mZVI)

Expt 2 (nPd/ZVI)
Pd/nZVI impact on plant growth

- No effect of aroclor or nZVI on growth of *Trifolium*
- nZVI significantly (p<0.01) enhanced growth of *Lolium* at all time points
Pd/nZVI impact on plant symbiosis

- Aroclor and nZVI significantly depressed nodulation in *Trifolium*
Effects on plant growth and symbioses may be due to perturbation of microbial soil N cycling

- nZVI appeared to enhance concentrations of soil plant available nitrogen and this was reflected in the N content of plant tissues
- Increased N availability could explain the growth response of *Lolium*
- Growth response in *Trifolium* was not evident due to compensation by symbiotic nitrogen fixation
  - Plants in control (low available N) soils had more nodules and obtained more nitrogen from symbiotic fixation
Summary of nZVI impacts on soil microbes

• In the short term, no negative effects of nZVI on total microbial activity were detected, however, nZVI did perturb the activity of microbes specifically involved in chloroaromatic biodegradation. This has possible implications for the efficacy of combined nZVI/microbial remediation approaches.

• In the longer term, Pd/nZVI had no effect on the activity of chloroaromatic degradative microbes in the developing clover rhizosphere but impacted plant growth and symbiotic N₂ fixation possibly through effects on soil nitrogen cycling.
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Environmental health and safety considerations related to the use of nZVI for remediation

- In the case of incomplete remediation, are the remediation products (e.g. partially reduced compounds) more bioavailable/ mobile/ toxic than the starting contaminant mix?
- What happens to the particles themselves after remediation?
  - [Results suggest that reactivity is quite quickly attenuated in the soil environment and soil microbial functions may be resistant, or, after an initial perturbation display resilience*.] Can particles transfer to other systems (e.g. enter aboveground foodchains via plant uptake) with ecological and human health implications

*We need to confirm if ‘sensitive’ functions such as ammonia oxidation conform to this statement!
Challenges for the development, commercialisation and application of nZVI for site remediation

- Developing a framework for demonstrating environmental and human health safety.
  - What ecotoxicology tests to use? Some standard soil assays are confounded by the reactivity of the particles.
  - What should we use as the control? (for example, should we use ZVI in microscale or a competing remediation technology)
- Challenges of scaling up
- Generalizing across environments, particles and contaminants
Acknowledgements

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- Environmental Nanoscience Initiative

+ Aroclor + nZVI

- Aroclor - nZVI

NATURAL ENVIRONMENT RESEARCH COUNCIL

Environment Agency

defra
Effects on soil pH and redox are consistent with Fe corrosion chemistry

- Addition of nZVI to soil (10 g Kg\(^{-1}\)) produced an immediate and significant elevation in soil pH of between 1 and 1.5 units
- pH declined thereafter
- Effects produced by fresh and aged nZVI were similar, the impact of mZVI was less pronounced

\[
2\text{Fe}^0 + 4\text{H}^+ + \text{O}_2 = 2\text{Fe}^{2+} + 2\text{H}_2\text{O}
\]

\[
\text{Fe}^0 + 2\text{H}_2\text{O} = \text{Fe}^{2+} + \text{H}_2 + 2\text{OH}^-
\]
ZVI impacts on ‘general’ microbial activity
-some background on the assays

Polymeric organic matter

Hydrolytic extracellular enzymes produced by cell

Bacterial cell

Endo and exo enzymes

Monomers

CO₂

Electron transport chain

½ O₂

H₂O

Oxidation
ZVI impacts on microbial activity

- some background on the assays

- Fluorescein diacetate (FDA) in soil is hydrolysed by extra- and intra-cellular hydrolases (includes lipases, proteases) to produce fluorescein

- The **FDA assay** estimates the global hydrolytic activity of soil in the depolymerisation reactions involved in organic matter breakdown

\[
\text{Fluorescein diacetate (colourless)} \quad \xrightarrow{\text{hydrolases}} \quad \text{Fluorescein (coloured)}
\]
ZVI impacts on microbial activity
-some background on the assays

- The **dehydrogenase assay** specifically estimates the activity of the membrane bound electron transport chain and therefore measures the activities of intact cells in the decomposition of organic matter.
nZVI impacts on chloroaromatic degrader number and activity

2,4-dichlorophenoxyacetic acid (2,4-D) chosen as model chloroaromatic since the pathway is convergent with that for chlorobenzoate biodegradation and $^{14}C$-UL-2,4-D is available

3-chlorobiphenyl

3-chlorobenzoate

3-chlorocatechol
nZVI impacts on dehydrogenase activity

- nZVI apparently stimulated the production of INTF, the dehydrogenase assay product
- There was no abiotic reduction of INT to INTF
- The stimulation of INTF production could be linked to the removal of O$_2$ as a competing electron acceptor (with the assay substrate) rather than a real stimulation of microbial activity
- Identify the extent of the possible environmental benefits from the application of those nanotechnologies;
- Consider the environmental health and safety implications related to the use of nanotechnology for environmentally beneficial purposes;
- Identify challenges for the development, commercialisation and application of nanotechnology for environmental benefit; and,
- Discuss policy measures to address challenges in the application of nanotechnology for environmental benefit.