PEPS – Scientifically and Field Proven Phytoremediation Systems for Petroleum and Salt Impacted Soil

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Partners: ConocoPhillips, Lone Pine, Baytex, TransEuro, Shell, Devon, Legacy, Tundra, Enbridge, Seaway Energy Services, NSERC
Presentation Outline

• Phytoremediation
  – What it is
  – How it works

• PEPS phytoremediation systems
  – Science behind PEPS
  – Commercial activities - laboratory and field

• Advantages of PEPS
Phytoremediation is the use of plants to extract, degrade, contain and immobilize chemicals, including salt, from the soil.

Rhizosphere processes create contaminant bioavailability
- Plant uptake soil $\rightarrow$ root
- Translocation: root symplast $\rightarrow$ xylem
- Chelation/compartment in leaves or roots
Phytoremediation

• One of the remedial techniques for treatment of contaminated soils
  – Dig and dump
  – Soil washing/flushing
  – Thermal desorption
  – Oxidation
  – Conventional Bioremediation (i.e. landfarming)
  – **Phytoremediation**
PEPS - Plant growth promoting rhizobacteria (PGPR) Enhanced Phytoremediation Systems

• PGPR applied to seeds prior to planting
• PGPR – natural, non-pathogenic strains; usually Pseudomonads
• Isolate PGPR from all soils (ON, AB, SK, NWT)
• Not bioaugmentation

Science  Experience  Results
• Thirteen years of lab and field research
• Full scale commercial remediation for >7 years
• Successfully deployed at >30 sites
  – 10+ sites remediated
  – PHC in AB, BC, MB, NWT, QC and ON
  – Salt sites in SK, AB, MB and NWT
• Research to continually improve the systems
The Science Behind PEPS
PEPS

Rhizosphere consists of:
- Soil
- Organic matter
- Bacteria
- Water
- Roots
- Contaminants

PEPS improves the rhizosphere which results in aggressive plant growth

Remediation

Rhizosphere consists of:
- Soil
- Organic matter
- Bacteria
- Water
- Roots
- Contaminants
Interaction of a PGPR Containing ACC Deaminase with a Root

- Stress ethylene
- Plant vigor
- Root development
- Rhizobacteria ➔ Consumption of PHC
- Leaves ➔ Salt and metals uptake

- Active rhizosphere
- Partitioning of contaminants
PHC Metabolism

A

PHC-degrading microbes
PHC-degrading microbes

oxidized PHC

oil droplet

PHC

O₂

1 µm

rhizosphere

cytosol

B

Cₙ PHC

[O] ω-oxygenase

OH

[O] dehydrogenase

H

[O] ω-oxygenase

OH

Cₙ fatty acid

β-oxidation

acetate

cell membrane

(width C₃₀ to C₄₀)
PEPS Performance

- PEPS creates abundant plant shoot and root growth
- Greater than 2X more plant biomass due to PGPR
- Very healthy rhizosphere – microbe level 10-100X greater
- Effective partitioning of contaminants
- PHC degraded in the soil
- PHC remediation - 30 to 40% per year
- Salt uptake – 0.5 – 1.5 dS/m per year
Commercial Projects
Laboratory and Field
PEPS

- Interpretation of remediation results
- PGPR isolation
- Seed preparation and shipping
- Site prep, sampling and seeding
- Plant growth and monitoring
- Fall sampling and site work
- Sample analysis (GC and QA/QC)
- PGPR regeneration & validation
PGPR Isolation

• Naturally occurring
• Isolated from site rhizospheres – adapted to impacted soils
• Continually isolating new strains
• DNA sequencing to identify them
• Biosafety Level I
• Non-GMO
• Currently have >10 strains
PEPS

Interpretation of remediation results

PGPR isolation

Seed preparation and shipping

PGPR regeneration & validation

Sample analysis (GC and QA/QC)

Fall sampling and fall site work

Plant growth and monitoring

Site prep, sampling and seeding
PGPR Regeneration & Validation

- Regenerate proven PGPR isolates for field use
- Confirm PGPR are healthy and retain key biological activities
- Assay for ACC deaminase
- Assay for auxin production
- Assay for plant growth
PEPS

Seed preparation and shipping

Site prep, sampling and seeding

Plant growth and monitoring

Fall sampling and fall site work

Sample analysis (GC and QA/QC)

PGPR isolation

PGPR regeneration & validation

Interpretation of remediation results

Seed preparation and shipping

PGPR regeneration & validation

Sample analysis (GC and QA/QC)

Plant growth and monitoring

Fall sampling and fall site work

Seed preparation and shipping

PGPR isolation

Interpretation of remediation results
Seed Treating

- Treat seeds with proven and regenerated PGPR
- Only proven grass and cereal species are used
- Mechanical seed treater efficiently and evenly coats the seeds
PGPR Seed Treatment QA/QC

- Aliquots of PGPR-treated seeds assayed for plant growth enhancement
Shipment

- Treated seeds shipped to sites after QA/QC
PEPS

Seed preparation and shipping

Site prep, sampling and seeding

Plant growth and monitoring

PGPR isolation

Interpretation of remediation results

PGPR regeneration & validation

Fall sampling and fall site work

Sample analysis (GC and QA/QC)
Seed Bed Preparation & Amendment Application

Compacted Clay Liner Construction

Sampling

Sow PGPR-treated Seed
Site prep, sampling and seeding

Plant growth and monitoring

Fall sampling and fall site work

Sample analysis (GC and QA/QC)

Interpretation of remediation results

PGPR isolation

PGPR regeneration & validation

Seed preparation and shipping
Edson, AB – Before site prep and seeding

All previous steps assure sites that looked like this.....

Soil Impact – PHC (Diesel Invert: 85% F3)
PEPS Deployment, Edson, AB

……Look like this

Soil Impact – PHC (Diesel Invert: 85% F3)
Weyburn, SK: Before PEPS

Soil Impact – Salt (ECe ~ 10 dS/m)
Weyburn, SK: After one month

Soil Impact – Salt (ECe ~ 10 dS/m)
Weyburn, SK: After three months

Average NaCl in leaf tissue = 23 g/kg

Soil Impact – Salt (ECe ~ 10 dS/m)
PEPS

Interpretation of remediation results

Sample analysis (GC and QA/QC)

PGPR isolation

Fall sampling and fall site work

PGPR regeneration & validation

Plant growth and monitoring

Seed preparation and shipping

Site prep, sampling and seeding
Swathing/Mowing

At Salt Sites, Cut Grass is Removed

Fall Sampling and Site Work

Baling

At Salt Sites, Cut Grass is Removed
PEPS

Site prep, sampling and seeding

Plant growth and monitoring

Fall sampling and fall site work

Sample analysis (GC and QA/QC)

PGPR isolation

PGPR regeneration & validation

Seed preparation and shipping

Interpretation of remediation results
Sample Analysis

- Analysis of PHC and salt impacted soils
- Soil PHC – CCME GC method
- Soil Salt – ECe, SAR, Na and Cl
- Tissue Salt – Analysis of plant samples to assess plant uptake of salt
QA/QC Analysis

- PHC and/or salt samples are analyzed in at least two laboratories
- Data sets compared to assure data quality
- Data correlations are reviewed to determine data acceptance
Before PEPS | After PEPS
---|---
0 | 500
500 | 1000
1000 | 1500
1500 | 2000

Jun '07 | Jun '08 | Oct '08
---|---|---

PHC (mg/kg)

All 10 sampling points below criteria after remediation

Average 1500 mg/kg F3 to 1000 mg/kg in 2 years
## Full Scale PEPS Deployment at Typical PHC Sites

### Completed Sites - 1st Generation

<table>
<thead>
<tr>
<th>Site</th>
<th>Analysis</th>
<th>Date</th>
<th>Average (mg/kg)</th>
<th>% Remediation</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edson</td>
<td>CCME F3</td>
<td>Spring 2007</td>
<td>1500</td>
<td>33.33%</td>
<td>5 of 10 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F3</td>
<td>Fall 2008</td>
<td>1000</td>
<td></td>
<td>All sample point met Tier 1 criteria</td>
</tr>
<tr>
<td>Hinton 2</td>
<td>CCME F3</td>
<td>Spring 2007</td>
<td>900</td>
<td>44.44%</td>
<td>6 of 15 sample points above criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F3</td>
<td>Fall 2008</td>
<td>500</td>
<td></td>
<td>All sample point met Tier 1 criteria</td>
</tr>
<tr>
<td>Dawson 1</td>
<td>EPH(C10-19)</td>
<td>Spring 2009</td>
<td>6500</td>
<td>91.54%</td>
<td>12 of 12 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C10-19)</td>
<td>Fall 2011</td>
<td>550</td>
<td></td>
<td>1 of 12 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Spring 2009</td>
<td>2500</td>
<td>72.00%</td>
<td>11 of 12 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Fall 2011</td>
<td>700</td>
<td></td>
<td>All sample point met Tier 1 criteria</td>
</tr>
<tr>
<td>Peace River</td>
<td>F3</td>
<td>Spring 2007</td>
<td>900</td>
<td>78.89%</td>
<td>4 of 11 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>Fall 2008</td>
<td>190</td>
<td></td>
<td>All sample point met Tier 1 criteria</td>
</tr>
<tr>
<td>Quebec City</td>
<td>F3</td>
<td>Spring 2009</td>
<td>550</td>
<td>49.09%</td>
<td>3 of 3 sample points above criteria</td>
</tr>
<tr>
<td></td>
<td>F3</td>
<td>Fall 2009</td>
<td>280</td>
<td></td>
<td>All sample point met Tier 1 criteria</td>
</tr>
</tbody>
</table>

### Sites in Progress - 2nd Generation

<table>
<thead>
<tr>
<th>Site</th>
<th>Analysis</th>
<th>Date</th>
<th>Average (mg/kg)</th>
<th>% Remediation</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hinton 1</td>
<td>CCME F2</td>
<td>Spring 2010</td>
<td>1100</td>
<td>77.27%</td>
<td>10 of 10 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F2</td>
<td>Fall 2010</td>
<td>250</td>
<td></td>
<td>6 of 10 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F3</td>
<td>Spring 2010</td>
<td>3200</td>
<td>56.25%</td>
<td>9 of 10 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F3</td>
<td>Fall 2010</td>
<td>1400</td>
<td></td>
<td>3 of 10 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td>Swan Hills</td>
<td>CCME F2</td>
<td>Spring 2009</td>
<td>1400</td>
<td>78.57%</td>
<td>8 of 8 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F2</td>
<td>Fall 2010</td>
<td>300</td>
<td></td>
<td>4 of 8 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F3</td>
<td>Spring 2009</td>
<td>2550</td>
<td>64.71%</td>
<td>7 of 8 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>CCME F3</td>
<td>Fall 2010</td>
<td>900</td>
<td></td>
<td>1 of 8 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td>Dawson 2</td>
<td>EPH(C10-19)</td>
<td>Spring 2009</td>
<td>6500</td>
<td>46.15%</td>
<td>15 of 15 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C10-19)</td>
<td>Fall 2011</td>
<td>3500</td>
<td></td>
<td>8 of 15 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Spring 2009</td>
<td>700</td>
<td>42.86%</td>
<td>3 of 15 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Fall 2011</td>
<td>400</td>
<td></td>
<td>All sample point met Tier 1 criteria</td>
</tr>
<tr>
<td>Dawson 3</td>
<td>EPH(C10-19)</td>
<td>Spring 2009</td>
<td>7000</td>
<td>81.43%</td>
<td>11 of 12 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C10-19)</td>
<td>Fall 2011</td>
<td>1300</td>
<td></td>
<td>5 of 15 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Spring 2009</td>
<td>3500</td>
<td>57.14%</td>
<td>12 of 12 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Fall 2011</td>
<td>1500</td>
<td></td>
<td>6 of 12 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td>Beaver River</td>
<td>EPH(C10-19)</td>
<td>Spring 2010</td>
<td>1600</td>
<td>25.00%</td>
<td>8 of 20 sample points above Tier 1 criteria</td>
</tr>
<tr>
<td></td>
<td>EPH(C10-19)</td>
<td>Fall 2010</td>
<td>1200</td>
<td></td>
<td>6 of 20 sample points above Tier 1 criteria</td>
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<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Spring 2010</td>
<td>850</td>
<td>35.29%</td>
<td>8 of 20 sample points above Tier 1 criteria</td>
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<tr>
<td></td>
<td>EPH(C19-32)</td>
<td>Fall 2010</td>
<td>550</td>
<td></td>
<td>3 of 20 sample points above Tier 1 criteria</td>
</tr>
</tbody>
</table>

### Average Remediation = 34 % per year
# Full Scale PEPS Deployment at Typical Salt Sites

<table>
<thead>
<tr>
<th>Site</th>
<th>Analysis</th>
<th>Date</th>
<th>Average (dS/m)</th>
<th>% Remediation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Completed Sites – 1st Generation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nota</td>
<td>ECe</td>
<td>Spring 2008</td>
<td>7.7</td>
<td>70.13%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2010</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Provost</td>
<td>ECe</td>
<td>Spring 2009</td>
<td>14.5</td>
<td>44.83%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2009</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Sites in Progress – 2nd Generation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weyburn</td>
<td>ECe</td>
<td>Fall 2010</td>
<td>13.5</td>
<td>22.22%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2011</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Weyburn</td>
<td>ECe</td>
<td>Fall 2010</td>
<td>6.9</td>
<td>14.49%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2011</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Weyburn</td>
<td>ECe</td>
<td>Fall 2010</td>
<td>13.5</td>
<td>10.37%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2011</td>
<td>12.1</td>
<td></td>
</tr>
<tr>
<td>Weyburn</td>
<td>ECe</td>
<td>Fall 2010</td>
<td>14.3</td>
<td>11.89%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2011</td>
<td>12.6</td>
<td></td>
</tr>
<tr>
<td>Red Earth</td>
<td>ECe</td>
<td>North, Sp 2010</td>
<td>5.2</td>
<td>13.46%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>North, F 2011</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>South, Sp 2010</td>
<td>4.2</td>
<td>9.52%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>South, F 2011</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Kindersley</td>
<td>ECe</td>
<td>Spring 2008</td>
<td>5.5</td>
<td>27.27%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2009</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Cannington Manor</td>
<td>ECe</td>
<td>Spring 2007</td>
<td>17.6</td>
<td>32.95%</td>
</tr>
<tr>
<td></td>
<td>ECe</td>
<td>Fall 2008</td>
<td>11.8</td>
<td></td>
</tr>
</tbody>
</table>

Approximately 1 ECe unit per year
Conclusions for Salt Remediation

Data derived from 12 commercial research project sites

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual Drop in Soil $E_{C_e}$</td>
<td>10% to 20%</td>
</tr>
<tr>
<td>NaCl Uptake into Foliage</td>
<td>29 g/kg dry weight</td>
</tr>
<tr>
<td>NaCl removed from the field in foliage</td>
<td>150 kg/ha</td>
</tr>
<tr>
<td>Change in $E_{C_e}$ accounted for by foliar uptake of salt</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Research Initiatives = Innovation

- NSERC: Optimization of PHC analysis for phytoremediation
- NSERC: Establishing Tier 2 SOPs for site closure
- ISTP: Phytoremediation of salt impacted soils in China
- IRAP: Product development platform for improved PEPS
Why Use PEPS?

- Peer reviewed science and performance
- Proven for PHC and/or salt impacted sites
- PHC - PEPS meets Tier I or II
- Salt – PEPS re-vegetates impacted sites & reduces soil salt levels to guideline values
- 100 % success rate at >30 sites
- Liability is reduced, not transferred and maintained
- Regulator support
Why Use PEPS?

• Environmentally responsible
  – Green technology
  – Driven by solar energy – northern vs. southern
  – Soil is conserved
  – Soil quality is improved
  – Greenhouse gas storage

• Cost effective
  – More cost effective at remote sites
  – Sites with large soil volumes – half the cost of landfilling
  – Costs spread out over 2 – 3 years

• It works!
Thank you

To get a publications list, please provide me with a business card