Ground Water Monitoring&Remediation

Biodegradation of Soybean and Castor Oil Biodiesel: Implications on the Natural Attenuation of Monoaromatic Hydrocarbons in Groundwater

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Abstract

The potential groundwater impacts of biodiesel releases have received limited attention despite the increasing probability of such events. In this work, microcosms were prepared with unacclimated sediment and groundwater from the Ressacada Experimental Site (Florianopolis, Santa Catarina, Brazil) and spiked with 54.8 mg/L of pure soybean or castor oil biodiesel (B100). Oxygen was purged from the microcosms to mimic commonly anoxic and hypoxic conditions at fuel-impacted sites; low background concentrations of nitrate (1.2 to 2.5 mg/L) and sulfate (2.2 to 3.0 mg/L) were present. Biodegradation was assessed by the removal of fatty acid methyl esters and hydrocarbon components relative to sterile controls. Approximately 80% of soybean biodiesel was biotransformed in 41 d, compared to only 40% of castor oil biodiesel removed in 90 d. The higher persistence of castor biodiesel was attributed to its higher viscosity and lower bioavailability. Additional microcosms were prepared similarly to assess the impact of biodiesel on hydrocarbon degradation. These microcosms were spiked with benzene (2.9 mg/L) and toluene (0.8 mg/L) with or without soybean biodiesel (54.8 mg/L). The biodiesel had an inhibitory effect, increasing the time required to remove toluene from 25 to 34 d. Similarly, 45% of benzene was removed in the presence of biodiesel within 34 d, compared to 90% in the absence of biodiesel. Overall, we postulate that the relatively high viscosity of biodiesel is conducive to limited migration potential and a smaller but longer lasting inhibitory region of influence, compared to that exerted by more soluble, more mobile, and readily degradable biofuels such as ethanol. However, controlled release studies are needed to test this hypothesis and characterize the complex dynamics of such releases.

Introduction

Renewable fuels are increasingly being used to curtail escalating oil prices and the carbon footprint of fossil fuel use, as well as to gain independence from imported oil (Hill et al. 2006). Brazil, the global frontrunner of biofuel production, implemented ethanol-blended gasoline as early as the 1930s and has considered the use of biodiesel since 1975 (Nass et al. 2007). In 2005, biodiesel was added to the official Brazilian Energy Matrix (Law 11.097) and since 2010, the mandatory blending percentage of biodiesel with petroleum diesel is 5% (Brazil 2009). Although the use of biodiesel reduces harmful volatile emissions and decreases oil dependence, the risks associated with releases that impact groundwater aquifers have received limited attention.

Biofuels are typically blended with gasoline or petroleum diesel, which contain toxic and relatively mobile monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene, and xylenes (BTEX). BTEX can pose a risk to public health in the event of a spill or a leaking underground storage tank containing biofuel blends. Benzene is of particular concern due to its carcinogenicity, relative persistence under anaerobic conditions that prevail in fuel-impacted aquifers, and high solubility in water (Alexander 1999; Alvarez and Illman 2006).

Biodegradation and natural attenuation are commonly used to mitigate the risk of hydrocarbon-contaminated aquifers. Recent research on the effects of biofuels on hydrocarbon-impacted groundwater has focused primarily on ethanol. However, unlike ethanol, biodiesel is composed of multiple components and is produced from various sources. Biodiesel is produced from the transesterification of oils, resulting in saturated or unsaturated C_{16}–C_{20} fatty acid methyl esters (FAMEs). The combination of FAMEs and respective percentages within biodiesel is a function of

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the initial feedstock. For example, the main component of rapeseed biodiesel is the methyl ester of erucic acid, making up approximately 50% of the mixture (Zhang et al. 1998); soybean biodiesel lacks the methyl ester of erucic acid and 55% of its content is the methyl ester of linoleic acid; and castor oil-based biodiesel is made up of approximately 80% methyl ricinoleate (Table 1).

In addition to environmental releases of biodiesel/diesel blends from leaking storage tanks or spills, pure biodiesel may be intentionally introduced into hydrocarbon-impacted sediment to enhance the biodegradation of crude oil and coal tar PAH (Miller and Mudge 1997; Mudge and Pereira 1999; Taylor and Jones 2001). The mechanisms for the putative stimulatory effect have not been elucidated, although based on previous studies, it is likely that they involve microbial growth promotion (Follis et al. 1995) and bioavailability (emulsion) enhancement (Taylor et al. 2001; DeMello et al. 2007; Owsianiak et al. 2009).

The increased probability of biodiesel co-occurring with hydrocarbons in contaminated aquifers underscores the need to assess the associated risks to groundwater quality. Critical knowledge gaps include the biodegradation characteristics of different types of biodiesel (and their constituents) under oxygen-limited conditions commonly encountered at fuel-impacted sites and their effect on BTEX natural attenuation. Toward this goal, this article compares the biodegradation of pure biodiesel methyl esters derived from soybean vs. castor oil, evaluates the influence of soybean biodiesel on the biodegradation of benzene and toluene, and considers the physicochemical properties of biodiesel to infer on its likely field-scale behavior and region of influence compared to the common biofuel, ethanol.

Materials and Methods

Groundwater Characterization

Groundwater used in the microcosms was collected from a monitoring well in an uncontaminated area of the Ressacada Experimental Site (27°41′04.09″S, 48°32′48.20″), Florianopolis, Santa Catarina, Brazil, at a depth of 2.5 m. A peristaltic pump and Teflon® tubing were used to transfer groundwater into capped sterile bottles without headspace. An MP20 MicroPurge Flow Cell (QED Environmental Systems, Ann Arbor, Michigan) was used to measure pH (4.7), redox potential (340 mV), dissolved oxygen (5.6 mg/L), and temperature (22 °C) at the field site. Groundwater samples were analyzed for nitrate (1.2 to 2.5 mg/L as NO_3^-), phosphate (0.2 mg/L as PO_4^{-3}), and sulfate (2.2 to 3.0 mg/L as SO_4^{-2}) using a Dionex Ion Chromatograph S-1000 (São Paulo, SP, Brazil) equipped with a conductivity detector and an AS14A column. The method used is described elsewhere (American Public Health Association 1992). Detection limits were 0.05 mg/L for nitrate, 0.01 mg/L for phosphate, and 0.01 mg/L for sulfate.

Sediment Characterization

Sediment samples were collected near the monitoring well at a depth of 1.5 to 2.5 m and were analyzed for calcium (30 mg/kg), magnesium (14 mg/kg), manganese (5.4 mg/kg), aluminum (69 mg/kg), potassium (18 mg/kg), sodium (32 mg/kg), iron (0.1% w:w), copper (0.2 mg/kg), sulfur (0.2 % w:w), zinc (1.5 mg/kg), and phosphorus (4.8 mg/kg). Analyses were performed by the Integrated Agricultural Development Company of Santa Catarina (CIDASC).

Biodiesel Characterization

Biodiesel from soybean oil and castor oil were provided by the Paraná Institute of Technology (TECPAR). The biodiesel samples were analyzed using a gas chromatograph (Trace GC Ultra, Thermo Scientific, San Jose, California) coupled to an ion trap mass spectrometer (Polaris Q 25 GC/MS) equipped with a CBP20 capillary column (30 m × 0.25 mm × 0.25 mm). One microliter of biodiesel extract was injected at a split ratio of 50:1. The heating program was 100 °C with an incremental rate of 10 °C/min to 240 °C for 20 min with an injector and detector temperature of 250 °C.

Biodiesel from castor oil consisted of approximately 75% methyl ricinoleate and 25% of other nonhydroxy methyl esters. The methyl ester of ricinoleate was not present in soybean biodiesel, which contained only nonhydroxy methyl esters, including linoleate at 42% (Table 1).

Table 1
Biodiesel Fatty Acid Methyl Ester Composition

FAME	Rapeseed Oil (%)	Soybean Oil (%)		Castor Oil (%)
Palmitate	2.2	9.9	12.1	2.6
Stereate	0.9	3.8	4.4	1.6
Oleate	12.6	19.0	28.1	6.7
Linoleate	12.1	55.7	42.0	12.0
Linolenate	7.0	10.2	13.3	1.2
Ricinoleate	—	—	—	76.0
Eicoseneate	7.4	0.2	—	—
Eurceate	49.8	—	—	—
Reference	Zhang et al. (1998)	Zhang et al. (1998)	This study	This study

Microcosm Preparation

Aquifer microcosms were prepared in 100-mL serum bottles with 20 g of wet sediment and 80 mL of groundwater (18 mL of headspace remaining), and sealed with Teflon-coated septa and aluminum crimp caps. The microcosms were purged with compressed nitrogen gas for 15 min. Teflon tubing was attached to the nitrogen tank valve and fitted with an 18-gauge needle to puncture the rubber septa and supply a constant stream of nitrogen gas to the microcosms. A second needle was used to relieve the buildup of purged gas within the microcosm. The microcosms were incubated in the dark at 25 °C. Microcosms were amended with soybean B100, castor oil B100, benzene and toluene, or soybean B100 plus benzene and toluene. Sets 1 (54.8 mg/L B100 soybean) and 2 (56.9 mg/L B100 castor oil) were prepared in duplicate, and sets 3 (2.9 mg/L benzene and 0.8 mg/L toluene) and 4 (2.9 mg/L benzene, 0.8 mg/L toluene, and 54.8 mg/L soybean biodiesel) in triplicate. Concentrations of benzene and toluene were based on concentrations near the source zone of a previous diesel experiment at Ressacada (Corseuil et al. 2003). Biodiesel concentrations were chosen as one order of magnitude higher. Abiotic control microcosms were poisoned with 1 g/L of mercuric chloride ($HgCl_2$). The incubation periods were 41 d for soybean oil biodiesel, 92 d for castor oil biodiesel, and 34 d for microcosms containing BTEX components. First-order biodegradation rate coefficients were determined as the slope of the linearized (semi-log) concentration vs. time data. Biotransformation rates were statistically compared using analysis of variance and p values less than 0.05 were considered significant.

Methyl Ester and Monoaromatic Hydrocarbon Analysis

Methyl esters were quantified by liquid-liquid extraction according to EPA Method 3510B (U.S. Environmental Protection Agency 1996a, 1996b) and analyzed on a Agilent 6890N II gas chromatograph (Agilent, São Paulo, SC, Brazil), equipped with a FID. Benzene and toluene were analyzed according to EPA Method 8015A (U.S. Environmental Protection Agency 1996a, 1996b) with a HP 6890 II gas chromatograph equipped with an FID detector and coupled to an HP Headspace Self Sampler–static (model 7694). Column and temperature settings were previously described (Gomes 2008). Detection limits were 1.0 μg/L for both benzene and toluene.

Results and Discussion

Biodegradation of Pure Biodiesel (B100) Derived from Soybean and Castor Oil

A significant disparity was observed in the persistence of soybean oil methyl esters (SME) vs. castor oil methyl esters (CME). Removal of total SME was 80% after 41 d, compared to only 40% of total CME in 92 d (Figure 1). Although methyl esters can be hydrolyzed abiotically, removal of methyl esters was not detected in the sterile control microcosms; therefore, the observed degradation was attributed to biological processes.

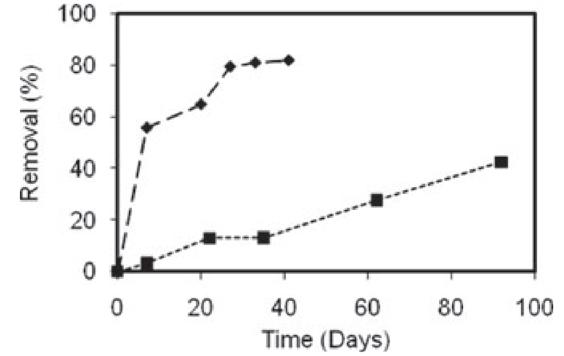

Figure 1. Percentage of transformation of the methyl esters was greater for (♦) SME (54.8 mg/L) vs. (■) CME (56.9 mg/L) at 25 °C and pH 4.7.

The significant difference between CME and SME biodiesel biodegradability could be explained by differences in their physicochemical properties, mainly viscosity. Higher viscosity results in decreased bioavailability and slower biodegradation. This relationship was previously established for the biodegradation of paraffinic oils (Haus et al. 2000), crude oil (Sugiura et al. 1996), and oleochemical esters (Andreas 1999). Castor-based biodiesel has a significantly higher viscosity (~13.5 mm^2/s at 40 °C) than soybean biodiesel (~4.1 mm^2/s at 40 °C) due to the presence of methyl ricinoleate, which makes up about 80% of CME and is not present in other biodiesel feedstocks (Tate et al. 2006; Conceição et al. 2007). Methyl ricinoleate is a unique ester with a mid-chain double bond and a highly reactive hydroxyl group. The latter is responsible for the three to five times greater viscosity when compared to other methyl esters found in CME or in SME (Table 2; Knothe and Steidley 2005). Previous studies have suggested that biodiesel feedstock does not affect its biodegradability. However, these studies only compared the aerobic biodegradation of ethyl and methyl esters from two feedstocks (rapeseed and soybean) both of which had similar viscosities (Zhang et al. 1998; Peterson and Moller 2005).

Anaerobic biodiesel degradation has been shown to proceed quickly (≤1 month) in salt and fresh water microcosms (Aktas et al. 2010). However, neither SME nor CME were completely removed within 41 or 92 d, respectively, in this work. Our experimental design allowed for some oxygen leakage into the microcosms, although it is unlikely that the amount infiltrated met the stoichiometric requirements for complete aerobic degradation. Therefore, the limited availability of terminal electron acceptors can be partially implicated in the relative persistence of the tested biodiesels. Anaerobic electron acceptor limitation is implied by the sequential depletion of the background nitrate and then sulfate (which is thermodynamically a less favorable as electron acceptor) during SME degradation (Figure 2A), although sulfate was not depleted in microcosms amended with the more persistent CME during the 34-d monitored incubation period (Figure 2B). Note that in the absence of electron acceptors, FAME hydrolysis followed by beta oxidation of the resulting fatty acids can occur and coenzyme NAD^+ (needed for sustained

Table 2
Composition of Soybean- and Castor Oil-Derived B100

Methyl Esters	Molecular Formula	SME (%)	CME (%)	Viscosity[1] (40 °C; mm^2/s)
Palmitate ($C_{16:0}$)	$C_{17}H_{34}O_2$	12.1	2.6	4.38
Stearate ($C_{18:0}$)	$C_{19}H_{38}O_2$	4.4	1.6	5.85
Oleate ($C_{18:1}$)	$C_{19}H_{36}O_2$	28.1	6.7	4.51
Linoleate ($C_{18:2}$)	$C_{19}H_{34}O_2$	42.0	12.0	3.65
Linolenate ($C_{18:3}$)	$C_{19}H_{32}O_2$	13.3	1.2	3.14
Ricinoleate ($C_{18:1\text{-}OH}$)	$C_{19}H_{36}O_3$	—	76.0	15.44

CX, length of chain; Y, the number of double bonds.

[1]Values obtained from Knothe et al. (2005).

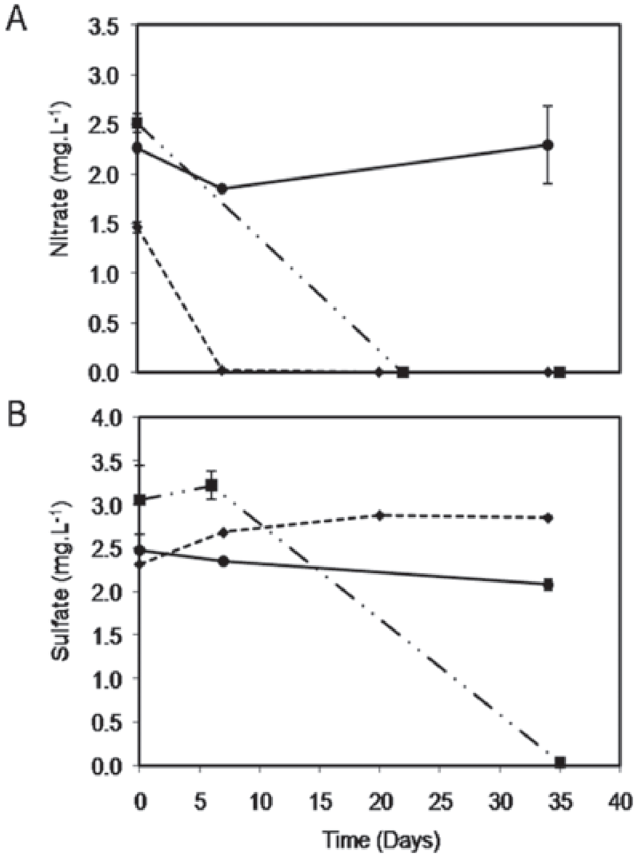

Figure 2. Nitrate (A) and sulfate (B) were sequentially reduced during the degradation of SME and only nitrate was utilized for the degradation of CME. (■) SME, (♦) CME, and (•) abiotic control. Error bars represent ± 1 standard deviation from the mean of triplicate measurements.

operation of related central metabolic pathways) can be fermentatively regenerated (Lengeler et al. 1999). Thus, electron balances based on electron acceptor consumption are precluded and no inference of biodiesel mineralization should be made.

The individual methyl ester components, palmitate ($C_{16:0}$), oleate ($C_{18:1}$), linoleate ($C_{18:2}$), and linolenate ($C_{18:3}$), of SME were transformed more rapidly than their corresponding methyl esters of CME (Figure 3). Results for each of the FAME constituents were normalized to total mass within the microcosm (separate and dissolved phases) because biodiesel was added into microcosms as a separate phase. First-order degradation rate coefficients for methyl esters of oleate, linoleate, and linolenate of SME were approximately one order of magnitude higher than their counterparts in CME (Table 3). The methyl ester of palmitate was six times faster in SME than in CME, whereas the degradation coefficient for the stearate methyl ester was similar for the two biodiesels.

In contrast to previous studies (Miller et al. 1997; DeMello et al. 2007), preferential transformation of methyl esters in SME or CME was not observed for either C_{16} or C_{18} except for the methyl ester of stearate, which had the slowest transformation rate of the C_{18}'s. First-order rate coefficients for the methyl ester components of CME were statistically undistinguishable, which suggests that the rate limiting step for CME and SME degradation was dissolution of the biodiesel nonaqueous phase liquid (NAPL). Compounds with a higher number of unsaturated bonds (the methyl esters of linolenate, linoleate, and oleate) had a greater (although not statistically significant) biotransformation rate in SME. Apparently, the biological oxidation of unsaturated acids is generally more favorable than that of saturated acids (Rhead et al. 1971; Miller et al. 1997; Lalman and Bagley 2001).

Effects of Soy Biodiesel on Hypoxic Biodegradation of Toluene and Benzene

The impact of SME on the degradation of toluene and benzene was evaluated over a 34-d incubation period. The presence of SME increased the time required for complete toluene removal from 25 to 34 d (Figure 4A). Similarly, at the end of the incubation period, 90% benzene removal occurred in microcosms lacking SME, compared to only 45% removal when amended with SME (Figure 4B). Considering the recalcitrance of benzene under anaerobic conditions and the relatively long acclimation times required for its anaerobic degradation (often requiring years) (Grbic-Galic and Vogel 1987; Edwards and Grbic-Galic 1992; Da Silva and Alvarez 2004), benzene removal was likely

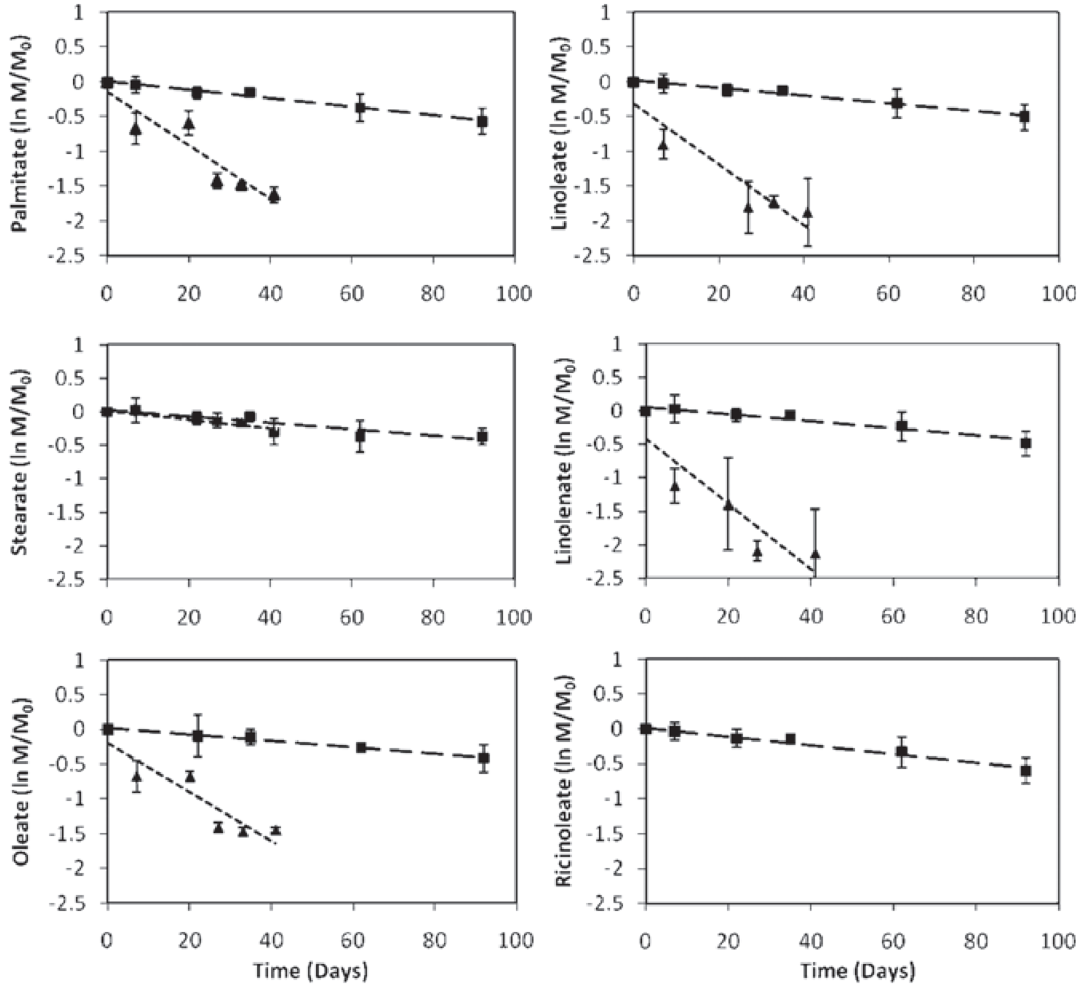

Figure 3. First-order degradation patterns for methyl esters present in (▲) soybean and (■) castor oil B100 show that soybean-derived B100 is more efficiently transformed.

Table 3
First-Order Biodegradation Rate Coefficients of FAMEs

	Degradation Coefficient k (Per Day)	
Methyl Ester	**Soybean Biodiesel**	**Castor Biodiesel**
Palmitate ($C_{16:0}$)	0.039 ± 0.013	0.006 ± 0.002[1]
Stearate ($C_{18:0}$)	0.006 ± 0.004	0.004 ± 0.001
Oleate ($C_{18:1}$)	0.043 ± 0.001	0.005 ± 0.002[1]
Linoleate ($C_{18:2}$)	0.062 ± 0.008	0.005 ± 0.002[1]
Linolenate ($C_{18:3}$)	0.062 ± 0.016	0.005 ± 0.001[1]
Ricinoleate ($C_{18:1\text{-}OH}$)	Not present	0.006 ± 0.002

[1]Denotes a statistically significant reduction in the degradation rate of castor biodiesel methyl ester compared to soybean biodiesel methyl ester.

associated with aerobic (possibly hypoxic) biotransformations followed by oxygen leaking into the microcosms (which were not incubated inside an anaerobic chamber). Oxygen intrusion into anaerobic contaminated plumes (e.g., due to natural recharge from surrounding groundwater and from the surface) is a common phenomenon, and BTEX catabolism initiated by oxygenases has previously been observed at very low levels of dissolved oxygen (0.1 mg/L) (Costura and Alvarez 2000). Accordingly, competition for oxygen by bacteria degrading alternative substrates hinders aerobic benzene biotransformations and the high oxygen demand exerted by SME (two times that of ethanol on a per liter basis (Gomes 2008)) likely contributed to a lower benzene removal efficiency.

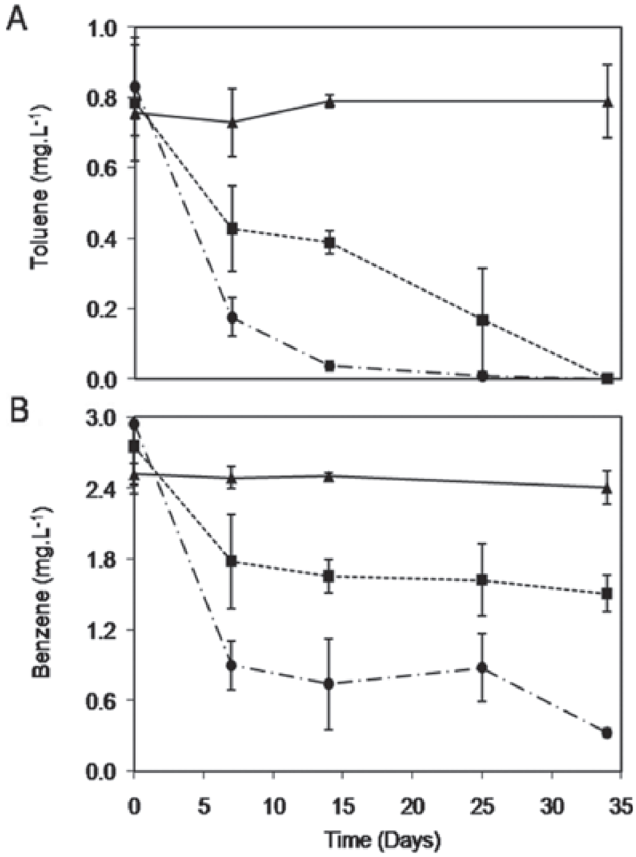

Figure 4. Biodegradation of toluene (A) and benzene (B) in the absence and presence of soy biodiesel. Error bars represent ± one standard deviation from the mean of triplicate measurements. (▲) Abiotic Control, (■) Hydrocarbon + Biodiesel, and (●) Hydrocarbon.

Implications on Biodiesel Region of Influence and Associated Inhibition of Hydrocarbon Natural Attenuation Relative to Ethanol

These results suggest that biodiesel could inhibit the biodegradation of co-occurring hydrocarbons, possibly through similar mechanisms as those reported for the common fuel additive, ethanol. Potential inhibitory mechanisms include accelerated electron acceptor depletion, preferential degradation (catabolite repression), metabolic flux dilution, and unfavorable microbial population shifts (Duetz et al. 1994; Da Silva and Alvarez 2002; Lovanh et al. 2002; Lovanh and Alvarez 2004; Capiro et al. 2008; Lawrence et al. 2009). However, the viscosity, solubility, and resulting migration properties of these two biofuels are significantly different, which would likely impact hydrocarbon natural attenuation and plume dynamics in different ways.

Ethanol is highly soluble in water and tends to quickly partition into groundwater and migrate away from the source zone (Heermann and Powers 1998; Cápiro et al. 2007), even though a small fraction can be retained in the pore water resulting in some mass transfer from the capillary zone into the groundwater (Stafford et al. 2009). Thus, ethanol is likely to primarily behave as a migrating source (Figure 5A). When dissolved at concentrations greater than about 10,000 mg/L, ethanol can exert cosolvency, which could result in faster hydrocarbon dissolution from the oily phase and faster migration (i.e., decreased sorption-related retardation) in the aquifer (Groves 1988; Corseuil et al. 2004; Cápiro et al. 2007; Gomez et al. 2008), thereby contributing to hydrocarbon plume elongation. The preferential biodegradation of ethanol and associated consumption of nutrients and dissolved oxygen that might otherwise be available for hydrocarbon degradation also contribute to inhibited natural attenuation. However, such impacts of ethanol are relatively short-lived (Gomez and Alvarez 2010) as ethanol is highly biodegradable and is rapidly removed from the source zone, allowing for upgradient recharge to reoxygenate the anaerobic source zone. A recent study reported the rapid (i.e., less than 1.5 months) return of aerobic conditions once ethanol was removed (biodegraded or transported) from an ethanol-hydrocarbon-impacted pilot-scale aquifer (Capiro et al. 2008). In contrast to ethanol, biodiesel is not readily miscible in groundwater and tends to remain near the source zone (Figure 5B). Although some FAME metabolites such

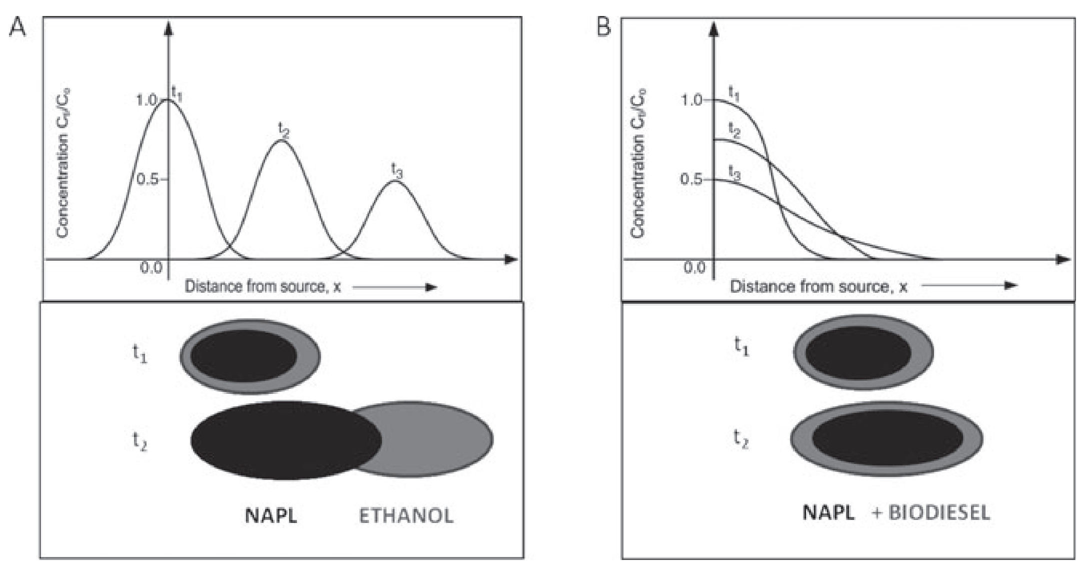

Figure 5. Hypothetical concentration profiles and NAPL plume impacts of (A) ethanol vs. (B) biodiesel.

as fatty acids would be sufficiently soluble to migrate away from the source zone, these would be readily biodegraded by indigenous microorganisms. Thus, the methyl esters of biodiesel may behave primarily as a fixed, decaying, yet long-lived, source with a relatively small region of influence compared to that of ethanol. Although the presence of the readily degradable FAMEs could support microbial growth, it is unknown whether this would contribute to the fortuitous proliferation of specific hydrocarbon degraders or mainly support the growth of incompetent species (i.e., genotypic dilution) (Da Silva et al. 2002).

Conclusions

In the race to alleviate oil dependence, renewable fuels are being investigated and promptly pushed into the commercial market. Although biofuels may reduce some emissions and offer other benefits, considerable uncertainty exists about their unintended environmental impacts.

This microcosm study demonstrated that biodiesel FAME composition can significantly influence its biodegradability and that the presence of biodiesel can hinder the degradation of benzene and toluene. Although this inhibition of natural degradation processes is similar to that exerted by ethanol, the potential field-scale behavior and inhibitory region of influence of these biofuels could be considerably different because of differences in their viscosity, solubility, and resulting mobility. The slower degradation and lower mobility of biodiesel are conducive to longer lasting inhibitory substrate interactions over possibly a smaller region of influence compared to ethanol, although field experiments are needed to test this hypothesis and discern the complex dynamics of such releases. These initial studies suggest that biodiesel degradation and the resulting impact on BTEX natural attenuation may be more complex than that observed for ethanol and underscores the importance of investigating potential impacts on groundwater quality as a function of the biodiesel composition and release scenario. To that end, soybean-derived B20 and B100 field-scale releases are currently underway at Ressacada.

Acknowledgments

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