INTERNATIONAL SYMPOSIUM ON

# BIOREMEDIATION AND SUSTAINABLE ENVIRONMENTAL TECHNOLOGIES

# FINAL PROGRAM

June 27-30, 2011 • Reno, Nevada • Peppermill Resort


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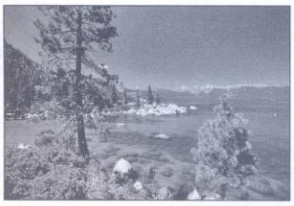
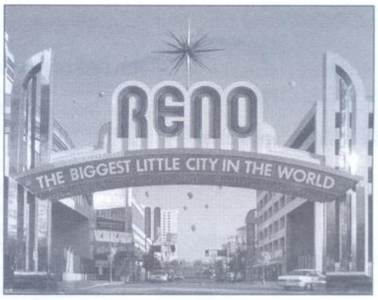
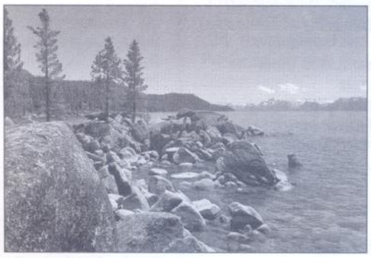
| D Sessions<br>Tuscany 9 | E Sessions<br>Tuscany 8 | F Sessions<br>Tuscany 7 | |
|---|---|---|---|
| **D10. Mitigating Vapor Intrusion Cost-Effectively and Sustainably** | **E7. Remedial System Optimization for Footprint Reduction** | **F8. Addressing Biofuel Releases** | |
| **Design of Passive and Sustainable Vapor Intrusion Mitigation Systems.** *D.J. Folkes.*<br>David J. Folkes (EnviroGroup, Ltd./USA | **State Perspectives on Green Considerations during Site Optimization: Interstate Technology Regulatory Council Findings.** *T.K. O'Neill, R. Bourdon, and S. Madabhushi.*<br>Sriram Madabhusi (Booz Allen Hamilton/USA) | **BREAK** | 3:30 |
| **Advanced Vapor Barrier Technology: A 15-Site Review.** *P. Grant.*<br>Peter Grant (Land Science Technologies/USA) | **Lifecycle Optimization: Bringing Sustainability into Evaluation of Short- and Long-Term Remedial Effectiveness.** *C.A. Hook, M.J. Maughon, K.W. Henn, D. Owens, M.A. Singletary, and T.A. Spriggs.*<br>Christopher A. Hook (Tetra Tech, Inc./USA) | **Assessment of Stimulatory Fermentative Processes to Enhance Natural Attenuation of Groundwater Contaminated with Biodiesel (B20).** *D. Toledo Ramos, H.S. Chiaranda, M.L. Busi da Silva, and H.X. Corseuil.*<br>Henry X. Corseuil (Federal University of Santa Catarina/Brazil) | 3:55 |
| **Mitigation of Soil Vapor Intrusion through Application of Zero-Valent Iron and Emulsified Vegetable Oil.** *C. Bickmore, W. Newman, K. Colberg, K. Rapp, W. Anthony, and R. Wojciak.*<br>Keith Rapp (Sustainable Resources Group, Inc./USA) | **Utilizing Advanced Diagnostic Tools to Minimize the Environmental Footprint of In Situ Remediation Projects.** *M. Burns and J.A. Simon.*<br>John Simon (WSP Environment & Energy/USA) | **In Situ Remediation of an E95 Ethanol Spill—A Case Study.** *L.P. Tousignant, E. Evrard, and I. Campin.*<br>Luc P. Tousignant (LPT Enviro Inc./Canada) | 4:20 |
| **VOC Diffusion Testing of Innovative Vapor Intrusion Mitigation Membrane.** *H. Nguyen and J. Di.*<br>Hieu Nguyen (CETCO/USA) | **Greening a Multimillion-Dollar Pump-and-Treat System: Remedial Process and Energy Process Optimization.** *T.L. McMonagle, R. Niederoest, D.S. Woodward, R.M. Fetzer, and J. Iannuzzo.*<br>Thomas L. McMonagle (AECOM/USA) | **Gasohol Biosparging in Fractured Bedrock: LNAPL to Drinking Water Standards in Less Than Three Years.** *L.L. Hartig, D.S. Woodward, and M.S. Heaston.*<br>Leslie L. Hartig (AECOM/USA) | 4:45 |

THURSDAY





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# PROGRAM AT A GLANCE

## MONDAY, JUNE 27, 2011

**8:00 A.M.–5:00 P.M. Short Course**
- Geochemical Evaluations of Metals in Environmental Media

**8:00 A.M.–Noon Short Courses**
- In Situ Chemical Reduction (ISCR) Technologies
- The SRT™ and SiteWise™ Sustainable Remediation Tools
- Measurement and Use of Mass Discharge and Mass Flux

**1:00–5:00 P.M. Short Courses**
- Utilization of Stable Isotopes in Bioremediation Studies
- Advances in PRB Design, Application, and Assessment

**Short Course Registration 7:00 A.M. and 12:00 Noon**

**Symposium Registration 2:00–8:30 P.M.**

**Exhibits, Welcome Reception, and Poster Group 1 Display 4:30–6:00 P.M.**

**Plenary Session 6:00–8:00 P.M.**

| | TUESDAY, JUNE 28, 2011 | WEDNESDAY, JUNE 29, 2011 | THURSDAY, JUNE 30, 2011 |
|---|---|---|---|
| | Exhibits, Registration, Poster Display 7:00 A.M.–7:00 P.M.<br>Continental Breakfast 7:00–8:00 A.M.<br>Group Lunch | Exhibits, Registration, Poster Display 7:00 A.M.–7:00 P.M.<br>Continental Breakfast 7:00–8:00 A.M.<br>Group Lunch | Poster Display 7:00 A.M.–1:00 P.M.<br>Exhibits 7:00 A.M.–1:30 P.M.<br>Registration 7:00 A.M.–5:00 P.M.<br>Continental Breakfast 7:00–8:00 A.M.<br>Group Lunch |
| | **Platform Sessions 8:00 A.M.–5:10 P.M.** | **Platform Sessions 8:00 A.M.–5:10 P.M.** | **Platform Sessions 8:00 A.M.–5:10 P.M.** |
| **A** | **A1.** Improvements to Subsurface Delivery Strategies<br>**A2.** Combining Thermal Treatments with Biological Polishing Approaches<br>**A3.** Designs for Bioremediation in Fractured Rock and Bedrock Environments | **A4.** Biobarrier Installation, Monitoring, and Renewal<br>**A5.** Strategies for DNAPL Site Remediation<br>**PANEL.** Integrated Approaches for DNAPL Sites | **A6.** Bioaugmentation<br>**A7.** Addressing the Impacts of pH on Aquifer Bioremediation<br>**A8.** Combining Chemical Treatments with Biological Polishing Approaches |
| **B** | **B1.** Biodegradation of Crude Oil Spills in the Terrestrial Environment<br>**B2.** Biodegradation of Crude Oil Spills in Marine Environments<br>**B3.** Advances in Phytoremediation<br>**B4.** Bioremediation of PAHs | **B5.** LNAPL Site Management Strategies<br>**B6.** Anaerobic Bioremediation of Petroleum Hydrocarbons<br>**B7.** Ex Situ Biological Treatment | **B8.** Enhancements to Aerobic Biodegradation Strategies<br>**B9.** Enhancements to Anaerobic Biodegradation Strategies<br>**B10.** Enhanced Chlorinated Ethene Degradation Strategies<br>**B11.** Substrate Enhancements for In Situ Remedies |
| **C** | **C1.** Biogeochemical Transformation Processes<br>**PANEL.** Biogeochemical Transformation Processes<br>**C2.** Applications of Compound-Specific Stable Isotope Analysis (CSIA)<br>**C3.** Identification and Evaluation of Novel Microorganisms for Contaminant Degradation | **C4.** Applying Molecular Methods to Understand the Microbial Communities Involved in Contaminant Degradation<br>**C5.** Aerobic and Anaerobic Degradation Pathways<br>**C6.** Abiotic Degradation Pathways<br>**C7.** Identifying and Modeling Biodegradative Pathways | **C8.** Use of Molecular Biological Tools (MBTs)<br>**C9.** Integrating Detection Methods for Bioremediation Assessment<br>**C10.** Development and Applications of Predictive Models for Bioremediation Assessment<br>**C11.** Assessment of Monitored Natural Attenuation (MNA) of Organics |
| **D** | **D1.** Bioremediation and Monitored Natural Attenuation (MNA) of Inorganics: Strategies, Implementation, and Assessment<br>**D2.** Fate and Persistence of Endocrine Disruptors and Emerging Contaminants That Threaten Aquatic Environments<br>**D3.** NDMA, 1,4-dioxane, and Other Emerging Contaminants | **D4.** Detection Methods for Emerging Contaminants<br>**D5.** Treatment Technologies for Emerging Contaminants<br>**D6.** Degradation Processes in the Vadose Zone | **D7.** Vapor Intrusion Conceptual Site Models (CSMs): Toxicity, Risk, Fate and Transport<br>**D8.** Sampling and Assessment at Vapor Intrusion Sites<br>**D9.** Spatial and Temporal Variability in Vapor Intrusion Data<br>**D10.** Mitigating Vapor Intrusion Cost-Effectively and Sustainably |
| **E** | **E1.** Estimating Remediation Impacts: Tools and Approaches<br>**E2.** Incentives for Implementing Green and Sustainable Remediation (GSR) Techniques<br>**E3.** Integrating Renewable Energy into Remedial Programs to Achieve Cost-Effective Solutions | **PANEL.** Integrating Society into Sustainable Remediation Decision Making<br>**E4.** Site End-Use Considerations to Maximize Net Environmental, Social, and Economic Benefits<br>**E5.** Incorporating Green and Sustainable Remediation (GSR) Practices into Remedy Selection and Design | **E6.** Technologies and Approaches to Achieve More Sustainable Remedies<br>**E7.** Remedial System Optimization for Footprint Reduction |
| **F** | **F1.** Innovative Site Characterization<br>**F2.** Remedial Risk Management<br>**F3.** Long-Term Monitoring Optimization | **F4.** Optimization and Evaluation of Remedy Effectiveness<br>**PANEL.** Performance-Based Remediation<br>**F5.** Microbial-Based Fuel Cells | **F6.** Biologically Based Alternative Energy<br>**F7.** Environmental Impacts from Biofuels<br>**F8.** Addressing Biofuel Releases |
| | **Poster Group 1 Presentations and Reception 5:15–6:45 P.M.**<br>**Session list on page 8** | **Poster Group 2 Presentations and Reception 5:15–6:45 P.M.**<br>**Session list on page 8** | **Symposium Adjourns 5:10 P.M.** |

# Assessment of Stimulatory Fermentative Processes to Enhance Natural Attenuation of Groundwater Contaminated with Biodiesel (B20)

Débora Toledo Ramos[1], Helen Simone Chiaranda[1], Márcio Luis Busi da Silva[2], ***Henry Xavier Corseuil***[1]
[1]Federal University of Santa Catarina, Department of Sanitary and Environmental Engineering, Florianópolis, Santa Catarina, Brazil
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While BTEX and PAHs have been reported to be degraded under sulfate-reducing/fermentative conditions little is known about how stimulation of fermentative processes could minimize time to achieve groundwater remediation goals. The aim of this work was to evaluate the feasibility of biostimulation of fermentative processes to remediate groundwater contaminated with diesel/biodiesel blends. A field-scale experiment is being conducted at the Ressacada Experimental Farm in Florianópolis, SC, Brazil. In July 2010, 100 L of B20 (80% diesel 20% biodiesel) was spilled in an area of 1 m x 1 m that was excavated up to 1.6 meters below ground surface. Ammonium acetate is being added weekly**,** since one month after the release in injection wells near the source zone to promote fermentative biostimulation. Results indicated that fermentative biostimulation could promote the establishment of strongly reducing conditions three months after the release of B20, as demonstrated by methanogenesis evidence and decreases in the redox potential. Comparatively, such conditions were only observed after 2 years in a similar control experiment under natural attenuation. Additionally, a decrease in BTEX concentration was noticed in the source zone, 8 months after the controlled release of B20, while in the control experiment, BTEX concentrations were still increasing two years after the release. Results indicate that the injection of ammonium acetate promoted the fortuitous growth of fermentative bacteria and methanogenic archaea, which rapidly promoted monoaromatic hydrocarbons biodegradation in the aqueous phase at the source zone.

KEY WORDS: Biodiesel, field-scale experiment, fermentative biostimulation

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## ABSTRACT

Bioremediation often relies on aerobic and anaerobic electron acceptors to accelerate natural attenuation of groundwater contaminated. The use of nitrate, iron and sulfate to promote the establishment of thermodynamically favorable conditions and thus, enhance anaerobic hydrocarbon biodegradation has been extensively demonstrated, but groundwater contaminated with biodiesel/diesel blends tends naturally towards methanogenic conditions as they exert significantly high electron acceptors demands. While BTEX and PAHs have been reported to be degraded under sulfate-reducing/fermentative conditions little is known about how stimulation of fermentative processes could minimize time to achieve groundwater remediation goals. The aim of this work was to evaluate the feasibility of biostimulation of fermentative processes to remediate groundwater contaminated with diesel/biodiesel blends. A field-scale experiment is being conducted at the Ressacada Experimental Farm in Florianópolis, SC, Brazil. In July 2010, 100 L of B20 (80% diesel 20% biodiesel) was spilled in an area of 1 m x 1 m that was excavated up to 1.6 meters below ground surface. Ammonium acetate is being added weekly**,** since one month after the release in injection wells near the source zone to promote fermentative biostimulation. Results indicated that fermentative biostimulation could promote the establishment of strongly reducing conditions three months after the release of B20, as demonstrated by methanogenesis evidence and decreases in the redox potential. Comparatively, such conditions were only observed after 2 years in a similar control experiment under natural attenuation. Additionally, a decrease in BTEX concentration was noticed in the source zone, 8 months after the controlled release of B20, while in the control experiment, BTEX concentrations were still increasing two years after the release. An inhibitory effect caused by thermodynamic constraints associated with acetate and hydrogen accumulation, might have prevented BTEX attenuation from occurring during the third month after the release and when such intermediates were consumed (8 months after the release), BTEX degradation started to proceed. Results indicate that the injection of ammonium acetate promoted the fortuitous growth of fermentative bacteria and

methanogenic archaea, which rapidly promoted monoaromatic hydrocarbons biodegradation in the aqueous phase at the source zone.

KEY WORDS: Biodiesel, field-scale experiment, fermentative biostimulation.

## INTRODUCTION

The worldwide increasing demands for energy and the environmental problems associated with the use of fossil fuels have led to the sought for alternative biofuels such as biodiesel. Biodiesel is being added to the official Brazilian Energy Matrix) since 2005. In 2010, biodiesel blending percentage with diesel increased to 5% (ANP, 2011). While biodiesel do not pose potential hazardous risks to the environment, when mixed with diesel fuels, the mixture will contain known groundwater contaminants such as BTEX and PAHs. In case of a biodiesel/diesel blend spill in groundwater, the high biochemical oxygen demand exerted by this blend, results in the development of strongly anaerobic (methanogenic) conditions. Therefore, anaerobic bioremediation strategies are considered more advantageous for the cleanup of organic pollutants releases, mainly near the source zone, as it is a predominantly anaerobic environment. The use of nitrate, iron and sulfate to enhance anaerobic hydrocarbon biodegradation has been extensively demonstrated (Cunningham et al., 2001 ; Anderson & Lovely, 2000 ; Schreiber & Bahr, 2002 ; Beller et al., 1992 ; Lovley & Lonergan, 1990).However, due to the fact that biodiesel exerts a higher biochemical oxygen demand, it contributes to significantly higher electron acceptors and nutrient requirements compared to BTEX and PAH.

The use of anaerobic electron acceptors can promote the establishment of thermodynamically favorable conditions but groundwater contaminated with biodiesel/diesel blends tends naturally towards methanogenic conditions. While BTEX and PAH have been reported to be degraded under sulfate-reducing/fermentative conditions (Ulrich et al., 2005 ; Anderson & Lovley, 2000 ; Weiner & Lovley, 1998; Lovley et al., 1995) little is known about how stimulation of fermentative processes could minimize time to achieve groundwater remediation goals under field conditions.

Fermentative biostimulation may contribute to the degradation of petroleum hydrocarbons and biodiesel esters by rapidly providing the redox conditions that would be naturally achieved in more than a year and also stimulating the growth of specific microorganisms that might accelerate degradation kinetics of the aromatic hydrocarbons. This study addresses the feasibility of fermentative biostimulation to remediate groundwater contaminated with diesel/biodiesel blends in a field-scale experiment.

## MATERIALS AND METHODS

**Experimental area.** The field-scale experiment is located at the Ressacada Experimental Farm in Florianópolis, SC, Brazil. The regional climate in Florianópolis is humid mesotermic with a mean annual precipitation of 1165mm. The measured groundwater temperature is approximately 26ºC in the summer and 22ºC in the winter.

The subsurface layer is composed by 80% of gray fine sand, approximately 5% of silt and less than 5% of clay. The average soil organic carbon varies between 0.16 and 0.68%. Groundwater velocity in the experimental area is 6 meters/year.

The source zone was established in an area of 1 m x 1 m that was excavated up to 1.6 m below the surface where 100 L of B20 (80% diesel 20% biodiesel) and 3kg of potassium bromide tracer were spilled in July 2010. The experimental area has $330m^2$ and is covered with 41 monitoring wells and 5 injection wells with five depths (2, 3, 4, 5 and 6 meters). Ammonium acetate is being added weekly since one month after the release in the injection wells near the source zone to promote fermentative biostimulation.

**Groundwater analyses.** Groundwater samples were analyzed for BTEX and methane using a gas chromatograph equipped with a flame ionization detector (FID). A Flow Cell MP20 was used to measure redox potential at the field site. Analyses of acetate ($CH_3COO^-$) were performed by a Dionex Ion Chromatograph S-1000 equipped with a conductivity detector. Molecular biology analyses by RT-qPCR (Real-time quantitative polymerase chain reaction) were carried out in a Mastercycler ep realplex (Eppendorf) thermocycler and samples are being monitored for total bacteria, iron and sulfate-reducing bacteria, methanogenic archaea and *bssA* gene copies through the use of primers and probes reported in several studies (Silva & Alvarez, 2004, Beller et al., 2008; Stults et al., 2001).

## RESULTS AND DISCUSSION

Geochemical analyses indicate that the system is becoming more reductive, according to what would be expected in fermentative processes. Significant changes in redox potential (from 179 to -14 mV) and increases in methane concentrations (from 1.5 to 17 mg/L) were noticed near the source zone 8 months after the release, while in the natural attenuation experiment such results were observed approximately 2 years after the release (Chiaranda and Corseuil, 2010). Aqueous BTEX concentrations also started to decrease in 8 months (Figure 1) while in the natural attenuation experiment BTEX concentrations were still increasing two years after the release. Total bacteria, iron reducers and methanogenic archaea increased, respectively, 4, 3 and 5 orders of magnitude, 8 months after the release indicating that biodiesel and diesel compounds, together with ammonium acetate amendment, stimulated microbial growth. The presence of *bssA* genes was also observed at the source zone indicating that aromatic hydrocarbons are being degraded under anaerobic conditions (Beller et al., 2008).

The present study demonstrated that fermentative biostimulation increased biomass concentration and established optimal conditions to enhance BTEX degradation preventing the negative influence of the biodiesel esters over aromatic compounds biodegradation. However, this fermentative biostimulation experiment is still in its initial stages and further results are needed to better understand the processes involved.

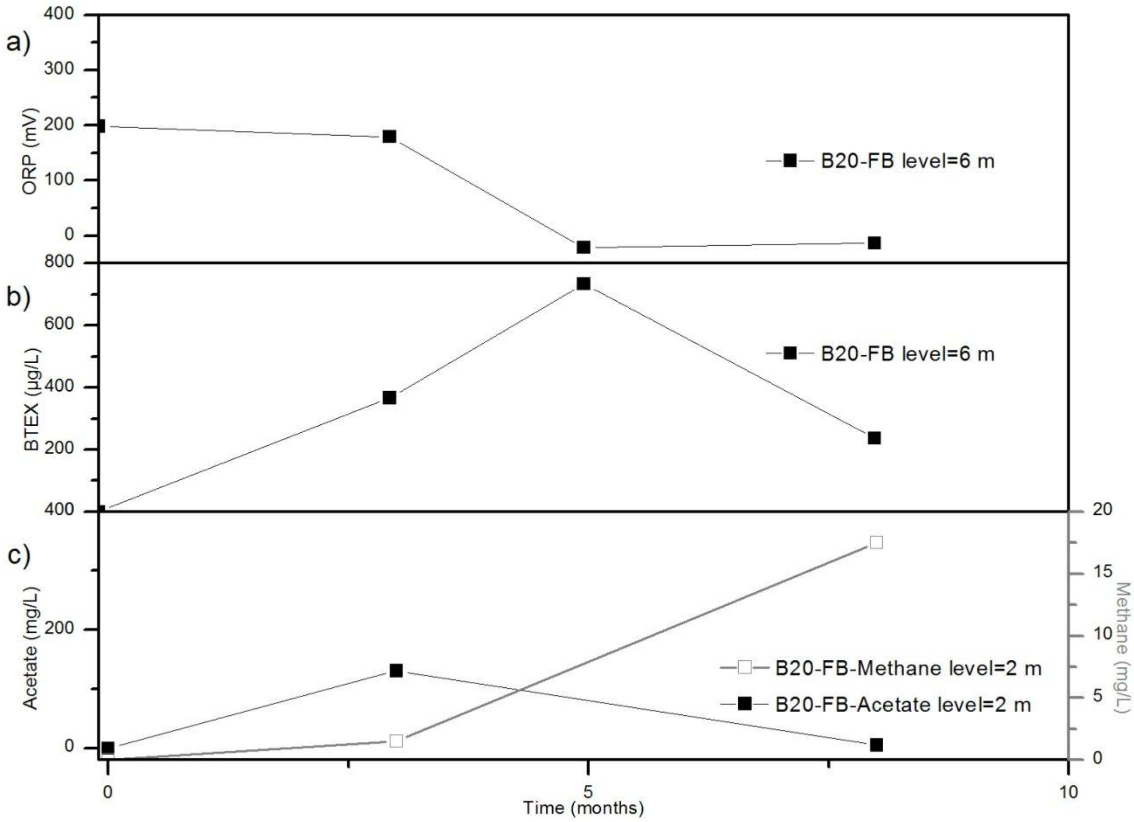


Figure 1. Redox potential values (mV) (a) and BTEX concentrations (µg/L) (b) at level 6 meters from FB (fermentative biostimulation) experiment. Acetate and methane concentrations (mg/L) at level 2 meters from FB experiment (c).

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