



## Biodegradation of Ethanol, n-Butanol, iso-Butanol, n-Propanol, 2,5-Dimethyfuran and B-100 Biodiesel in Aquifer Sediment From Fuel Spill Sites

## **Problem Definition**

Previous EPA research has shown that ethanol can inhibit natural anaerobic biodegradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) in ground water, making for longer plumes of BTEX compounds. It can produce potentially explosive concentrations of hydrogen and methane gas at gasoline spill sites. Little is known about the potential impact of other alternative biofuels (such as normal propanol, normal butanol, isobutanol, or 2,5-dimethyfuran) on BTEX biodegradation or about their capacity to produce methane and hydrogen in ground water.

Petroleum diesel has had less impact on ground water than gasoline because the content of water soluble components, such as BTEX, in petroleum diesel is much less. The BTEX plumes are smaller and don't go very far. Most biodiesel is composed of methyl esters of long-chain fatty acids. These compounds should be readily degradable under sulfate-reducing conditions and, much like ethanol, would consume available sulfate and allow the BTEX plumes associated with a biodiesel spill to expand. They should also ferment to form methane and hydrogen gas.

## **Project Objective**

This investigation will involve preparation of laboratory microcosms to:

- Evaluate the sulfate demand of alternative biofuels in aquifer sediment
- Evaluate the biofuels' capacity to produce methane and hydrogen
- Determine the biofuels' impact on the rate of anaerobic biodegradation of BTEX compounds

Laboratory microcosm experiments will be conducted on sediment from two fuel spill sites. One is a fresh spill of B-100 biodiesel in Minnesota. The second is an old spill of JP-4 jet fuel in North Carolina. The second is well acclimated for anaerobic biodegradation of organic materials, including BTEX compounds.

The experiments are intended to determine the:

- Kinetics of natural biodegradation of ethanol, n-butanol, iso-butanol, n-propanol, 2,5-dimethylfuran, and biodiesel when adequate sulfate is and is not available
- Effect of ethanol, n-butanol, iso-butanol, n-propanol, 2,5-dimethylfuran, and biodiesel on the natural anaerobic biodegradation of the BTEX compounds when adequate sulfate is and is not available
- Potential for production of methane from ethanol, n-butanol, iso-butanol, n-propanol, 2,5-dimethylfuran, and biodiesel when adequate sulfate is and is not available

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## **Experimental Protocol**

Nineteen experimental treatments will be applied to each of the two sediment samples:

- 1. 2,000 milligrams per liter (mg/L) sulfate, 200 mg/L ethanol, 1 mg/L each of the BTEX compounds
- 2. No added sulfate, 200 mg/L ethanol, 1 mg/L each of the BTEX compounds
- 3. 2,000 mg/L sulfate, 200 mg/L butanol, 1 mg/L each of the BTEX compounds
- 4. No added sulfate, 200 mg/L butanol, 1 mg/L each of the BTEX compounds
- 5. 2,000 mg/L sulfate, 200 mg/L iso-butanol, 1 mg/L each of the BTEX compounds
- 6. No added sulfate, 200 mg/L iso-butanol, 1 mg/L each of the BTEX compounds
- 7. 2,000 mg/L sulfate, 200 mg/L n-propanol, 1 mg/L each of the BTEX compounds
- 8. No added sulfate, 200 mg/L n-propanol, 1 mg/L each of the BTEX compounds
- 9. 2,000 mg/L sulfate, 20 mg/L 2,5-dimethylfuran, 1 mg/L each of the BTEX compounds
- 10. No added sulfate, 20 mg/L 2,5-dimethylfuran, 1 mg/L each of the BTEX compounds
- 11. 2,000 mg/L sulfate, 200 mg/L biodiesel emulsion, 1 mg/L each of the BTEX compounds
- 12. No added sulfate, 200 mg/L biodiesel emulsion, 1 mg/L each of the BTEX compounds
- 13. 2,000 mg/L sulfate, 1 mg/L each of the BTEX compounds
- 14. No added sulfate, 1 mg/L each of the BTEX compounds
- 15. Autoclaved sterile sediment, 200 mg/L ethanol, butanol, iso-butanol, n-propanol, 20 mg/L 2,5-dimethylfuran, 1 mg/L each of the BTEX compounds, and 2,000 mg/L sulfate (The amendments should also be sterile.)
- 16. Autoclaved sterile sediment, 200 mg/L biodiesel emulsion, 1 mg/L each of the BTEX compounds and 2,000 mg/L sulfate (The amendments should also be sterile.)
- 17. Sediment with 2,000 mg/L sulfate
- 18. Sediment with no amendments as provided from Minnesota or North Carolina
- 19. Container control with sterile water containing 200 mg/L ethanol, butanol, iso-butanol, n-propanol, 20 mg/L 2,5-dimethylfuran, 2,000 mg/L sulfate, and 1 mg/L each of the BTEX compounds, but no sediment

Water samples will be collected from the microcosms after 1 day and after 1, 2, 4, 6, 8, and 12 months. Water samples will be analyzed for:

- Concentration of chemicals of concern, including BTEX, ethanol, n-butanol, iso-butanol, n-propanol, and 2,5-dimethylfuran
- Degradation products, including formic acid, lactic acid, acetic acid, propionic acid, butyric acid, and methane
- Geochemical parameters, including sulfate, dissolved organic carbon, and dissolved inorganic carbon

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