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Enhancing Natural Attenuation
1,4-Dioxane Degradation using Butane Biostimulation

Abstract:

1,4-dioxane (dioxane) is used as a solvent for cellulose esters, oils, waxes, resins, and numerous organic and inorganic substances. It is also used in coatings and as a stabilizer in chlorinated solvents. Dioxane is a colorless liquid with a faint ethereal odor. It has a density of 1.033 at 20 C and boils at 101 C. The toxicity of dioxane is low in test animals by all routes of exposure. However, in humans the toxicity of this compound is severe. The target organs are the liver, kidneys, lungs, skin, and eyes. Exposure to dioxane vapors as well as absorption of the compound through the skin or by ingestion can cause poisoning, the symptoms of which include drowsiness, headache, respiratory distress, nausea, and vomiting. It causes depression of the central nervous system. There are reports of human deaths from subacute and chronic exposures to dioxane vapors at concentration levels ranging between 500 and 1000 parts per million (ppm). Serious health hazards may arise from its injurious effects on the liver, kidneys, and brain. Rabbits died of kidney injury resulting from repeated inhalation of dioxane vapors for 30 days (Smyth 1956).

Environmental Fate – Biodegradation

The aqueous aerobic half-life of dioxane is estimated between 672-4320 hours, based on data obtained using unacclimated aerobic aqueous screening test data (Sasaki 1978; Kawasaki 1980; Howard et al. 1991). The aqueous anaerobic half-life of dioxane is estimated between 2688-17280 hours, based on estimated aqueous aerobic biodegradation half-life (Howard et al. 1991).

Groundwater Sample Collection Method

Global BioSciences, Inc. (GBI) received a groundwater sample from a Brownfield location in Massachusetts. The Brownfield site was impacted by various chemical releases in the past and low concentrations of dioxane remain in the groundwater at the site. GBI was engaged to determine whether dioxane was amenable to enhanced biological degradation using butane biostimulation.

Isolation Methods and Enrichment Transfers

The groundwater sample collected at the Brownfield location was enriched using standard microbiological subculturing techniques. The enrichment serum bottles were incubated at an average groundwater temperature (approximately 10 C) for a four week period.

The medium used for isolating, maintaining and conducting degradation experiments with butane-oxidizing bacteria for the groundwater sample was a modification of the mineral salt medium (MSM) of Whittenbury et al. The medium consisted of the following chemicals:

$MgSO_4$–$7H_2O$, 1.0 g; $CaCl_2$, 0.2 g; NH_4Cl, 0.5 g; $FeCl_3$– $6H_2O$, 4.0 mg; trace elements solution, 0.5 ml; distilled water, 1000 ml. The trace elements solution was: $ZnCl_2$, 5.0 mg; $MnCl_2$–$4H_2O$, 3.0 mg; H_3BO_4, 30.0 mg; $NiCl_2$–$6H_2O$, 2.0 mg; $(NH_4)_6Mo_7O_{24}$–$4H_2O$, 2.25 mg; distilled water, 1000 ml.

The pH of the MSM was adjusted to 6.8 before autoclaving (20 min at 121 C) with 20.0 ml of a phosphate buffer solution comprising 3.6 g of Na_2HPO_4 and 1.4 g of KH_2PO_4 in 100 ml of distilled water. After autoclaving the MSM and the buffer solution, another 20 ml of the buffer solution was added to the MSM when the temperature of the media reached 60 C.

The enrichments for the groundwater sample, Brown-1 and Brown-1-Dup, were conducted in 125-ml serum bottles with 50 ml of MSM and 10 milliliters of untreated Site groundwater. Subsequent culture transfers (5.0 ml) were conducted with sterilized plastic syringes. The bottles were capped with red rubber plugs and crimped with aluminum seals. Each sample was handled aseptically, and all glassware, materials and supplies were sterilized by autoclaving. Butane and air were replaced in the headspace of each serum bottle using a dedicated, sterile, gas tight syringe with inert sampling valve (on/off lever). Butane was added at a concentration of 4% (vol/vol). A serum bottle of groundwater (heat sterilized) was maintained as a control.

Microcosm Study

A microcosm study using static headspace methods was conducted to evaluate butane consumption and dioxane degradation rates for the groundwater sample. Static headspace involves a partitioning of volatile components between the aqueous and vapor phases enclosed in a gas-tight vessel (serum bottle). A concentration of research grade butane in the range of 175 to 400 parts per million (ppm) and dioxane in the range of 5.0 to 10.0 ppm were added to the headspace of each microcosm (Brown-1 and Brown-1-Dup). Two serum bottles of dead cells (heat sterilized) were maintained as controls (Control-1 and Control-1-Dup). The serum bottles were inverted and shaken to allow for equilibration. All bottles were incubated at an average groundwater temperature (approximately 10 C) for the five day study.

The microcosm study evaluated butane and dioxane disappearance at times 0, +1, +2, +4 and +5 days. Twenty serum bottles (10 positive and 10 control samples) were spiked with the butane and dioxane concentrations at time zero. Two positive sample serum bottles and two control serum bottles were sacrificed immediately for GC analysis. At time +1 day, two additional positive samples and two control samples were sacrificed for GC analysis. The process repeated itself at time +2, +4, and +5 days.

The serum bottles were capped with gray butyl rubber plugs coated with Teflon® and crimped with aluminum seals. A 1.0-ml headspace sample from each serum bottle was analyzed using a SRI 8610-C gas chromatograph (GC) equipped with a flame ionization detector (FID). Gas-tight syringes with inert sampling valves were used for on-column injections. The method was calibrated using external standards.

Microcosm Study Results

Enrichment Transfers

After two weeks of enrichment transfers, the liquid suspension for the two samples (Brown-1 and Brown-1-Dup) demonstrated a significant increase in turbidity with the exception of the control bottles. The cell suspension color in the microcosm was off white.

Microcosm Evaluations

The two samples Brown-1 and Brown-1-Dup demonstrated dioxane losses of 6.73 and 7.66 ppm, respectively, over a five day period while the control serum bottles (Control-1 and Control-1-Dup) demonstrated losses of 1.35 and 2.07 ppm, respectively (abiotic loss). Abiotic losses can be attributed to leaks through a serum bottle septum. At day two, the dioxane concentrations in the serum bottles Brown-1 and Brown-1-Dup dropped below the laboratory detection limit of 0.1 ppm. *The difference between the total loss and abiotic loss is attributed to microbial activity, direct metabolism or cometabolism, and demonstrates that dioxane was completely degraded within a 48 hour period.*

During the five day study, samples Brown-1 and Brown-1-Dup consumed 96 and 132 ppm of butane, respectively, while the control samples Control-1 and Control-1-Dup demonstrated losses of 4 and 18 ppm. Again, the difference between the total loss for each sample and the control or abiotic loss can be attributed to direct microbial activity and butane oxidation.

The increase in butane consumption and dioxane degradation (direct metabolism or cometabolism) is a direct result of the aerobic microbial community shifting toward a higher percentage of butane-oxidizers after butane enrichment.

Discussion

The degradation potential using butane biostimulation for dioxane appears to be high based on the results of the four week enrichment project and five day microcosm evaluation. Butane and air injection have already proven to be highly effective for the remediation of another ether; that is, methyl tertiary butyl ether or MTBE. As referenced earlier in this report, the aqueous aerobic half-life of dioxane is estimated between 672-4320 hours. The accelerated half-life of dioxane, using butane biostimulation, drops to 24 hours since the laboratory

microcosm evaluation demonstrated complete degradation within a 48 hour period (while still taking into consideration the abiotic losses).

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