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# Microbial processes influencing the transport, fate and groundwater impacts of fuel ethanol releases

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Fuel releases that impact groundwater are a common occurrence, and the growing use of ethanol as a transportation biofuel is increasing the likelihood of encountering ethanol in such releases. Microorganisms play a critical role in the fate of ethanol-blended fuel releases, often determining their region of influence and potential impacts. This review summarizes current understanding on the biogeochemical footprint of such releases and the factors that influence their natural attenuation. Implications for site investigation, risk assessment and remediation strategies are also addressed along with research priorities.

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## Introduction

The use of renewable transportation fuels (biofuels) is rapidly growing to alleviate dependence on imported oil and enhance energy security, as well as to mitigate air pollution and greenhouse gas emissions by fossil fuel combustion [1••,2,3•]. Currently, the major commercialized biofuel products include ethanol and biodiesel. Ethanol holds a much larger global market share than biodiesel (23 483 vs. 5510 million gallons/year) [4].

Incidental and accidental fuel releases that impact groundwater are a common occurrence and the likelihood of encountering biofuels (mainly ethanol) in such releases is increasing. Thus, it is important to understand how such releases behave and affect groundwater geochemistry, and how indigenous microorganisms respond and affect their migration, fate, and overall impact. This information is critical to optimize site characterization, risk assessment and remediation practices when dealing with releases of current and future biofuel blends.

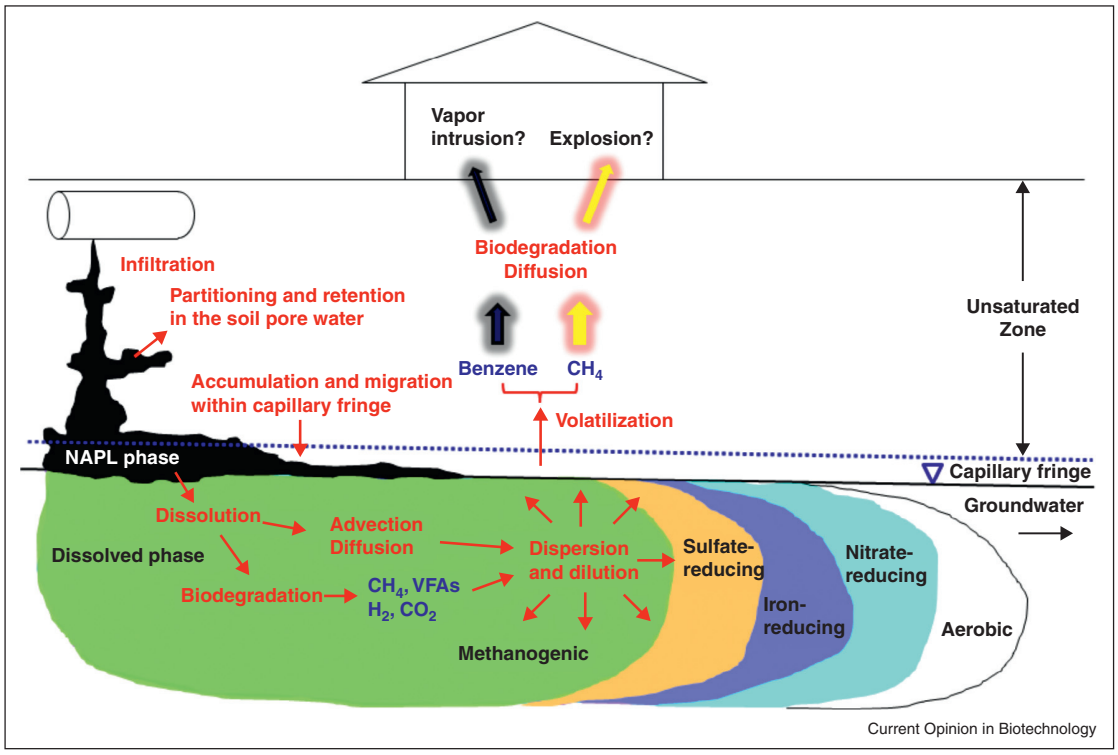
## Physical behavior of ethanol-blended fuel releases

When an ethanol-blended fuel release occurs, it infiltrates as a non-aqueous phase liquid (NAPL) through the unsaturated zone to the water table and forms a floating NAPL pool at the water table when a sufficient volume is spilled (Figure 1). Ethanol will partition into pore water throughout the unsaturated zone [5–7] and will tend to accumulate at the water table interface and the capillary fringe owing to its buoyancy [6,8–11]. For high content ethanol fuels (e.g. E95, which has 95% ethanol and 5% gasoline by volume), the fuel will probably migrate through this interface, initially as a water miscible phase, and then separate into two phases as the fuel becomes diluted, precipitating a new NAPL phase along its path [9,10,12]. Pore water containing high ethanol concentrations will also be enriched in hydrocarbons owing to their enhanced solubility in the presence of ethanol (cosolvent effect) [13–16]. Thus, different domains of microbial activity are likely to develop: a region of anaerobic activity in the core of a contaminant plume in the saturated zone (where the biochemical oxygen demand [BOD] exerted by the release exceeds the available dissolved oxygen) with aerobic degradation occurring at the fringes of the plume; a second region of high anaerobic activity in the capillary zone (except in cases when ethanol concentrations are sufficiently high to be toxic to microbial processes); and a third region in the unsaturated zone where aerobic degradation of methane (emanating from the anaerobic fermentation of ethanol in the capillary zone) is predominant (Figure 1).

## Biodegradation of ethanol-blended fuel

Direct exposure to ethanol in drinking water has minimal adverse impacts on human health, but ethanol may increase the exposure potential of toxic fuel constituents (i.e. benzene, toluene, ethylbenzene and xylenes [BTEX]) by hindering their biodegradation and increasing their region of influence [1••]. Because ethanol generally biodegrades faster than BTEX, the latter tend to form larger and more persistent plumes than ethanol. Therefore, substrate interactions during ethanol and BTEX degradation and their effect on plume dynamics (range and longevity) have received considerable attention [17–25,26•].

**Figure 1**




Fate, transport, and potential impacts of ethanol-blended fuel releases.

During transport in groundwater, ethanol and BTEX can undergo a series of biotransformations which can be performed by a variety of microorganisms in aerobic or anaerobic environments [1••]. The relatively high concentration of ethanol found in recently-impacted groundwater exerts a high BOD that rapidly consumes the available dissolved oxygen and other terminal electron acceptors in the vicinity of the source zone, which results in the development of strongly anaerobic, fermentative methanogenic conditions (Figure 1). Nevertheless, aerobic microbial activity might be important for the natural attenuation of the leading edge of the plume.

Under aerobic conditions, BTEX are activated by oxygenases to form catechol or structurally related compounds, which subsequently undergo ring fission to byproducts such as acetyl-CoA, acetaldehyde and pyruvic acid that enter central metabolic pathways such as Krebs' cycle (for final mineralization to $CO_2$) or glycolysis [27]. Ethanol can also be aerobically metabolized to the pivotal intermediate acetyl-CoA via acetaldehyde and acetate [1••].

Under anaerobic conditions, BTEX are initially transformed via different pathways (*fumarate addition*, *$O_2$-independent hydroxylation*, *and carboxylation*) to a common aromatic intermediate, benzyl-CoA, which subsequently undergoes ring reduction followed by hydrolytic cleavage [28••]. Further anaerobic transformations in anaerobic (methanogenic) food webs eventually produce acetate, which is finally mineralized by acetoclastic methanogens to produce $CH_4$ and $CO_2$. BTEX fermentation also generates $H_2$, which is consumed by different commensal anaerobes, including hydrogenotrophic methanogens. Ethanol is similarly transformed to acetate and $H_2$, which are subsequently metabolized by methanogens to produce $CH_4$ and $CO_2$ [1••]. Depending on the available electron acceptors, sulfate reducers, iron reducers, and denitrifiers could also participate in the anaerobic degradation of ethanol-blended fuel, and spatially distinctive redox zones could form in plume (Figure 1).

## How will ethanol affect BTEX biodegradation?

The major impact from ethanol may be related to its inhibitory effect on BTEX biodegradation (Table 1), which (depending on the release scenario) may increase the likelihood of BTEX to reach receptors (longer plumes) as well as the potential duration of exposure (more persistent plumes).

Benzene, which is the most toxic compound of the BTEX and often drives the need for cleanup action, is relatively resistant to degradation under anaerobic conditions [29], while ethanol and its degradation byproducts (e.g. volatile fatty acids [VFAs]) are easier to degrade under both

**Table 1**

**Mechanisms by which ethanol affects BTEX degradation**

| Mechanisms | System affected | Effects on BTEX degradation |
|---|---|---|
| Catabolite repression | Gene expression | – |
| Metabolic flux dilution | Metabolism | – |
| pH decrease | Cell physiology | – |
| Ethanol toxicity | Physiology and metabolism | – |
| Fortuitous growth of BTEX degraders | Community structure | + |
| Genotypic dilution | Community structure | – |
| Growth of syntrophic microorganisms | Community structure | + |
| Increase richness and diversity | Community structure | + |
| Electron acceptor/nutrients depletion | Metabolism, kinetics | – |
| Thermodynamic inhibition due to VFAs accumulation | Metabolism, kinetics | – |

aerobic and anaerobic conditions [1••]. The preferential degradation of ethanol and its degradation byproducts may deplete available $O_2$ that would otherwise be available for aerobic benzene degraders, hindering their activity. Therefore, accelerated oxygen depletion is one of the most important inhibitory mechanisms of ethanol on benzene degradation [17,23,24].

Although the initial steps of ethanol and BTEX degradation (including both aerobic and anaerobic pathways) are catalyzed by different enzymes under different pathways, their degradation may eventually converge to common intermediates (e.g. acetate and acetyl Co-A) that enter central metabolic pathways (e.g. Krebs cycle) for final mineralization. Fast degradation of ethanol may result in the accumulation of acetyl-CoA (inside the cell) and acetate (mainly secreted in groundwater), which may hinder BTEX degradation by both intracellular mechanisms (e.g. catabolite repression and/or metabolic flux dilution) and abiotic constraints (decreased pH and/or thermodynamic inhibition) as discussed below.

### Gene expression

Ethanol is metabolized by constitutive enzymes through a central metabolic pathway, while the initial step of BTEX degradation is usually catalyzed by inducible enzymes. To save the energy associated with the synthesis of inducible catabolic enzymes, which are not needed when ethanol is available, microorganisms are likely to consume ethanol preferentially [30]. Therefore, the presence of ethanol could repress the synthesis of inductive enzymes required for BTEX degradation [1••], thus hindering BTEX degradation at the transcription level [31••]. Two independent experiments using different detection methods reported that ethanol (or its byproduct acetate) could repress the *tod* gene (coding for toluene dioxygenase) and that the degree of *tod* repression increased with the ethanol concentration [31••,32]. It should be noted that catabolite repression is unlikely to occur under carbon-limiting conditions that are conducive to simultaneous utilization of multiple substrates [33].

### Metabolic flux dilution

Ethanol could hinder BTEX degradation by 'metabolic flux dilution' [31••,34]. The metabolic flux of a specific compound is analogous to the specific degradation rate and can be defined as the rate at which the compound is metabolized per unit biomass [33]. Metabolic flux dilution is a form of non-competitive inhibition in which the utilization rate of one substrate decreases owing to the metabolism of another that is not necessarily degraded by the same enzymes. For example, BTEX and ethanol are initially transformed by different pathways that eventually converge into common metabolic intermediates (e.g. acetyl-CoA). This could create a bottleneck that exerts feedback inhibition and decreases the degradation rate of a target compound (e.g. benzene). Whereas the utilization of ethanol would decrease the specific BTEX degradation rates, this does not preclude a potential enhancement in overall degradation rates owing to additional (fortuitous) growth of BTEX degraders on ethanol [34]. To illustrate simplistically, ten bacteria degrading BTEX at 20% capacity would be faster than one bacterium working at 100% capacity.

### Thermodynamic inhibition

The build-up of ethanol-derived acetate could thermodynamically hinder benzene degradation under methanogenic [35•] and sulfate reducing [36] conditions. The degradation of BTEX under anaerobic fermentative conditions is endergonic under standard conditions [35•,36], as illustrated for benzene: $C_6H_6 + 6H_2O \rightarrow 3CH_3$-$3CH_3COO^- + 3H^+ + 3H_2$; $\Delta G^{o\prime} = +190.19$ kJ mol$^{-1}$.

Therefore, syntrophic consumption of acetate and hydrogen is needed for the reaction to proceed, and the accumulation of ethanol-derived acetate at concentrations greater than about 64 mg/L makes this reaction thermodynamically unfavorable [35•]. The effects of ethanol on the dynamics of commensal populations that

produce and consume acetate and hydrogen remain poorly understood.

#### Cell physiology

High concentrations of ethanol are toxic to microorganisms. Ethanol could dissolve phospholipids and disintegrate the cell membrane [37]. As the cell membrane loses its structural integrity, ethanol could enter the cell and denature enzymes. High concentrations of ethanol could inhibit the synthesis of DNA [38], RNA [39] and proteins [40], thus leading to loss of functions or even cell death [37]. The inhibitory threshold of ethanol ranges between 10 000 and 100 000 mg/L for various microorganisms [37]. Concentrations in this range are possible only near the source of relatively recent releases.

In poorly buffered aquifers, ethanol-derived VFAs could significantly decrease groundwater pH (pH $< 5$ in the core of the plume) [41]. Some microorganisms are very sensitive to pH changes. For example, the growth of methanogens is generally inhibited at pH $< 6$ [1••]. Because methanogens consume thermodynamically inhibitory byproducts (e.g. $H_2$ and acetate) and play a key role in the fermentative/methanogenic mineralization pathway, low pH could adversely affect anaerobic BTEX degradation.

#### Community structure

Ethanol could be consumed by a wide variety of microorganisms, including some BTEX degraders. Thus, ethanol could fortuitously stimulate the growth of BTEX degraders and enhance the potential for BTEX degradation [34,41–43]. Increases in the abundance of catabolic genes for aromatic hydrocarbons degradation, such as *bssA* (coding for benzylsuccinate synthase) [44•] and PHE (coding for phenol hydroxylase) [41,42], were reported in systems exposed to ethanol-blended fuel. However, more microbial species can feed on ethanol than on BTEX, which is conducive to a greater proliferation of commensal microorganisms and a decrease in relative abundance of BTEX degraders (genotypic dilution) [23]. While genotypic dilution decreases specific BTEX degradation rates, overall degradation rates may increase owing to higher total concentration of BTEX degraders [34,45], especially after ethanol is removed and its inhibitory effects have waned while a higher concentration of BTEX degraders remains.

Ethanol could also influence BTEX degradation kinetics by affecting the growth and activity of syntrophic microorganisms. Anaerobic biodegradation of organic compounds is usually a syntrophic process which involves the interaction and cooperation of different microbial groups. Ethanol blend releases could stimulate the growth of commensal syntrophs that consume inhibitory fermentation byproducts (e.g. $H_2$ and acetate), thereby enhancing anaerobic bioremediation [41].

Pristine aquifer ecosystems usually have very low biomass concentration because substrates are scarce [46]. Ethanol blend releases increase substrate concentrations and the available metabolic niches, thus stimulating the growth of diverse species [41]. Ecological resilience is generated by diverse but functionally overlapping species [47], and phylogenetic diversity and ecological resilience are usually positively correlated [48]. Thus, the resulting increases in phylogenetic diversity enhance the resilience of groundwater ecosystems to bioremediate hydrocarbons remaining after ethanol is consumed as well as for recurring releases [41].

Table 2 summarizes the most widely used quantitative real-time PCR (qPCR) primer sets for catabolic genes involved in aerobic and anaerobic degradation of BTEX. As a sensitive and reliable method to detect and quantify genes, qPCR may be very useful in establishing the presence of specific biodegradation potential and assessing biodegradation activities and bioremediation performance [27].

#### Overall effect of ethanol on BTEX plume dynamics

Several laboratory [17,23,24,31••,43] and field studies [26•,35•,49] showed that ethanol could inhibit BTEX degradation and result in longer plumes. However, results from other laboratory [50,51], pilot-scale [52,53], and field studies [19] indicate that BTEX plume elongation may be insignificant under certain site conditions (e.g. small volume of spill, significant retention of ethanol in the unsaturated zone, high replenishment rate of electron acceptors and nutrients, and fortuitous proliferation of BTEX degraders).

Several mathematical models have simulated the fate and transport of BTEX and ethanol as well as potential effects of ethanol on BTEX plume dynamics (Table 3). These model simulations predict that the presence of ethanol would elongate benzene plumes by 17–150% [19,20,24,45,54,55•,56]. However, the risk of exposure depends not only on plume length but also on persistence (plume lifespan), both of which can be affected by the content of ethanol in the fuel blend. Simulations for higher ethanol content blends yielded shorter-lived benzene plumes because of decreased mass of benzene present in the source zone NAPL and increased benzene degradation rates associated with fortuitous growth (and higher concentration) of BTEX degraders [45] (Figure 2). Accordingly, a release of a high ethanol content blend (e.g. E85) may pose a lower overall risk than a comparable size release of a low ethanol content blend (e.g. E10) [45].

## Other impacts from ethanol-blended fuel releases

#### Will ethanol-derived $CH_4$ be an explosion hazard?

Biodegradation of ethanol could result in relatively high $CH_4$ concentrations in groundwater (23–47 mg/L)

**Table 2**

**qPCR primer/probe sets for BTEX biodegradation**

| Primer | Target enzyme | Sequence | Function | Reference |
|---|---|---|---|---|
| TOD | Toluene dioxygenase | 5′-ACCGATGARGAYCTGTACC-3′<br>5′-CTTCGGTCMAGTAGCTGGTG-3′ | Aerobic degradation of BTEX | [70] |
| TOL | Xylene monooxygenase | 5′-TGAGGCTGAAACTTTACGTAGA-3′<br>5′-CTCACCTGGAGTTGCGTAC-3′ | Aerobic degradation of toluene or xylene | [70] |
| RMO | Toluene monooxygenase | 5′-TCTCVAGCATYCAGACVGACG-3′<br>5′-TTKTCGATGATBACRTCCCA-3′ | Aerobic degradation of toluene | [70] |
| PHE | Phenol monooxygenase | 5′-GTGCTGACSAAYCTGYTGTTC-3′<br>5′-CGCCAGAACCAYTTRTC-3′ | Aerobic degradation of BTEX in $O_2$ limited environments | [70] |
| *cat23* | Catechol 2,3-dioxygenase | 5′-AAGAGGCATGGGGGCGCACCGGTTCGATCA-3′<br>5′-AACAAADGCGCSGTCATGCGG-3′ | Aerobic degradation of BTEX in $O_2$ limited environments | [71] |
| *cat23* | Catechol 2,3-dioxygenase | 5′-CTCGTTGCGGTTGCCGCTSGGGTCGTCGAAGAAGT-3′<br>5′-ATCGAGGCCTGGGGTGTGAAGACCACCATGCT-3′ | The same as above | [72] |
| *cat23* | Catechol 2,3-dioxygenase | 5′-AGGTGCTCGGTTTCTACCTGGCCGA-3′<br>5′-ACGGTCATGAATCGTTCGTTGAG-3′ | The same as above | [73] |
| *bssA* | Benzylsuccinate synthase | 5′-ACGACGGYGGCATTTCTC-3′<br>5′-GCATGATSGGYACCGACA-3′<br>FAM-5′CTTCTGGTTCTTCTGCACCTTGGACACC3′-TAMRA | Anaerobic degradation of toluene and xylene (denitrifying) | [51] |
| *bssA* | Benzylsuccinate synthase | 5′-TCGAYGAYGGSTGCATGGA-3′<br>5′-TTCTGGTTYTTCTGCAC-3′ | Anaerobic degradation of toluene and xylene (iron-reducing) | [74] |
| *bssA* | Benzylsuccinate synthase | 5′-GTSCCCATGATGCGCAGC-3′<br>5′-CGACATTGAACTGCACGTGRTCG-3′ | Anaerobic degradation of toluene and xylene (sulfate-reducing) | [44•] |
| *bssA* | Benzylsuccinate synthase | 5′-CCTATGCGACGAGTAAGGTT-3′<br>5′-TGATAGCAACCATGG AATTG-3′<br>FAM-5′TCCTGCAAATGCCTTTTGTCTCAA3′-TAMRA | The same as above | [75] |
| *bssA* | Benzylsuccinate synthase | 5′-GGCTATCCGTCGATCAAGAA-3′<br>5′-GTTGCTGAGCGTGATTTCAA-3′<br>FAM-5′CTACTGGGTCAATGTGCTATGCATG3′-TAMRA | The same as above | [75] |
| *bamA* | 6-oxocyclohex-1-ene-1-carbonyl-CoA hydrolase | 5′-GCAGTACAAYTCCTACACSACYGABATGGT-3′<br>5′-CCRTGCTTSGGRCCVGCCTGVCCGAA-3′ | Anaerobic degradation of aromatic hydrocarbons including BTEX | [74] |

*Note*: This table uses standard code for mixed base sites: R = A, G; Y = C, T; M = A, C; K = G, T; S = G, C; W = A, T; H = A, C, T; B = G, T, C; V = G, C, A; D = G, A, T; N = A, C, G, T.

[57•,58] and in subsurface deep soil gas (68% v:v) [59]. Under ignitable conditions, $CH_4$ can pose an explosion risk when it accumulates in air at 50 000 to 150 000 $ppm_v$ [60], and explosion accidents have been reported at landfill sites [61,62]. During $CH_4$ transport through the vadose zone, both aerobic degradation (by methanotrophs) and physical dispersion and dilution could attenuate the $CH_4$ flux and decrease the concentration of $CH_4$ reaching the surface [63]. However, the explosion risk cannot be dismissed when source-zone methanogenic activity is sufficiently high to induce pressure-driven advective flow through a shallow unsaturated zone. No study has investigated the advective contribution to $CH_4$ fluxes through the vadose zones overlying ethanol blend releases.

### Will $CH_4$ generation enhance BTEX vapor intrusion?

High concentrations of BTEX and $CH_4$ usually coexist in aquifers impacted by ethanol-blended fuel [35•,57•]. $CH_4$ aerobic degradation by methanotrophs in the vadose zone may deplete the available $O_2$ and hinder the aerobic degradation of BTEX vapors, thus increasing their intrusion potential [59]. Simulations with an analytical model inferred that methanotrophic activity could decrease the thickness of the aerobic layer in the vadose zone and increase benzene vapor concentrations at the soil surface by more than $10^5$-fold [63].

### How do ethanol-derived volatile fatty acids affect groundwater quality?

Ethanol-derived VFAs generate odor that could compromise groundwater aesthetic quality. As one of fifteen regulated contaminants in the U.S. National Secondary Drinking Water Regulations (NSDWR), odor significantly affects the public's perception of the safety of drinking water [64]. A pilot-scale ethanol blend release experiment showed that the odor level in the impacted groundwater (calculated based on measured concentrations of VFAs) during summer months was 350 times higher than the secondary maximum contaminant level (SMCL) for odor, and butyric acid was the major odor contributor [52]. However, this aesthetic problem was relatively short-lived owing to the fast biodegradability of VFAs and their slower production in cooler seasons [52].

**Table 3**

**Fate and transport models**

| Model name Reference | Conceptual model | Mathematical model | Biodegradation | Increased benzene plume length |
|---|---|---|---|---|
| Heerman and Power 1996 [76] | 2D (X-Z); Focus on cosolvent and interphase mass transfer | Analytical | Not included | Benzene not modeled (≤10% for xylene) |
| McNab *et al.*, 1999 [77] | 3D aqueous transport from a finite source zone | Analytical | First-order decay of ethanol and benzene; | +100% |
| Molson *et al.*, 2002 [20] | 3D; Consider microbial growth (Monod kinetics) and $O_2$ competition; Cosolvency is not considered | Numerical | Monod kinetics; Fermentation pathway. BOD comes from ethanol and its degradation byproducts | ≤ +150% |
| Deeb *et al.*, 2002 [24] | 2D (X-Y) transport from a gasoline pool | Numerical | First-order decay of ethanol and benzene Benzene is not biodegraded when $C_{ethanol} > 3mg/L$ | 17–34% |
| Gomez *et al.*, 2008 [45,54] | 3D model based on RT3D; Consider $O_2$ competition, catabolic repression, metabolic flux dilution and microbial population shifts | Numerical | Multiplicative Monod kinetics | ≤40% |
| BONAPL/3D 2011 [56] | 3D multi component NAPL dissolution with dissolved-phase reactive transport | Numerical | First-order, Monod ($O_2$ limited) kinetics, Monod partial mineralization | E95 inhibits benzene degradation while E 10 does not. |
| BONAPL/3D 2011 [19] | The same model as [56]. Consider ethanol retention in the unsaturated zone | Numerical | The same as [56] | 40% |

### How do fuel ethanol releases affect groundwater geochemistry?

Depending on the amount released, ethanol-blended fuels can greatly alter groundwater geochemistry. The high BOD exerted by ethanol creates strongly anaerobic (reducing) conditions [26•] under which VFAs accumulate and cause a decrease in pH [41]. These conditions promote the dissolution of redox-sensitive and/or pH-sensitive metals from the aquifer matrix (e.g. iron, manganese, and arsenic), thus exacerbating groundwater contamination [65,66]. Although no studies of metal mobilization by ethanol-blend releases has been reported in the literature, elevated arsenic concentrations have been detected in groundwater contaminated by petroleum hydrocarbons [66]. Because of higher dissolved concentrations and faster anaerobic degradation, ethanol is more likely to induce reducing and acidic conditions that mobilize metals than petroleum hydrocarbons. Therefore more drastic changes in groundwater geochemistry and higher risk for metal mobilization may be expected in groundwater impacted by ethanol-blended fuel than regular fuel.

## Do we need to modify site characterization and remediation practices when dealing with ethanol-blend releases?

Differences in the environmental behavior and potential impacts of ethanol-blended versus conventional fuel suggest that the following modifications to site investigation and remediation practices should be considered:

(1) High concentration of acetate could hinder the thermodynamic feasibility of anaerobic BTEX degradation and could also repress inducible enzymes associated with aerobic BTEX metabolism. Therefore, acetate in groundwater should be monitored.

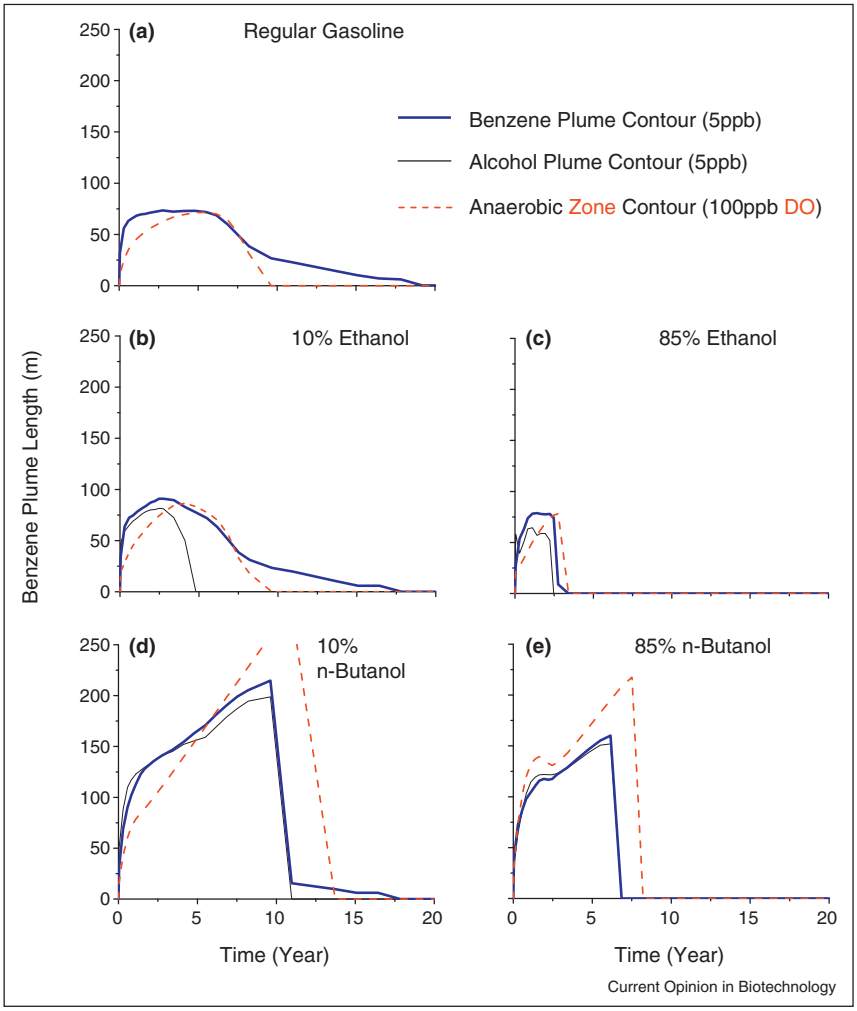
(2) Ethanol degradation has the potential to produce $CH_4$ that could cause an explosion risk, and $CH_4$ generation may continue after the apparent disappearance of source ethanol (owing to the presence of acetate). Therefore, long-term monitoring of $CH_4$ in groundwater and soil gas near the source zone should be considered.

(3) The release of ethanol-blended fuel may result in lower aerobic attenuation of BTEX vapors through the vadose zone (owing to $O_2$ depletion by methanotrophic activity) and thus, higher potential for BTEX vapor intrusion into overlying buildings. Therefore, monitoring fuel hydrocarbons in soil gas and the corresponding vapor intrusion risk should be considered.

(4) Since the near-source ethanol accumulates and migrates horizontally mainly within the capillary fringe, monitoring of ethanol should focus on this zone. Sampling wells with shorter screen interval, multi-level sampling well, and soil coring may be effective approaches to collect samples from the capillary fringe [67].

(5) Ethanol could hinder BTEX natural attenuation and may be persistent in the source zone for years, thus engineered remediation techniques such as source excavation, anaerobic biostimulation [18,21,68], and bioaugmentation with anaerobic BTEX degraders

**Figure 2**




Simulated benzene plume dynamics (centerline reach) resulting from a 30-gal release of regular gasoline or various fuel alcohol blends. Adapted from Gomez and Alvarez 2010 [55•]. The anaerobic zone was arbitrarily defined at the 0.1 ppm dissolved oxygen (DO) contour.

[69] should be considered for source removal. Monitored natural attenuation could be used as a long-term polishing approach [3•,35•,41,42].

## Conclusions

A primary concern about ethanol blend releases is exacerbating the potential impact of co-occurring or pre-existing BTEX contamination. Ethanol (and other biofuels) could increase potential exposure to BTEX in groundwater (i.e. causing longer BTEX plumes), either by enhancing BTEX dissolution and migration or by hindering biodegradation. The significance of these complex effects will be site-specific, and insufficient data are available to determine how ethanol might affect BTEX remediation time and costs or the number of sites that will require corrective action. In most cases, the presence of ethanol should not pose a serious threat to drinking water resources because BTEX plume elongation is unlikely to exceed a few hundred feet, while drinking water wells are often located beyond one mile from fuel stations.

Recently, there has been an increased focus on vapor exposure pathways. This has improved understanding of the effect of ethanol on methane generation, which could limit the attenuation of BTEX in the unsaturated zone (owing to oxygen depletion by methanotrophic bacteria) and enhance BTEX vapor intrusion in above-ground enclosed spaces. Ethanol-derived methane could also pose a potential explosion risk above-ground when ignitable conditions exist. Other ethanol degradation byproducts such as volatile fatty acids can also be problematic, generating odor and facilitating heavy metal dissolution into groundwater.

Metagenomic tools are currently being used to advance quantitative understanding of the dynamics and functional diversity of impacted microbial communities. This may lead to improved characterization of the biogeochemical processes that attenuate such releases, and discernment of the associated microbial adaptation mechanisms and metabolic niches. The integration of this knowledge with site-specific information on pertinent hydrogeologic and geochemical processes will undoubtedly enhance risk assessment, remedial design and performance assessment practices.

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- • of special interest
- •• of outstanding interest


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