RICE UNIVERSITY

Effects of Fuel Alcohols on BTEX Plume Dynamics: An Assessment of Natural Attenuation Using RT3D with a General Substrate Interaction Module

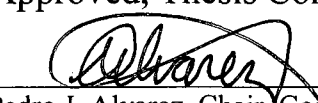
by

Diego E. Gomez

A DISSERTATION SUBMITTED
IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE

Doctor of Philosophy
(Environmental Engineering and Sciences)

Approved, Thesis Committee:



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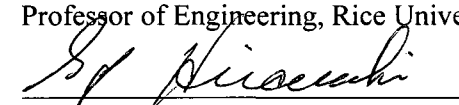

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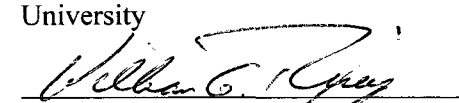

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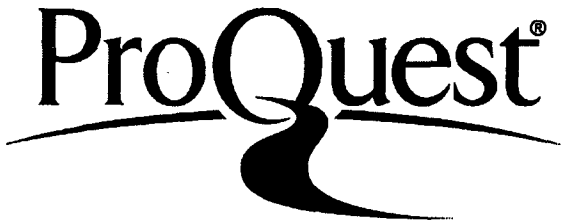

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ABSTRACT

Effects of Fuel Alcohols on BTEX Plume Dynamics: An Assessment of Natural Attenuation Using RT3D with a General Substrate Interaction Module

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A numerical model was developed to evaluate the effect of fuel alcohols present in reformulated gasoline on BTEX natural attenuation and groundwater plume elongation. The model, developed as a module for the RT3D (Reactive Transport in 3-Dimensions) model, includes commonly considered fate and transport processes (advection, dispersion, adsorption, biodegradation and depletion of electron acceptors during biodegradation) and substrate interactions previously not considered (e.g., a decrease in the specific benzene utilization rate due to metabolic flux dilution and/or catabolite repression) as well as microbial populations shifts, cosolvency effects, alcohol toxicity and source zone depletion dynamics that affect groundwater concentrations of gasoline constituents. The model was used to (1) evaluate the relative importance of benzene plume-elongation mechanisms, (2) how the concentration of ethanol in reformulated gasoline affects the length and longevity of benzene plumes, and (3) the effects of five fuel alcohols (methanol, ethanol, 1-propanol, iso-butanol and n-butanol) on the natural attenuation of benzene in fuel contaminated groundwater. Model simulations showed that all fuel alcohols can hinder the natural attenuation of benzene, due mainly to accelerated

depletion of dissolved oxygen during their biodegradation (leading to strongly anaerobic methanogenic conditions) and a decrease in the specific degradation rate for benzene (due to catabolite repression and metabolic flux dilution). Thus, releases of alcohol-blended gasoline should result in longer benzene plumes compared to regular gasoline. However, the simulated lifespan of benzene plumes was shorter for blends with higher alcohol contents, due to a lower mass of benzene released, and increased microbial activity associated with fortuitous growth of BTEX degraders on fuel alcohols. Benzene plume elongation and longevity were more pronounced in the presence of alcohols that biodegrade slower (e.g., propanol and n-butanol), forming longer and more persistent alcohol plumes. In general, our model indicates that higher alcohols blends have a lower impact on BTEX natural attenuation, while more recalcitrant alcohols have a higher impact. Thus, E85 (85% Ethanol) had the lowest impact on BTEX plume elongation and B10 (10% n-Butanol) had the highest impact. However, simulations were highly sensitive to site-specific biokinetic coefficients for alcohol degradation, which forewarns against generalizations about the level of impact of specific fuel alcohols on benzene plume dynamics, and calls for further pilot-scale and field research to validate the assumptions and results from this model.

ACKNOWLEDGEMENTS

I would like to acknowledge:

Dr. Pedro Alvarez, my advisor, for the ideas, discussions and insights behind this work; and for motivation, encouragement and demand for rigor on my work;

Prof. Philip Bedient, Prof. George Hirasaki and Prof. Bill Rixey for their constant help and support on the development of this dissertation, and helpful discussion on the technical aspects of this research; and Dr. Phillip De Blanc for his contributions in the initial development of this model.

All my friends in Houston, without whom I couldn't have completed this long effort: Marcio Silva, Jacques Matthew, Amy Monier, Vanessa Luna, Katherine Zodrow, Sandra Baylor and Guyton Durnin.

And lastly, and most importantly, I wish to thank my parents, Maria Angelica Jorquera and Walter Gomez, for their constant support, guidance and love at the distance.

This work was financially supported in part by The American Petroleum Institute (API) and BP Global.

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VARIABLE GLOSSARY

f_S : Metabolic flux dilution and catabolite repression factor (dimensionless)
S_{TOC} : Substrate concentration as total organic carbon (mg/l)
T_{TOC} : Total organic carbon concentration (mg/l)
U_b : Specific substrate utilization rate of benzene (g/g-d)
U_e : Specific substrate utilization rate of ethanol (g/g-d)
S : substrate concentration (mg/L)
O : Oxygen concentration (mg/l)
X_{Aer} : aerobic biomass concentration (mg/L)
X_{An} : anaerobic biomass concentration (mg/L)
γ : fraction of total porosity available to microbial growth (dimensionless)
$r_{S,Aer}$: rate of substrate aerobic biodegradation (mg/L-d)
$r_{S,An}$: rate of substrate anaerobic biodegradation (mg/L-d)
$\mu_{mS,Aer}$: maximum specific growth rate of aerobic biomass on substrate S (day^{-1})
$\mu_{mS,An}$: maximum specific growth rate of anaerobic biomass on substrate S (day^{-1})
$Y_{S,Aer}$: aerobic biomass yield coefficient for substrate (g-biomass/g-substrate)
$Y_{S,An}$: anaerobic biomass yield coefficient for substrate (g-biomass/g-substrate)
$K_{S,Aer}$: half-saturation coefficient of substrate under aerobic metabolism (mg/L)
$K_{S,An}$: half-saturation coefficient of substrate under anaerobic metabolism (mg/L)
K_O : half-saturation coefficient of oxygen (mg/L)
$E_{S,Aer}$: stoichiometric oxygen utilization requirement for substrate mineralization under aerobic conditions (dimensionless)
b_{Aer} : endogenous decay coefficient of aerobic biomass (day^{-1})
b_{An} : endogenous decay coefficient of anaerobic biomass (day^{-1})
R_S : retardation factor of substrate (dimensionless)
R_O : retardation factor of oxygen (dimensionless).
n : total porosity (dimensionless)
$I_{An,O}$: empirical oxygen inhibition factor for anaerobic metabolism
X_{sat} : total biomass saturation (volume of biomass per volume of pore space)
ρ_{Aer} : aerobic biomass density (mass of cells/volume of biomass)
ρ_{An} : anaerobic biomass density (mass of cells/volume of biomass)
X_1 : ethanol aerobic degrader population (mg/l)

X_2 : ethanol and benzene aerobic degrader population (mg/l)
X_3 : ethanol anaerobic degrader population (mg/l)
X_4 : ethanol and benzene anaerobic degrader population (mg/l)
X_t : Total microbial population (mg/l)
X_b : Benzene degrader population (mg/l)
α_e : fraction of ethanol available for fortuitous growth of benzene degraders (dimensionless)
λ : first-order rate coefficient for substrate degradation (1/d)
P_e : Peclet stability number (dimensionless)
C_r : Courant stability number (dimensionless)
v : average linear flow velocity (m/d)
Δt : time step difference (d)
Δx : spatial step difference (m)
D : coefficient of hydrodynamic dispersion (m^2/d)
α : dispersivity (m)

ACRONYM GLOSSARY

API	: American Petroleum Institute
B10	: 10% Butanol/Regular Gasoline blend (v:v)
B85	: 85% Butanol/Regular Gasoline blend (v:v)
BTEX	: Benzene, Toluene, Ethylbenzene and Xylene mixtures
BTEX	: Benzene, Toluene, Ethylbenzene and Xylenes
CR	: Catabolite Repression
DOD	: Dissolved oxygen depletion
DOE	: Department of Energy
E10	: 10% Ethanol/Regular Gasoline blend (v:v)
E85	: 85% Ethanol/Regular Gasoline blend (v:v)
EPA	: Environmental Protection Agency
IB10	: 10% Iso-Butanol/Regular Gasoline blend (v:v)
IB85	: 85% Iso-Butanol/Regular Gasoline blend (v:v)
LNAPL	: Light Non-Aqueous Phase Liquid
LUST	: Leaking Underground Storage Tanks
M10	: 10% Methanol/Regular Gasoline blend (v:v)
M85	: 85% methanol/Regular Gasoline blend (v:v)
MC50	: Microbial Cytotoxicity 50%
MFD	: Metabolic Flux Dilution
MODFLOW model	: MODular three-dimensional finite-difference ground-water FLOW
ODE	: Ordinary Differential Equations
P10	: 10% Propanol/Regular Gasoline blend (v:v)
P85	: 85% Propanol/Regular Gasoline blend (v:v)
RT3D	: Reactive Transport in 3-Dimensions
TEAP	: Terminal Electron Acceptor Pathway
VFA	: Volatile Fatty Acids

1. Introduction and Problem Statement

Groundwater contamination by accidental or incidental releases of petroleum products is a widespread occurrence. One particular concern is the contamination of drinking water sources by the toxic hydrocarbons benzene, toluene, ethylbenzene and xylenes (BTEX). Bioremediation and monitored natural attenuation (MNA), which rely on microbial degradation of these priority pollutants, are the most cost-effective approaches to manage soil and groundwater contamination by BTEX (*Alvarez and Illman,* 2006). However, *in situ* biodegradation of BTEX compounds is not ubiquitous, and some BTEX compounds can persist in the environment at levels exceeding regulatory thresholds. Several factors such as electron acceptor conditions, microbial community structure and adaptation, temperature, pH, availability of inorganic nutrients, and bioavailability, influence the rate and extent of BTEX biodegradation. Although these factors have been recognized, limited attention has been placed on the ability of other gasoline constituents and additives to stimulate or inhibit BTEX natural attenuation and plume dynamics.

Ethanol and other biomass-derived fuels (i.e., biofuels) are increasingly being used to meet Energy Independence and Security Act [*U.S. Cong.*, 2007] and Clean Air Act requirements [*U.S. EPA*, 2009]. The widespread use of ethanol in gasoline has lead to an increase in its potential presence in groundwater contaminated with other gasoline constituents such as benzene, toluene, ethylbenzene, and xylenes (BTEX). Preferential degradation of ethanol and the accelerated depletion of oxygen that would otherwise be

available for BTEX biodegradation have been reported to hinder BTEX degradation [*Corseuil et al.*, 1998]. As a result, longer BTEX plumes may form [*Ruiz-Aguilar et al.*, 2002], increasing the risk of exposure for potential downgradient receptors [*Powers et al.*, 2001a]. This concern is particularly important for benzene, which is the most hazardous of the gasoline constituents and the one that often dictates the need for remedial action [*Alvarez and Illman, 2006*]. However, many confounding factors that influence plume length could not be considered due to data limitations (e.g., age and amount of spill, hydraulic conductivity, and redox conditions). Thus, considerable uncertainty remains about the magnitude of the plume elongating effect of ethanol. Furthermore, the relative influence of different substrate interactions and geochemical footprints resulting from the presence of ethanol or other fuel alcohols has not been investigated.

Ethanol is the most commonly used fuel alcohol in North America followed by methanol, which respectively account for 3.4% and 2.3% of the total transportation fuel consumption [*U.S. DOE, 2009*; *Lynn, 1999*], and the most common ethanol blend used in the US is E10 (i.e., gasoline with 10% ethanol v:v) [*Yacobucci*, 2007]. However, groundwater contamination by multiple ethanol blends, including E20 which is likely to replace E10 by 2013 in some states [*Kittelson et al.*, 2007], and E85 which is increasingly being used for flexible fuel vehicles or high-compression engines, is possible. Furthermore, interest in higher-molecular-weight fuel alcohols such as propanol and butanol has grown recently due to logistic considerations. These higher-molecular-weight alcohols have higher energy density, improving fuel economy [*U.S. EPA, 2009b*]; they have lower vapor pressure resulting in decreased atmospheric pollution; and their lower

hygroscopicity and water solubility allows for storage and distribution using existing infrastructure [*U.S. EPA, 2009b*] without concern to absorb excessive moisture, which requires special handling of ethanol and dedicated pipelines [*U.S. DOE, 2009b*]. This creates a complex scenario where several blends of alternative fuel alcohols could be present.

The goal of this thesis work is to discern the effect that fuel alcohols have on natural attenuation of BTEX and associated plume dynamics (e.g., length and longevity). A computer module - designated the "General Substrate Interaction Module" (GSIM) - was developed for use with RT3D (Reactive Transport in 3-Dimensions) model [*Clement et al.*, 1998]. GSIM considers common fate and transport processes, and includes additional important substrate interactions: dilution of benzene metabolic flux, catabolite repression, microbial growth/population shifts, cosolvency, toxicity and electron acceptor availability and their sequential utilization on BTEX natural attenuation.

This thesis describes the development of GSIM and presents several simulations aimed at discerning the relative importance of various plume elongation mechanisms, under different contaminant source conditions (constant and decay). Work developed chronologically in five phases: (a) GSIM model development; (b) evaluation of relative importance of processes involved in benzene plume elongation due to ethanol; (c) evaluation of the impact ethanol content has on benzene plume elongation; (d) assessment of the effect of alternative fuel alcohols on benzene natural attenuation.

Specific goals associated with each task are:

(a) Develop an advanced computer module - designated the "General Substrate Interaction Module" (GSIM) - for use with the RT3D (Reactive Transport in 3-Dimensions) model [*Clement et al.,* 1998]. Model was developed to be modular, allowing future implementation of different contaminants and alcohols interactions and to be compatible with the complex 3D scenarios possible with RT3D. **[Chapter 4]**

(b) Evaluate the relative importance of substrate interactions (benzene/ethanol) and the resulting microbial metabolic and population shifts that influence the natural attenuation of E10 releases and the resulting benzene plume length. **[Chapter 5]**

(c) Assess how the availability of alternative anaerobic electron acceptors (Nitrate, Sulfate and Iron) and TEX constituents in gasoline affect benzene plume dynamics. **[Chapter 6]**

(d) Build on the GSIM numerical model to include cosolvency and microbial toxicity exerted by high ethanol blends near the source zone, and evaluate the effect of ethanol content in gasoline on the natural attenuation of benzene plumes. **[Chapter 7]**

(e) Apply the GSIM module to perform a theoretical comparative analysis of the advantages and disadvantages of several alcohol alternatives: Ethanol, methanol, 1-propanol and n-butanol. **[Chapter 8]**

2. Literature Review

One of the principal sources of groundwater contamination are accidental and incidental gasoline releases from underground storage tanks. These metal containers are prone to corrosion and leaking, giving rise to a nationwide problem: leaking underground storage tanks (LUST). Most of the petroleum contamination that reaches groundwater aquifers originates from these leaking storage tanks [*Squillace et al.*, 1996], which has lead 479,000 cases of fuel release in the US with over 377,000 of them requiring some form of remediation efforts [*U.S. EPA*, 2008]. Although fuel spills from LUST can vary in magnitude from a few gallons to tens of thousands of gallons [*NDEQ*, 2005]. The majorities of these spills have constant low volume leaks that are hard to detect and could be present for many years before remedial action is taken. *Dakhel et al.* [2003] performed field experiments with small ethanol releases that indicate that groundwater impacts in these cases should be minimal. The effect of large ethanol releases from LUST has been left largely overlooked [*Zhang et al.*, 2006].

Direct health and environmental effects of ethanol releases in the environment are very unlikely to have strong adverse consequences. This is mainly due to the facts that: (1) fast degradation of ethanol, through aerobic and anaerobic processes, readily occurs in the environment [*Ulrich*, 1999; *Corseuil et al.*, 1998; *Suflita and Mormile*, 1993]; (2) due to its fast degradation and short life in the environment, exposure of humans to toxic ethanol levels is not expected [*Armstrong*, 2000]; (3) literature on ethanol methabolism by humans, and the related health effects of ethanol ingestion, indicate that environmental

exposures to ethanol have a minimal adverse health impact with no symptoms observed below 1000 ppm [*ACGIH*, 1991; *Clayton and Clayton*, 1994]; (4) the human body metabolizes and eliminates ethanol very fast [*Pohorecky and Brick*, 1987; *Holford*, 1987]; and (5) ethanol is not persistent in the environment, with a surface water half-life of 6.5 to 26 hours [*Howard et al.*, 1991].

On the other hand, ethanol has the potential to affect natural attenuation and transport processes of other target pollutants, like BTEX (Benzene, toluene, ethylbenzene and xylenes). The preferential biodegradation of ethanol and associated accelerated depletion of dissolved oxygen and nutrients in aquifers may hinder BTEX degradation. Decreased natural attenuation would in turn increase the length of BTEX plumes, which raises a concern for increased downgradient exposure [*Da Silva and Alvarez*, 2002; *Ruiz-Aguilar et al.*, 2002; *Lovanh et al.*, 2002]. A statistical study of benzene plumes resulting from regular versus E10 (10% v/v ethanol/gasoline) gasoline spills shows increased benzene plume length (an average of 36%) when ethanol is blended with the gasoline. [*Ruiz-Aguilar et al.*, 2003]. Laboratory experiments [*Da Silva and Alvarez*, 2002; Lovanh et al., 2002; *Lovanh and Alvarez*, 2004; *Ruiz-Aguilar et al.*, 2002] and modeling studies [*Heermann and Powers*, 1996; *McNab et al.*, 1999; *Molson et al.*, 2002; *Gomez et al.*, 2008, *Deeb et al.*, 2002] have also shown this elongation for benzene, with changes ranging from 10% to 150%. This is of particular importance as benzene is potentially the most toxic of the BTEX hydrocarbons and its presence in gasoline-contaminated sites often dictates the need for remediation.

However, significant questions remain regarding the relative importance of the processes involved on BTEX plume elongation, the behavior of different ethanol blends and the impact of replacing ethanol with alternative fuel alcohols. We present the literature and previous efforts relevant to answering these questions and that provides the foundation to build our theoretical model and that simulates the underlying processes and interactions.

2.1. Previous Modeling Efforts

Previous modeling efforts (**Table 1**) have simulated the effect of ethanol in E10 on benzene plume length. These models have typically considered important fate and transport processes that form the basis for our work, such as advection, dispersion, sorption, aerobic and anaerobic biodegradation, and ethanol-driven O_2 depletion. *Heermann and Powers* [1996] considered 2D transport, with focus on cosolvency and mass transfer effects, and obtained a 10% increase in the length of a simulated *m*-xylene plume. *McNab et al.* [1999] considered 3D aqueous transport from a finite source release zone and assumed that no anaerobic benzene degradation would occur following oxygen depletion exerted by ethanol, which resulted in a benzene plume elongation on the order of 100%. *Molson et al.* [2002], considered 3D transport and microbial growth following Monod kinetics, including competition for oxygen between ethanol and hydrocarbon degraders. These simulations showed benzene plume elongation of up to 150%.

Table 1 – Modeling Efforts to Assess the effect of Ethanol on Benzene Plume Length

Conceptual model	Increase in benzene plume length	Citation
- 2-D transport from a pool of gasoline. - Focus on cosolvency and interface mass transfer. - Biodegradation not included.	< + 10% (for xylene not benzene)	Heermann and Powers (1996)
- Steady-State, 2-D transport from a gasoline pool. - First-order decay of benzene when CEtOH<3 mg l-1. - First-order decay of ethanol.	+ 17-34 %	Malcom Pirnie Inc. (1998)
- 3-D aqueous transport. - Continuous slow release of gasoline (up to 3 gpd) to a growing NAPL pool at the water table. - First-order decay of ethanol and benzene. - Benzene degradation rate constant defined by inverse correlation to BOD conc. at the source.	~ + 100 %	McNab et al. (1999)
- 3-D transport from a gasoline source at the water table at a residual saturation. - Aerobic decay with O2 as the sole electron acceptor quantified by Monod kinetics. - Microbial growth incorporated.	+ 10-150 %	Molson et al. (2002)

Although past models provided valuable insight into how ethanol influences hydrocarbon plume dynamics, including competitive inhibition processes [*Lu et al.*, 1999], most have not simulated potentially important substrate interactions that influence catabolic enzyme induction (i.e., the synthesis of an enzyme by the cell, when in the presence of a specific substrate) and the metabolic flux of the target pollutants (i.e., the rate at which a pollutant such as benzene is metabolized per unit of biomass, which is analogous to the specific utilization rate). These interactions can cause slower BTEX degradation rates at sites with high ethanol concentrations [*Lovanh and Alvarez*, 2004], although this negative effect can be offset by higher microbial concentrations resulting from the presence of ethanol as an additional substrate [*Lovanh et al.,* 2002]. However, it is unknown how the content of ethanol in different blends, or the use of alternative fuel alcohols that are rapidly entering the market will affect benzene natural attenuation and the resulting plume lifespan and maximum length, which is important to assess the potential likelihood and duration of exposure.

Furthermore, previous research on the effect of ethanol on benzene plume dynamics suggest the potential for similar impacts by other fuel alcohols, which exhibit similar physico-chemical characteristics as well as other properties that might accentuate the hindrance of the natural attenuation of benzene. These include: (1) higher microbial toxicity [*Kaiser and Devillers*, 1994*; Dutka and Kwan*, 1981], which could hinder biodegradation; (2) higher cosolvency power, which could result in faster hydrocarbon dissolution and faster migration (i.e., decreased sorption-related retardation) [*Poulsen et al.,* 1991*; Paan et al.,* 2006]; and (3) slower biodegradation rates [*Howard et al.*, 1991],

which is conducive to longer and more persistent inhibitory substrate interactions. However, the effect of alternative fuel alcohols on benzene biodegradation and natural attenuation has not been addressed in the literature, and it is unknown whether their presence may increase or decrease the potential for benzene plume elongation relative to ethanol.

2.2. Behavior of Ethanol on the unsaturated zone

Ethanol can exert cosolvent effects that influence blended gasoline migration in the unsaturated zone. First, reduced surface and interfacial tension due to ethanol results in a more complete drainage of gasoline, leaving less residual chemicals entrapped in the unsaturated zone [*Powers*, 2001b]. Second, a significant fraction of ethanol partitions and is retained by residual water in the capillary zone. As this residual ethanol infiltrates into the lower gasoline pool, it creates a non-uniform distribution of ethanol on the LNAPL pool. This heterogeneous LNAPL lens complicates the calculation and behavior of BTEX dissolution from the source [*Powers*, 2001b]. Finally, the infiltration rate of residual ethanol towards the capillary fringe and the gasoline pool is limited by the increased viscosity and, therefore, reduced unsaturated hydraulic conductivity of this phase [*Powers*, 2001b]. Another important property of ethanol is that in high concentrations it partitions from fuel ethanol blends due to its higher buoyancy. This leads to a phase separation and accumulation of ethanol on the capillary fringe, resulting in lower groundwater concentrations near the source than would be expected if ethanol were considered completely miscible [*Cápiro et al.*, 2007].

2.3. Enzyme Induction and Repression

Easily degraded substrates, like ethanol, are often preferentially degraded by microorganisms over more important target contaminants like benzene. One of the mechanisms for this is enzyme repression, where the presence of the preferred substrate inhibits the production of the enzyme required to degrade the target pollutant. [*Duetz et al.*, 1994; *Monod*, 1949]. This repression of benzene degrading enzymes in the presence of ethanol was reported by *Hunt et al.* [1997] during aerobic degradation experiments where benzene degradation was delayed. Furthermore, microcosm studies by Corseuil et al. [1998], indicate that this mechanism might lead to slower in situ BTEX biodegradation. This mechanism, known as catabolic repression, prevents microorganisms capable of degrading benzene from utilizing their full potential, hindering BTEX degradation and natural attenuation [*Madigan et al.*, 2005].

Other studies also point to carbon-limiting conditions as responsible for multi-substrate utilization [*Egli*, 1995], where a decrease in the specific benzene utilization rate is due to the presence of ethanol, which is degraded simultaneously, a phenomenon also known as metabolic flux dilution [*Lovanh and Alvarez*, 2004].

2.4. Stimulation of Microbial Growth

One of the advantages of ethanol is that it promotes the growth of a wide variety of microbial populations, including those that can degrade BTEX compounds [*Alvarez and Hunt*, 1999; *Cápiro et al.*, 2008]. Proliferation of BTEX degraders on ethanol (also known as fortuitous growth) would result in faster BTEX degradation rates. Unfortunately, this positive effect of ethanol is likely to be offset by its preferential degradation through catabolic repression and metabolic flux dilution. Ethanol degrading enzymes are associated with central metabolic pathways, which can be utilized by many species that cannot degrade BTEX. Furthermore, favorable thermodynamics lead to faster microbial growth on ethanol than on BTEX compounds, with an increase in maximum specific growth rate of ~45% *[Hunt*, 1999; *McCarty*, 1969]. The overall result of these processes is a significant increase in BTEX degrading microbial populations due to ethanol presence. However, ethanol can stimulate the growth of other bacteria faster than BTEX degraders, which decreases their relative abundance [*Da Silva and Alvarez*, 2002; *Cápiro et al.*, 2007], a phenomenon known as genotypic dilution.

2.5. Microbial Toxicity of Ethanol

Ethanol has been shown to have high concentration toxicity values. Several sources report an EC50 concentration (when microbial activity has been reduced by 50% of its maximum) between 31,000 mg/l and 57,000 mg/l [*Dutka and Kwan*, 1981]. Ethanol concentrations higher than 40,000 mg/l are toxic to most microorganisms, as shown

during aerobic degradation experiments reported by Hunt et al. [1997a]. Ethanol is toxic to microorganisms through disruption of the cellular permeability barrier [*Brusseau et al.*, 1991; *Ingram and Buttke*, 1984; *Harold*, 1970]. In the presence of soil, microorganisms can find some protection, increasing effective toxicity values in the field. Microbial activity can occur at concentrations up to 100,000 mg/l [*Araujo et al.*, 1998]. The majority of BTEX degrading microorganisms have toxicity values to ethanol in the range of 10,000 to 100,000 mg/l. Alternative fuel alcohols can have a wide range toxicity values ranging from ~2000 mg/l (butanol) to ~42,000 mg/l (methanol).

2.6. Depletion of Nutrients and Electron Acceptors

Compared to BTEX and other gasoline components, ethanol exerts a significantly higher biochemical oxygen demand in groundwater. This results in an accelerated consumption of dissolved oxygen within the ethanol plume [*Corseuil et al.*, 1998]. Fast oxygen depletion hinders aerobic BTEX degradation, and particularly of benzene, as it degrades at a much slower rate under anaerobic conditions [*Alvarez and Vogel*, 1995; *Anderson et al.*, 1998; *Weiner and Lovley*, 1998]. Anaerobic degradation of BTEX is also affected. Ethanol can be anaerobically degraded under most common electron-acceptor conditions and this will lead to the depletion of other important dissolved electron acceptors (i.e. ferric iron). Field studies were conducted by Barker et al. [1992] using methanol, which presents environmental impacts similar to those of ethanol. These studies involved releasing controlled amounts of BTEX and methanol mixtures. The

experiment showed, over the course of 476 days, that BTEX degradation is hindered by the presence of methanol in the gasoline plume.

2.7. Accumulation of Volatile Fatty Acids

Ethanol degradation by mixed anaerobic cultures can result in the production of VFAs (acetic, propionic and butyric acid), which can accumulate and decrease the groundwater pH [*Lasko et al.*, 1997; *Speece*, 1983] and contribute undesirable taste and odor to the groundwater. This change in site conditions can also adversely affect some microbial populations that perform BTEX natural attenuation. Methanogens can be inhibited by pH lower than 6 [*McCarty*, 1964], resulting in lower degradation rates of BTEX under such conditions. It is not known if VFAs would accumulate in the field to the levels required to significantly decrease the pH, inhibit microbial growth and result in decreased natural attenuation rates. These effects are likely to vary locally and be specific to site characteristic. Another potential impact of this anaerobic souring effect is the reductive dissolution of metals that can further contribute to water quality degradation.

2.8. Impact of Microbial Processes on Aquifer Permeability

Microbial growth is highly stimulated by ethanol presence. This enhanced microbial growth could affect the hydrodynamic properties of the aquifer, through the formation of biofilm and microbial cell aggregates that can reduce the available pore space and become a potential clogging mechanism [*Taylor and Jaffe*, 1990; *Vandevivere and*

Baveye, 1992]. Microorganisms could also affect aquifer permeability by increasing mineral dissolution (for example, CaCO3) and precipitation (for example, FeS). These opposing processes could affect soil pore space, affecting the available area for contaminant sorption, thus affecting hydraulic conductivity and darcy velocity among other properties. However, laboratory column studies suggest that such effects are minimal [*Da Silva and Alvarez*, 2002].

2.9. Sorption and Cosolvency

Ethanol can have two effects on BTEX concentrations due to cosolvency effects. First, the presence of ethanol in the water phase can decrease sorption-related retardation and is likely to increase BTEX plume lengths. The effect of a cosolvent on BTEX has been described by [*Rao et al.*, 1985]. Cosolvent effects on sorption at ethanol concentrations expected from gasohol spills should be minor, as shown by *Powers* [2001c] and model simulations [*Gomez et al.,* 2008].

The second effect is how ethanol will change the equilibrium partitioning of BTEX compounds between the LNAPL phase and the water phase, which would have a direct impact on dissolution rates of BTEX from spills into the ground and pore water and the resulting plume concentrations. Batch-equilibrium experiments were performed by Heermann and Powers [*1998*] and compared with three mathematical models. Results of these experiences show an overall increase in partition coefficients as a function of increasing ethanol content in the aqueous phase. Heermann and Powers developed a

model to predict BTEX concentrations using a linear relationship for low ethanol volume fractions and a log-linear model for higher concentrations, which showed that changes in gasoline-water partition due to ethanol can be significant.

2.10. Alternative Fuel Alcohols

For the past 50 years methanol and ethanol have been intensively studied resulting in the current use of ethanol as transportation fuel, with E10 expected to be the nationwide standard in the next few years [*U.S. EPA*, 2009b]. Interest in alternatives to ethanol, like propanol and butanol, has grown recently as research shows that longer chained alcohols could offer significant advantages over ethanol and methanol as gasoline substitutes. Higher alcohols have higher energy density improving fuel economy [*U.S. EPA*, 2009b]; they have lower vapor pressure resulting in decreased atmospheric pollution; they have lower hygroscopicity allowing them to be stored and distributed in existing infrastructure [*U.S. EPA*, 2009b]; they can be blended with regular gasoline at concentrations higher than 10% for operation with regular engines [*U.S. EPA*, 2009b]; and they can be synthesized in large-scale by microbiological processes from renewable resources [*Atsumi et al.*, 2008b*; Atsumi et al.*, 2008b*; Lin and Blaschek*, 1983*; Formanek et al.*, 1997*; Shen and Liao*, 2008].

However, these alcohols also have several characteristics that increase their potential environmental impact: they have higher microbial toxicity [*Kaiser and Devillers*, 1994*; Dutka and Kwan*, 1981], which could result in lower degradation activity of BTEX

contaminants; they have higher cosolvency power, resulting in lower soil/water partition coefficients of BTEX constituents [*Poulsen et al.*, 1991; *Paan et al.*, 2006]; and lower biodegradation rates, based on groundwater half-lives of the compounds [*Howard et al.*, 1999], leading to potentially longer alcohol plumes.

3. Theoretical Background

3.1. Contaminant Fate & Transport

The principal mechanisms of contaminant transport in groundwater are *advection* (transport of contaminant due to groundwater flow), *dispersion* (random contaminant movement due to turbulence and molecular movement), *diffusion* (contaminant migration along a concentration gradient in the groundwater) and *adsorption* to aquifer material (accumulation of contaminant on the surface of organic material in soil). These mechanisms were simulated using existing validated models: *Reactive Transport in 3-Dimensions* [RT3D, *Clement et al.,* 1998] and the *USGS MODular three-dimensional finite-difference ground-water FLOW model* [MODFLOW, *Harbaugh et al.,* 2000]. These models present several advantages to handle transport processes over developing our own: (1) developing time can be focused on degradation processes in this work; (2) original versions of the models have been rigorously validated by their authors [*Clement et al.,* 1998; *Harbaugh et al.,* 2000]; (3) commercially available version are widely used by private, educational and governmental institutions, making them the standard for such simulations; (4) both models are freely available for educational/research purposes use. Source code and executables can be found at the USGS MODFLOW website and the Battelle RT3D homepage:

USGS : http://water.usgs.gov/nrp/gwsoftware/modflow2000/modflow2000.html

Battelle : http://bioprocess.pnl.gov/rt3d.htm

MODFLOW describes the movement of constant density groundwater through porous soil using the following partial-differential equation [*McDonald and Harbaugh, 1988*]:

$$\frac{\partial}{\partial x}\left(K_{xx}\frac{\partial h}{\partial x}\right)+\frac{\partial}{\partial y}\left(K_{yy}\frac{\partial h}{\partial y}\right)+\frac{\partial}{\partial z}\left(K_{zz}\frac{\partial h}{\partial z}\right)-W=S_S\frac{\partial h}{\partial t} \qquad (1)$$

Where,

K_{XX}, K_{yy} *and* Kzz = hydraulic conductivity along the x, y, and z coordinate axes (m/d);

h = potentiometric head (m);

W = water sources/sinks volumetric flux per unit volume (1/d);

S_S, is the specific storage of the porous material (1/m);

t = time (d).

This equation is solved by the model using the finite-differences method to obtain an approximate solution for h. Solving this equation together with boundary conditions for groundwater flow and/or initial heads, represents the behavior of an aquifer and can be used to estimate groundwater flow velocities [*McDonald and Harbaugh, 1988*].

RT3D is a model that describes reactive-flow and transport of multiple mobile and/or immobile contaminant species in groundwater flowing through a porous media. It does so by solving the 3D reactive advection dispersion equation that governs these processes [*Clement et al., 1998*]:

$$\frac{\partial C}{\partial t} = \left[D_x \frac{\partial^2 C}{\partial x^2} + D_y \frac{\partial^2 C}{\partial y^2} + D_z \frac{\partial^2 C}{\partial z^2} \right] - \left[\bar{v}_x \frac{\partial C}{\partial x} + \bar{v}_y \frac{\partial C}{\partial y} + \bar{v}_z \frac{\partial C}{\partial z} \right] + r \qquad (2)$$

Where,

D_i = coefficient of hydrodynamic dispersion along the i axis (m^2/d)

C = contaminant aqueous-phase concentration (mg/l)

$\bar{v}_i$ = seepage velocity along the i axis (m/d)

r = reactions that occur in the aqueous and solid phases (mg/l-d).

RT3D uses the solvers for advection and dispersion from the 1997 Department of Defense version of MT3D, and requires MODFLOW to compute variations in groundwater head distribution (groundwater flow $\bar{v}_i$). Several biological reaction modules are included with RT3D, with the option to develop custom reaction modules by the user. RT3D was chosen as the platform for our simulations due to this feature.

3.2. Substrate Interactions and Biodegradation

One of the main advantages of RT3D is that it has a user-defined reaction option that can be used to simulate any type of user-specified reaction kinetics [*Clement.,* 1997]. This capability allows the development of custom biodegradation reaction modules without changing the coded flow and transport processes.

A unique feature of the GSIM biodegradation module for RT3D is that it incorporates metabolic flux dilution (MFD) and catabolite repression (CR) (**Figure 1**). The metabolic flux of a compound is defined as the rate at which it is metabolized per unit biomass. Therefore, the specific substrate utilization rate (i.e., the degradation rate per unit biomass, U (g-substrate g-cells^{-1} hr^{-1}), is a direct measure of metabolic flux. Metabolic flux dilution is a form of non-competitive inhibition in which the rate of utilization of one substrate decreases due to the utilization of another substrate [*Lovanh and Alvarez,* 2004]. Previous laboratory studies have shown that the metabolic flux of a compound in a mixture is proportional to its relative availability, expressed as a fraction of the available organic carbon [*Egli et al.,* 1993; *Lovanh et al.,* 2002]. Ethanol may also act as a cosolvent, increasing BTEX mobility [*Groves,* 1988]. Other fuel alcohols might also have these cosolvent properties. When available, literature data was used to estimate their effect.

Limitations to benzene biodegradation rates caused by MFD are incorporated into GSIM through the variable f_S, which is calculated as the aqueous concentration of a substrate S (benzene in this case) divided by the total concentration of other dissolved organic species, expressed on a total organic carbon (TOC) basis and excluding biomass:

$$f_S = \frac{S_{TOC}}{T_{TOC}} \tag{3}$$

where f_S is the metabolic flux dilution factor (dimensionless), S_{TOC} is the substrate concentration as total organic carbon (mg/l) and T_{TOC} is the total organic carbon concentration (mg/l). The specific substrate utilization rate of the substrate in the absence

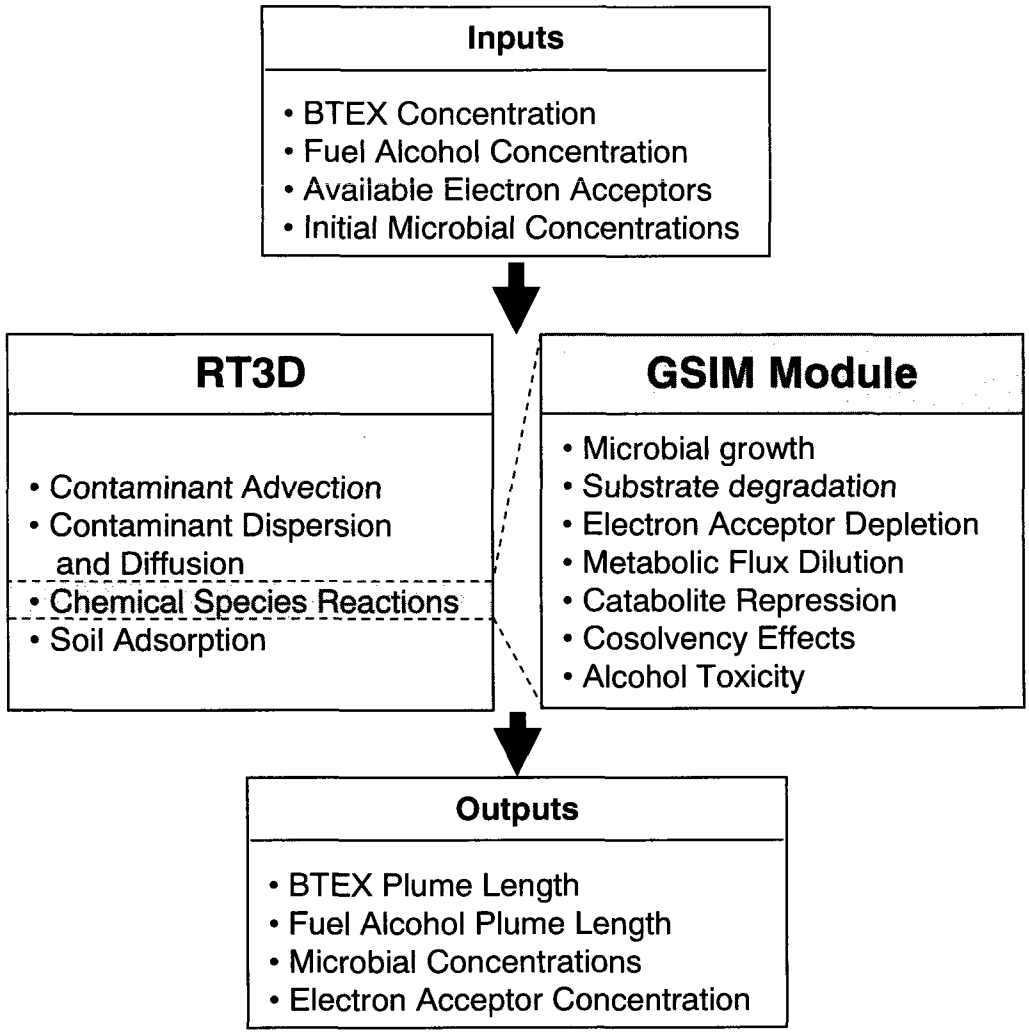

Figure 1 – Processes considered by RT3D and GSIM for the simulation of benzene and ethanol fate and transport.

of ethanol (U_S, [g/g-d]) is multiplied by f_s [*Lovanh and Alvarez*, 2004] to obtain the corrected rate (U_S^*, [g/g-d]). That is,

$$U_S^* = f_S \cdot U_S \tag{4}$$

Thus, as the concentration of ethanol increases, f_S decreases, and the specific substrate utilization rate of benzene is increasingly diminished, potentially leading to longer plumes.

Catabolite repression (CR) is the repression of inducible enzymes by the presence of a preferred carbon source (e.g., ethanol) [*Madigan et al.,* 2005]. CR was modeled as a modulated mechanism in which the induction of a hydrocarbon catabolic gene decreases with increasing concentrations of ethanol. A simple empirical equation was previously used to combine the effects of MFD and CR, based on the assumption that catabolic gene expression increases with increasing relative availability of the inducer (i.e., benzene) in the mixture, as shown by *Lovanh and Alvarez* [2004]. Thus, when MFD and CR act concurrently, substrate degradation rates are considerably reduced, through the use of the square of f_S,

$$U_S^* = f_s^2 \cdot U_S \tag{5}$$

Substrate biodegradation is modeled using a system of equations based on multiplicative Monod kinetics that incorporate MFD plus CR (eq. 4 and 5), recognizing

that the overall degradation rate (r) is the product of the specific degradation rate (U) and the microbial concentration (X). Thus, degradation rate equations are derived for both aerobic (eq. 6) and anaerobic conditions (limited to methanogenic conditions in the latter case) (eq. 7). Oxygen consumption (eq. 8) [*Borden and Bedient,* 1986], aerobic biomass growth (eq. 9) and anaerobic biomass growth (eq. 10) are also considered. The reaction term (r) in equation 2, translates directly into equations 6 to 8, while microbial growth is represented in equations 9 and 10:

$$r_{S,Aer} = \left[\frac{dS}{dt}\right]_{Aer} = -\frac{f_S{}^2}{R_S}\left[\frac{\mu_{mS,Aer} X_{Aer}}{Y_{S,Aer}}\left(\frac{S}{K_{S,Aer}+S}\right)\left(\frac{O}{K_O+O}\right)\right] \tag{6}$$

$$r_{S,An} = \left[\frac{dS}{dt}\right]_{An} = -\frac{f_S{}^2}{R_S}\left[\frac{\mu_{mS,An} X_{An}}{Y_{S,An}}\left(\frac{S}{K_{S,An}+S}\right)\left(\frac{I_{An,O}}{I_{An,O}+O}\right)\right] \tag{7}$$

$$r_O = \frac{dO}{dt} = \left[r_{S,Aer} F_S\right] \tag{8}$$

$$r_{X,Aer} = \frac{dX_{Aer}}{dt} = -\left[r_{S,Aer} Y_{S,Aer}\right]\left(1-\frac{\eta_{bio}}{\gamma \cdot n}\right) - b_{Aer} X_{Aer} \tag{9}$$

$$r_{X,An} = \frac{dX_{An}}{dt} = -\left[r_{S,An} Y_{S,An}\right]\left(1-\frac{\eta_{bio}}{\gamma \cdot n}\right) - b_{An} X_{An} \tag{10}$$

where S is the substrate concentration (mg/l), where $\mu_{mS,Aer}$ and $\mu_{mS,An}$ are the maximum specific growth rate of aerobic biomass and anaerobic biomass respectively (day^{-1}), $Y_{S,Aer}$ and $Y_{S,An}$ are the aerobic and anaerobic biomass yield coefficients (g-biomass/g-substrate), and $K_{S,Aer}$ and $K_{S,An}$ are the half-saturation coefficients of the substrate under aerobic and anaerobic metabolism (mg/l), X_{Aer} and X_{An} are the aerobic and

anaerobic microbial populations (mg/l), f_S is the metabolic flux dilution factor (dimensionless), R_S is the retardation factor (dimensionless), O is the oxygen concentration (mg/l), F_S *is* the stoichiometric oxygen use factor (mg/mg), $I_{An,O}$ is an empirical factor representing inhibition of anaerobic processes by oxygen (mg/l), η_{bio} is the total biomass saturation (volume of biomass per volume of pore space), n is the total porosity, and γ is the maximum pore space utilization factor (non-dimensional)

Equations 6 and 7 describe the loss of substrates due to aerobic and anaerobic biodegradation. Catabolite repression and metabolic flux dilution, as well as soil adsorption, are accounted for through the f_S terms and retardation factor R_S. Equation 8 describes the loss of oxygen by aerobic biodegradation processes. Equations 9 and 10, describe aerobic and anaerobic biomass growth (limited to methanogenic growth for E10 release scenarios). The new values of substrate, electron acceptor, and biomass concentrations at the end of each time step in each grid block are then returned to RT3D as initial values for the subsequent time step. This process is repeated for each time step of simulation.

Since both aerobic and anaerobic (methanogenic) processes are considered, the change from aerobic to anaerobic conditions is simulated by implementing a "switching" function [*Widdowson et al.,* 1988]. This function uses an empirical factor $I_{An,O}$ that gradually initiates anaerobic metabolism as oxygen concentration decreases:

$$\left(\frac{I_{An,O}}{I_{An,O}+O}\right) \tag{11}$$

where O is the oxygen concentration. The anaerobic substrate utilization rate is multiplied by the switching function for simulation of anaerobic biodegradation to limit anaerobic metabolism when oxygen is present.

GSIM also provides mechanisms to control total microbial biomass through a maximum pore space utilization factor γ. The biomass growth expressions of equations 8 and 9 are multiplied by a term to limit the volume of the biomass [*de Blanc et al.,* 1996]:

$$\left(1-\frac{\eta_{bio}}{\gamma \cdot n}\right) \tag{12}$$

where η_{bio} is the total biomass saturation (volume of biomass per volume of pore space) and n is the total porosity. The value of η_{bio} is calculated as:

$$\eta_{bio} = \frac{X_{Aer,T} + X_{An,T}}{\rho} \tag{13}$$

where ρ = biomass density (mass of cells/volume of biomass), $X_{Aer,T}$ is the total aerobic biomass concentration (mg/l), and $X_{An,T}$ is the total anaerobic biomass concentration (mg/l). At low biomass concentrations, the growth limiting expression of Equation 12 has a negligible effect on biomass growth and substrate utilization rates because the biomass

occupies a relatively small volume of the total pore space. As the biomass increases, the growth limiting expression (eq. 13) approaches zero.

All biomass in this work model is assumed to be attached in the form of immobile micro-colonies that behave as fully-penetrated biofilms [*Chen et al.*, 1992], which is the case for at least 99% of subsurface microorganisms [*Harvey et al.*, 1984; *Lehman et al.*, 2001].

3.3. Microbial population shifts

Simultaneous BTEX and fuel alcohol utilization was implemented as several different degradation processes involving 15 separate microbial populations: oxygen (O2) reducers, nitrate (as NO_3^-) reducers, sulfate (as SO_4^{2-}) reducers, iron (as Fe_3^+ immobile in the solid phase) reducers and methanogens, using benzene, alcohol and TEX as substrates. Toluene, ethylbenzene and xylene were grouped into 1 chemical species to simplify the model setup. **Table 2** shows the 15 different degradation processes considered by the final GSIM module. Each column represents a possible degradation pathway that results in rate of changes (r) for the involved species, with the symbols indicating its associated substrate (S), electron acceptor (EA) and microbial population (M).

Table 2 – Substrate/Electron Acceptor/Microbial Population Matrix.

	Degradation Pathway														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fuel Alcohol	S	S	S	S	S										
Benzene						S	S	S	S	S					
TEX											S	S	S	S	S
O2	EA					EA					EA				
Nitrate		EA					EA					EA			
Sulfate			EA					EA					EA		
Iron				EA					EA					EA	
Alcohol Aerobes	M														
Alcohol Denitrifiers		M													
Alcohol Sulfate Reducers			M												
Alcohol Iron Reducers				M											
Alcohol Methanogens					M										
Benzene Aerobes	M					M									
Benzene Denitrifiers		M					M								
Benzene Sulfate Reducers			M					M							
Benzene Iron Reducers				M					M						
Benzene Methanogens					M					M					
TEX Aerobes	M										M				
TEX Denitrifiers		M										M			
TEX Sulfate Reducers			M										M		
TEX Iron Reducers				M										M	
TEX Methanogens					M										M

Additional microbial activity due to fortuitous growth is considered for degradation pathways 7 to 15, where some BTEX degraders can grow fortuitously on fuel alcohols. However, alcohols can stimulate the growth of other bacteria faster than hydrocarbon degraders, which decreases the relative abundance of BTEX degraders [*Da Silva and Alvarez,* 2002; *Cápiro et al.,* 2007]. This phenomenon is coined as "genotypic dilution". For example, in the case of ethanol and benzene aerobic and methanogenic degradation only (Pathways 1, 5, 6 and 10 in **Table 2**), the pertinent equations are described below:

Biodegradation of Ethanol:

$$r_E = \left[\frac{dE}{dt}\right] = -\frac{f_E}{R_E}(r_{E,1} + r_{E,2} + r_{E,3} + r_{E,4})$$

$$r_{E,1} = \frac{\mu_{E,Aer1} X_1}{Y_{E,Aer1}} \left(\frac{E}{K_{E,Aer1} + E}\right)\left(\frac{O}{K_O + O}\right) \quad (aerobic)$$

$$r_{E,2} = \frac{\mu_{E,Aer2} X_2}{Y_{E,Aer2}} \left(\frac{E}{K_{E,Aer2} + E}\right)\left(\frac{O}{K_O + O}\right) \quad (aerobic - Fortuitous\_growth)$$

$$r_{E,3} = \frac{\mu_{E,An1} X_3}{Y_{E,An1}} \left(\frac{E}{K_{E,An1} + E}\right)\left(\frac{I_{An,O}}{I_{An,O} + O}\right) \quad (methanogenic)$$

$$r_{E,4} = \frac{\mu_{E,An2} X_4}{Y_{E,An2}} \left(\frac{E}{K_{E,An2} + E}\right)\left(\frac{I_{An,O}}{I_{An,O} + O}\right) \quad (methanogenic - Fortuitous\_growth) \qquad (14)$$

Biodegradation of Benzene:

$$r_B = \left[\frac{dB}{dt}\right] = -\frac{f_B{}^2}{R_B}(r_{B,2} + r_{B,4})$$

$$r_{B,2} = \frac{\mu_{B,Aer} X_2}{Y_{B,Aer}}\left(\frac{B}{K_{B,Aer} + B}\right)\left(\frac{O}{K_O + O}\right) \quad (aerobic) \tag{15}$$

$$r_{B,4} = \frac{\mu_{B,An} X_4}{Y_{B,An}}\left(\frac{B}{K_{B,An} + B}\right)\left(\frac{I_{An,O}}{I_{An,O} + O}\right) \quad (methanogenic)$$

Oxygen Consumption:

$$r_O = \frac{dO}{dt} = \left[r_{E,1}F_E + r_{E,2}F_E + r_{B,2}F_B\right] \tag{16}$$

Aerobic Ethanol Degraders (X_1) Growth:

$$r_{X,1} = \frac{dX_1}{dt} = -\left[r_{E,1}Y_{E,Aer1}\right]\left(1 - \frac{\eta_{bio}}{\gamma n}\right) - b_{Aer}X_1 \tag{17}$$

Aerobic Ethanol and Benzene Degraders (X_2) Growth:

$$r_{X,2} = \frac{dX_2}{dt} = -\left[r_{E,2}Y_{E,Aer2} + r_{B,2}Y_{B,Aer}\right]\left(1 - \frac{\eta_{bio}}{\gamma n}\right) - b_{Aer}X_2 \tag{18}$$

Anaerobic Methanogenic Ethanol Degraders (X_3) Growth:

$$r_{X,3} = \frac{dX_3}{dt} = -\left[r_{E,3}Y_{E,An1}\right]\left(1 - \frac{\eta_{bio}}{\gamma n}\right) - b_{An}X_3 \tag{19}$$

Anaerobic Methanogenic Ethanol and Benzene Degraders (X_4) Growth:

$$r_{X,4} = \frac{dX_4}{dt} = -\left[r_{E,4}Y_{E,An2} + r_{B,4}Y_{B,An}\right]\left(1 - \frac{\eta_{bio}}{\gamma n}\right) - b_{An}X_4 \qquad (20)$$

Where X_1 is ethanol aerobic degraders (mg/l), X_3 is ethanol methanogenic degraders (mg/l), X_2 is benzene aerobic degraders that can also grow on ethanol (mg/l) and X_4 is benzene methanogenic degraders that can also grow on ethanol (mg/l). Equation 14 shows the fortuitous growth of benzene degraders. If we consider a system where ethanol is being degraded along with benzene and TEX, and considering all possible electron acceptors, the number of equations increases significantly. **Figure 2** shows a Venn diagram of the 4 microbial populations described and their overlapping roles.

3.4. Cosolvency, Volatilization and Toxicity

Ethanol and other fuel alcohols may also act as a cosolvent if present in groundwater at concentrations greater than 10,000 mg/l [**Table 3**, *Da Silva and Alvarez*, 2002; *Powers et al.*, 2001c] increasing BTEX dissolution and mobility [*Groves*, 1988]. This potential effect is incorporated into the GSIM model by considering cosolvency effects both on the source zone dissolution of LNAPL and changes in retardation factor due to soil/water partitioning of benzene. The model was modified to consider ethanol toxicity to microbial populations. The model inhibits growth of either benzene or ethanol degraders when ethanol reaches concentrations higher than 38,000 mg/l average. This is based on values from several sources reported by *Dutka and Kwan* [1981].

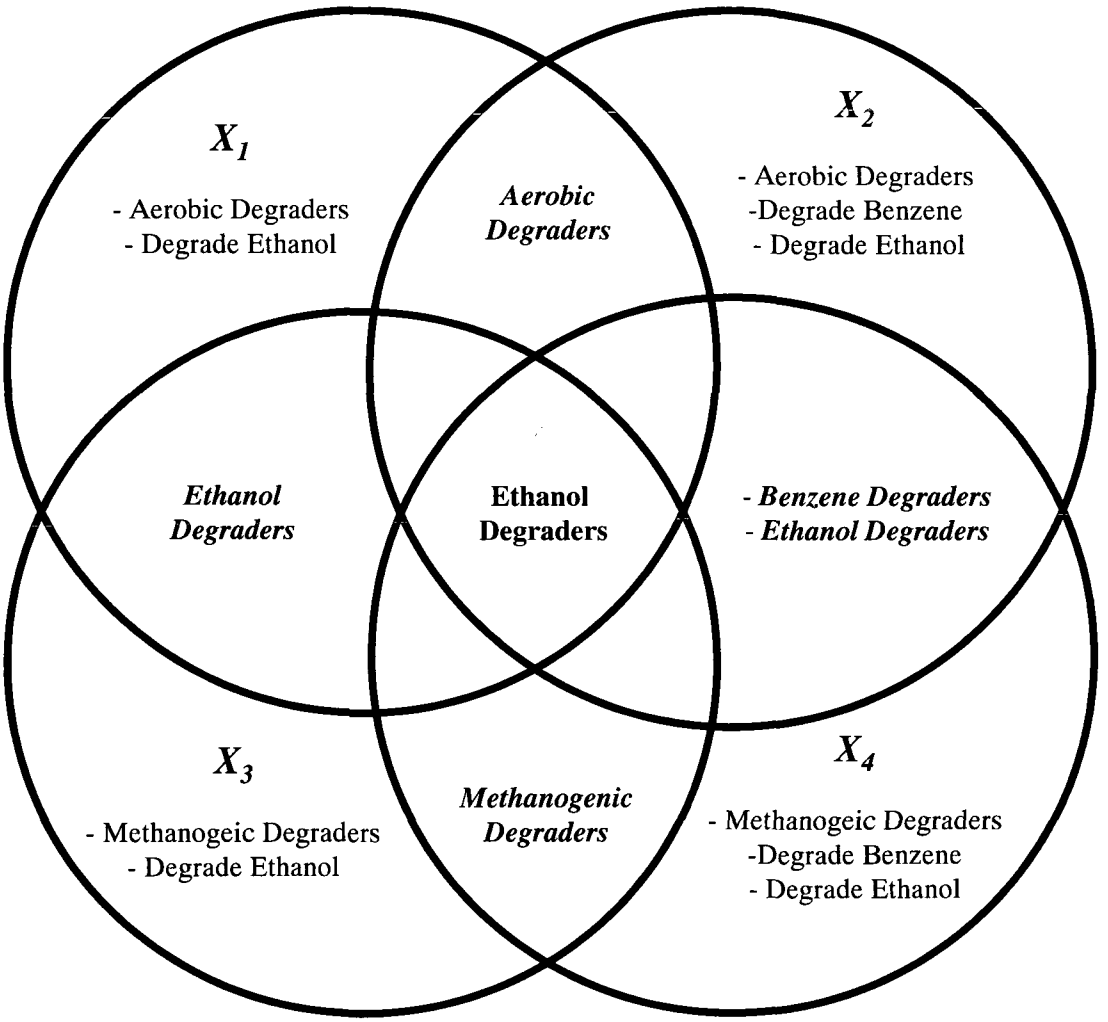

Figure 2 – Venn diagram showing the roles of ethanol and benzene degrading microbial populations X_1, X_2, X_3 and X_4.

Benzene concentrations in groundwater equilibrated with the LNAPL source zone are calculated using an excel spreadsheet model, developed for this research (**Appendix 1**), based on the American Petroleum Institute's (API) LNAPL Dissolution and Transport Screening Tool (LNAST) [*Huntley and Beckett,* 2002] and that considers both source zone dissolution into groundwater and volatilization into the atmosphere of organic compounds. This spreadsheet incorporates the cosolvency effect of aqueous ethanol, which enhances benzene dissolution. Ethanol concentrations cannot be calculated using Raoult's law (equation 21) because ethanol is infinitely soluble in water. Concentrations can be estimated using a mass transfer limitation factor of 20 (factor of 2 due to fuel/groundwater mixing and of 10 due to subsurface dilution factors), and assuming that >99% of the ethanol dissolves in water. This leads to ethanol concentrations in the groundwater interface with the LNAPL of 0.5% - 1% by volume [*Malcolm Pirnie*, 1998].

$$C_i = C_i^w X_i^o \tag{21}$$

Where C_i is chemical concentration in water phase, C_i^w is the maximum solubility of chemical i in the water phase and X_i^o is the molar fraction of chemical i in the organic phase. Equation 21 can usually provide a reasonable estimate for benzene concentrations in groundwater from regular gasoline LNAPL [*Mackay et al.*, 1991]. In the case of ethanol, gasoline does not follow thermodynamically ideal behavior and the organic phase activity coefficients become important (can't be assumed as unity). Under these conditions, and in the presence of high concentrations of ethanol and benzene in the water phase, Raoult's law cannot be used to calculate benzene concentrations [*Heerman*

and Powers, 1998]. A linear/log-linear model developed by *Heerman and Powers* [1998] is used instead, for calculation of benzene concentrations:

$$C_b = (1-\frac{f_c}{\beta})C_b^w \gamma_b^o X_b^o + \frac{f_c}{\beta} C_b^\beta \gamma_b^o X_b^o \qquad f_c < \beta \qquad (22)$$

$$\ln C_b = (1-\frac{f_c-\beta}{1-\beta})\ln(C_b^\beta \gamma_b^o X_b^o) + \frac{f_c-\beta}{1-\beta}\ln(C_b^e f_b^o) \qquad f \geq \beta \qquad (23)$$

Where, C_b is the benzene concentration in the aqueous phase (mg/l), f_c is ethanol content in the water phase (v/v), β is the volume fraction of ethanol in the aqueous phase coinciding with the breakpoint between the two segments of the model (v/v), C_b^w is the benzene solubility in pure water (mg/l), C_b^β is the benzene solubility at β ethanol fraction in water (mg/l), γ_b^o is the benzene activity coefficient in the organic phase, X_b^o is the benzene molar fraction in the organic phase, C_b^e is the benzene solubility in ethanol (mg/l), and f_b^o is the organic phase volume fraction of benzene (v/v).

Equations 22 and 23 calculate the dissolved concentration of benzene for two ranges of ethanol fraction in the water phase (lower and higher than β), account for ethanol being an infinitely soluble organic compound, and consider activity coefficient of ethanol due to its non-ideal solution behavior in the organic phase. Blends with lower ethanol content than E10 (resulting in less than 10,000 mg/l of ethanol in the water phase) have a negligible increase in benzene solubility in water (< 1%).

Changes in molar fraction composition of the different LNAPL components over time, due to different diffusion coefficients and LNAPL mass depletion, are also considered. These processes generate ethanol and benzene concentrations in the boundary between the NAPL and water phases. Mass transfer rates for the different constituents are used to calculate mass depletion based on Fick's second law and groundwater flow characteristics [*Clark*, 1996]:

$$M_i^w = 2C_i^w \sqrt{\frac{D_i v L t^2}{\pi}} \tag{24}$$

Where M_i^w is the total mass transferred per unit of width of the NAPL source zone interface with water (mg), C_i^w is the *i*-component water phase concentration (mg/l), D_i is the *i*-component diffusivity (m^2/d), v is the groundwater pore space velocity (m/d), L is the length of the NAPL source zone interface with water (m) and t is time elapsed (d).

Assuming that gasoline constituent concentrations just below the LNAPL/Water interface decrease rapidly to non-detect levels within two to three meters [*Huntley and Beckett,* 2002], source cell concentrations that represent the average between the interface value at the top of the source cells and zero (i.e., the value at the bottom of the source cells) are used as direct input for transient RT3D simulations.

The GSIM model also incorporates variations in retardation factor of benzene due to changes in the soil-water partition coefficient equilibrium. The effect of a cosolvent on

the BTEX components partitioning can be described by the relationship [*Rao et al.*, 1985]:

$$Log(K_m) = Log(K_w) - \alpha \cdot \sigma \cdot f_c \qquad (25)$$

Where K_m is the distribution ratio in presence of the cosolvent, K_w is the distribution ratio with pure water. This relationship was later refined, [*Rao et al.*, 1991]:

$$Log(\frac{K_d'}{K_d}) = -\alpha \cdot \beta \cdot \sigma \cdot f_c \qquad (26)$$

Where K_d is the distribution ratio for pure water, and K_d' accounts for the presence of ethanol. f_c is the cosolvent content as volume fraction in the water phase, and σ is the cosolvent power of ethanol on any given BTEX compound. This relationship is valid for ethanol volume fractions of 1 to 40%.

The product $\alpha\beta$ in equation 26, is measured empirically and depends on various molecular interactions between cosolvent and sorbent (α), and cosolvent and solute (β). There is no documented relationship for these values and soil parameters, so they have to be measured experimentally in a case by case basis. In the case of α, the more it deviates from 1, the more the cosolvent interacts with the sorbent (soil). If the soil is relatively inert and low in organic content, then this value should approach 1. α and β have been assumed to be 1 for simplicity (conservative approach), and the value for σ for benzene

taken as 2.96 [*Poulsen et al.*, 1991]. Simplifying equation 26 for benzene, assuming inert soil with low organic content ($\alpha\beta = 1$):

$$K_d^{'} = K_d * 10^{-\sigma \cdot f_c} \tag{27}$$

Which can be transformed into a retardation factor relationship of the form [*Li et al.*, 2000]:

$$R' = \frac{R-1}{10^{\sigma f_c}} + 1 \tag{28}$$

R can be calculated using the standard linear model ($R = 1 + \frac{\rho_b K_d}{n}$ [*Charbeneau*, 2000]) or the dual equilibrium desorption model presented Chen [*et al.*, 2002]. In our case, we use the linear sorption model incorporated into RT3D to calculate the value of R. For E10 blends, f_c is usually less than 1% (<10,000 mg/l) and the resulting reduction of R is negligible, as previously documented in laboratory studies [*Da Silva and Alvarez,* 2002; *Powers et al.,* 2001c].

Volatilization of organic compounds from the LNAPL source zone was considered. This assumes that the LNAPL spill site is open to the atmosphere and not covered by hard surfaces like asphalt or concrete, and follows a Fick's law behavior. For a permeable ground surface, and considering the unsaturated zone of the soil as the diffusion distance, then [*Kim and Corapcioglu,* 2003]:

$$M_i^a = -\left(\theta_g D_g^{'} + \theta_{wr} D_{wr}^{'} K_H\right)\frac{(C_a - C_g^s)}{d} \tag{29}$$

Where, C_a is the organic compound concentration in the atmosphere (mg/l, assumed cero); C_g^s is the organic compound saturation concentration in the vapor phase (mg/l); d is the unsaturated zone depth (m); K_H is the dimensionless Henry's constant; θ_g and θ_{wr} are the volumetric content of air and water in the soil (vol/vol); D_g is the effective diffusion coefficient in the vapor phase and D_{wr} is the effective diffusion coefficient in the vadose zone pore water (m^2/d). Effective diffusion coefficients in the unsaturated zone air and water phases can be calculated from air and water diffusion coefficients by [*Millington and Quirk*, 1961]:

$$D_g^{'} = \frac{\theta_g^{\frac{7}{3}}}{n^2} D_g \tag{30}$$

$$D_{wr}^{'} = \frac{\theta_{wr}^{\frac{7}{3}}}{n^2} D_{wr} \tag{31}$$

Where n is the soil porosity. The final loss of mass due to volatilization and dissolution from the LNAPL source zone considers both equation 24 and 29.

3.5. Electron Acceptor Utilization Sequence and Stoichiometry

The GSIM module is designed with the potential to include several common electron acceptors that better characterize domain characteristics: oxygen, nitrogen, sulfate and ferric iron; and several substrates like benzene, toluene, ethylbenzene and xylenes. TEX compounds were not considered on previous modeling studies using GSIM [*Gomez et al., 2008; Gomez and Alvarez, 2009*].

Maximum specific growth rate and microbial biomass yield coefficient for all species considered in this thesis were obtained from literature sources (**Table 4**). Aerobic and methanogenic values were readily available for BTEX contaminant species. Significant knowledge gaps exist concerning Monod degradation kinetics for alcohols. To fill this gap and to obtain stoichiometric electron acceptor use, *McCarty's* [*2007*] Electron Equivalent Model for Bacterial Yield Prediction was used. With this model, the balanced reaction equation representing substrate and electron acceptor use required to generate cell mass is obtained, based on the involved species' half reaction equations and their associated Gibbs standard free energy (ΔG^o). Half reaction equations for electron acceptors considered in our model are (*Rittmann and McCarty, 2001*):

Water-oxygen : $\frac{1}{4}O_2 + H^+ + e^- = \frac{1}{2}H_2O$ (ΔG^o=-78.72 kJ/e-eq)

Nitrogen-Nitrate : $\frac{1}{2}NO_3^- + H^+ + e^- = \frac{1}{2}NO_2^- + \frac{1}{2}H_2O$ (ΔG^o=-72.20 kJ/e-eq)

Ferrous-Ferric : $Fe^{3+} + e^- = Fe^{2+}$ (ΔG^o=-74.27 kJ/e-eq)

Sulfide-Sulfate : $\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^- = \frac{1}{16}H_2S + \frac{1}{16}HS^- + \frac{1}{2}H_2O$ (ΔG^o=20.85 kJ/e-eq)

Carbon Dioxide : $\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$ (ΔG^o=-24.13 kJ/e-eq)

All reactions were assumed to follow ammonia-based cell synthesis, with the exception of denitrification which uses nitrate based cell synthesis. Electron acceptor donor for all reactions considered is the initial substrate (BTEX and alcohols), with the exception of methanogenic reactions were the substrate is assumed to be transformed into acetate, which is then readily available for methanogenic degradation. Electron donor half reaction equations are [*Rittmann and McCarty, 2001*]:

Benzene : $\frac{1}{30}C_6H_6 + \frac{2}{5}H_2O = \frac{1}{5}CO_2 + H^+ + e^-$ (ΔG^o=28.34 kJ/e-eq)

Toluene : $\frac{1}{36}C_7H_8 + \frac{7}{18}H_2O = \frac{7}{36}CO_2 + H^+ + e^-$ (ΔG^o=27.85 kJ/e-eq)

Methanol : $\frac{1}{6}CH_3OH + \frac{1}{6}H_2O = \frac{1}{6}CO_2 + H^+ + e^-$ (ΔG^o=36.84 kJ/e-eq)

Ethanol : $\frac{1}{12}C_2H_4OH + \frac{1}{4}H_2O = \frac{1}{6}CO_2 + H^+ + e^-$ (ΔG^o=31.18 kJ/e-eq)

Propanol : $\frac{1}{17}C_3H_6OH + \frac{5}{17}H_2O = \frac{3}{17}CO_2 + H^+ + e^-$ (ΔG^o=29.94 kJ/e-eq)

Butanol : $\frac{1}{24}C_4H_9OH + \frac{7}{24}H_2O = \frac{4}{24}CO_2 + H^+ + e^-$ (ΔG^o=29.26 kJ/e-eq)

Acetate : $\frac{1}{8}C_2H_3OO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCOO^{3-} + H^+ + e^-$ (ΔG^o=27.40 kJ/e-eq)

Cell synthesis was assumed ammonia based and follows the following half-reaction (R_c) [*Rittmann and McCarty, 2001*]:

Cell synthesis: $\frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O = \frac{1}{5}CO_2 + \frac{1}{20}HCOO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^-$

Based on these half reaction equations and their free energies, we use the following equations [*McCarty, 2007; Rittmann and McCarty, 2001*] to estimate the yield and the maximum specific growth rates:

$$Y = \frac{\gamma_d}{\gamma_x} f_s^0 \tag{32}$$

$$\mu_m = Yk \tag{33}$$

$$k = k_m \frac{1+A}{A} \tag{34}$$

$$\gamma_d = \frac{P_{donor}}{C_{donor}} \tag{35}$$

$$\gamma_x = \frac{P_{cells}}{C_{cells}} \tag{36}$$

$$f_s^0 = \frac{1}{1+A} \tag{37}$$

$$A = \frac{-\Delta G_S}{\varepsilon \Delta G_r} = \frac{\frac{\left(\Delta G_{fa} - \Delta G_d\right)}{\varepsilon^m} + \frac{\left(\Delta G_{in} - \Delta G_{fa}\right)}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon\left(\Delta G_a - \Delta G_d - \frac{q}{p}\Delta G_{xy}\right)} \tag{38}$$

Where f_s^0 is the true yield expressed as a fraction of electron donor converted for synthesis (e-eq cells/e-eq donor), ΔG_r is the Gibbs free energy released by energy reaction (kJ/e-eq), ΔG_s is the Gibbs free energy required for synthesis (kJ/e-eq), ΔG_a is the reduction potential for electron acceptor half-reaction (kJ/e-eq), ΔG_d is the reduction potential for electron donor half-reaction (kJ/e-eq), ΔG_{xy} is the reduction potential for NADH oxidation (kJ/e-eq), ΔG_{in} is the reduction potential for acetyl-CoA half-reaction (kJ/e-eq), ΔG_{fa} is the reduction potential for formaldehyde half reaction (kJ/e-eq), ΔG_{pc} is the Gibbs free energy for intermediate conversion to cells (kJ/e-eq), ε is the energy transfer efficiency, p is the number of electron equivalents per mole of substrate from half reaction reduction equation, q is the number of oxygenase reactions per mole of substrate, and γ_d and γ_x are the degree of reduction of electron donor and cells respectively [*McCarty, 2007*]. These equations yield the values shown in **Tables 5 and 6**, assuming no oxygenase is involved (q=0) and no Cl compounds are involved (ΔG_{fa} = 0, m = n). ΔG_{in} = 30.9 kJ/e-eq and ΔG_{pc} = 18.8 kJ/e-eq and ΔG_{xy} = -219.2 kJ/e-eq [*McCarty, 2007*]. Using the calculated f_s values, we can estimate the stoichiometric electron acceptor use for all degradation pathways (**Tables 5 and 6**):

$$R_f = R_d - f_e R_a - f_s R_d \tag{39}$$

$$f_e = 1 - f_s \tag{40}$$

Table 3– Physicochemical Properties of Chemical Species

Property	Methanol	Ethanol	1-Propanol	n-Butanol	IBA	Benzene	TEX	Toluene	Ethylbenzene	Xylene	Source
Formula	CH_4O	C_2H_6O	C_3H_8O	$C_4H_{10}O$	$C_4H_{10}O$	C_6H_6	-	C_7H_8	C_8H_{10}	C_8H_{10}	
CAS Number	67-56-1	64-17-5	71-23-8	71-36-3	78-83-1	71-43-2	-	108-88-3	100-41-4	1330-20-7	
Molecular Weight (g/mole)	32.04	46.07	60.10	74.12	74.12	78.11	101.51	92.14	106.20	106.20	*Hilal et al. 2003*
Solubility (mole fraction in water)	Miscible	Miscible	1.0×10^{-1}	1.8×10^{-2}	3.1×10^{-2}	3.4×10^{-4}	4.9×10^{-5}	9.1×10^{-5}	2.9×10^{-5}	2.7×10^{-5}	*Hilal et al. 2003*
Vapor pressure (atm)	1.9×10^{-1}	1.1×10^{-1}	3.6×10^{-2}	1.2×10^{-2}	1.7×10^{-2}	1.3×10^{-1}	2.4×10^{-2}	4.4×10^{-2}	1.5×10^{-2}	1.3×10^{-2}	*Hilal et al. 2003*
(adimensional)	2.9×10^{-4}	3.9×10^{-4}	4.8×10^{-4}	6.5×10^{-4}	6.0×10^{-4}	3.0×10^{-1}	3.6×10^{-1}	3.5×10^{-1}	3.7×10^{-1}	3.7×10^{-1}	
Water Diffusivity (cm^2/s)	1.6×10^{-5}	1.3×10^{-5}	1.1×10^{-5}	9.6×10^{-6}	9.6×10^{-6}	9.8×10^{-6}	8.3×10^{-6}	8.8×10^{-6}	8.1×10^{-6}	8.1×10^{-6}	*Hilal et al. 2003*
Air Diffusivity (cm^2/s)	1.6×10^{-1}	1.2×10^{-1}	1.0×10^{-1}	8.6×10^{-2}	8.6×10^{-2}	9.1×10^{-2}	7.3×10^{-2}	7.8×10^{-2}	7.0×10^{-2}	7.0×10^{-2}	*Hilal et al. 2003*
Partitioning Coefficient (L/Kg)	3.0×10^{-4}	1.2×10^{-3}	3.6×10^{-3}	1.4×10^{-2}	7.6×10^{-3}	1.4×10^{-1}	1.2×10^{0}	5.1×10^{-1}	1.5×10^{0}	1.7×10^{0}	*Hilal et al. 2003*
Cosolvency Power	2.79	2.96	3.18	3.23	3.23	-	-	-	-	-	*Poulsen et al., 1991; Paan et al., 2006*
Bacterial MC50 (mg/l)	42,237	31,000	9,862	2,056	2,467	-	-	-	-	-	*Kaiser and Devillers, 1994; Dutka and Kwan, 1981, Atsumi et al, 2008*
Density (g/cm^3)	0.79	0.79	0.8	0.81	0.80	0.87	0.86	0.86	0.86	0.86	*Hilal et al. 2003*
Carbon content fraction (mol/mol)	0.38	0.52	0.60	0.65	0.65	0.92	0.91	0.91	0.90	0.90	*Calculated from chemical formula*

Table 4 – Degradation Properties of Chemical Species

	First-order degradation rate (1/d)		Maximum specific growth rate (1/d)	
Ethanol				
Aerobic	0.35	*Corseuil et al., 1998*	11.04	*Lovanh et al., 2002*
Methanogenic	0.20	*Corseuil et al., 1998*	1.10	*Lovanh et al., 2002*
Nitrate Reducing	0.53	*Corseuil et al., 1998*	0.35	*Calculated*[2]
Sulfate Reducing	0.10	*Corseuil et al., 1998*	0.21	*Calculated*[2]
Iron Reducing	0.17	*Corseuil et al., 1998*	0.21	*Calculated*[2]
Benzene				
Aerobic	0.68	*Alvarez et al., 1991*	3.24	*Alvarez et al., 1991*
Methanogenic	0.0030	*Wilson et al, 1990; Kazumi et al, 1997*	0.30	*Ulrich and Edwards, 2003*
Nitrate Reducing	0.0075	*Burland and Edwards, 1999*	4.80	*Schreiber and Bahr, 2002*
Sulfate Reducing	0.016	*Kazumi et al., 1997; Wiedemeier et al., 1996*	1.25	*Godeke et al, 2008*
Iron Reducing	0.0035	*Wilson et al, 1996; Rifai et al., 1995*	0.15	*Lovley and Lonergan, 1990*
TEX				
Aerobic	0.12	*Nielsen et al, 1996*	6.47	*Goudar & Strevett, 1998*
Methanogenic	0.0327	*Wiedemeier et al., 1995; Wilson et al., 1990*	1.42	*Chaudhuri and Wiesmann, 1996*
Nitrate Reducing	0.326	*Hilton et al, 1992; Reinhard et al., 1997*	4.80	*Schreiber and Bahr, 2001*
Sulfate Reducing	0.046	*Wiedemeier et al., 1996; Reinhard et al., 1997*	1.25	*Godeke et al, 2008*
Iron Reducing	0.0094	*Rifai et al., 1995; Wilson et al., 1996*	0.15	*Lovley and Lonergan, 1990*
Methanol				
Aerobic	0.19	*Calculated*[1]	1.72	*Calculated*[2]
Methanogenic	0.108	*Calculated*[1]	0.09	*Calculated*[2]
Nitrate Reducing	0.29	*Calculated*[1]	0.35	*Calculated*[2]
Sulfate Reducing	0.054	*Calculated*[1]	0.18	*Calculated*[2]
Iron Reducing	0.092	*Calculated*[1]	0.18	*Calculated*[2]
Butanol				
Aerobic	0.095	*Calculated*[1]	2.51	*Calculated*[2]
Methanogenic	0.0542	*Calculated*[1]	0.09	*Calculated*[2]
Nitrate Reducing	0.144	*Calculated*[1]	0.51	*Calculated*[2]
Sulfate Reducing	0.0271	*Calculated*[1]	0.26	*Calculated*[2]
Iron Reducing	0.0460	*Calculated*[1]	0.27	*Calculated*[2]
Propanol				
Aerobic	0.190	*Calculated*[1]	2.42	*Calculated*[2]
Methanogenic	0.108	*Calculated*[1]	0.09	*Calculated*[2]
Nitrate Reducing	0.29	*Calculated*[1]	0.49	*Calculated*[2]
Sulfate Reducing	0.054	*Calculated*[1]	0.25	*Calculated*[2]
Iron Reducing	0.092	*Calculated*[1]	0.26	*Calculated*[2]

[1] *First order degradation rates estimated based on ethanol values and relative groundwater half-lives of the compounds [Howard et al., 1999].*

[2] *Values obtained from stoichiometry using the Thermodynamic Electron Equivalents Model for Bacterial Yield Prediction [McCarty, 2007] and the resulting reactions.*

[3] *Estimated on the basis of the relationship l = (mX/Y Ks) [Alvarez and Illman, 2006]*

	Microbial cell yield (g/g)		Half-saturation constant (mg/l) [3]
Ethanol			
Aerobic	0.50	*Heulekian and Manganelli, 1951*	63.09
Methanogenic	0.07	*Lawrence and McCarty, 1969*	78.86
Nitrate Reducing	0.26	*Calculated* [2]	2.52
Sulfate Reducing	0.18	*Calculated* [2]	11.43
Iron Reducing	0.18	*Calculated* [2]	6.72
Benzene			
Aerobic	0.39	*Grady et al. 1989*	7.63
Methanogenic	0.05	*O'Rourke, 1968*	21.58
Nitrate Reducing	0.62	*Calculated* [2]	10.31
Sulfate Reducing	0.43	*Calculated* [2]	1.80
Iron Reducing	0.14	*Calculated* [2]	3.05
TEX			
Aerobic	0.40	*Goudar & Strevett, 1998*	133.37
Methanogenic	0.08	*Calculated* [2]	0.56
Nitrate Reducing	0.57	*Calculated* [2]	0.26
Sulfate Reducing	0.40	*Calculated* [2]	0.68
Iron Reducing	0.11	*Calculated* [2]	1.46
Methanol			
Aerobic	0.52	*Calculated* [2]	17.59
Methanogenic	0.08	*Calculated* [2]	10.26
Nitrate Reducing	0.21	*Calculated* [2]	5.81
Sulfate Reducing	0.15	*Calculated* [2]	23.07
Iron Reducing	0.15	*Calculated* [2]	13.57
Butanol			
Aerobic	1.06	*Calculated* [2]	25.03
Methanogenic	0.08	*Calculated* [2]	20.52
Nitrate Reducing	0.37	*Calculated* [2]	9.69
Sulfate Reducing	0.23	*Calculated* [2]	42.23
Iron Reducing	0.23	*Calculated* [2]	24.90
Propanol			
Aerobic	1.00	*Calculated* [2]	12.81
Methanogenic	0.08	*Calculated* [2]	10.26
Nitrate Reducing	0.35	*Calculated* [2]	4.90
Sulfate Reducing	0.22	*Calculated* [2]	21.23
Iron Reducing	0.22	*Calculated* [2]	12.52

[1] *First order degradation rates estimated based on ethanol values and relative groundwater half-lives of the compounds [Howard et al., 1999].*

[2] *Values obtained from stoichiometry using the Thermodynamic Electron Equivalents Model for Bacterial Yield Prediction [McCarty, 2007] and the resulting reactions.*

[3] *Estimated on the basis of the relationship* $l = (mX/Y\,Ks)$ *[Alvarez and Illman, 2006]*

Table 5 – Thermodynamic Properties for McCarty's Model

Benzene					
	Oxygen Reduction	Nitrogen Reduction	Sulfate Reduction	Iron Reduction	Methanogenic
ΔG_d (kJ/e-eq)	28.34	28.34	28.34	28.34	27.40
ΔGa (kJ/e-eq)	-78.72	-72.20	20.85	-74.27	-24.13
ε	0.57	0.38	0.60	0.12	0.22
A	0.67	1.50	7.92	14.48	8.96
f_s^0	0.60	0.40	0.11	0.07	0.10
Y (gr/gr)	**1.30**	**0.62**	**0.24**	**0.14**	**0.08**
k (gr/gr)	**2.49**	**1.67**	**1.13**	**1.07**	**1.11**
E_{o2} (mol/mol)	**3.02**	**4.03**	**3.00**	**28.05**	-
μ_{max} (1/d)	**3.24**	**1.03**	**0.27**	**0.15**	**0.09**
TEX					
	Oxygen Reduction	Nitrogen Reduction	Sulfate Reduction	Iron Reduction	Methanogenic
ΔG_d (kJ/e-eq)	27.85	27.85	27.85	27.85	27.40
ΔGa (kJ/e-eq)	-78.72	-72.20	20.85	-74.27	-24.13
ε	0.60	0.38	0.26	0.11	0.22
A	0.60	1.50	4.02	17.28	8.99
f_s^0	0.62	0.40	0.20	0.06	0.10
Y (gr/gr)	**1.25**	**0.57**	**0.40**	**0.11**	**0.08**
k (gr/gr)	**2.66**	**1.67**	**1.25**	**1.06**	**1.11**
E_{o2} (mol/mol)	**3.39**	**4.83**	**3.60**	**34.02**	-
μ_{max} (1/d)	**3.32**	**0.96**	**0.50**	**0.12**	**0.09**
Ethanol					
	Oxygen Reduction	Nitrogen Reduction	Sulfate Reduction	Iron Reduction	Methanogenic
ΔG_d (kJ/e-eq)	31.18	31.18	31.18	31.18	27.40
ΔGa (kJ/e-eq)	-78.72	-72.20	20.85	-74.27	-24.13
ε	0.63	0.24	0.18	0.16	0.21
A	0.43	3.00	7.00	7.01	9.91
f_s^0	0.70	0.25	0.13	0.13	0.09
Y (gr/gr)	**1.03**	**0.26**	**0.18**	**0.18**	**0.07**
k (gr/gr)	**3.33**	**1.33**	**1.14**	**1.14**	**1.10**
E_{o2} (mol/mol)	**0.90**	**1.91**	**1.31**	**10.50**	-
μ_{max} (1/d)	**3.44**	**0.35**	**0.21**	**0.21**	**0.08**

Table 6 – Thermodynamic Properties for McCarty's Model (Continued)

Methanol					
	Oxygen Reduction	Nitrogen Reduction	Sulfate Reduction	Iron Reduction	Methanogenic
ΔG_d (kJ/e-eq)	36.84	36.84	36.84	36.84	27.40
ΔGa (kJ/e-eq)	-78.72	-72.20	20.85	-74.27	-24.13
ε	0.51	0.28	0.19	0.17	0.22
A	0.43	1.50	4.00	4.00	8.99
f_s^0	0.70	0.40	0.20	0.20	0.10
Y (gr/gr)	**0.52**	**0.21**	**0.15**	**0.15**	**0.08**
k (gr/gr)	**3.34**	**1.67**	**1.25**	**1.25**	**1.11**
E_{o2} (mol/mol)	**0.45**	**0.81**	**0.60**	**4.80**	-
μ_{max} (1/d)	**1.72**	**0.35**	**0.18**	**0.18**	**0.09**
Propanol					
	Oxygen Reduction	Nitrogen Reduction	Sulfate Reduction	Iron Reduction	Methanogenic
ΔG_d (kJ/e-eq)	29.94	29.94	29.94	29.94	27.40
ΔGa (kJ/e-eq)	-78.72	-72.20	20.85	-74.27	-24.13
ε	0.51	0.28	0.19	0.17	0.22
A	0.70	2.45	6.67	6.56	8.99
f_s^0	0.59	0.29	0.13	0.13	0.10
Y (gr/gr)	**1.00**	**0.35**	**0.22**	**0.22**	**0.08**
k (gr/gr)	**2.43**	**1.41**	**1.15**	**1.15**	**1.11**
E_{o2} (mol/mol)	**1.74**	**2.59**	**1.85**	**14.79**	-
μ_{max} (1/d)	**2.42**	**0.49**	**0.25**	**0.26**	**0.09**
Butanol					
	Oxygen Reduction	Nitrogen Reduction	Sulfate Reduction	Iron Reduction	Methanogenic
ΔG_d (kJ/e-eq)	29.26	29.26	29.26	29.26	27.40
ΔGa (kJ/e-eq)	-78.72	-72.20	20.85	-74.27	-24.13
ε	0.51	0.28	0.19	0.17	0.22
A	0.73	2.56	6.96	6.83	8.99
f_s^0	0.58	0.28	0.13	0.13	0.10
Y (gr/gr)	**1.06**	**0.37**	**0.23**	**0.23**	**0.08**
k (gr/gr)	**2.37**	**1.39**	**1.14**	**1.15**	**1.11**
E_{o2} (mol/mol)	**2.52**	**3.70**	**2.61**	**20.88**	-
μ_{max} (1/d)	**2.51**	**0.51**	**0.26**	**0.27**	**0.09**

4. Model Development

4.1. General Substrate Interaction module

The model used on this work is based on a MODFLOW/RT3D coupled system, working under the GMS (Groundwater Modeling System, Version 6.5.4, 2007, Aquaveo, LLC, Provo, UT) user interface. RT3D uses the solvers for advection and dispersion from the DOD_1.5 [1997] version of MT3D, and requires the groundwater flow code MODFLOW [*Harbaugh et al.*, 2000] to compute variations in groundwater head distribution (groundwater flow). RT3D has been previously validated in the literature by comparing the code results against various numerical and analytical solutions [*Clement et al.,* 1998; *Sun and Clement*, 1998; *Sun et al.* 1998]. One of the main advantages of RT3D is that it has a user-defined reaction option that can be used to simulate any type of user-specified reactive transport systems. [*Clement*, 1997]. This allows the development of custom biodegradation reactions without changes to flow and transport processes. GSIM is one such user-defined reactive module.

GSIM incorporates the biodegradation equations already presented, which comprise a system of ordinary differential equations (ODEs) that must be solved at each grid block and each time step after the advection and dispersion terms are calculated by RT3D. The ODEs are solved in RT3D using reaction solvers contained in MT3D [*Zheng*, 1990] or using a custom module, in this case, GSIM. Equations 6 to 10 are implemented and solved by GSIM to calculate microbial growth, substrate degradation and electron donor

consumption. The solution to these degradation kinetics result in rates of change (r) in equation 2. GSIM then passes the value of these rates to RT3D to be solved along with the transport equation. GSIM was coded using FORTRAN programming language (Digital Visual FORTRAN Professional Edition 5.0.A, Digital Equipment Corporation, Maynard, MA). **Appendix 2** contains the final FORTRAN source code for the GSIM module and **appendix 3** a directory of electronic resources included with this dissertation. The final concentration results of the system can be seen in graphical format in GMS.

Figure 3 shows a flow diagram of the model and the interactions between MODFLOW, RT3D, GSIM and GMS.

Although the GSIM module is very versatile allowing for multiple substrates, biological species and electron acceptors, the module itself has several limitations and incorporates assumptions that are important to highlight:

a) Biodegradation reactions occur independently without mutual effects unless explicitly linked through competition or inhibition terms.

b) All microbial growth is assumed to occur attached to the aquifer matrix with no consideration of detachment kinetics.

c) We assume all substrate biodegradation to take place in the liquid phase (groundwater) and potential decay of sorbed contaminants is conservatively ignored.

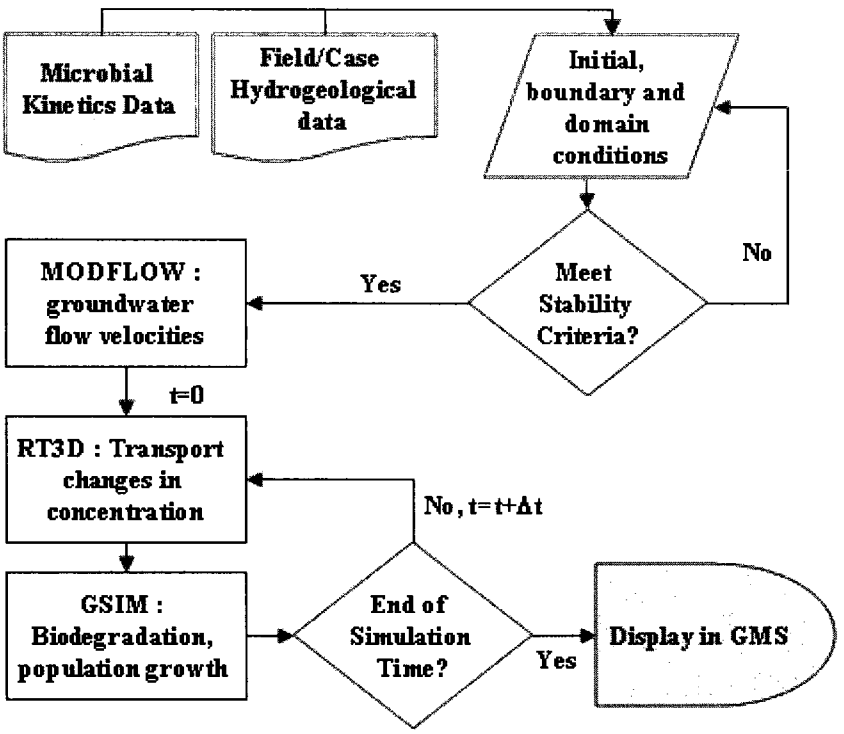

Figure 3 – Flowchart of MODFLOW/RT3D/GSIM model.

d) Bacteria are assumed to have complete access to dissolved total organic carbon.

e) High alcohol content blends could present deviations in transport behavior not considered by the model, such as alcohol buoyancy and phase partitioning, which could result in complex capillary-zone transport [*Sutton et al.*, 2009].

f) Our model also assumes all microbial activity to occur in the form of fully penetrated biofilms (i.e., immobile micro-colonies) [*Chen et al.*, 1992] attached to the aquifer solid matrix, based on the fact that about 99% of subsurface microorganisms are attached [*Harvey et al.*, 1984; *Lehman et al.*, 2001].

4.2. Model Stability & Code Optimization

Numerical stability of the combined flow and biodegradation system simulation was ensured by applying the Peclet and Courant convergence and stability criteria to the model. These criteria affect the time step Δx and the space discretization of the grid in RT3D respectively, and minimize the numerical errors due to round-off and truncation of derivatives that occur when derivatives are replaced by finite differences [*Holzbecher and Sorek*, 2005]. The criteria are:

Peclet number criterion: $$P_e = \frac{v * \Delta x}{D} \leq 2 \qquad (41)$$

Courant number criterion: $$C_r = \frac{v * \Delta t}{\Delta x} \leq 1 \quad (42)$$

Where, v = average linear flow velocity, Δt = time step, Δx = grid spacing and D = coefficient of hydrodynamic dispersion. Using these two constraints when designing the grid size and time step for the simulation ensures a minimal numerical error for the RT3D simulation.

RT3D (Reactive Transport in 3-Dimensions; *Clement et al.,* 1998) and GSIM (General Substrate Interaction Module; *Gomez et al.*, 2008) needs to solve a set of multiplicative Monod equations representing the rate of change of the different species present in the system, at the same time as the fate and transport equation is solved. RT3D offers several solvers for this, but they all approach the problem in the same manner, solving the 3D reactive advection dispersion equation (equation 2) using operator splitting methods. The GSIM model uses multiplicative Monod degradation kinetics, as described by *Gomez et al.,* [2008] to calculate the rates (r) in equation 2. Briefly, GSIM consists of a system of equations representing the rate of change of substrates, electron acceptors and microbial populations, as defined by equations 6 to 10.

Solving equation 2 imposes a heavy computational load (slow simulation times) and requires small time steps to ensure convergence and minimize errors. All these processes are handled directly by RT3D using one of several solvers available [*Clement et al.,* 1998]: Third order TVM Scheme, Standard finite differences, Method of characteristic,

Modified method of characteristics and Hybrid MOC/MMOC. When running RT3D, the model imposes a time step smaller than 0.01 days to avoid numerical errors. However this resulted in prohibitively long simulation times of 2 days in some cases.

We propose a method to speed the solution of this system of equations, by decoupling equation 2 and solving the reaction term (r) separately. To this end, when GSIM is called during a RT3D simulation, it returns a value of $r = 0$ to RT3D. RT3D then solves a simple tracer transport problem without a reaction term, significantly simplifying the problem and simulation times. A similar method, with variable time step, was implemented by Bordent and Bedient [*1986*] to improve simulation times. Limitations with the modular connectivity between GSIM and RT3D, hinder the ability to implement such variable time step techniques in our case. The reaction terms are solved explicitly by GSIM by transforming the differential terms (r) into time differences. For example, for alcohol degradation:

Differential term:
$$r_{A,Aer} = \left[\frac{dA}{dt}\right]_{Aer} = -\frac{f_{SA}^{\ 2}}{R_A}\left[\frac{\mu_{mA,Aer} X_{A,Aer}}{Y_{A,Aer}}\left(\frac{A}{K_{A,Aer}+A}\right)\left(\frac{O}{K_O+O}\right)\right] \qquad (43)$$

Time Difference:
$$\Delta A = -\frac{f_{SA}^{\ 2}}{R_A}\left[\frac{\mu_{mA,Aer} X_{A,Aer}}{Y_{A,Aer}}\left(\frac{A}{K_{A,Aer}+A}\right)\left(\frac{O}{K_O+O}\right)\right]\Delta t \qquad (44)$$

With equation 44, the change for alcohol concentration for a known Δt, can be calculated explicitly and then directly applied to the alcohol concentration. However, this approximation inserts a significant error into our solution, due to mass balance

considerations. This can be addressed by calculating the total change on a given chemical species by all the metabolic combinations present in the system. If the problem has n different degradation pathways (aerobic degradation, anaerobic degradation, etc), then we can express the total time difference of a species S as:

$$\frac{\Delta S_T}{\Delta t} = \frac{\Delta S_1}{\Delta t} + \frac{\Delta S_2}{\Delta t} + \ldots + \frac{\Delta S_n}{\Delta t} \tag{45}$$

However, mass available for all these metabolic processes is limited, and given a sufficiently large Δt, equation 44 will result in an erroneous value that surpasses this limit. A mass limitation factor is implemented to each of the terms in equation 45, so that mass balance is maintained:

$$\frac{\Delta S_T^*}{\Delta t} = \phi_{S,1}\frac{\Delta S_1}{\Delta t} + \phi_{S,2}\frac{\Delta S_2}{\Delta t} + \ldots + \phi_{S,n}\frac{\Delta S_n}{\Delta t} \tag{46}$$

$$\Delta S_T^* \leq S \tag{47}$$

$$S_{t+\Delta t} = S_t + \Delta S_T^* \tag{48}$$

Where S is the available concentration of species S in the system. If the species consumption (equation 45) exceeds the available concentration in the system (S), GSIM calculates the mass limiting factors required in equation 46 to maintain the condition

imposed by equation 47. Final concentration of S is defined by equation 48. Otherwise, all ϕ factors are assumed to be 1. ϕ is estimated by GSIM as:

$$\phi_{S,i} = \frac{S}{\Delta S_T} \tag{49}$$

In the metabolic processes involved in the GSIM module, consumption of available substrates and electron acceptors are related by stoichiometry and by microbial growth. This means that mass limitations affecting one species might also have an impact on the other species associated with that specific mechanism. In our case, equation 46 can be extended to the system:

Substrate Utilization

$$\frac{\Delta S_T^*}{\Delta t} = \phi_{S,1}\frac{\Delta S_1}{\Delta t} + \phi_{S,2}\frac{\Delta S_2}{\Delta t} + \ldots + \phi_{S,n}\frac{\Delta S_n}{\Delta t} \tag{50}$$

Electron Acceptor Consumption

$$\frac{\Delta EA_T^*}{\Delta t} = \phi_{EA,1}\frac{\Delta EA_1}{\Delta t} + \phi_{EA,2}\frac{\Delta EA_2}{\Delta t} + \ldots + \phi_{EA,n}\frac{\Delta EA_n}{\Delta t} \tag{51}$$

Microbial degrader generation

$$\frac{\Delta X_T^*}{\Delta t} = \phi_{X,1}\frac{\Delta X_1}{\Delta t} + \phi_{X,2}\frac{\Delta X_2}{\Delta t} + \ldots + \phi_{X,n}\frac{\Delta X_n}{\Delta t} \tag{52}$$

Where S is the substrate, EA is the electron acceptor and X is the microbial population, associated with a specific degradation mechanism (1 to n). We then define the mass balance for a given metabolic combination, as the smallest factor from all those involved in the different species. We can do this, because multiplicative Monod kinetics use the same form for all species, with only multiplicative differences between expressions based on microbial yield or electron acceptor utilization stoichiometry (except for the additive term b for microbial decay, for which the GSIM code accounts for). Hence,

$$\phi_n = \min(\phi_{S,n}, \phi_{EA,n}, \phi_{X,n}) \tag{53}$$

Finally, the change on the chemical species involved in the system, considering equation 53 can be written as:

$$\frac{\Delta S_T^*}{\Delta t} = \phi_1 \frac{\Delta S_1}{\Delta t} + \phi_2 \frac{\Delta S_2}{\Delta t} + \ldots + \phi_n \frac{\Delta S_n}{\Delta t} \tag{54}$$

$$\frac{\Delta EA_T^*}{\Delta t} = \phi_1 \frac{\Delta EA_1}{\Delta t} + \phi_2 \frac{\Delta EA_2}{\Delta t} + \ldots + \phi_n \frac{\Delta EA_n}{\Delta t} \tag{55}$$

$$\frac{\Delta X_T^*}{\Delta t} = \phi_1 \frac{\Delta X_1}{\Delta t} + \phi_2 \frac{\Delta X_2}{\Delta t} + \ldots + \phi_n \frac{\Delta X_n}{\Delta t} \tag{56}$$

Equations 54 to 56 are solved by GSIM with a different time step than the transport time step used by RT3D for the advection dispersion equation. This allows the system to

solve the reaction part of the problem with a small time step that maintains accuracy without having to solve the transport processes with each time step. This solution scheme has a significant impact on simulation speed times with a minimum impact on accuracy. 10 year simulations considering a constant (fixed concentration) source LNAPL regular gasoline and 10% ethanol blended gasoline, were performed with the original GSIM solver (Slow) and compared to the improved GSIM solver (fast). No dissolution or volatilization dynamics were considered to avoid input artifacts. Improved transport time steps of 0.2 days (0.07 for degradation solution), with faster execution times were achieved with the improved solver, resulting in a ~600% increase in model speed (a decrease from 22 hours to 3 hours total simulation time). A statistical t-test was used on the available data to assess whether the means of the two solver's output were statistically similar.

Table 7 shows the values, mean, standard deviation, T-test results and errors (difference) for both sets of data, for benzene plume length, ethanol plume length and microbial population degraders. Mean differences (error) for all data sets were consistently between 0.1% and 1%, with the exception of benzene aerobic degraders in the presence of ethanol, which were underestimated (-18.3%). However, values were in the same order of magnitude and did not have a significant impact on benzene plume length. T-test results indicate that data sets are statistically similar ($p < 0.005$) for benzene plume lengths, the relevant parameter of this study, for both regular gasoline and E10. Microbial populations exhibited a more pronounced difference but stayed in the same order of magnitude for both solvers.

Table 7– Statistical Analysis of GSIM (Fast Version)

Time (days)	Benzene Plume length (m)				Ethanol Plume length (m)	
	Regular Gasoline (Slow)	Regular Gasoline (Fast)	E10 (Slow)	E10 (Fast)	E10 (Slow)	E10 (Fast)
30	48.1	48.1	48.1	48.1	2.1×10^{8}	2.1×10^{8}
330	56.0	56.0	59.3	59.1	2.5×10^{8}	2.5×10^{8}
630	59.8	59.8	63.7	63.5	2.8×10^{8}	2.7×10^{8}
930	63.7	63.7	67.9	67.7	3.0×10^{8}	2.9×10^{8}
1230	67.5	67.5	71.9	71.7	3.1×10^{8}	3.1×10^{8}
1530	70.3	70.3	75.9	75.6	3.3×10^{8}	3.3×10^{8}
1830	71.9	71.9	79.6	79.1	3.4×10^{8}	3.4×10^{8}
2130	74.2	74.0	82.9	82.2	3.5×10^{8}	3.5×10^{8}
2430	75.2	75.0	84.8	84.1	3.6×10^{8}	3.6×10^{8}
2730	75.2	75.0	87.1	86.4	3.7×10^{8}	3.7×10^{8}
3030	75.2	75.0	88.0	87.8	3.8×10^{8}	3.7×10^{8}
Mean	**67.0**	**66.9**	**73.6**	**73.2**	$\mathbf{3.2\times10^{8}}$	$\mathbf{3.1\times10^{8}}$
Standard Deviation	**9.1**	**9.0**	**12.8**	**12.6**	$\mathbf{5.4\times10^{7}}$	$\mathbf{5.3\times10^{7}}$
T-Test	**99%**		**95%**		**86%**	
error	**-0.1%**		**-0.5%**		**-1.3%**	
Time (days)	**Benzene Anaerobes (mg/L)**				**Ethanol Anaerobes (mg/L)**	
	Regular Gasoline (Slow)	Regular Gasoline (Fast)	E10 (Slow)	E10 (Fast)	E10 (Slow)	E10 (Fast)
30	2.0×10^{3}	2.0×10^{3}	4.0×10^{3}	2.5×10^{3}	1.4×10^{8}	1.4×10^{8}
330	1.8×10^{6}	1.8×10^{6}	5.0×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
630	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
930	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
1230	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
1530	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
1830	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
2130	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
2430	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
2730	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
3030	1.8×10^{6}	1.8×10^{6}	4.9×10^{6}	4.0×10^{6}	2.4×10^{8}	2.4×10^{8}
Mean	$\mathbf{1.6\times10^{6}}$	$\mathbf{1.6\times10^{6}}$	$\mathbf{4.4\times10^{6}}$	$\mathbf{3.6\times10^{6}}$	$\mathbf{2.3\times10^{8}}$	$\mathbf{2.3\times10^{8}}$
Standard Deviation	$\mathbf{5.4\times10^{5}}$	$\mathbf{5.5\times10^{5}}$	$\mathbf{1.5\times10^{6}}$	$\mathbf{1.2\times10^{6}}$	$\mathbf{2.8\times10^{7}}$	$\mathbf{2.8\times10^{7}}$
T-Test	**100%**		**17%**		**94%**	
error	**0.1%**		**-18.3%**		**0.4%**	

No significant differences between plume shape and concentration distribution were appreciated. However, the error associated with this improved method is expected to increase in stiffer systems. Higher alcohol/substrate concentrations at the source zone, larger hydraulic gradients, faster microbial degradation kinetics, etc., will increase the impact that the chosen Δt has on the system solution. As such, it is important to calibrate the correct time steps used in the model, for the hydrogeological characteristics of each scenario.

4.3. Model Calibration

GSIM was tested to ensure that correct solutions to the biodegradation equations are produced. GSIM solutions of biodegradation problems were compared to analytical solutions calculated in spreadsheets (**Appendix 3**) for aerobic and anaerobic populations degrading benzene and benzene with ethanol (**Figure 4**), to validate the correct implementation of such equations in GSIM. RT3D was run without transport modules to assess only microbial activity and oxygen consumption. In both cases tested, the GSIM solution and spreadsheet solution matched nearly exactly, indicating that the GSIM biodegradation model correctly solves the biodegradation equations.

GISM was further tested by comparing the output of MODFLOW/RT3D/GSIM with BIOSCREEN [*Newell et al.*, 1996] and field data. GSIM simulations considered flow, transport and biodegradation of BTEX, under the same set of parameters as BIOSCREEN. The field case used for comparison was the Keesler Air Force Base (SWMU 66), where groundwater contamination by BTEX has been extensively

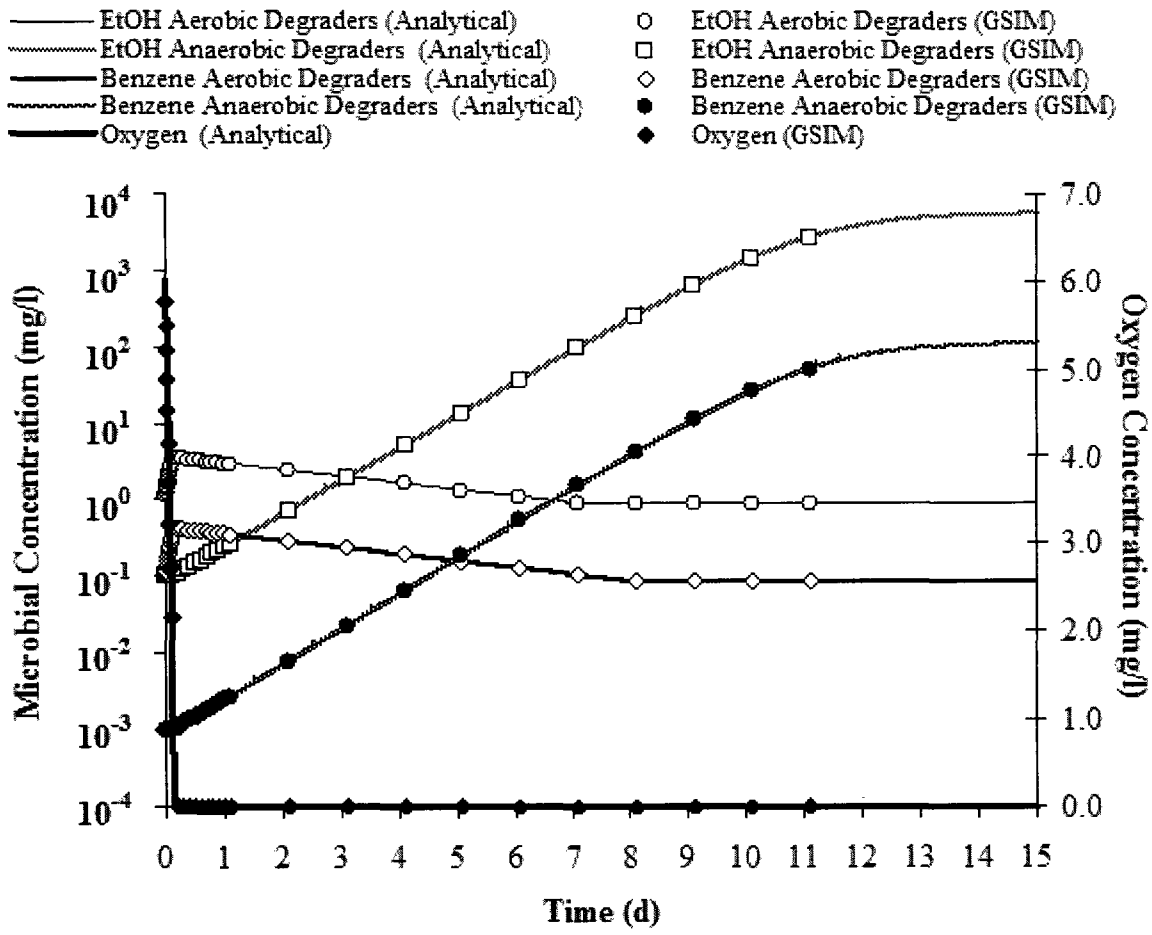

Figure 4– Analytical solution and GSIM/RT3D solution comparison for microbial population growth (aerobic and anaerobic) on ethanol and benzene as substrates.

characterized. Hydrogeological data and biokinetic parameters used to model this site are readily available from the BIOSCREEN User's manual [*Newell et al.,* 1996]. Since this case presents total BTEX concentrations and the GSIM module tracks aromatic components separately, other literature sources were used to obtain biodegradation parameters. This simulation was also used to calibrate the domain simulation parameters for stability (cell size and time step), and to validate the first order degradation values used for benzene (as shown in **Table 4**).

BIOSCREEN reports total BTEX concentrations, so the sum of the concentrations of all BTEX species obtained from GSIM were used. Simulation time considered was 6 years. The BTEX source concentration was simulated to remain constant at 13.68 mg/L [*Newell et al.,* 1996]. Since BIOSCREEN is a first order model, to accurately compare both, the values of the biokinetic parameters were changed. $\mu_{mB,Aer}$ and $\mu_{mB,An}$ were increased 10,000-fold, as well as $K_{B,Aer}$ and $K_{B,An}$, and microbial concentrations kept constant at 1 mg/l. This effectively transforms equations 1 and 2, into first-order kinetic expressions, of the form:

$$r_{S,An} = -\frac{f^2}{R_S}\left[\frac{\mu_{S,An}S}{Y_{S,An}K_{S,An}}\right] = -\frac{f^2}{R_S}\lambda * S \qquad (57)$$

Where λ is the first-order rate coefficient for substrate degradation. Values of λ for BTEX degradation used were obtained from the literature (**Table 4**).

Figure 5 compares the output of the GSIM module (numerical model), with BIOSCREEN (analytical model) output and field data from sampling wells. As can be seen from the figure, the time versus concentration profile is close, with an R^2 of 0.976. As compared to BIOSCREEN (R^2 of 0.963), GSIM module gives a better goodness of fit. This comparison indicates that the combined flow and biodegradation system is accurately simulated by MODFLOW/RT3D with the GSIM module.

Further validation of the microbial kinetics module was done by comparing simulated benzene and ethanol concentrations with results from laboratory microcosm studies by *Hunt et al.* [1997] (**Figure 6**). The simulations matched ethanol data with an R^2 of 0.96, and benzene data with a R^2 of 0.94. Thus, model outputs for benzene degradation in the presence of ethanol closely matched laboratory data.

4.4. Sensitivity Analysis

Sensitivity analysis of the model was done in two stages. First, a elasticity analysis was used to analyze the effect of the most relevant model variables in the model output. This analysis includes hydrogeological parameters like hydraulic conductivity and porosity. A elasticity analysis was used because it is a fast method and doesn't consider variable interdependency. Since there is no certainty of such dependence between hydrological parameters and biodegradation parameters, this approach was better suited for a global analysis. The second analysis, based on a multiple regression method, focused only on the biodegradation parameters of the model. Since growth rate, biomass

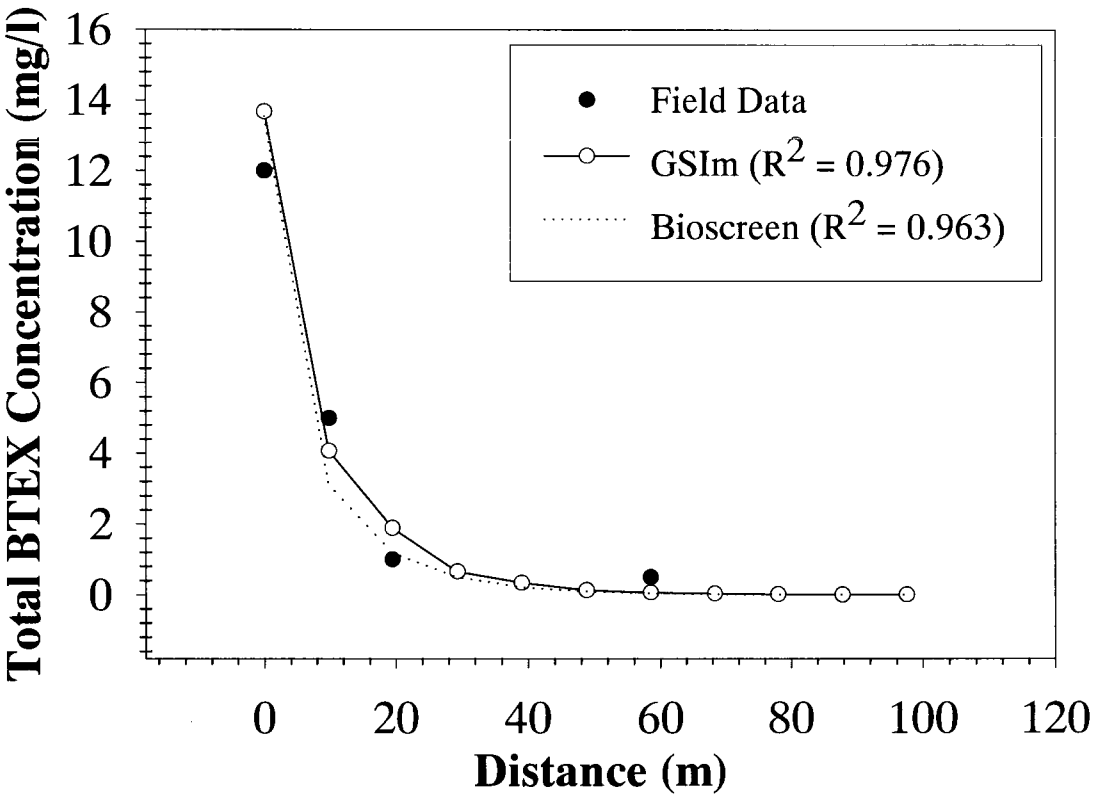

Figure 5– Centerline BTEX concentration as a function of distance for a constant source. (Keesler AFB data)

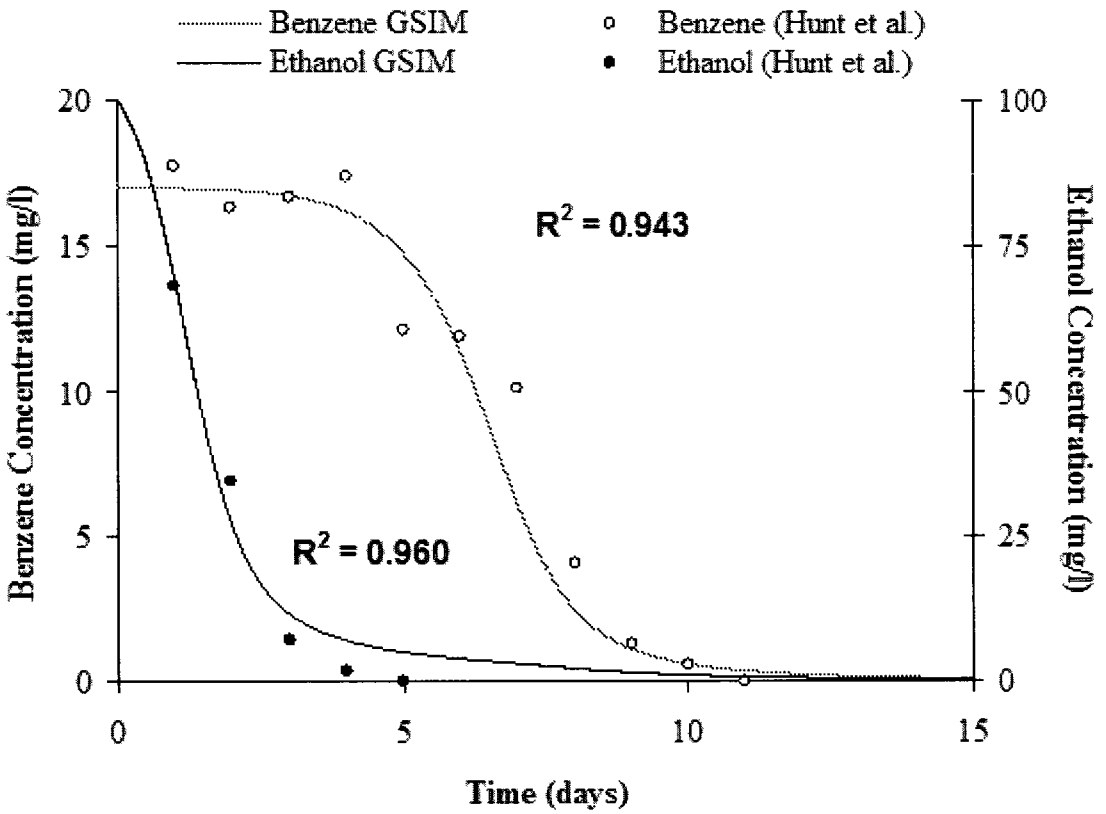

Figure 6– Comparison of GSIM and microcosm benzene and ethanol concentrations during aerobic degradation.

yield and half-saturation coefficients are related by the stoichiometry and thermodynamics of the reactions involved, we can be certain that these variables are interdependent and a more advanced methodology is appropriate.

4.4.1. Independent Variable Elasticity Analysis

A sensitivity analysis of the system (**Appendix 4**) was performed to assess the most influential variables involved in the system. Benzene plume length simulations without ethanol were used, and a baseline was set using parameters from **Table 4**. Plumes were simulated in a 300 by 80 meter domain for a simulation time of 20 years. The analysis consisted of several different simulations of the ethanol/benzene E10 system, changing one variable at a time by -50% and +50% (or 2 and 4 orders of magnitude in the case of hydraulic conductivity), and then comparing the point elasticity of the benzene centerline plume length after 10 years under each variable. Point elasticity is defined as the percent change of a function (plume length in this case) under a percent change of a variable, $E(f(x)) = (dx/dy)(y/x)$ [*Case,* 1999].

Results (**Figure 7**) indicate that soil hydraulic parameters: porosity (n), hydraulic conductivity (k) and hydraulic gradient (i), are the most relevant (0.76, 0.86 and 0.55 point elasticity, respectively), consistent with water flow being the primary process involved in the fate and transport of contaminants in groundwater. Source-zone benzene concentration and biofilm density are also important with point elasticities of 0.33 and 0.26 respectively. At benzene concentrations below 1 mg/l, electron acceptor depletion

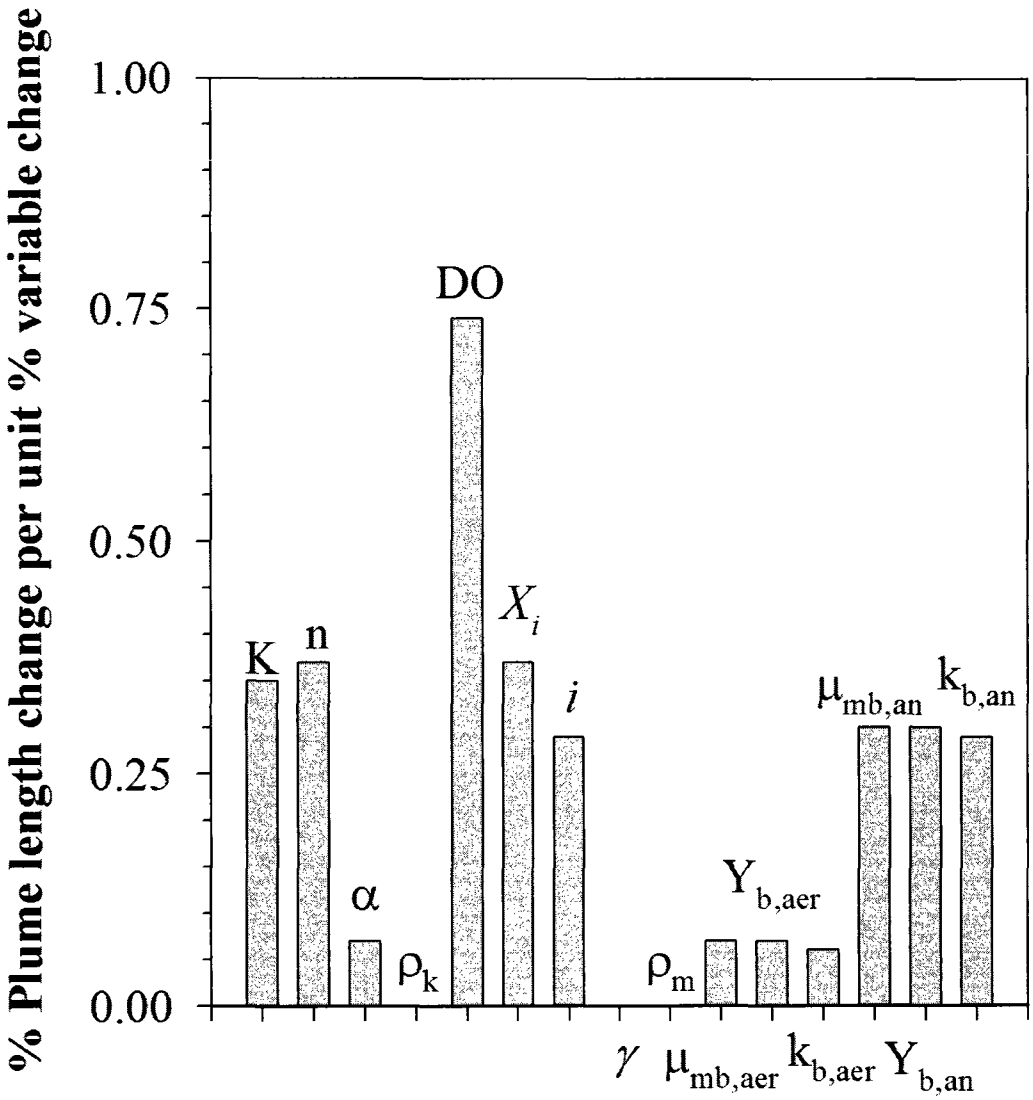

Figure 7– Sensitivity analysis of maximum benzene plume length (steady state) simulations. Figure shows the plume length increase (mean, in percentage) per unit percentage change of variable value.

by ethanol increases point elasticity of benzene concentration up to ~0.55. Benzene and ethanol aerobic microbial growth kinetics follow in importance (0.13 to 0.54 elasticity), as they define the rate at which the plume fringe aerobic degradation occurs. Benzene and ethanol anaerobic kinetics are third in importance with elasticity up to 0.20; significantly lower due to the low degradation rates of anaerobic processes relative to aerobic degradation. It is interesting to note that none of the values obtained in the analysis is larger than one, which indicates that the system is largely inelastic to changes in a single variable.

4.4.2. Latin Hypercube Sampled Stepwise Multiple Regression Analysis

The variability of reported biokinetic coefficients (**Table 4**) are a source of uncertainty for the model output. Reported groundwater half-lives of n-butanol, for example, range from 96 to 1296 h [*Howard et al.*, 1991]. Thus, a sensitivity analysis of biokinetic coefficients was conducted to address this uncertainty and identify the most influential parameters requiring the most effort to properly characterize. A combined probabilistic method was used for this purpose. A formal procedure based on Latin Hypercube sampling and stepwise multiple regression analysis was chosen. This approach is suited to complex geophysical models [*McWilliams*, 1987], such as reactive contaminant flow through porous media. The advantage of this method is that it allows to simultaneously vary all variables considered in the analysis, minimizing the number of simulations required on complex (long simulation time) models [*McWilliams*, 1987], and consider possible interdependency between variables. Numerical details of the

implementation of this method using MATLAB (Version R2008a, October 9 2008, The MathWorks, Inc., 3 Apple Hill Drive, Natick, MA) tools are presented in **Appendix 5**.

Specifically, a group of 17 parameters were chosen as inputs for GSIM model and grouped in vector form ($\bar{x}$). A Latin Hypercube Sampling method [*McKay et al.*, 1979; *Stein,* 1987], as presented by *McWilliams* [1987] was used to generate 100 different input vectors ($\bar{x}_i$) with the aid of MATLAB. Each input vector was used in a different 1 year simulation scenario for the GSIM model. Benzene plume length (i.e., the centerline distance from the source to the 5 ppb contour, which represents the drinking water MCL for benzene [*U.S. EPA*, 2003]) was used as the indicator output of the GSIM model (L).

Results of the Latin Hypercube sampling were analyzed using a Stepwise Multiple Linear Regression method [*Neter et al.*, 1983; *Draper and Smith*, 1981], as presented by *McWilliams* [1987]. This method identifies which input variables contribute the most to variability in the model indicator output. Multiple linear regression methods consider several possible scenarios to explain model variability based on a linear combination of the variables considered. The parameter from the input vector $\bar{x}$ that best explains model variability is the first to enter the linear regression. The next most relevant variable is chosen amongst the remaining, and so on, until all variables have entered the linear regression [*McWilliams*, 1987]. Results and analysis of this method are presented in chapter 7.

5. Evaluation of Benzene Plume Elongation Mechanisms Exerted by Ethanol

[Extract from Gomez et al., 2008 – Published in Water Resources Research]

Although past BTEX fate and transport models provided valuable insight into how ethanol influences hydrocarbon plume dynamics, including competitive inhibition processes [*Lu et al.*, 1999], most have not simulated potentially important substrate interactions that influence catabolic enzyme induction (i.e., the synthesis of an enzyme by the cell, when in the presence of a specific substrate) and the metabolic flux of the target pollutants (i.e., the rate at which a pollutant such as benzene is metabolized per unit of biomass, which is analogous to the specific utilization rate). These interactions can cause slower BTEX degradation rates at sites with high ethanol concentrations [*Lovanh and Alvarez*, 2004], although this negative effect can be offset by higher microbial concentrations resulting from the presence of ethanol as an additional substrate [*Lovanh et al.*, 2002].

This chapter evaluates the importance of substrate interactions (benzene/ethanol) and the resulting microbial metabolic and population shifts that influence the natural attenuation of E10 releases and the resulting benzene plume length. An advanced computer module - designated the "General Substrate Interaction Module" (GSIM) - was developed for this purpose for use with the RT3D (Reactive Transport in 3-Dimensions) model [*Clement et al.*, 1998]. Three mechanisms were considered separately and

simultaneously to evaluate their relative importance on benzene plume elongation, under both constant and decreasing source scenarios. These mechanisms are: (1) metabolic flux dilution (MFD), which is defined as a decrease in the specific benzene utilization rate due to non-competitive inhibition when ethanol is present [*Lovanh and Alvarez*, 2004]; (2) catabolite repression, which is defined as the repression of inducible enzymes that degrade the target pollutant (e.g., benzene) by the presence of a preferred carbon source (e.g., ethanol) [*Madigan et al.*, 2005]; and (3) proliferation of different microbial populations in response to changes in oxygen and substrate availability.

5.1. Initial, Boundary and Domain Conditions

The simulation domain for all model tests in this section consists of a single, 3-m thick layer that is 80 m wide by 300 m long. A constant seepage velocity of 0.9 cm/d was created using constant boundary conditions at the two ends of the model domain (H = 2 m measured from bottom on left boundary, H = 1.4 m measured from bottom on right boundary), and the top and bottom of the domain were specified as no-flow boundaries. Other properties of the model domain are shown in **Table 8**.

Table 8– Model Domain Properties

Parameter	Value	Reference
Hydrogeology		
Hydraulic Conductivity (K)	3.0 m/d	Fine-Medium Sand, LNAST Soils database*
Hydraulic Gradient (i)	0.003 m/m	*Newell et al.*, 1996
Darcy water velocity (v)	0.9 cm/d	Fine-Medium Sand, LNAST Soils database*
Total Porosity (n)	0.3	*Newell et al.*, 1996
Groundwater dissolved oxygen (O)	6 mg/l	*Newell et al.*, 1996
Pore space utilization factor (γ)	0.2	*Vandevivere et al.*, 1995; *Thullner et al.*, 2002
Dispersivity		
Longitudinal	7 m	*Newell et al.*, 1996**
Transverse	0.7 m	
Adsorption		
Soil Bulk Density (ρ_b)	1.7 kg/l	*Newell et al.*, 1996
Partitioning coefficient, K_{dE} (Ethanol)	0.001 l/kg	
Retardation factor, Ethanol, R_E	1.01	Calculated, $R_E = 1 + \rho_b K_{dE}/n$
Partitioning coefficient, K_{dB} (Benzene)	0.095 l/kg	
Retardation factor, Benzene, R_B	1.54	Calculated, $R_B = 1 + \rho_b K_{dB}/n$
General simulation		
Modeled Area length	300 m	
Modeled Area Width	80 m	
X space discretization	75 units	
Y space discretization	100 units	
Cell width	0.8 m	
Cell length	4 m	
Simulation Time	30 years	
Time step	0.02	

*[*Huntley and Beckett*, 2002]

** Modified to fit initial benzene plume lengths measurements of *Ruiz-Aguilar et al.* [2003)]

All simulations were based on the same set of microbial kinetic and hydrogeological parameters. The initial dissolved oxygen concentration was set at 6 mg/l, and groundwater entering the model domain contained this same dissolved oxygen concentration. For anaerobic processes, the system was assumed to become strongly anaerobic (methanogenic conditions), which commonly occurs as a result of the rapid depletion of thermodynamically-more-favorable electron acceptors [*Da Silva and Alvarez,* 2002]. Initial microbial concentrations for all ethanol aerobic populations and benzene aerobic populations on the domain were set to 1 mg/l (~10^6 cells/g-soil) and 0.1 mg/l (~10^5 cells/g-soil), respectively. Maximum pore space occupation by microbial species during growth was set at 20%, corresponding to a porosity reduction of 80% of the initial value [*Vandevivere et al.,* 1995; *Thullner et al.,* 2002]. Ethanol anaerobic population and benzene anaerobic population initial concentrations were set to 0.1 mg/l (~10^5 cells/g-soil) and 0.001 mg/l (~10^3 cells/g-soil), respectively.

Two types of source zones were simulated: a constant concentration source and a decreasing concentration source. For both release scenarios, benzene and ethanol in the groundwater were assumed to originate from a spill of non-aqueous phase liquid (NAPL). For the constant concentration scenario, an ethanol concentration of 1,000 mg/l [*Wilson and Adair.,* 2006] and a benzene concentration of 10 mg/l were assumed to exist at the source as a result of a relatively large NAPL release.

For the decreasing concentration source scenario, concentrations of benzene and ethanol in the groundwater directly in contact with the source NAPL were estimated

using the American Petroleum Institute's (API) LNAPL Dissolution and Transport Screening Tool (LNAST) model [*Huntley and Beckett,* 2002] (**Appendix 6**). A release of 2,000 kg of an ethanol/benzene mixture (E10) was considered. Spill volume was chosen to match model grid cell size and mass, resulting in a LNAPL spill on a volume 4 m wide by 4.8 m long by 0.79 m thick above groundwater level. Parameters used to estimate source concentrations were those shown in **Table 8**. The average depth to the top of the LNAPL was considered to be 1.2 meters. E10 composition used, in mole fraction, was 0.015 for benzene, 0.172 for ethanol, 0.158 for TEX and 0.824 for other compounds [*Poulsen et al.,* 1991]. LNAST predicted initial ethanol and benzene concentrations of 63,000 and 25 mg/l, respectively, decaying over time.

5.2. Results and Discussion

The model was used first to evaluate the dissolved benzene groundwater plume from a constant source. Plume length was defined as the distance from the source to the 5 μg/l contour, corresponding to the drinking water MCL (maximum concentration level) for benzene [*U.S. EPA,* 2003], along the flow direction. Simulated plumes were allowed to reach steady state, which generally occurred after approximately 10 years of simulation. Seven different scenarios (**Table 9**) were implemented. For both constant-concentration and decreasing concentration sources, the MODFLOW/RT3D/GSIM system produced plume lengths within the range reported by *Ruiz-Aguilar et al.* [2003] for plumes from gasoline stations (80 m median and 152 m maximum).

Table 9- Simulation Scenarios for Constant Concentration Source Simulations

Scenario	Conditions
Baseline (No Ethanol)	Only benzene present, considering O_2 consumption during benzene degradation
EAD	Benzene and ethanol, considering O_2 depletion during ethanol degradation
EAD + FG	Benzene and ethanol, with fortuitous growth of benzene degraders
EAD + CR	Considers both O_2 depletion and catabolite repression
EAD + MFD	Considers both O_2 depletion and metabolic flux dilution
EAD + FG + MFD + CR	Considers O_2 depletion, fortuitous growth, metabolic flux dilution and catabolite repression
EAD + MFD + CR + O_2	Considers metabolic flux dilution and catabolite repression, with unlimited O_2 supply.

The constant source simulations yielded steady state plumes after ~30 years (**Figures 8 and Figure 9**). In these simulations, the biochemical oxygen demand exerted by benzene alone near the source was higher than the available dissolved oxygen (as is often the case in contaminated sites), leading to anaerobic conditions in the center of the plume. Fortuitous growth of benzene degraders on ethanol contributed to higher anaerobic degradation rates and resulted in a decrease of 48% in benzene plume length (without MFD and catabolite repression). Benzene/ethanol simulations with no substrate interactions considered resulted in a 7% plume length increase due to ethanol-driven oxygen depletion. Catabolite repression increased benzene plume length by 49%, compared to a 123% increase for MFD. Metabolic Flux dilution was thus the most influential plume elongation mechanism for this constant E10 release scenario.

Simulations considering a decreasing source (**Figure 10**) show smaller increases in the maximum benzene plume length due to the presence of ethanol and a sharp decline in plume length once ethanol is completely depleted in the system. The baseline scenario with benzene alone reached a maximum length of 35.5 m. In the presence of ethanol, electron acceptor depletion increased plume length by 13%, catabolite repression by 23% and MFD by 46%. All substrate interactions resulted in a combined plume length increase of 22%. Metabolic flux dilution was thus the most influential factor in this E10 release scenario.

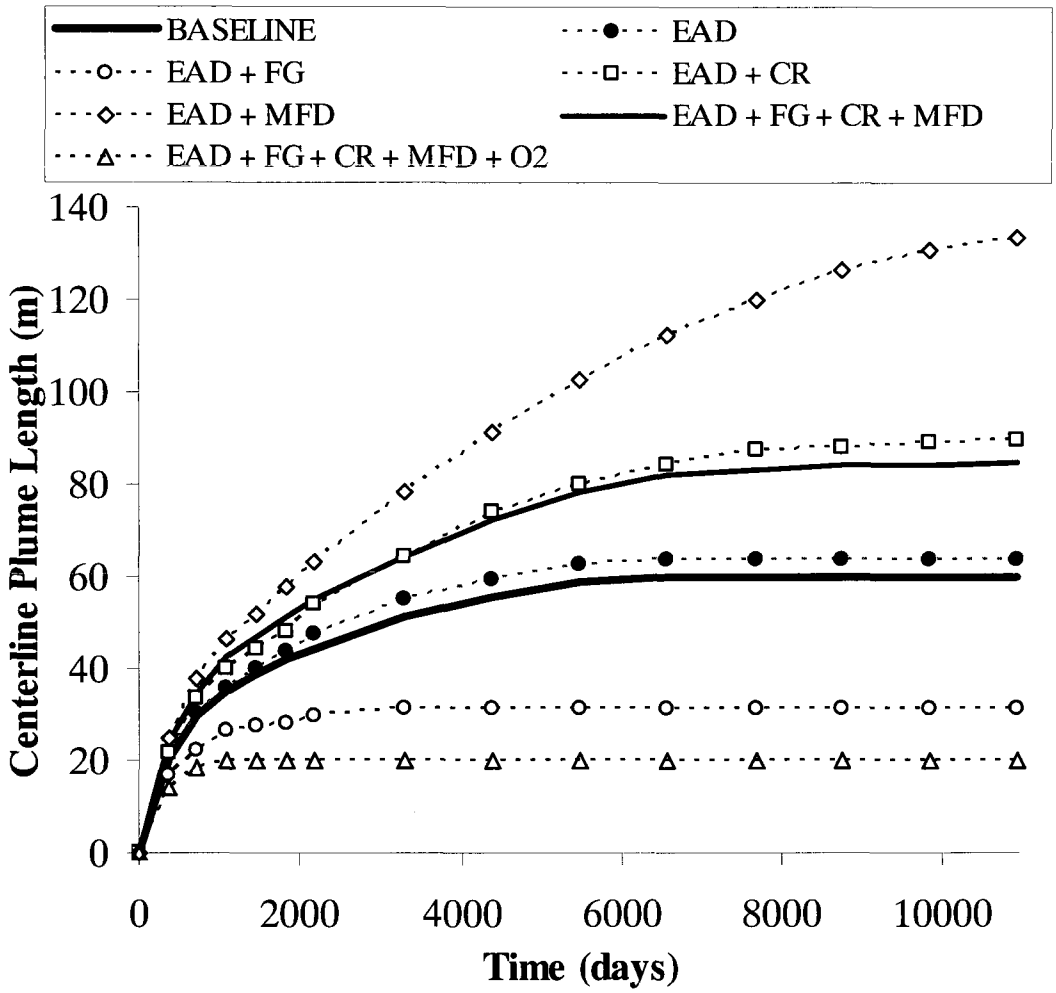

Figure 8– Influence of various inhibitory mechanisms (dissolved oxygen depletion, metabolic flux dilution [MFD] and catabolite repression [CR]) on the elongation of a simulated benzene plume emanating from a constant benzene/ethanol source (Model parameters are given in Tables 1 and 2).

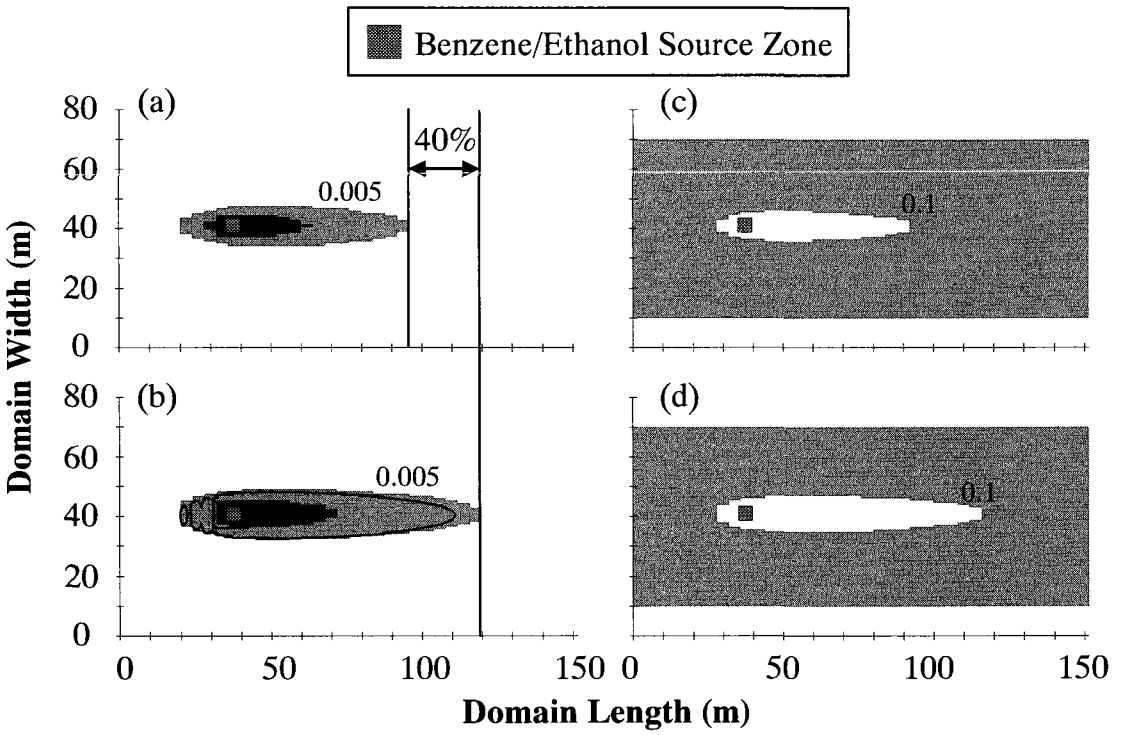

Figure 9 – 30-year, steady state benzene and ethanol plumes, showing the effects of: (a) baseline with source zone benzene concentration of 10 mg/l [1 and 0.005 mg/l contours]; (b) 40% benzene plume length increase with source zone ethanol concentration of 1,000 mg/l [1 and 0.005 mg/l benzene contours and 0.005 mg/l ethanol (Solid line)]; (c) anaerobic shadow caused by benzene degradation [0.1 mg/l dissolved oxygen contour]; and (d) anaerobic shadow caused by ethanol plus benzene degradation [0.1 mg/l dissolved oxygen contour].

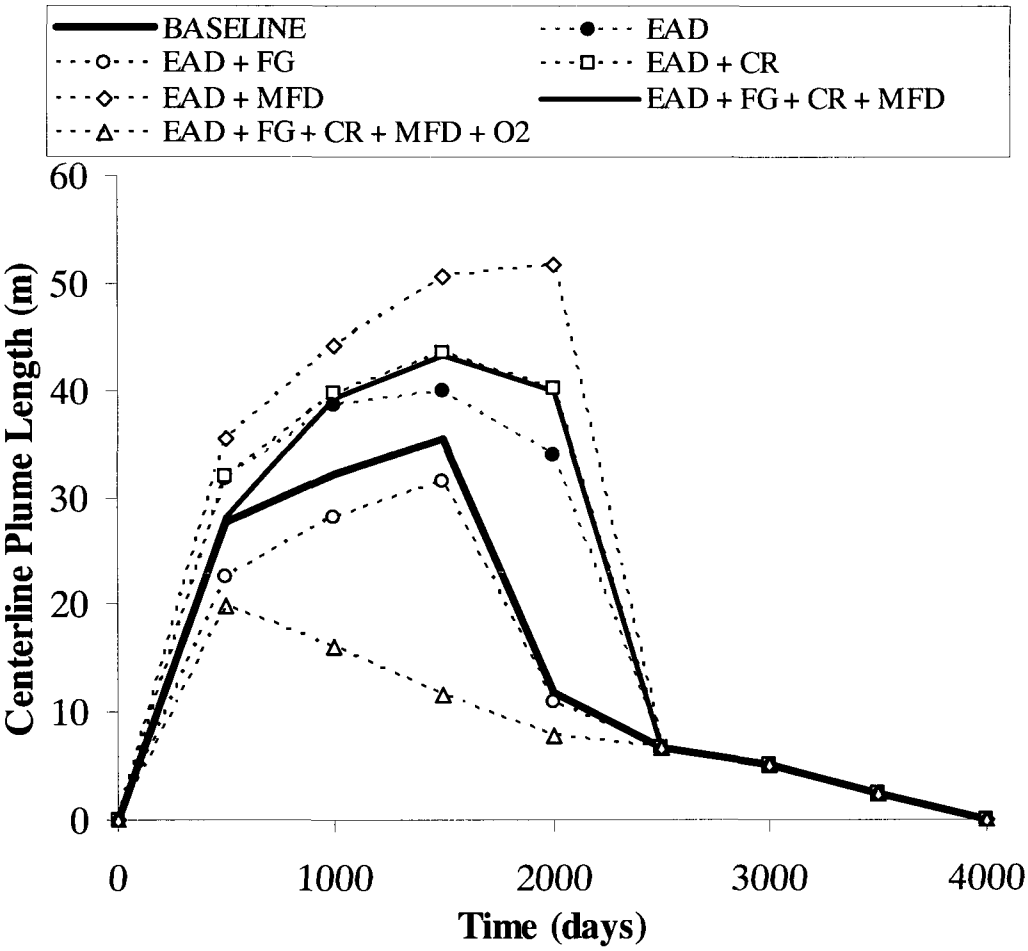

Figure 10– Influence of various inhibitory mechanisms (dissolved oxygen depletion, metabolic flux dilution [MFD] and catabolite repression [CR]) on the elongation of a simulated benzene plume emanating from a decreasing source (Model parameters are given in Tables 1 and 2).

Regarding microbial populations, when dissolved oxygen was allowed to deplete, as is the case under natural attenuation conditions, anaerobic microorganisms reached consistently higher concentrations than aerobic populations. **Figure 11** shows the spatial distribution of microbial populations after 30 years of simulation (steady state), with anaerobic population thriving in the anaerobic source zone, and some aerobic activity still taking place on the plume fringes.

Anaerobic degradation is the main substrate consumption mechanism at this point in the plume life cycle, while aerobic degradation dominated early in the simulations (<1 year). For constant source simulations, microbial growth associated with the consumption of ethanol increased total microbial populations near the source zone (0.5 meters downgradient) from 10^6 to 10^8 cells/g-soil (**Figure 7**), and up to 10^{10} cells/g-soil at the source zone, resulting in increased benzene degrader populations (+180%), while decreasing the ratio of benzene degraders to total degraders (25% to 2%). **Figure 12** shows that for a decreasing source scenario, total microbial populations decreased faster than benzene degrader populations, resulting in an increase in the ratio of benzene to total degraders during the first ~800 days of simulation, then decreasing until reaching equilibrium at about 1%. This ratio agrees in order of magnitude with previous studies [*Cápiro et al.,* 2007]. In both cases, benzene degrader populations were higher with ethanol, while their fractions relative to the total populations were smaller. This reflects that ethanol is a preferred substrate for most microbial communities and that genotypic dilution is taking place.

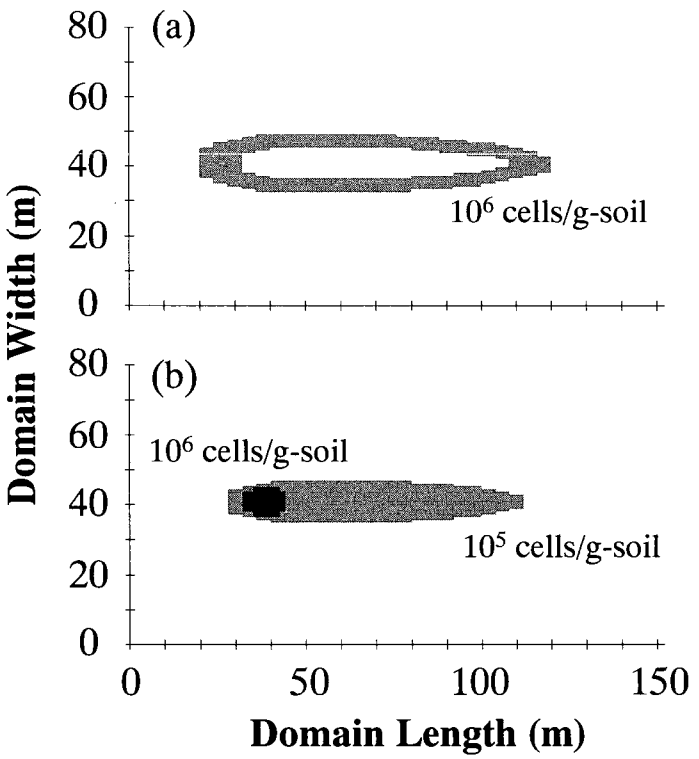

Figure 11 – 30-year, steady state microbial concentration contours (cells/g-soil): (a) Aerobic benzene degraders, (b) Anaerobic benzene degraders. Shaded cells indicate populations larger than background concentrations.

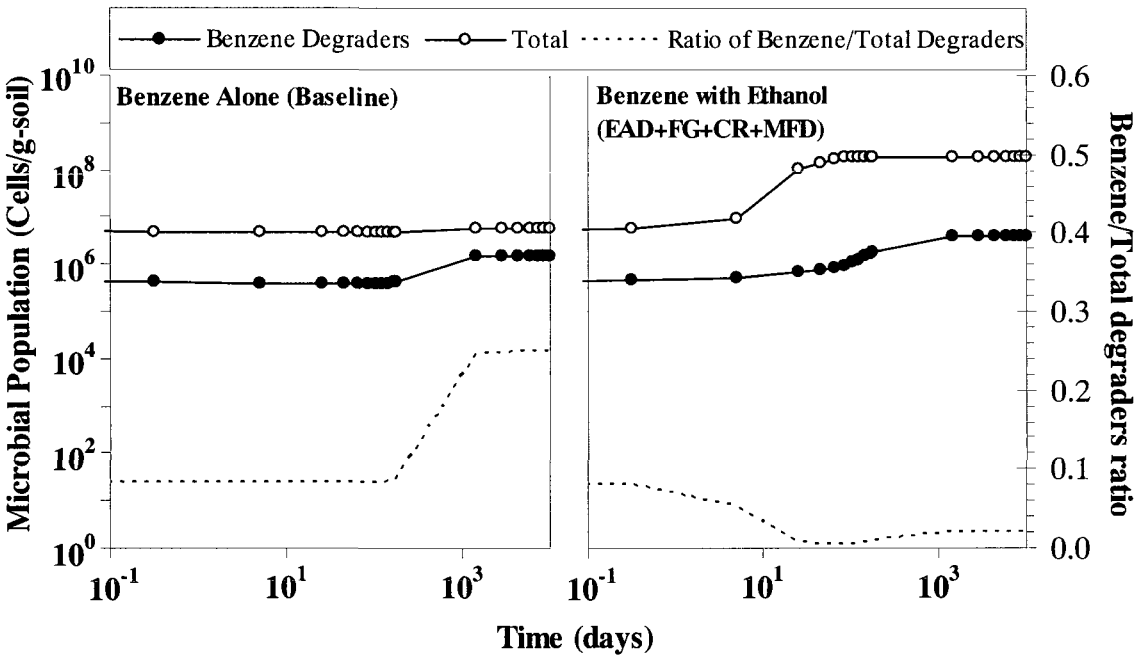

Figure 12- Influence of various inhibitory mechanisms (dissolved oxygen depletion, metabolic flux dilution [MFD] and catabolite repression [CR]) on benzene degraders and total microbial populations (0.1 m downgradient from source) for a benzene/ethanol constant source (Model parameters are given in Tables 1 and 2).

To illustrate the potential benefits of oxygen addition as a bioremediation technique and discern the potential enhancement of aerobic benzene degradation due to additional growth of benzene degraders on ethanol, an unlimited supply of oxygen was provided to the scenario that considers all substrate interactions (**Figure 4 and Figure 6**). Simulations with a constant source resulted in a plume length decrease of 67% compared to the baseline without ethanol. Total microbial population reached the highest simulated values, generating an increased degradation potential that offset the elongating effects of negative substrate interactions. When applying an unlimited oxygen supply to the decreasing source simulations, benzene plume length decreased by 44%. However, the high oxygen demand exerted by typically high ethanol concentrations may make aerobic stimulation a prohibitively expensive alternative.

6. Evaluation of Alternative Electron Acceptor Availability and Source Zone Fuel Composition on the Natural Attenuation of Benzene Plumes

[Basis for future research, unpublished]

This section builds on the General Substrate Interaction Module (GSIM), previously developed for evaluating benzene plume elongation due to the presence of ethanol [*Gomez et al.*, 2008]. The GSIM model was further refined and setup to include degradation of TEX compounds and reduction of several additional anaerobic electron acceptors. Literature values for degradation kinetics of ethanol, benzene and TEX under all reducing conditions were used, with McCarty's revised Thermodynamic Electron Equivalents Model [*McCarty*, 2007] used to fill knowledge gaps.

The mechanisms considered by the model include [*Gomez and Alvarez*, 2009]: common fate and transport processes (e.g., advection, dispersion, adsorption, depletion of molecular oxygen during aerobic and anaerobic biodegradation), substrate interactions that decrease the specific utilization rate for BTEX in the presence of alcohol fuels (e.g., metabolic flux dilution and catabolite repression), microbial populations shifts, toxicity and cosolvency effects, several common electron acceptors that better characterize domain characteristics: oxygen, nitrogen, sulfate and ferric iron. BTEX compound oxidation is a thermodynamically feasible process, which occurs through the use of electron acceptors by microorganisms. When considering terminal electron acceptor pathways (TEAP), we assume the following sequence in GSIM: oxygen > nitrate > ferric

iron > sulfate > carbon dioxide (methanogenic). This sequence is based on a decreasing oxidation potential for these compounds [*Corseuil and Alvarez*, 1996]. TEX compounds and additional anaerobic electron acceptors were not considered on previous modeling studies using GSIM [*Gomez et al.*, 2008; *Gomez and Alvarez*, 2009], and can provide important insights on the impact that electron acceptor heterogeneity can have on BTEX natural attenuation, ethanol impacts and GSIM model behavior.

6.1. Initial, Boundary, and Domain Conditions

Soil and hydraulic properties for model simulations were based on site characterization of the Hill AFB [*Newell et al.*, 1996; *Lu et al.*, 1999] as it provides a well characterized site which includes all the electron acceptors considered in our work. These properties were implemented on a simulation domain similar to that described by *Gomez* [et al., 2008]. The domain consists of a single 60 m wide by 200 m long layer (2-D) with a seepage water velocity set to a constant 9 cm/d by establishing a hydraulic head difference of 0.6 m between the two ends of the domain. Simulation and hydrogeological parameters are listed on **Table 10**.

Soil characteristics include a variety of available electron acceptors: 6 mg/l of dissolved oxygen (O_2), 17 mg/l of dissolved nitrogen (as NO_3^-), 98 mg/l of dissolved sulfate (as SO_4^{2-}), and 50.5 mg/l of solid ferrous iron (as Fe_3^+ immobile in the solid phase). Availability of all electron acceptors considered in the model, provide a better domain characterization to compare the effect of the different terminal electron acceptor processes and their impact on BTEX and ethanol degradation. Background groundwater

Table 10- Simulation Setup Parameters

Parameter	Value	Reference
Hydrogeology		
Hydraulic Conductivity (K)	9.0 m/d	Fine-Medium Sand, LNAST Soils database*
Hydraulic Gradient (i)	0.003 m/m	*Newell et al.*, 1996
Darcy water velocity (v)	2.7 cm/d	Fine-Medium Sand, LNAST Soils database*
Total Porosity (n)	0.3	*Newell et al.*, 1996
Groundwater dissolved oxygen (O)	6 mg/l	**
Groundwater dissolved nitrogen (N)	17 mg/l	**
Groundwater dissolved sulfate (S)	98 mg/l	**
Ferrous Iron Present in the Soil (F)	50.5 mg/l	**
Pore space utilization factor (γ)	0.2	*Vandevivere et al.*, 1995; *Thullner et al.*, 2002
Dispersivity		
Longitudinal	7 m	
Transverse	0.7 m	
Adsorption		
Soil Bulk Density (ρ_b)	1.7 kg/l	*Newell et al.*, 1996
Retardation factor, Ethanol, R_E	1.01	Calculated, $R_E = 1 + r_b K_{dE}/n$
Retardation factor, Benzene, R_B	1.81	Calculated, $R_B = 1 + r_b K_{dB}/n$
Retardation factor, TEX, R_T	7.98	Calculated, $R_T = 1 + r_b K_{dT}/n$
General simulation		
Modeled Area length	200 m	
Modeled Area Width	60 m	
X space discretization	50 units	
Y space discretization	75 units	
Cell width	0.8 m	
Cell length	4 m	
Simulation Time	15 years	
Simulation Time Step (Transport)	0.2 days	
Simulation Time Step (Degradation)	0.067 days	
Source Zone Concentrations		
Benzene (Baseline Simulation)	22.54 mg/l	
TEX (Baseline Simulation)	42.00 mg/l	
Alcohol (10% Simulation)	1975.00 mg/l	
Benzene (10% Simulation)	18.93 mg/l	
TEX (10% Simulation)	35.37 mg/l	
Alcohol (85% Simulation)	16787.50 mg/l	
Benzene (85% Simulation)	2.17 mg/l	
TEX (85% Simulation)	4.14 mg/l	
Background Microbial Populations		
Alcohol aerobic degraders	10^6 cells/g-soil	*Chen et al., 1992*
Alcohol anaerobic degraders	10^5 cells/g-soil	10% of alcohol degrading aerobic populations
BTEX aerobic degraders	10^5 cells/g-soil	10% of total populations
BTEX anaerobic degraders	10^3 cells/g-soil	1% of BTEX degrading aerobic populations

**[Huntley and Beckett, 2002]*

*** Based on soild and hydraulic characteristics of Hill AFB (Newell et al., 1996; Lu et al., 1999)*

flow into the domain provides a constant recharge of dissolved electron acceptors (O_2, NO_3^- and SO_4^{2-}). All thermodynamically favorable electron acceptors were assumed to rapidly deplete when alcohol biodegradation is present resulting in a quick transition to anaerobic methanogenic degradation [*Da Silva and Alvarez*, 2002].

Initial microbial concentrations were defined as: (a) 1 mg/L (10^6 cells/g-soil) for ethanol aerobic degrading populations [*Chen et al.*, 1992]; (b) 0.1 mg/L (10^5 cells/g-soil), 10% of total, for benzene aerobic degrading populations; (c) 0.1 mg/L (10^5 cells/g-soil), 10% of total, for ethanol anaerobic degrading populations; and (d) 0.001 mg/L (10^3 cells/g-soil), 1% of benzene aerobic degraders, for benzene anaerobic degraders.

Source zone concentrations were calculated assuming a 20 gallon gasohol LNAPL spill resting on top of the groundwater table, with its constituents dissolving into the groundwater phase following different dissolution rates and mass transfer limitations. The composition of E10 (10% ethanol with regular gasoline blend) in mole fractions was used as standard reference: 0.015 for benzene, 0.172 for alcohol, 0.158 for toluene, ethylbenzene, and xylenes, and 0.655 for other compounds (calculated from *Poulsen* [et al., 1991]). The resulting dissolved concentrations at the groundwater-LNAPL interface, in equilibrium with the LNAPL phase, can be reasonably estimated using Raoult's law [*Mackay et al.*, 1991] and modified by the cosolvent effects of alcohols using a linear/log linear model developed by *Heermann and Powers* [1998]. Details of this calculation were previously presented by *Gomez and Alvarez* [2009]. **Table 10** indicates the initial

dissolved groundwater concentrations of Benzene, TEX and alcohol, for the baseline (regular gasoline), 10% alcohol and 85% alcohol blends.

For analysis, the following simulation scenarios were implemented: (1) Benzene only; (2) Baseline (Regular gasoline); (3) 10% Ethanol blend; and (4) 85% Ethanol blend. All scenarios had a simulation time of 15 years with a transport time step of 0.2 days and a degradation time step of 0.067 days. RT3D model was setup to track 3 substrate chemical species (Benzene, TEX, and ethanol), 4 electron acceptor chemical species (Oxygen, Nitrate, Sulfate and Ferrous Iron; with Ferrous Iron being immobile), and 15 independent microbial populations (1 aerobic and 4 anaerobic populations for 3 different substrate combinations), as described in **Table 2**.

6.2. Results and Discussion

Simulation results for 10% ethanol gasoline blend scenario corroborate previous research indicating that the presence of ethanol may hinder benzene natural attenuation [*Corseuil et al.*, 1998*; Da Silva and Alvarez*, 2002*; Cápiro et al.*, 2007]. **Figure 13** shows how ethanol has a significant impact on BTEX plume elongation, with plume length defined as the centerline length of the 5 mg/L contour plume (drinking water MCL, maximum concentration level, for benzene [*U.S. EPA,* 2003]).

Figure 13 shows the evolution of benzene, TEX and ethanol plume length of gasoline blended with ethanol (10% and 85% mixtures) when compared to a regular gasoline

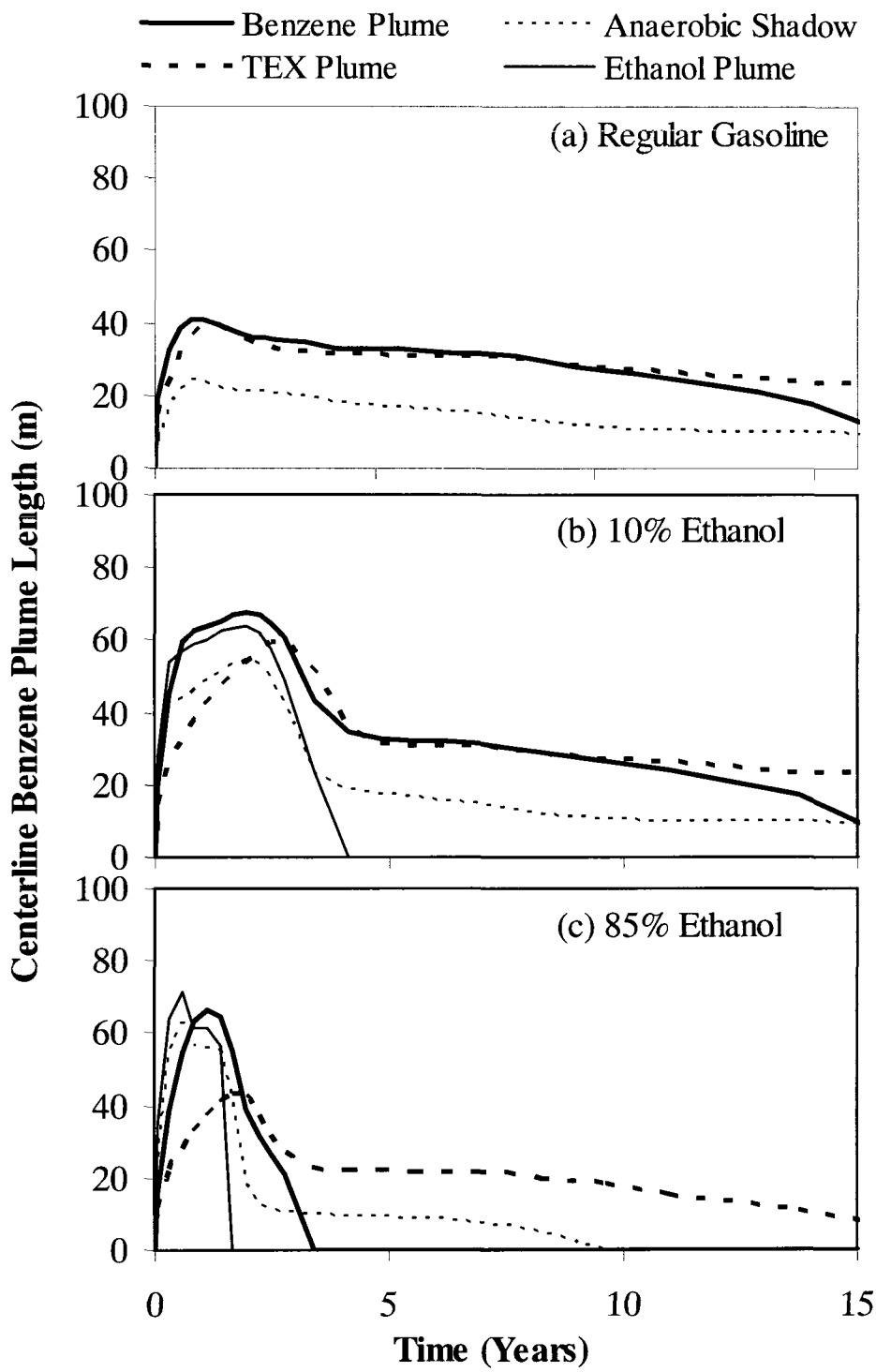

Figure 13 – Benzene, ethanol, TEX and anaerobic shadow centerline plume length (m) of (a) regular gasoline, (b) 10% ethanol blend and (c) 85% ethanol blend over a 15 year simulation period.

baseline, over a 15 year simulation period. Plumes go through an elongation period (an average of 2.5 years) reaching a maximum length, then retreating until the LNAPL source zone is depleted. This phenomenon has been previously observed in the field for BTEX plumes under the effects of ethanol during natural attenuation in a sulfate-reducing aquifer by *Mackay* [et al., 2006]. **Figure 13a** shows that a regular gasoline spill would reach a maximum of 41 meter benzene plume length. Centerline plume length would reach 68 meters (65% increase) when in the presence of 10% ethanol (**Figure 13b**), and 66 meters (62% increase) when in the presence of 85% ethanol (**Figure 13c**). For TEX the impact is lower (**Figure 13**) for E85 with 9% elongation and similar for E10 with 52% elongation. **Figure 14** shows the benzene, TEX and ethanol plumes after 1 year of simulation. As expected, both E10 and E85 (**Figures 14 a.2 and a.3**) generate longer benzene plumes, and result in a faster depletion of source zone LNAPL. The impact of ethanol on TEX is significantly lower than on benzene natural attenuation, particularly for E85.

Benzene plume behavior is related to microbial population growth. **Figure 15** shows the distribution of total microbial degraders under different electron acceptor conditions after 120 days of simulation. Aerobic degradation (**Figure 15a**) is most active in the fringe of the plume, where oxygen rich conditions are maintained by mixing with uncontaminated groundwater. With increasing ethanol content, the distribution of aerobic degraders migrates outwards as oxygen is quickly depleted near the center of the plume. The center of the plume harbors diverse anaerobic conditions, ranging from nitrate reducing degradation close to the aerobic fringe (**Figure 15b**), to methanogenic

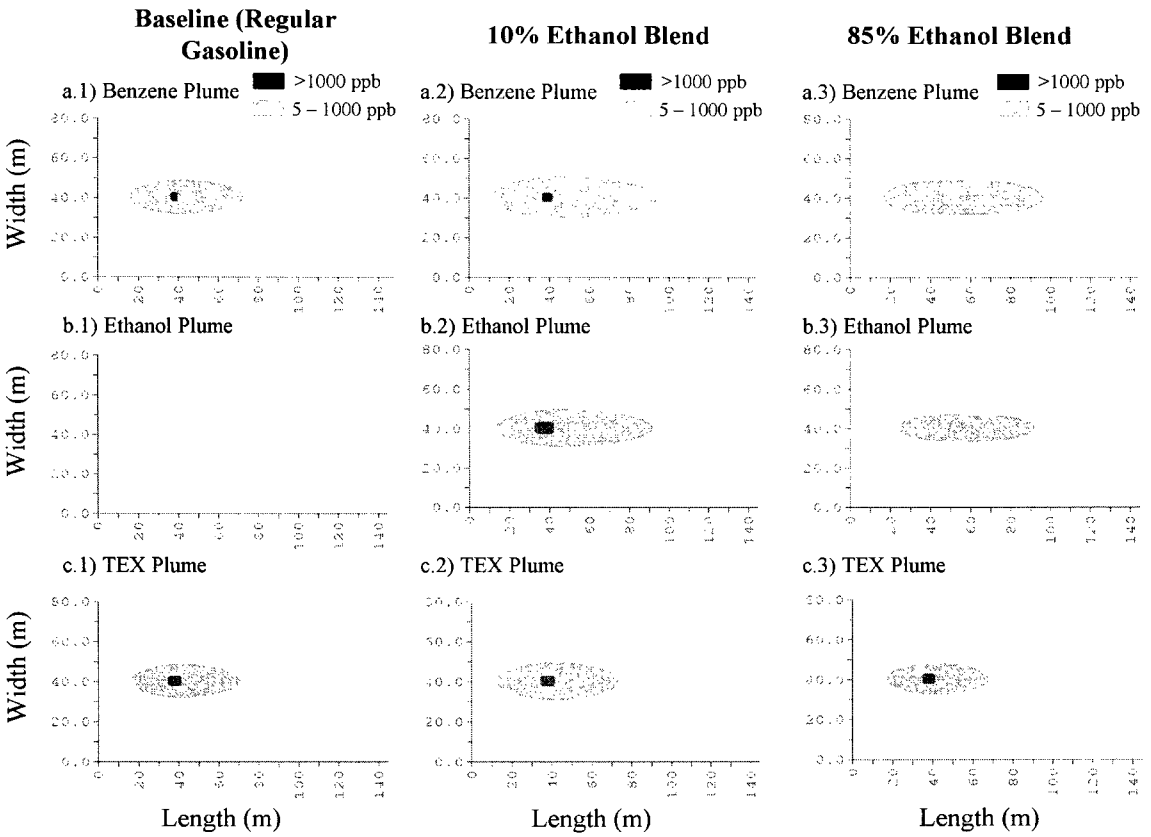

Figure 14 – Benzene (a), ethanol (b), and TEX (c) plumes (> 5 ppb) for regular gasoline, 10% ethanol blended gasoline and 85% ethanol blended gasoline after 1 year of simulation.

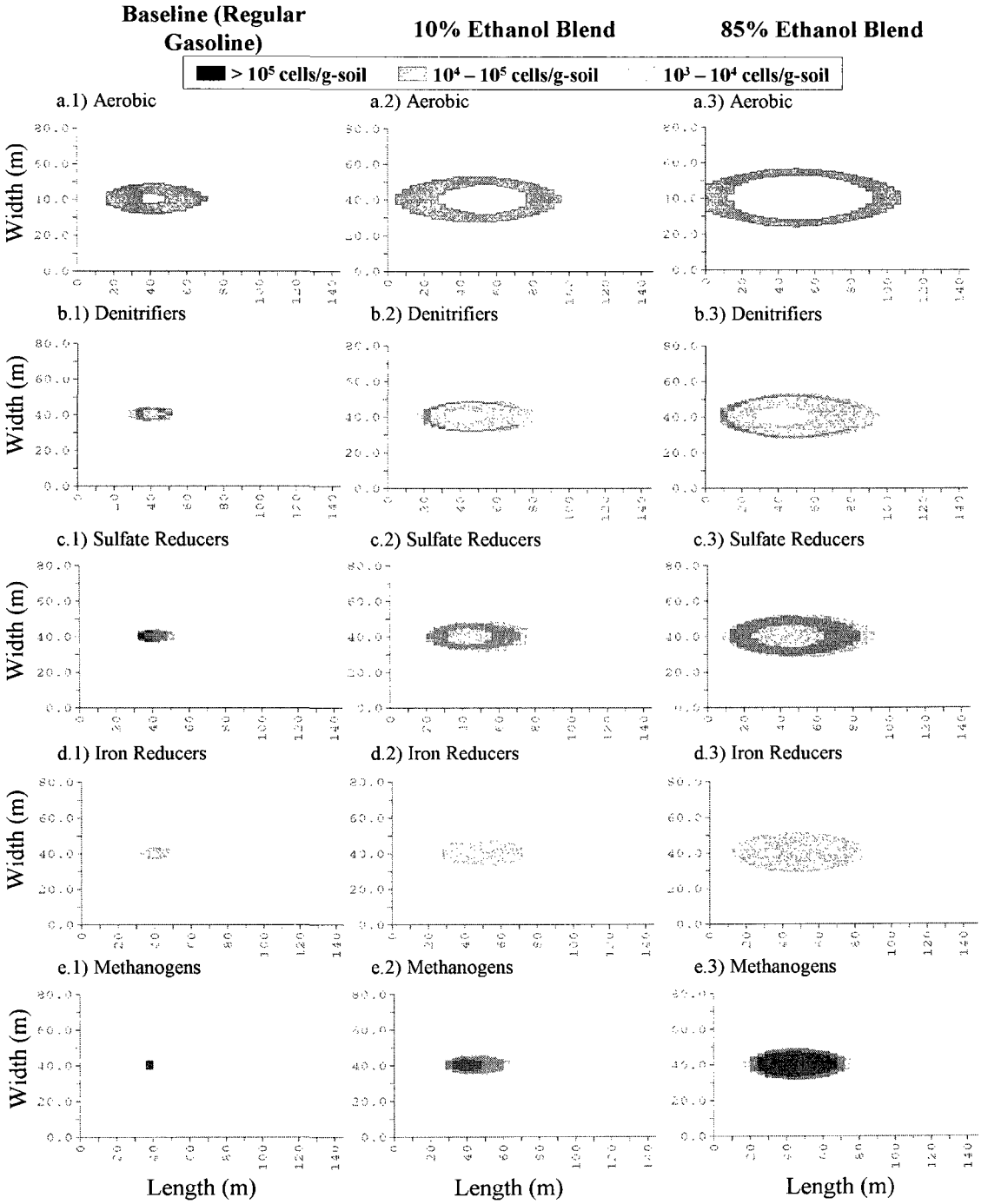

Figure 15 – Total microbial degrading populations (activity above background concentrations) after 120 days for regular gasoline, 10% ethanol blended gasoline and 85% ethanol blended gasoline. Microbial population plumes show: (a) aerobes; (b) denitrifiers, (c) sulfate reducers, (d) iron reducers and (e) methanogens.

conditions near the source zone (**Figure 15d**), as is commonly observed in naturally attenuated hydrocarbon plumes in the field [*Alvarez and Illman*, 2006].

Although benzene is generally recalcitrant under anaerobic conditions [*Lovley*, 1997], anaerobic benzene degradation linked to nitrate reduction has been observed in laboratory studies [*Burland and Edwards*, 1999], and it is associated with low degradation rates, with field measurements of 0.0043 1/d first order degradation rates [*Borden et al.*, 1997; *Morgan et al.*, 1993]. Due to its very low rates, we have opted to remove this mechanism from our simulations. Benzene degradation under sulfate reducing conditions (**Figure 15c**) has been observed, and is usually associated with marine and coastal sediments [*Lovley et al.*, 1995; *Edwards et al.*, 1992; *Roychoudhury*, 2006]. Although Iron reducing benzene degradation has been reported [*Lovley et al.*, 1996; *Rooney-varga et al.*, 1996; *Anderson et al.*, 1998], and the process is considered in our simulations, **Figure 15d** shows the lowest microbial activity in all scenarios. Ferric Iron, being an immobile electron acceptor that does not replenish through groundwater flow, is quickly depleted by anaerobic conditions inside the plume. Methanogenic activity shows a sharp increase in the presence of ethanol (**Figure 15e.2 and 15e.3**) compared to regular gasoline (**Figure 15e.1**). This is important, as it is the basis of one of our model assumptions: that in the presence of ethanol, the system is quickly driven to anaerobic methanogenic degradation conditions. Such methanogenic degrading conditions have been widely observed and reported [*Da Silva and Alvarez*, 2004]. BTEX degradation activity can be boosted (fortuitous growth) by the presence of an alternative food source like an alcohol.

Simulation results (**Figure 15**) shows an increase in degradation activity for all reducing conditions under the presence of ethanol. This fortuitous growth resulting from the presence of an alcohol as an additional substrate can partially offset the negative effects of ethanol [*Lovanh et al.*, 2002; *Cápiro et al*, 2008]. Ethanol, however, can also stimulate the growth of other bacteria, resulting in a significant increase of total populations in the system. This process results in genotypic dilution [*Da Silva and Alvarez*, 2002; *Cápiro et al.*, 2008], where BTEX degrader populations increase due to the presence of ethanol, but their abundance relative to total degraders, decreases.

Figure 16 shows the evolution of the total microbial degrading populations (measured as total mass (g) in the system). Different terminal electron acceptor using populations grow and peak in sequence: 1) aerobic, 2) nitrate reducing, 3) ferric iron reducing, 4) sulfate reducing, and 5) methanogenic, in accordance with the electron acceptor chain described in the literature [*Corseuil and Alvarez*, 1996; *Lovley*, 1997]. After about 5 years of simulation an increase in aerobic activity is detected as oxygen is recharged due to groundwater flow, and the electron acceptor demand load of ethanol is no longer present. As expected of iron reducers, they reach a peak and then disappear as iron is depleted from the soil matrix and no recharge occurs (considered insoluble). Iron reducers appear to have the lowest impact in BTEX natural attenuation.

The GSIM model has shown to have the capability to simulate several decreasing oxidation potential electron acceptors. The sequential use of these electron acceptors and

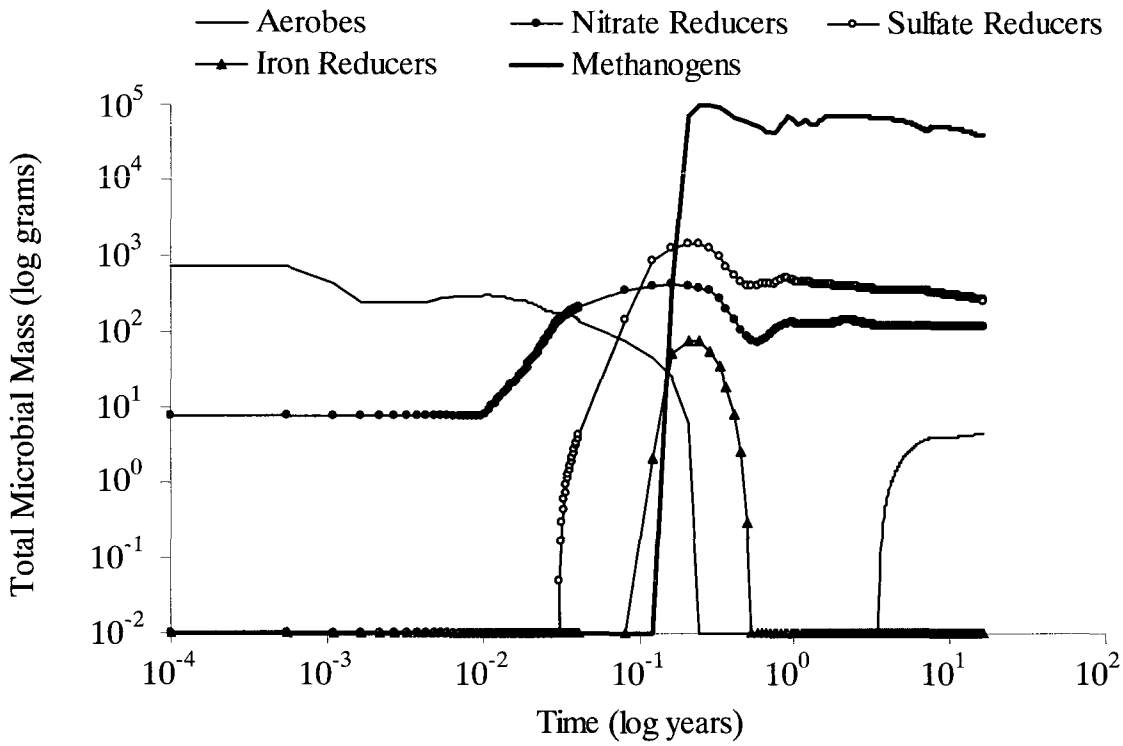

Figure 16 – Total degrader population evolution over time (as total mass – g) for E10 degradation under aerobic, nitrate reducing, sulfate reducing, iron reducing and methanogenic conditions.

the distribution of their associated microbial populations is in accordance to that reported by the literature [*Corseuil and Alvarez*, 1996; *Lovley*, 1997].

Figure 17 shows a comparison of the results of this chapter (degradation considering TEX and additional electron acceptors) to simulations which consider only benzene degradation (no TEX) under aerobic and methanogenic conditions under the same domain characteristics. Considering TEX in the simulations, results in slightly longer benzene plumes due to the additional electron acceptor demand exerted by them, resulting in decreased degradation rates for benzene. This was observed in all three scenarios. Considering additional anaerobic electron acceptors, results in shorter benzene plumes for the baseline case, where the availability of these additional electron acceptors counteracts the increased demand exerted by TEX. However, in the presence of ethanol, benzene plume lengths are longer. This is due to ethanol using all the available electron acceptors, and at the same time, having a longer electron acceptor chain leading to methanogenic conditions, resulting in more chemical species inhibiting this final degradation process. For the case without TEX and additional TEAPs, presence of ethanol results in 35.7% benzene plume elongation; for the case with additional TEX, the effect is of 37.3%; and for the scenario with TEX and additional TEAPs, benzene plume elongation is 62%. This shows that considering additional TEX has little influence on benzene plume elongation; however, additional anaerobic TEAPs can have a significant impact. Distribution and availability of such anaerobic electron acceptors as nitrogen, sulfate and iron, is highly site specific; for this reason, we have opted to not consider them, or TEX, in the next chapters to simplify our simulation scenarios.

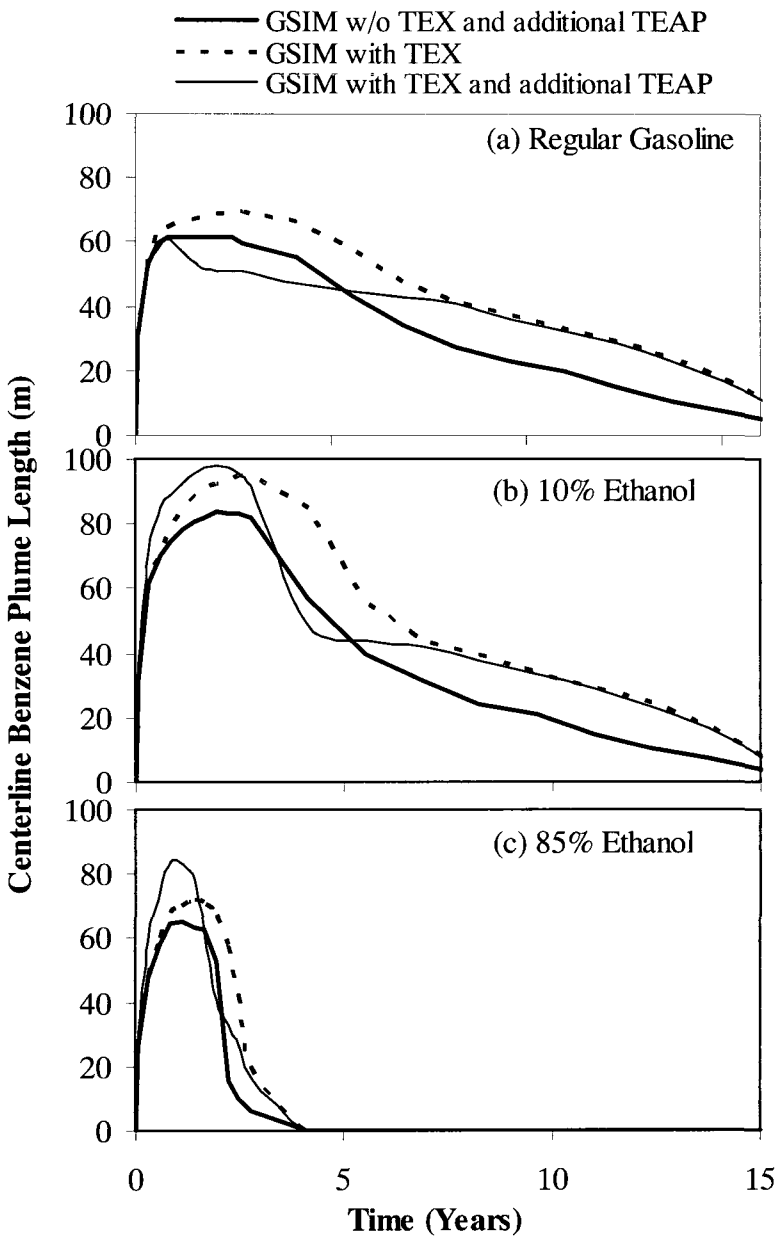

Figure 17 – Comparison of Benzene plume length with and without TEX and additional anaerobic TEAPs for regular gasoline, E10 and E85.

7. Effect of Ethanol Content on the Lifespan and Maximum Length of Benzene Plumes

[Extracted from Gomez and Alvarez, 2009 – Published in Water Resources Research]

The mechanisms responsible for benzene plume elongation were analyzed in Chapter 5 by the General Substrate Interactions Model (GSIM), which considered common fate and transport processes (e.g., advection, dispersion, adsorption, depletion of molecular oxygen during aerobic biodegradation, and anaerobic biodegradation), as well as previously overlooked substrate interactions that decrease the specific utilization rate for benzene in the presence of ethanol (e.g., metabolic flux dilution and catabolite repression) and the resulting microbial populations shifts [*Gomez et al.*, 2008]. However, it is unknown how the content of ethanol in different blends that are rapidly entering the market will affect benzene natural attenuation and the resulting plume lifespan and maximum length, which is important to assess the potential likelihood and duration of exposure.

This chapter builds on the GSIM numerical model to include cosolvency and microbial toxicity exerted by high ethanol blends near the source zone, and evaluates the effect of ethanol content in gasoline on the natural attenuation of benzene plumes. We consider groundwater contamination by multiple ethanol blends, including E20 which is likely to replace E10 by 2013 in some states [*Kittelson et al.*, 2007] and E85 which is

increasingly being used for flexible fuel vehicles or high-compression engines, and report differences in the maximum length and persistence (i.e., lifespan) of benzene plumes relative to regular gasoline without ethanol.

7.1. Initial, Boundary and Domain Conditions for Simulations

Aqueous ethanol/BTEX concentrations at the source zone were calculated by considering a finite mass of LNAPL, with ethanol fractions ranging from 5% to 95%, which is dissolved and depleted over time. E10 composition in mole fractions was used as standard reference for calculating dissolved benzene concentrations at the groundwater LNAPL interface for other ethanol blends (**Figure 18**), and was set as 0.015 for benzene, 0.172 for ethanol, 0.158 for TEX and 0.655 for other compounds [calculated from *Poulsen et al.,* 1991].

Benzene concentration in groundwater equilibrated with the LNAPL source zone was calculated using an excel spreadsheet model developed for this research (**Appendix 1**). Previous models have considered the changing composition of the source zone as its constituents dissolve, (e.g., the American Petroleum Institute's (API) LNAPL Dissolution and Transport Screening Tool (LNAST) [*Huntley and Beckett,* 2002]), but have not considered the cosolvency effects of ethanol on BTEX components. **Figure 19** shows the resulting depleting source zone concentrations for E10 and E85.

The simulations domain was the same as described by Gomez et al [2008]. Briefly, the domain consisted of a single 60 m wide by 200 m long layer (2D) with a seepage water velocity set to a constant 9 cm/d by establishing a hydraulic head difference of 0.6

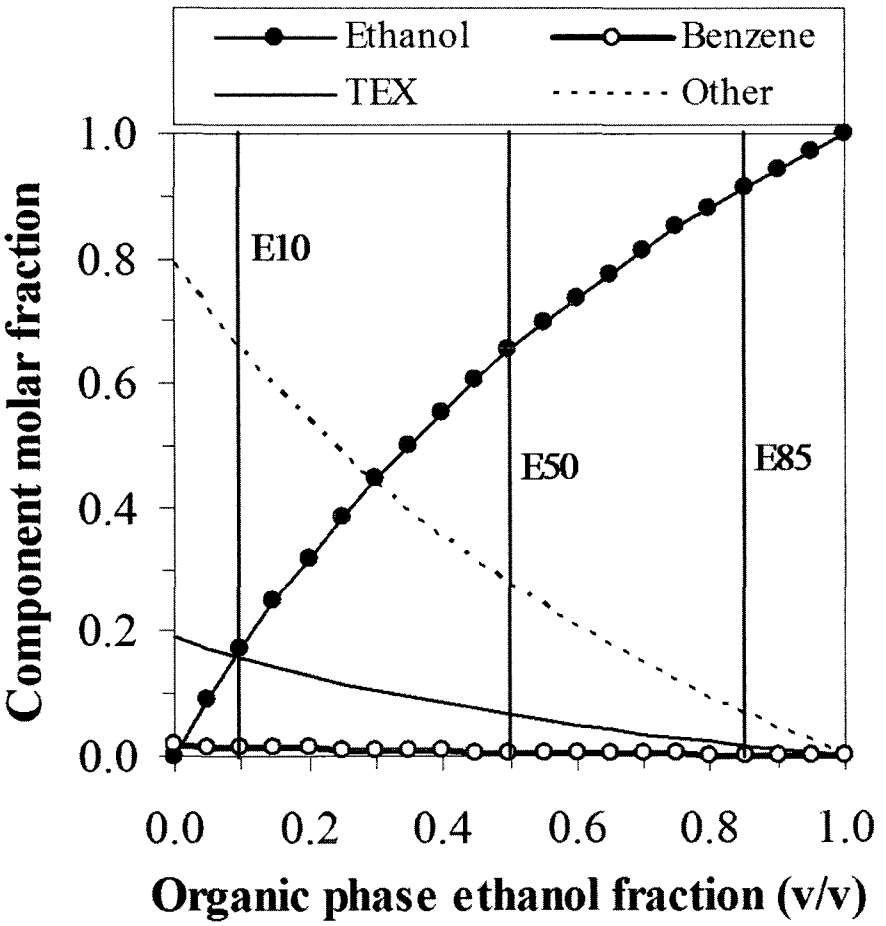

Figure 18- Composition of gasoline blended with ethanol, for different fractions of ethanol v:v in the organic phase (LNAPL).

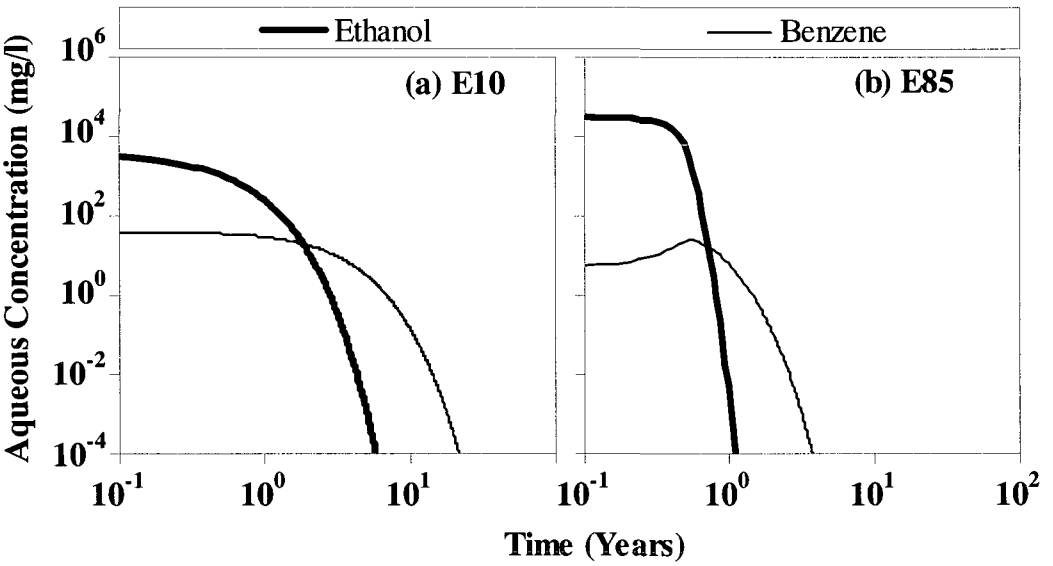

Figure 19– Ethanol and Benzene concentrations at the groundwater/LNAPL interface, for an (a) E10 release and (b) an E85 release (~85 kg NAPL total), considering Fick's second law of diffusion, and changes in source NAPL composition, enhanced dissolution effect of ethanol and mass transport due to advection.

m between the two ends of the domain. **Table 11** lists the hydrogeological domain parameters used.

The initial dissolved oxygen concentration was set at 6 mg/l, and background groundwater entering the model domain contained this same dissolved oxygen concentration. The system was assumed to become strongly anaerobic (methanogenic), which commonly occurs in ethanol-impacted systems as a result of the rapid depletion of thermodynamically-more-favorable electron acceptors [*Da Silva and Alvarez,* 2002]. Similar to previous simulations [*Gomez et al.,* 2008], initial microbial concentrations for aerobic populations that degrade ethanol or benzene were set at 1 mg/l (~10^6 cells/g-soil) [*Chen et al.*, 1992] and 0.1 mg/l (~10^5 cells/g-soil, 10% of aerobes), respectively. Initial concentrations for anaerobic populations that degrade ethanol or benzene were assumed as 10% of total and 1% of benzene aerobic degraders, or 0.1 mg/l (~10^5 cells/g-soil) and 0.001 mg/l (~10^3 cells/g-soil), respectively.

7.2. Results and Discussions

Figure 20 shows how the equilibrium concentration of benzene at the water/LNAPL interface changes for different fractions (v:v) of ethanol present in the LNAPL, for both the Heermann and Powers linear/log-linear model (equations 22 and 23) and for Raoult's law (equation 21). **Figure 20** also shows that ethanol increases the aqueous concentration of benzene, due to its cosolvent effects, by more than 40% when considering an E5 spill and up to 60% when E95 is considered. This leads to increased

Table 11– Model hydrogeological parameters.*

Parameter	Value	Reference
Hydrogeology		
Hydraulic Conductivity (K)	9.0 m/d	Fine-Medium Sand, LNAST Soils database**
Hydraulic Gradient (i)	0.003 m/m	*Newell et al.*, 1996
Darcy water velocity (v)	2.7 cm/d	Fine-Medium Sand, LNAST Soils database**
Total Porosity (n)	0.3	*Newell et al.*, 1996
Groundwater dissolved oxygen (O)	6 mg/l	*Newell et al.*, 1996
Pore space utilization factor (γ)	0.2	*Vandevivere et al.*, 1995; *Thullner et al.*, 2002
Dispersivity		
Longitudinal	7 m	*Newell et al.*, 1996***
Transverse	0.7 m	
Adsorption		
Soil Bulk Density (ρ_b)	1.7 kg/l	*Newell et al.*, 1996
Partitioning coefficient, K_{dE} (Ethanol)	0.001 l/kg	
Retardation factor, Ethanol, R_E	1.01	Calculated, $R_E = 1 + \rho_b K_{dE}/n$
Partitioning coefficient, K_{dB} (Benzene)	0.095 l/kg	
Retardation factor, Benzene, R_B	1.54	Calculated, $R_B = 1 + \rho_b K_{dB}/n$
General simulation		
Modeled Area length	200 m	
Modeled Area Width	60 m	
X space discretization	50 units	
Y space discretization	75 units	
Cell width	0.8 m	
Cell length	4 m	
Cell Depth	3 m	
Simulation Time	25 years	
Simulation Time Step	0.02	

* For a detailed description of the use of these parameters in the model, and a sensitivity analysis of selected parameters, please refer to *Gomez et al.* [2008].

** [*Huntley and Beckett*, 2002]

*** Modified to fit initial benzene plume lengths measurements of *Ruiz-Aguilar et al.* [2003]

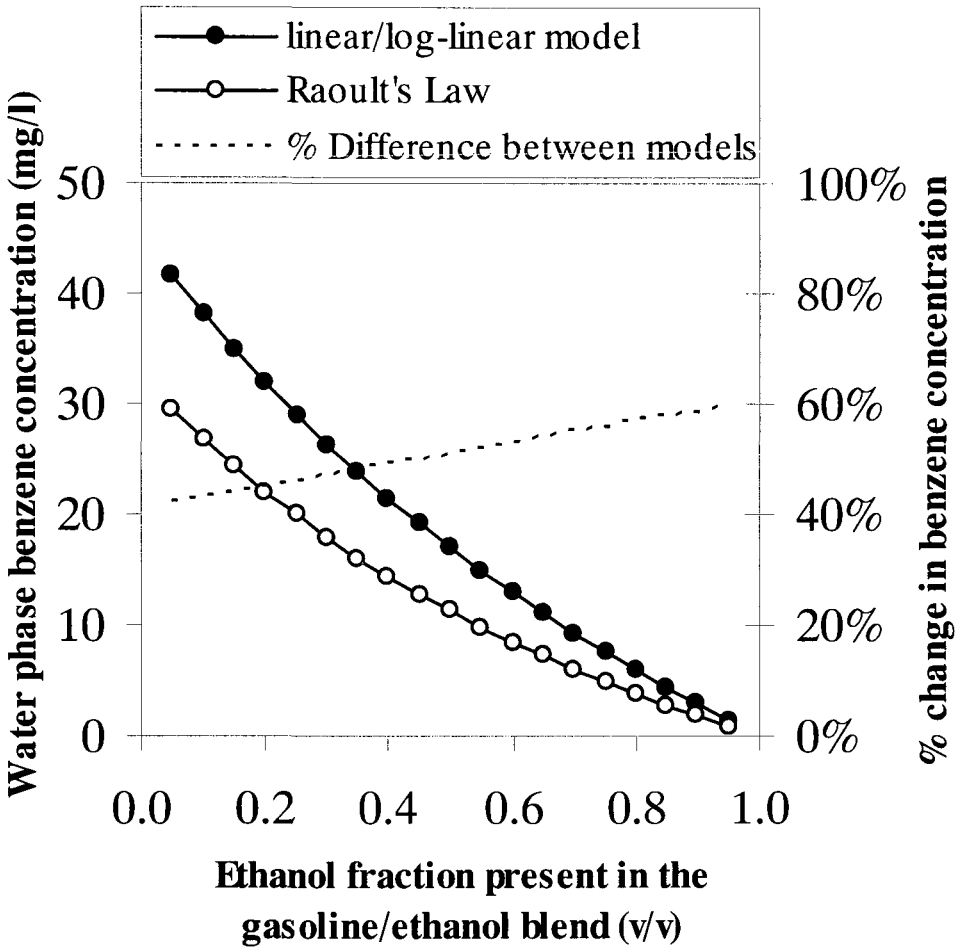

Figure 20– Equilibrium benzene concentrations at the water/LNAPL interface considering Heermann and Powers [1998] linear/log-linear model for gasoline/ethanol blends taking into account fugacity and cosolvency and Raoult's law (without cosolvency), for a range of ethanol blends.

mass transfer rates and faster dissolution when under the effects of ethanol. However, as the ethanol content in the LNAPL increases, both the mass of benzene available for dissolution and the dissolved benzene concentrations decrease.

When using equation 27 to evaluate the cosolvent effect of ethanol on benzene water-soil partitioning (sorption), there is a decrease in retardation for BTEX as the water-phase ethanol fraction increases, which could lead to longer BTEX plumes. Xylene and ethyl-benzene are the most hydrophobic of the BTEX and the most impacted by cosolvency with ~2% decrease in retardation for E10, 5-7% for E50 and 8-13% for E85. Benzene on the other hand, has a change in retardation of ~0.4% for E10, ~1.8% for E50 and 3% for E85 (**Figure 21**). These calculations consider a sandy soil with 0.2% organic matter.

Natural attenuation simulations for ethanol blends ranging from E5 to E95 were also performed. **Figure 22** shows the (a) benzene plumes formed after two years of LNAPL release (Regular Gasoline, E10 and E85), as well as the (b) oxygen depletion profile at 0.1 mg/l of dissolved oxygen, and the distribution of (c) aerobic and (d) anaerobic microorganisms that degrade benzene. Simulations show benzene plume elongation by 40% for the common blend E10 relative to the baseline release without ethanol (i.e., 250 vs. 180 ft). This is in excellent agreement with a statistical analysis of E10-impacted sites, which reported that the average benzene plume length was 36% loner than for regular gasoline (i.e., 263±103 ft versus 193±135) [*Ruiz-Aguilar et al.,* 2003].

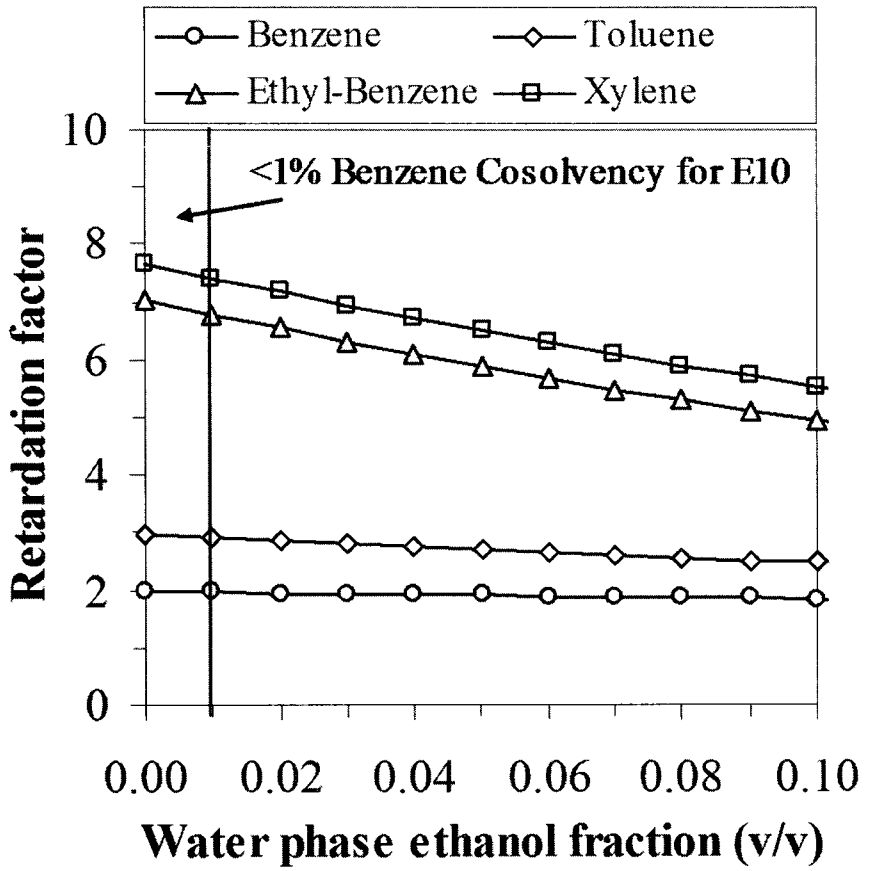

Figure 21- Retardation factor for benzene, ethylbenzene, xylene and toluene for different fractions of ethanol (v:v) in the water phase .

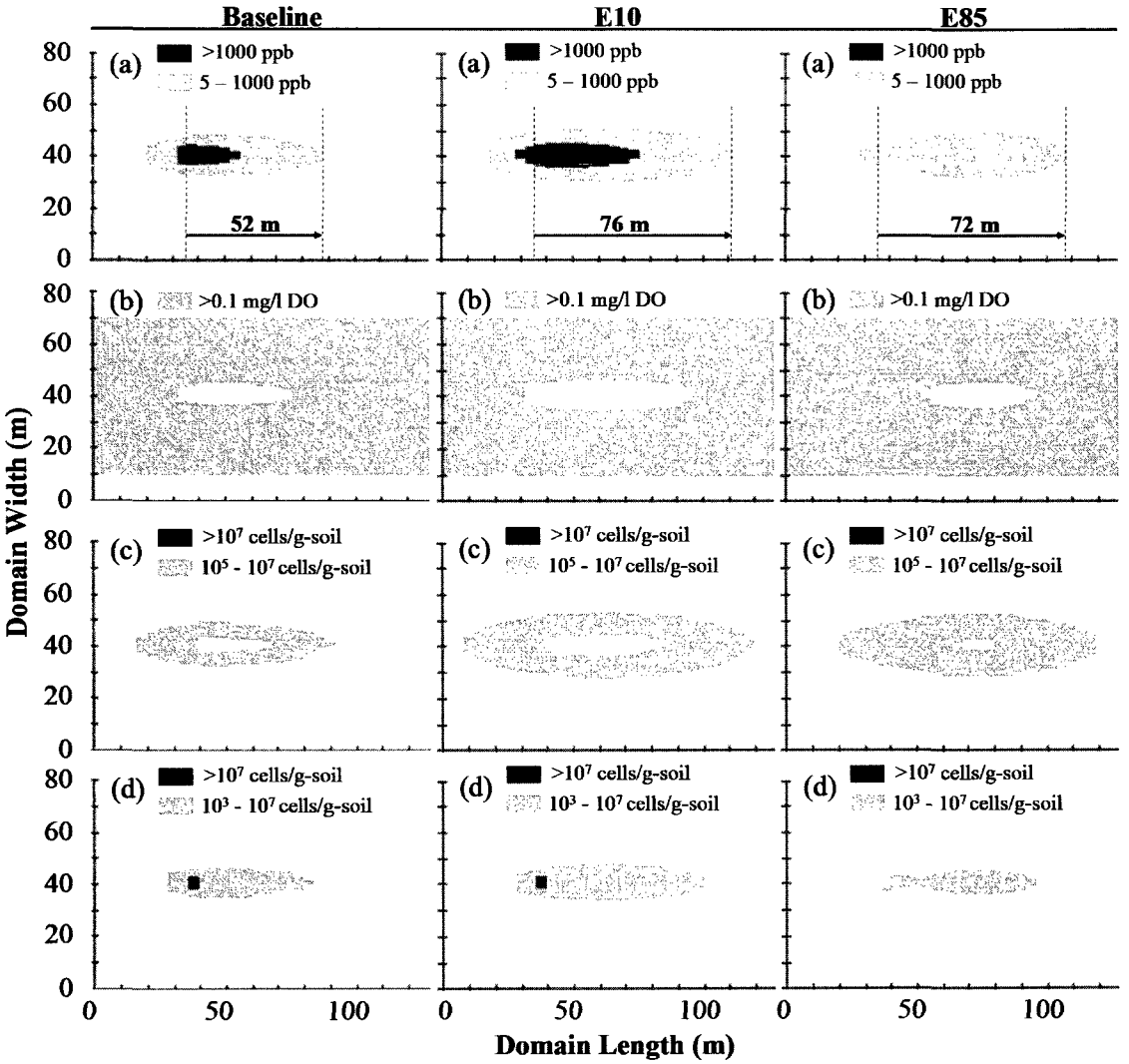

Figure 22– Simulated releases of regular gasoline (baseline), E10 and E85 after 2 years, showing: (a) benzene plume [1.0 and 0.005 mg/l contours]; (b) Oxygen depletion profile, and the distribution of (c) aerobic and (d) anaerobic benzene degraders.

Aerobic biodegradation of both ethanol and benzene quickly depletes the available dissolved oxygen inside the plume, causing a transition to anaerobic conditions. Then, aerobic benzene degraders prevail only on the fringe of the plume, where oxygen is being recharged by mixing with uncontaminated groundwater. The simulation reflects that the center of the plume harbors a dominantly anaerobic microbial community (**Figure 22d**), as is commonly observed in hydrocarbon plumes undergoing natural attenuation [*Alvarez and Illman*, 2006].

One important aspect to consider is microbial population changes in response to different ethanol blend releases. Some benzene degraders can grow fortuitously on ethanol, increasing the potential benzene degradation activity (*Cápiro et al.,* 2008). However, ethanol can stimulate the growth of other bacteria faster than hydrocarbon degraders, which decreases the relative abundance of benzene degraders (i.e., genotypic dilution) [*Da Silva and Alvarez,* 2002; *Cápiro et al.,* 2008]. Benzene degradation in the baseline case without ethanol increases the total microbial concentration near the source (aerobic plus anaerobic) to about 5×10^7 cells/g-soil. When ethanol is present, its consumption increases total microbial concentrations by 2 to 3 orders of magnitude, reaching ~10^9 cells/g-soil for E10 and ~10^{10} cells/g-soil for E50 and E85. The latter also results in shorter lived populations that undergo endogenous decay after the earlier depletion of available substrates (**Figure 23**).

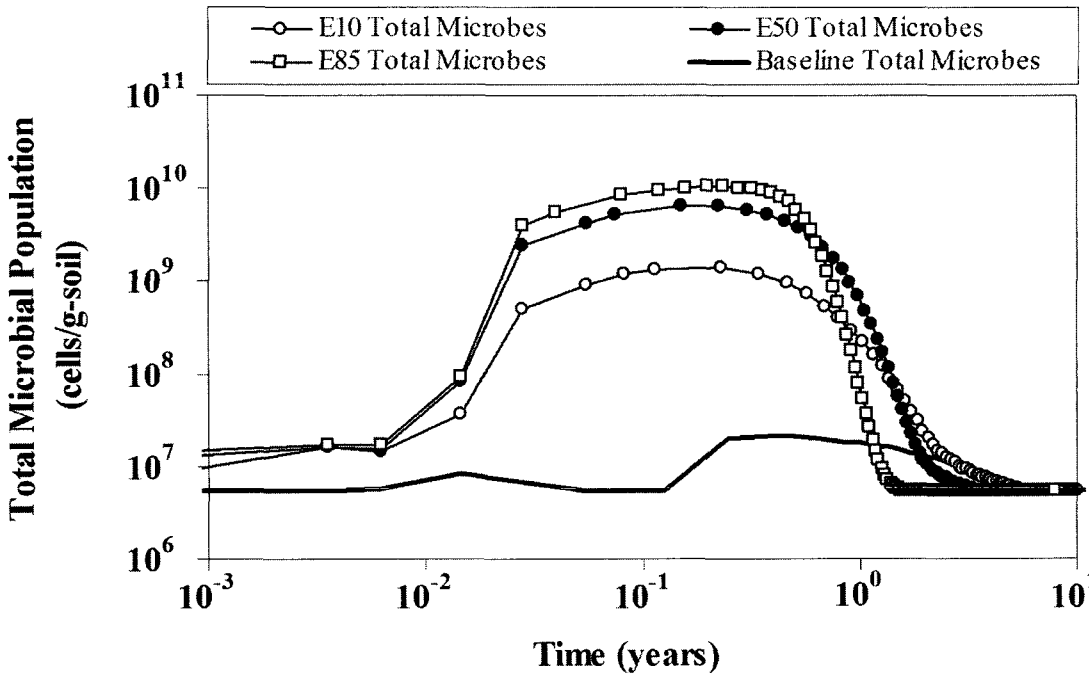

Figure 23- Near-source zone model total microbial population evolution over time, for four different gasoline/ethanol blends: no ethanol, E10, E50 and E85.

The maximum benzene plume length for the different ethanol contents in the released fuel was determined as the maximum downgradient distance from the spill source to the MCL (5 μg/l) contour (**Figure 24**). Ethanol had a significant elongation effect on benzene plumes, which is most pronounced for E10 – E20 blends (up to 59% elongation relative to the 56 m baseline). This elongation effect is similar for higher ethanol blends up to E45, and then plume elongation decreases to almost no impact for E95. This trend reflects competing processes that increase elongation versus those that offset it. As the ethanol content increases, processes that hinder the natural attenuation of benzene due to the presence of ethanol are accentuated, such as electron acceptor depletion, metabolic flux dilution and catabolite repression [*Gomez et al.*, 2008]. At the same time, the mass of benzene available for dissolution decreases for higher ethanol blends, due to the higher content of ethanol, resulting in lower benzene concentrations.

Furthermore, higher ethanol concentrations result in larger overall microbial populations that contribute to benzene degradation (**Figure 22**). Between E10 and E45 these competing plume elongation and attenuation processes are in relative balance. Above E45 ethanol content, a decrease in the mass of benzene released and increased biodegradation dominate and the maximum plume length decreases more abruptly (**Figure 25**).

A comparison of benzene plume lifecycles for four different blends (E10, E50, E95 and no ethanol) shows that, although all ethanol blends resulted in longer plumes than the baseline scenario for regular gasoline without ethanol, the benzene plume

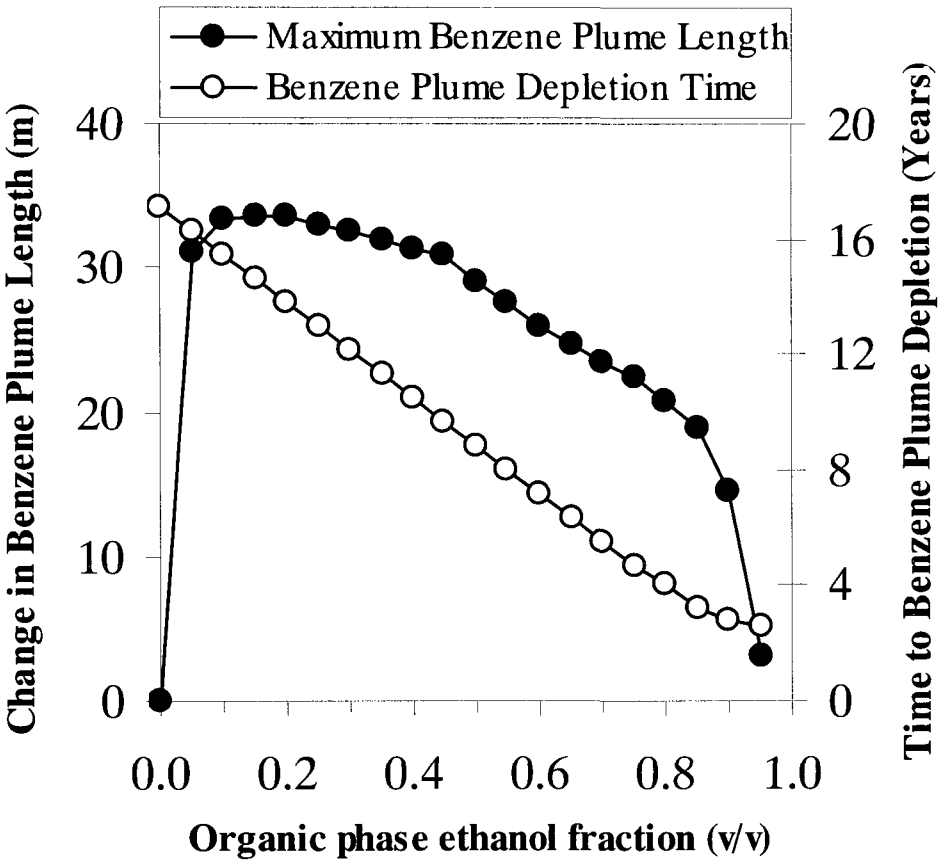

Figure 24– Maximum benzene centerline plume length (to 5 ppb contour) change (% of baseline) and time to benzene plume depletion, for blended fuels with varying ethanol fractions (v:v organic phase).

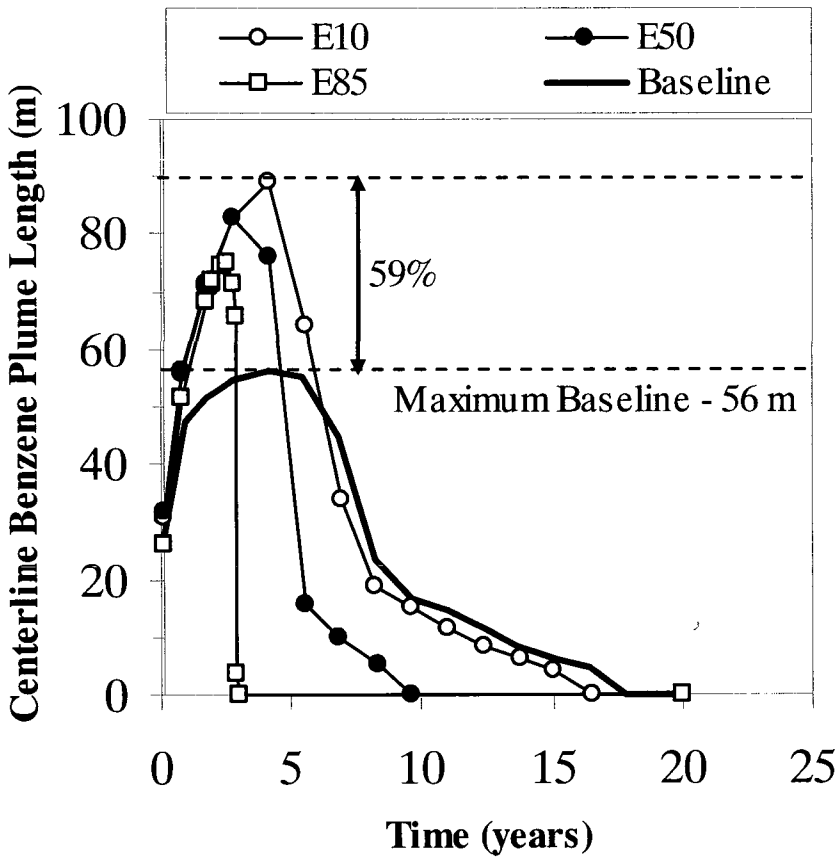

Figure 25 – Effect of ethanol volumetric content (10% for E10, 50% for E50 and 85% for E85) in released fuel on resulting benzene plume life cycle, compared to regular gasoline without ethanol (baseline).

lifespan (time until plume is degraded below MCL) decreases almost linearly as ethanol content in the blend increases (and thus the mass of benzene released decreases) (**Figure 25**).

Benzene transport may be influenced by site-specific heterogeneity. Thus, additional simulations were conducted to consider how heterogeneity in hydraulic conductivity (K) influences the effect of ethanol on benzene plume elongation. Spatially correlated hydraulic conductivity random fields were generated using an existing model, HYDRO_GEN [*Bellin & Rubin*, 1996] with a correlation scale of 5 times the spatial cell size in the x and y directions. HYDRO_GEN was run using a Gaussian distribution with a mean of 9 m/s and a variance ranging from 0 (baseline, homogeneous) to 8 m^2/d^2 (most heterogeneous case). Heterogeneity decreased simulated benzene plume lengths relative to the homogeneous baseline, by 7% (E10) to 9% (E85) for 2 m^2/d^2 of variance, 10% (E10) to 14% (E85) for 4 m^2/d^2, and 19% (E10) to 20% (E85) for 8 m^2/d^2. However, benzene plume elongation exerted by ethanol was not significantly affected by heterogeneity, compared to the homogeneous baseline (**Figure 26**).

Since the potential for exposure to benzene in groundwater depends on both plume length and persistence (i.e., lifespan), we arbitrarily combined these factors into an empirical index to compare the risk associated with groundwater contamination by different ethanol blends. This Potential Impact Index (PII) was defined as the area under the plume length versus lifespan curve (**Figure 25**), normalized to the corresponding area for the baseline case without ethanol. The PII is 1.16 for E10, 1.07 for E20, 0.78 for E50

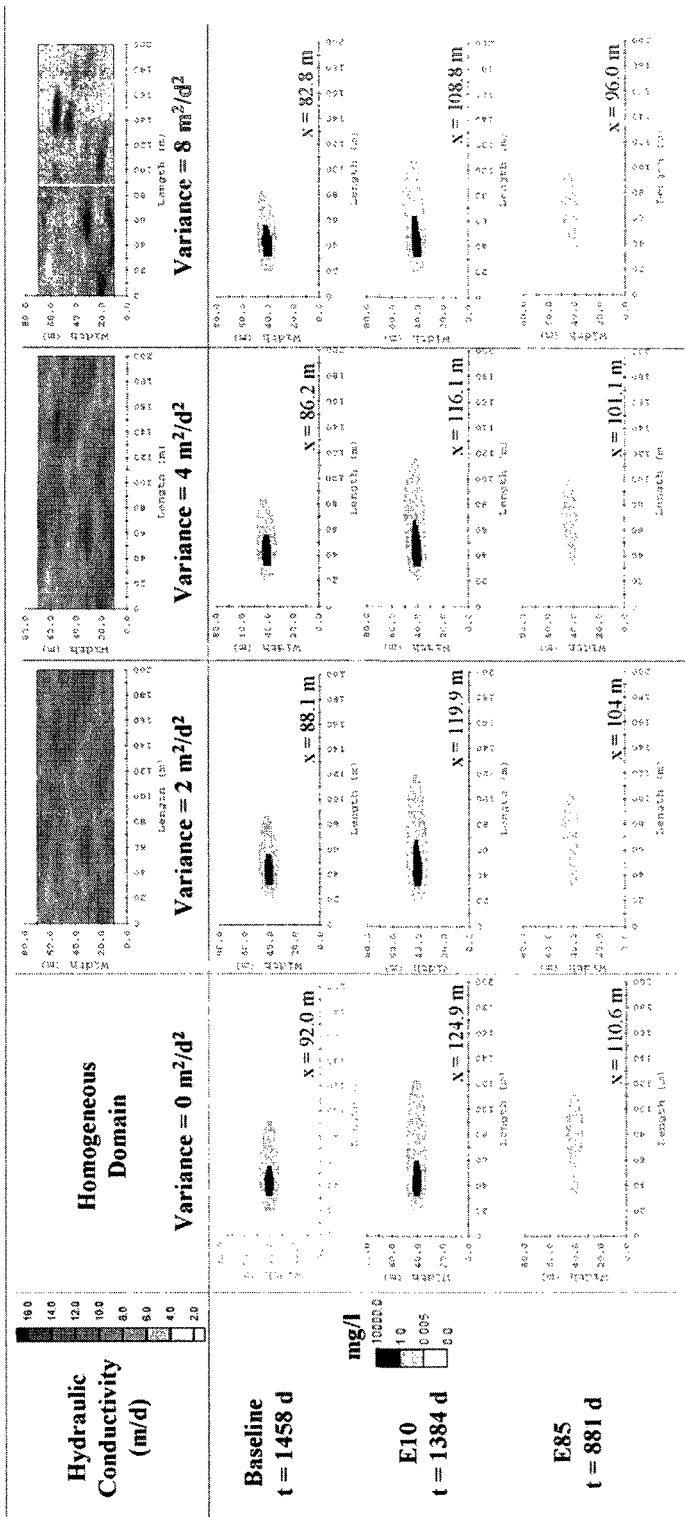

Figure 26 –Benzene plume elongation for E10 and E85 compared to the no ethanol baseline, in a homogeneous hydraulic conductivity domain (9 m/d); a random hydraulic conductivity field with 9 m/d mean and 2 m2/d2 variance; a random hydraulic conductivity field with 9 m/d mean and 4 m2/d2 variance; and a random hydraulic conductivity field with 9 m/d mean and 8 m2/d2 variance.

and 0.29 for E85. Thus, E10 and E20 spills represent a greater potential for benzene exposure (i.e., length × persistence) than regular gasoline without ethanol. Interestingly, E50 and E85 releases represent a lower PII than the baseline, even though their maximum benzene plume lengths are larger. In this case, longer plumes are offset by a shorter lifespan. A similar inference can be made by considering the maximum benzene plume area of influence for a given spill, normalized to the corresponding area for the baseline, as a metric of potential exposure. This ratio increases from 1.60 for E10 to 1.70 for E20, and then decreases to 1.50 for E50 and 0.91 for E85, inferring that E85 releases would result in smaller maximum benzene plume area of influence than both E10 and regular gasoline spills.

8. Comparison of the Effects of Various Fuel Alcohols on the Natural Attenuation of Benzene Plumes

[Extracted from Gomez and Alvarez, 2010 – In review Journal of Contaminant Hydrology]

Previous research on the effect of ethanol on benzene plume dynamics suggest the potential for similar impacts by other fuel alcohols, which exhibit similar physico-chemical characteristics as well as other properties that might hinder the natural attenuation of benzene. These include: (1) higher microbial toxicity [*Kaiser and Devillers*, 1994; *Dutka and Kwan*, 1981], which could hinder biodegradation; (2) higher cosolvency power, which could result in faster hydrocarbon dissolution and faster migration (i.e., decreased sorption-related retardation) [*Poulsen et al.*, 1991; *Paan et al.*, 2006]; and (3) slower biodegradation rates [*Howard et al.*, 1991], which is conducive to longer and more persistent inhibitory substrate interactions. However, the effect of alternative fuel alcohols on benzene biodegradation and natural attenuation has not been addressed in the literature, and it is unknown whether their presence may increase or decrease the potential for benzene plume elongation relative to ethanol.

An early evaluation of the potential groundwater impacts of alternative fuel alcohols is important for risk assessment and to determine the need to adjust current site management and remediation practices. This chapter presents a comparative modeling study of the effects of five fuel alcohols (methanol, ethanol, 1-propanol, iso-butanol and

n-butanol) on the natural attenuation of benzene. Eleven different alcohol/gasoline blends were considered: Regular Gasoline without fuel alcohol (Baseline), 10% ethanol (E10), 85% ethanol (E85), 10% methanol (M10), 85% methanol (M85), 10% 1-propanol (P10), 85% 1-propanol (P85), 10% iso-butanol (IB10), 85% iso-butanol (IB85), 10% n-butanol (B10) and 85% n-butanol (B85). We build on a previously developed model, General Substrate Interaction Module (GSIM) [*Gomez et al.*, 2008; *Gomez and Alvarez*, 2009], which considers common fate and transport processes (e.g., advection, dispersion, adsorption, depletion of molecular oxygen during aerobic and anaerobic biodegradation), as well as substrate interactions that decrease the specific degradation rate of benzene in the presence of fuel alcohols (e.g., metabolic flux dilution and catabolite repression). Resulting microbial population shifts, microbial toxicity at high alcohol concentrations, and cosolvency effects are also integrated into the model. A probabilistic sensitivity analysis of the principal biokinetic parameters used in the model was also conducted to account for uncertainty associated with such site-specific variables.

8.1. Initial, Boundary, and Domain Conditions

Aquifer material and hydraulic properties for model simulations were based on site characterization of the Hill AFB [*Newell et al.*, 1996; *Lu et al.*, 1999]. These properties were implemented on a simulation domain similar to that described by *Gomez et al.* [2008]. The model domain is composed of 3750 cells in a 60 m wide by 200 m long 2D layer. Groundwater seepage velocity of 9 cm/d is established by a hydraulic head difference of 0.6 m along the length of the domain. The model considers 6 mg/l of

dissolved oxygen (O_2) with a constant recharge through the background groundwater flow into the domain. Fast depletion of oxygen and other electron acceptors often occurs in aquifers contaminated with ethanol [*Da Silva and Alvarez*, 2002] and is assumed to take place in our simulations. Simulation and hydrogeological parameters are listed on **Table 12**.

Consistent with previous simulation efforts [*Gomez et al.*, 2008; *Gomez and Alvarez*, 2009], initial microbial concentrations were defined as: (a) 1 mg/L (about 10^6 cells/g-soil) for aerobic ethanol degraders [*Chen et al.*, 1992]; (b) 0.1 mg/L (about 10^5 cells/g-soil), or 10% of total, for aerobic benzene degraders; (c) 0.1 mg/L (about 10^5 cells/g-soil), or 10% of total, for anaerobic ethanol degraders; and (d) 0.001 mg/L (about 10^3 cells/g-soil), or 1% of aerobic benzene degraders, for anaerobic benzene degraders.

Depleting source zone concentrations were calculated assuming an 84 kg mass (30 gal) release of light non-aqueous phase liquid (LNAPL) resting on top of the groundwater table, as described in *Gomez and Alvarez* [2009]. Spill constituents (e.g., benzene and fuel alcohol) are assumed to dissolve into the groundwater at different rates depending on their LNAPL molar fractions and water diffusivity. The composition of E10 (10% ethanol with regular gasoline blend) in mole fractions was used as reference: 0.015 for benzene, 0.172 for alcohol, 0.158 for toluene, ethylbenzene, and xylenes, and 0.655 for other compounds (calculated from *Poulsen et al.* [1991]). The resulting dissolved concentrations at the groundwater-LNAPL interface can be reasonably estimated using Raoult's law [*Mackay et al.*, 1991] and modified by the cosolvent effects

Table 12- Simulation Parameters

Parameter	Value	Reference
Hydrogeology		
Hydraulic Conductivity (K)	9 m/d	Fine-Medium Sand, LNAST Soils database [*Huntley and Beckett, 2002*]
Hydraulic Gradient (i)	0.003 m/m	*Newell et al., 1996; Lu et al., 1999*
Darcy water velocity (v)	2.7 cm/d	Fine-Medium Sand, LNAST Soils database [*Huntley and Beckett, 2002*]
Total Porosity (n)	0.3	*Newell et al., 1996; Lu et al., 1999*
Groundwater dissolved oxygen (O)	6 mg/l	*Newell et al., 1996; Lu et al., 1999*
Dispersivity		
Longitudinal	7 m	*Newell et al., 1996; Lu et al., 1999*
Transverse	0.7 m	10% of Longitudinal Dispersivity
Adsorption and Dissolution		
Soil Bulk Density (ρ_b)	1.7 kg/l	*Newell et al., 1996; Lu et al., 1999*
Retardation factor (R) (Methanol, ethanol, 1-propanol, n-butanol, isobutanol, benzene)	1.00; 1.01; 1.02; 1.08; 1.04; 1.81	Calculated, $R = 1+ \rho_b K_d/n$
Water Diffusivity (D_i) (Methanol, ethanol, 1-propanol, n-butanol, isobutanol, benzene)	1.6×10^{-5}; 1.3×10^{-5}; 1.1×10^{-5}; 9.6×10^{-6}; 9.6×10^{-6}; 9.8×10^{-6} (cm2/s)	*Hilal et al. 2003*
Water Solubility (Methanol, ethanol, 1-propanol, n-butanol, isobutanol, benzene)	Miscible; Miscible; 0.105; 0.018; 0.031; 0.0003 (mole/mole)	*Hilal et al. 2003*
Cosolvency Power (σ_i) (Methanol, ethanol, 1-propanol, n-butanol, isobutanol, benzene)	2.79; 2.96; 3.18;3.23; 3.23; n/a	*Poulsen et al., 1991; Paan et al., 2006*
General simulation		
Modeled Area length	200 m	
Modeled Area Width	60 m	
X space discretization	50 units	
Y space discretization	75 units	
Cell width	0.8 m	
Cell length	4 m	
Simulation Time	20 years	
Simulation Time Step (Transport)	0.2 days	
Simulation Time Step (Degradation)	0.067 days	
Initial Source Zone Concentrations		
Benzene (Baseline Simulation)	45 mg/l	
Alcohol (10% Simulation)	3,800 mg/l	
Benzene (10% Simulation)	38 mg/l	
Alcohol (85% Simulation)	33,000 mg/l	
Benzene (85% Simulation)	5 mg/l	

of alcohols using a linear/log linear model developed by *Heermann and Powers* [1998]. Volatilization rates based on Fick's first law of diffusion were also considered, as presented by *Kim and Corapcioglu* [2003]. **Table 12** provides the initial dissolved groundwater concentrations of benzene and fuel alcohols for three scenarios: baseline (regular gasoline without alcohol), 10% alcohol and 85% alcohol blends.

8.2. Results and Discussion

The lifecycle of a plume, including longevity and plume length, is an important consideration for site investigation and remedial action decisions. **Figure 27** shows the simulated life cycle of benzene plumes for releases of gasoline blended with various alcohols. Simulations for E10 corroborate previous laboratory, pilot, field, and modeling studies showing that the presence of ethanol may hinder the natural attenuation of benzene [*Cápiro et al.*, 2007; *Corseuil et al.*, 1998; *Da Silva and Alvarez*, 2002; *Gomez et al.*, 2009; *Ruiz-Aguilar et al.*, 2003]. The model predicts that four years after the 30-gallon release to a sandy aquifer, a regular gasoline spill would emanate a benzene plume with a maximum length of 73.4 ±3.0 m, compared to 91.0 ±3.7 m (24% longer) for E10 **(Figures 27a and 27b)**. This is in reasonable agreement with a survey of benzene plumes at sites contaminated with regular gasoline versus E10, which found longer benzene plumes for the latter (80 ±31 m vs. 59 ± 41 m, or 36% longer) [*Ruiz-Aguilar et al.*, 2003].

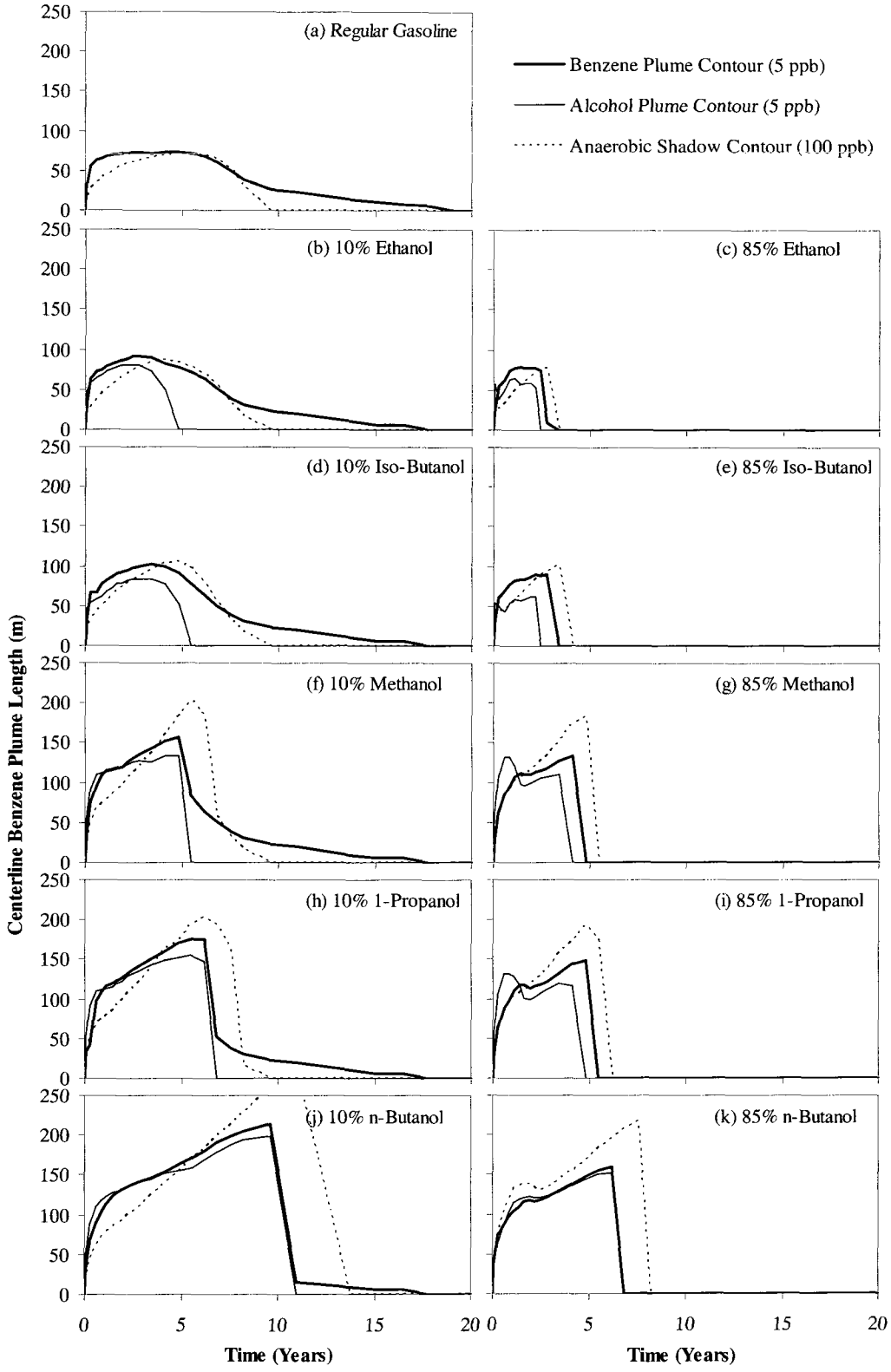

Figure 27– Simulated benzene plume dynamics (centerline plume length) resulting from a 30-gallons release of regular gasoline or various fuel alcohol blends.

In all scenarios, benzene plumes expand for the first 2 to 10 years, reaching a maximum length, and then recede as the source LNAPL mass is depleted until they disappear. However, both the type and content fuel alcohol can have a significant impact on the benzene plume life cycle. For example, maximum centerline benzene plume lengths were 91.0 ±3.7 m for E10, 102.0 ±4.2 m for IB10, 156.3 ±6.4 m for M10, 176.1 ±7.2 m for P10 and 214.8 ±8.8 m B10 (**Table 13**). Furthermore, benzene plumes where smaller and shorter-lived for higher blends of fuel alcohols, due mainly to the smaller content of benzene in the simulated release. Life span change due to alcohol content is most pronounced for ethanol and iso-butanol blends, showing a significant decrease in benzene plume duration from 17.8 years for E10 and IB10 (**Figures 27b and 27d**) to 3.4 years for E85 and IB85 (**Figure 27c and 27e**).

The simulated alcohol plumes were relatively short-lived and smaller than benzene plumes (**Figure 27**), reflecting faster degradation rates under the prevailing anaerobic conditions. The anaerobic shadows (defined as the 0.1 mg/L dissolved O_2 contours, which is commonly the limit of detection) are also depicted in **Figure 27**. These reflect a geochemical footprint associated with the biochemical oxygen demand of the release, which results in faster oxygen consumption than recharge. The anaerobic shadow generally reaches a maximum extension shortly after the alcohol plumes, and the contaminated zone remain mainly anaerobic for about 5 to 10 years until natural recharge of oxygen exceeds the decreasing oxygen consumption rate.

Table 13– Summary of Simulation Results

	Maximum Benzene Plume Length (m) [95% Conf.]	Percent Increase in Benzene Plume Length (%)	Time to Maximum Benzene Plume Length (Years)	Time to Benzene Plume Depletion (Years)	Potential Impact Index (PII)
Benzene Plume Length Results					
Baseline (Regular Gasoline)	73.4 ± 3.0	-	4.8	19.2	1.00
10% Ethanol (E10)	91.0 ± 3.7	24%	2.8	17.8	1.01
85% Ethanol (E85)	78.8 ± 3.2	7%	2.2	3.4	0.26
10% Iso-Butanol (IB10)	102.0 ± 4.2	39%	4.1	17.8	1.07
85% Iso-Butanol (IB85)	89.8 ± 3.7	22%	2.8	3.4	0.34
10% Methanol (M10)	156.3 ± 6.4	113%	4.8	17.8	1.35
85% Methanol (M85)	134.2 ± 5.5	83%	4.1	4.8	0.69
10% 1-Propanol (P10)	176.1 ± 7.2	140%	6.2	17.8	1.58
85% 1-Propanol (P85)	149.6 ± 6.1	104%	4.8	5.5	0.86
10% n-Butanol (B10)	214.8 ± 8.8	193%	9.6	17.8	2.47
85% n-Butanol (B85)	160.2 ± 6.6	118%	6.2	6.9	1.15

	Near Source Zone Maximum Benzene Degrader Population (cells/g-soil)	Increase in benzene degrading population (% of Baseline)	Near Source Zone Maximum Total Degrader Population (cells/g-soil)	Benzene degrader population (% of Baseline)
Microbial Population Results				
Baseline (Regular Gasoline)	1.6×10^{7}	-	2.0×10^{7}	78.28%
10% Ethanol (E10)	2.4×10^{7}	52%	4.9×10^{9}	0.48%
85% Ethanol (E85)	2.1×10^{6}	-87%	2.4×10^{10}	0.01%
10% Iso-Butanol (IB10)	2.4×10^{7}	55%	1.5×10^{9}	1.68%
85% Iso-Butanol (IB85)	1.9×10^{6}	-88%	6.3×10^{8}	0.30%
10% Methanol (M10)	2.2×10^{7}	40%	5.5×10^{8}	4.04%
85% Methanol (M85)	4.4×10^{6}	-72%	5.4×10^{8}	0.83%
10% 1-Propanol (P10)	1.7×10^{7}	8%	6.9×10^{8}	2.46%
85% 1-Propanol (P85)	5.5×10^{5}	-97%	5.1×10^{6}	10.84%
10% n-Butanol (B10)	5.6×10^{6}	-64%	2.7×10^{8}	2.11%
85% n-Butanol (B85)	4.0×10^{5}	-97%	1.6×10^{8}	0.25%

Larger benzene plumes and longer life spans were predicted for blends with 1-propanol and n-butanol, which were more persistent than the other alcohols considered (**Figure 27**). Although these higher molecular-weight alcohols tend to be more toxic and exert higher cosolvency power (**Table 4**), a sensitivity analysis indicates that anaerobic alcohol degradation rates (and associated persistence) are more influential on benzene plume elongation (**Table 14**). Specifically, n-butanol and 1-propanol generally exhibit slower dissolution and degradation rates than the other fuel alcohols considered, and persist longer in the aquifer exerting negative substrate interactions (e.g., catabolite repression and metabolic flux dilution) that hinder benzene natural attenuation for longer periods of time (**Figure 27**). Note that iso-butanol, which has been reported to degrade relatively fast under both aerobic and anaerobic conditions [*Pelz et al.*, 2009], was inferred to hinder the natural attenuation of benzene to a much lower extent than its isomer n-butanol. This illustrates the significant effect that a small difference in chemical structure can have on biodegradation, and corroborates the high sensitivity of the model to site-specific alcohol biokinetic parameters.

Figure 28 illustrates how a more persistent alcohol (n-butanol) promotes longer benzene plumes. After 150 days, both n-butanol and benzene plumes grow steadily from a LNAPL source zone with high alcohol and benzene concentrations. This stage, where n-butanol strongly hinders benzene degradation (**Figure 28a and 28b**), coincides with the period of benzene plume elongation (**Figure 27j**). After about 7 years, n-butanol has been depleted from the LNAPL and a residual butanol plume mobilizes downgradient, hindering biodegradation of the front end of the benzene plume (**Figure 28c and 28d**).

Table 14– Sensitivity Analysis

Model parameter	Relevance to model variabilty[1]	p-value
$Y_{B,Aer}$ (mg/mg)	1	6.1×10^{-6}
b_{An} (1/d)	2	4.5×10^{-7}
$m_{mB,Aer}$ (1/d)	3	3.6×10^{-6}
$K_{B,Aer}$ (mg/l)	4	3.2×10^{-6}
$m_{mE,An}$ (1/d)	5	7.3×10^{-6}
$Y_{E,Aer}$ (mg/mg)	6	6.7×10^{-2}
$K_{E,An}$ (mg/l)	7	8.8×10^{-2}
Ian,O (mg/l)	8	1.4×10^{-1}
$K_{B,An}$ (mg/l)	9	2.7×10^{-1}
b_{Aer} (1/d)	10	4.1×10^{-1}
K_O (mg/l)	11	4.4×10^{-1}
$Y_{B,An}$ (mg/mg)	12	5.1×10^{-1}
$K_{E,Aer}$ (mg/l)	13	5.8×10^{-1}
$Y_{E,An}$ (mg/mg)	14	6.2×10^{-1}
g (vol/vol)	15	6.8×10^{-1}
$m_{mB,An}$ (1/d)	16	8.1×10^{-1}
$m_{mE,Aer}$ (1/d)	17	8.7×10^{-1}
Output statistics (1 year simulations)		
Mean		40.92 m
Standard Deviation		8.56 m
95% Confidence		1.68 m (4.1%)

[1] *As calculated by the multilinear regression algorythm in MATLAB software (Supplemental material 3)*

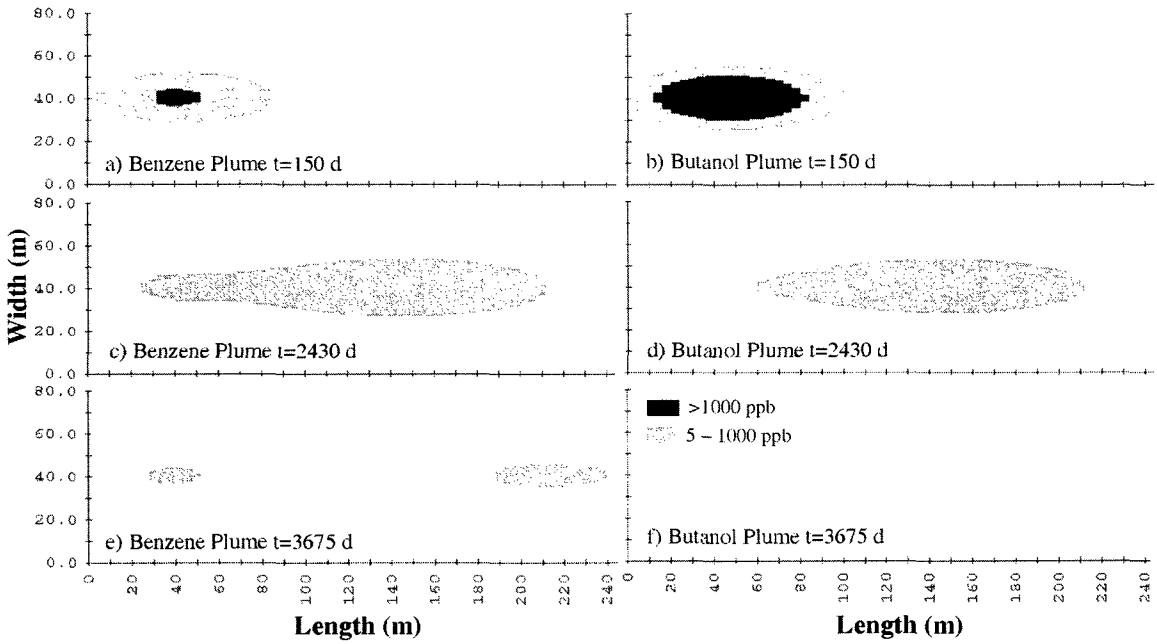

Figure 28– Simulated benzene and n-butanol contours (5 and 1,000 ppb) for a release of a 10% n-butanol blend, after 150, 2,430 and 3,675 days.

With n-butanol no longer present (**Figure 28f**), benzene degradation rates near the source zone increase. **Figure 28e** shows a split benzene plume after 9 years, where the central, lower concentration region of the original plume has been completely attenuated and no n-butanol remains in the system (**Figure 28f and Figure 27j**). The formation and eventual attenuation of the front end of the discontinued benzene plume results in the sharp decrease in benzene plume length depicted in **Figure 27 (j and k)**. Faster degrading alcohols like ethanol and iso-butanol are attenuated closer to the source zone and do not form a migrating residual plume. This results in a significantly smaller region of influence and shorter times for inhibition of benzene natural attenuation (**Figure 27b to 27e**).

We previously defined the Potential Impact Index (PII) of a plume as an empirical parameter that considers both plume length (which is relates to the possibility of the contaminant reaching a receptor) and lifespan (which relates to the potential duration of exposure) [*Gomez and Alvarez*, 2009]. Briefly, the PII is determined as the area under a given benzene plume life-cycle curve (**Figure 27**), normalized to the corresponding area for the regular gasoline baseline (**Figure 27a**). The estimated PII values (**Table 13**) infer that E10 and IB10 have relatively low groundwater impacts when considering benzene plume length and persistence, while blends with (more persistent) 1-propanol and n-butanol have a greater impact potential, particularly B10 and P10.

In most simulations, higher alcohol content resulted in higher total microbial populations. For example, near-source-zone total bacteria increased from 2×10^7 for

regular gasoline to 4×10^9 for E10 and 2×10^{10} cells/g-soil for E85 (**Table 13**). A similar trend is simulated for other alcohols, although lower population values than for ethanol are obtained despite their higher yield coefficients (**Table 4**), due to slower degradation rates and toxicity at high alcohol concentrations near the source zone. High concentrations of n-butanol (e.g., for B85) result in the lowest increase in total microbial populations (3×10^8 cells/g-soil), while 1-propanol (85%) results in a reduction in total microbial populations (5×10^6 cells/g-soil), reflecting their higher toxicity as indicated by lower MC50 (midpoint cytotoxicity) values (**Table 3**).

Although alcohols contribute to the fortuitous growth of benzene degraders, the higher alcohol blends resulted in lower total benzene degrader populations (**Table 13**). This is due to lower benzene mass available for their growth, which offsets the higher extent of fortuitous growth for higher alcohol content. For example, a 52% increase in benzene degraders is simulated for E10 (38 mg/l initial source zone concentration of benzene) compared to regular gasoline (45 mg/l initial source zone concentration of benzene), due to fortuitous growth on ethanol. However, for E85, the initial concentration of benzene is only 5 mg/l, which supports a smaller benzene degrader population (87% decrease) despite the growth-enhancing effect of ethanol. In all cases, genotypic dilution [*Da Silva and Alvarez*, 2002; *Cápiro et al.*, 2008] was observed; i.e., benzene degrader populations increase to a lower extent than other commensal microorganisms, and their relative abundance decreases. Genotypic dilution results in a decrease of the percentage of benzene degraders in the total population, from 78% for regular gasoline to 1% for E10, 4% for M10, 3% for P10, 2% for IB10, and 2% for B10 (**Table 13**).

A sensitivity analysis evaluated 17 biodegradation parameters: $\mu_{mB,Aer}$, $\mu_{mA,An}$, $\mu_{mA,Aer}$, $\mu_{mB,An}$, $Y_{B,Aer}$, $Y_{A,Aer}$, $Y_{A,An}$, $Y_{B,An}$, $K_{B,Aer}$, $K_{A,An}$, $K_{B,An}$, $K_{A,Aer}$, b_{An}, b_{Aer}, $I_{an,O}$ and γ (See Equations 6-10). One-year model simulations yielded a benzene plume length mean of 41m with a standard deviation of 8.5 m and a 95% confidence interval of 1.68 m (4.1%). **Table 14** lists model parameters in order of most to least relevant for model sensitivity, as given by the Multiple Linear Regression Analysis and the MATLAB software. Variables with lower *p*-values have a higher probability of impact on the model output, depending on their linear coefficients and standard errors. The most influential parameters are those related to aerobic benzene degradation and anaerobic ethanol degradation. However, for the simulated rapid depletion of molecular oxygen, anaerobic degradation becomes very important to control alcohol plume size and life span. Larger, longer lived alcohol plumes result in longer benzene plumes due to their extended inhibitory effect. The sensitivity analysis also indicates that aerobic benzene degradation, and by association dissolved oxygen concentrations and oxygen recharge rates, play a very important role in controlling benzene natural attenuation. Overall, the sensitivity analysis indicates that the two most important mechanisms that hinder benzene natural attenuation are (1) faster depletion of oxygen due to alcohols degradation, and (2) extended inhibitory effects associated with the more persistent alcohols (e.g., 1-propanol and n-butanol).

9. Conclusions

A custom reaction module for RT3D was developed to evaluate the effects of fuel alcohols (with focus on ethanol) on BTEX plume elongation and the relevance of the plume elongating processes involved. Previously overlooked mechanisms like sequential depletion of electron acceptors during ethanol degradation, the dilution of BTEX metabolic flux, catabolite repression, cosolvency, microbial population dynamics and toxicity were considered.

As with any model, there are limitations imposed by the assumptions made, including parameter estimation and process simplifications. Under the conditions to which this model is applicable, we can draw the following conclusions:

- Model results indicate that the presence of ethanol in E10 ethanol blend can cause benzene plume elongation between 25% and 59%, which agrees with previous statistical studies of benzene/ethanol plume lengths.

- Electron acceptor depletion during alcohol degradation is the principal mechanism hindering BTEX natural attenuation, followed by metabolic flux dilution and catabolite repression.

- Fuel alcohols stimulate an increase in microbial populations (including those that degrade BTEX), which can offset negative substrate interactions, although the relative abundance of BTEX degraders is decreased (genotypic dilution).

- Model simulations suggest that fuel alcohol content in the released blend has a significant impact on BTEX fate and transport, with longer benzene plumes compared to releases of regular gasoline without ethanol.

- Higher alcohol content leads shorter lived benzene plumes due to higher microbial concentrations and enhanced biodegradation rates for both BTEX and alcohol; decreased mass of benzene present in the source zone LNAPL; and increased benzene dissolution rates in the source zone LNAPL due to cosolvency.

- Within the assumptions and limitations of this model, we can conclude that high alcohol content blends (e.g., E85) might have a lower and shorter-lived impact on benzene groundwater contamination compared to low alcohol content blends like E10.

- Model simulations performed using the GSIM model suggest that all five renewable fuel alcohols considered (methanol, ethanol, 1-propanol, iso-butanol and n-butanol) can significantly hinder benzene natural attenuation, mainly due to depletion of available electron acceptors, inhibitory substrate interactions, and microbial toxicity near the source zone.

- More persistent alcohols (e.g., 1-propanol and n-butanol) have the greatest potential to exert inhibitory effects. Simulations infer that ethanol and iso-butanol have a lower propensity to hinder benzene natural attenuation, and that higher alcohol blends will result in smaller, shorter lived benzene plumes.

- There is considerable uncertainty associated with site-specific biokinetic coefficients for alcohol degradation, which are very influential parameters on simulated benzene plume dynamics as shown by a probabilistic sensitivity analysis. This forewarns against generalizations about the level of impact of specific fuel alcohols on benzene plume elongation, and calls for further laboratory and field research to enable model calibration and validation.

Overall, the findings of this research indicate that the use of fuel alcohols blended with regular gasoline could result in increased risk of exposure to BTEX contaminants present in groundwater LNAPL spills. The preferential use of such alcohols has the potential to quickly deplete the groundwater and soil matrix of available electron acceptors resulting in adverse conditions for the natural attenuation of BTEX. However, the uncertainty associated with the processes involved in benzene plume elongation is significant. That, coupled with the diverse heterogeneous site conditions that characterize each spill scenario, indicates that this model should be used for qualitative assessment of the impacts of fuel alcohols. Such qualitative assessments can be useful to guide future research, groundwater protection and renewable energy policies, environmental

regulation, and aid regulatory agencies. Furthermore, the ability to compare and evaluate the processes modeled in GSIM individually, can provide a tool to asses possible enhanced natural attenuation and remediation schemes, thanks to the complexity allowed by the RT3D model. Further research is required on several aspects of this topic, particularly on validation of the behavior of fuel alcohol blends in the field, to use the GSIM model quantitatively with an adequate degree of accuracy.

10.Recommendations for Future Research

Research results presented in this dissertation shed important insight into the processes involved in the effects alcohols have on BTEX natural attenuation. However, significant areas of interest remain for future laboratory, field and modeling research, including:

- Consider complex vadose zone processes and transport, phase partitioning, source zone dynamics of fuel alcohol blends and capillary zone movement of alcohol. Particularly for high alcohol content fuel blends, these processes can have a significant impact on source zone dynamics and alcohol migration.

- There is a great need for complete sets of data characterizing fuel alcohol migration in the environment, either through field experiments or pilot-scale setups. Although some such data exists for E10 ethanol blends, novel alcohols like butanol have been poorly characterized. Validation of this model with such data is an important step for future research.

- Although an alternative solution method was presented in this dissertation to achieve faster computational times on the GSIM module, the need still exists to develop a better, more accurate solver to handle the stiff conditions these simulation setups impose. A significant effort is required for this goal, as it

requires changing the code for RT3D. In our work, we have limited ourselves to working with the external module options RT3D provides.

- On the last chapter of this dissertation we considered iron(III)-reducing conditions, where iron(III) is present in immobile form in the soil matrix. It would be important to evaluate the effect of possible dissolved metals on degradation processes and as remediation schemes (enhanced reduction processes).

- The GSIM module is capable of calculating formation of byproducts of reactions, as it was coded originally. We have opted to leave such complex processes outside of this dissertation work. However, future work with this model could focus on formation of metabolic products like methane, acetate, volatile fatty acids, etc, that can be useful for field data comparison and for dynamic changing degradation conditions within the plume, including pH variations. These byproducts can also have potential aesthetic impacts, like odor and groundwater taste, that might be important to monitor.

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APPENDICES

Appendix I– Source Zone Dissolution Spreadsheet

Equations presented in sections 3.4 and 3.5 were implemented into a spreadsheet that calculates the depletion over time of a source LNAPL given its starting mass. Equations 24 and 29 are used to calculate the mass flow from the LNAPL phase to the groundwater and the atmosphere, based on the physicochemical properties of the LNAPL constituents. This results in decreasing concentrations at the interface between groundwater and LNAPL, that are used as inputs to the GSIM model simulations in this thesis.

Figures (a) to (c) show the input page of the spreadsheet, with graphical results for E0, E10 and E85 cases. Values in red are required from the user and are usually obtained from the literature. The spreadsheet will calculate intermediate values and return results (in green). Some important information of the spill the spreadsheet gives: total mass dissolved, total mass volatilized, time to source zone depletion, depletion rates, NAPL volume, NAPL mass, molar fraction composition of blend.

Volatilization can be activated/deactivated at will, using a simple binary switch in the spreadsheet. Figure (d) shows the spreadsheet for E10 without volatilization.

Figure (e) shows an example of raw data output, to be used in RT3D as transient simulation inputs.

Figure (a) – E0 Input Sheet

Source Zone Dissolution Spreadsheet

In Red - User Input Parameters

In Green - Model Outputs

Chemical Properties	Fuel Alcohol		Benzene		TEX	
Density	0.79	g/cc	0.87	g/cc	0.86	g/cc
MW	46.07	g/mol	78.11	g/mol	101.51	g/mol
Water Diffusivity	1.26E-05	cm^2/s	9.81E-06	cm^2/s	8.33E-06	cm^2/s
Air Diffusivity	0.122	cm^2/s	0.091	cm^2/s	0.073	cm^2/s
Vapor Pressure	0.112	atm	0.135	atm	0.024	atm

Fuel Alcohol Fraction in Organic Phase = 0.00%

Total Moles					Molar Fraction			
Fuel Alcohol	Benzene	TEX	Other	Total	Fuel Alcohol	Benzene	TEX	Other
0.0000	0.1645	1.7379	7.2557	9.1581	0.0000	0.0180	0.1898	0.7923

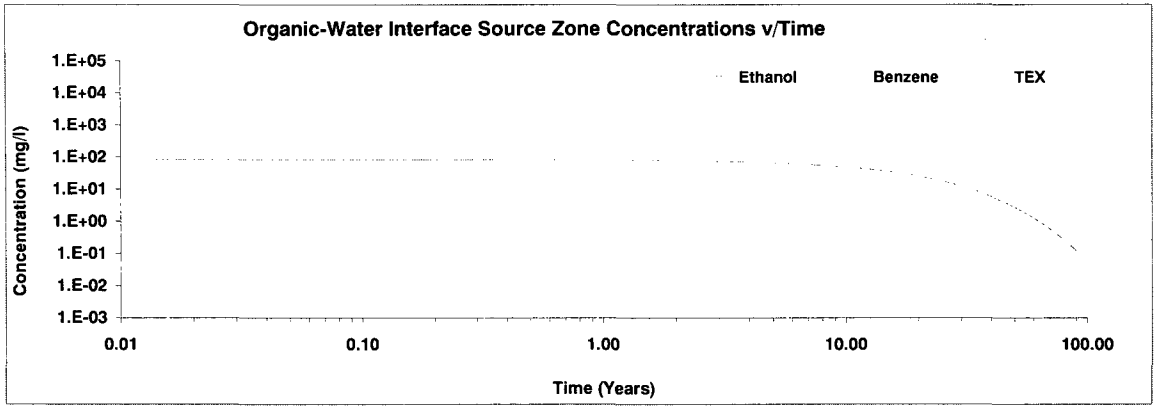
Spill Characteristics		
Depth (cm) (< max)	5.0	17.9
Width (m)	4.0	
Length (m)	4.0	
Total NAPL Mass (Kg)	82	
NAPL Density (g/cc)	0.73	
Volume (l)	419	
NAPL Vol (l)	113.10	

Hydrology	
I (m)	0.669
Kh (m/d)	9.000
i	0.003
q (m/d)	0.027
v (m/d)	0.090
Dt (days)	5.000

Benzene Linear/Log-linear Model	
Ci*W (Solubility) (Ben)	1780.00
γio (Activity) (Ben)	1.41
Ci*βγio (Ben)	4420.00
β (Ben)	0.27
Ci*cfio/xio (Ben)	963000.00
Ci*W (Solubility) (TEX)	282.00
γio (Activity) (TEX)	1.57
Ci*βγio (TEX)	761.00
β (TEX)	0.21
Ci*cfio/xio (TEX)	1156333.00

Volatilization	Activated?	1
Theta_g	0.27	
Theta_wr	0.030	
Ca (mg/l)	0	
d (m)	9.144	

Adimensional Henry's Constant	
Kh (Fuel Alcohol)	3.90E-04
Kh (Benzene)	2.96E-01
Kh (TEX)	3.64E-01



Results	
Total Mass Dissolved	
Ethanol (Kg)	0.00
Benzene (Kg)	0.50
TEX (Kg)	6.25
Total Mass Volatilized	
Ethanol (Kg)	0.00
Benzene (Kg)	0.96
TEX (Kg)	13.68
Time to Depletion (< MCL)	
5 Ethanol (years)	0.01
6750 Benzene (years)	18.49
TEX (years)	0.00
Depletion Rates	
Ethanol (Kg/y)	0.00
Benzene (Kg/y)	0.08
TEX (Kg/y)	#DIV/0!
Initial Fringe Concentrations	
Ethanol (mg/l)	0.00
Benzene (mg/l)	45.09
BTEX (mg/l)	84.02
Initial Cell Concentrations	
Ethanol (mg/l)	0.00
Benzene (mg/l)	22.55
BTEX (mg/l)	42.01

Soil Poperties	
Type	Fine Sand
Soil Grain Size (mm)	0.08
Sow (dyne/cm)	25.60
Porosity	0.300
Effective Porosity	0.27

Figure (b) - E10 Input Sheet

Source Zone Dissolution Spreadsheet

In Red - User Input Parameters

In Green - Model Outputs

Chemical Properties					
	Fuel Alcohol		*Benzene*		*TEX*
Density	0.79 g/cc	Density	0.87 g/cc	Density	0.86 g/cc
MW	46.07 g/mol	MW	78.11 g/mol	MW	101.51 g/mol
Water Diffusivity	1.26E-05 cm^2/s	Water Diffusivity	9.81E-06 cm^2/s	Water Diffusivity	8.33E-06 cm^2/s
Air Diffusivity	0.122 cm^2/s	Air Diffusivity	0.091 cm^2/s	Air Diffusivity	0.073 cm^2/s
Vapor Pressure	0.112 atm	Vapor Pressure	0.135 atm	Vapor Pressure	0.024 atm

Fuel Alcohol Fraction in Organic Phase =	10.00%			
Total Moles				
Fuel Alcohol	Benzene	TEX	Other	Total
1.7148	0.1481	1.5641	6.5301	9.9571

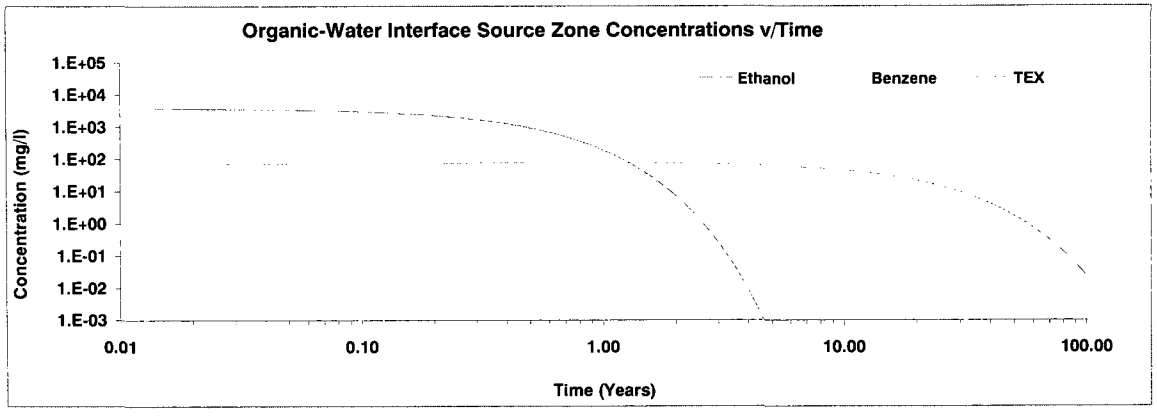
Molar Fraction			
Fuel Alcohol	Benzene	TEX	Other
0.1722	0.0149	0.1571	0.6558

Spill Characteristics		
Depth (cm) (< max)	5.0	17.9
Width (m)	4.0	
Length (m)	4.0	
Total NAPL Mass (Kg)	82	
NAPL Density (g/cc)	0.73	
Volume (l)	419	
NAPL Vol (l)	113.10	

Hydrology	
I (m)	0.669
Kh (m/d)	9.000
i	0.003
q (m/d)	0.027
v (m/d)	0.090
Dt (days)	5.000

Benzene Linear/Log-linear Model	
Ci*W (Solubility) (Ben)	1780.00
γio (Activity) (Ben)	1.41
Ci*βγio (Ben)	4420.00
β (Ben)	0.27
Ci*cfio/xio (Ben)	963000.00
Ci*W (Solubility) (TEX)	282.00
γio (Activity) (TEX)	1.57
Ci*βγio (TEX)	761.00
β (TEX)	0.21
Ci*cfio/xio (TEX)	1156333.00

Volatilization	Activated?	1		
Theta_g	0.27	*Adimensional Henry's Constant*		
Theta_wr	0.030	Kh (Fuel Alcohol)	3.90E-04	
Ca (mg/l)	0	Kh (Benzene)	2.96E-01	
d (m)	9.144	Kh (TEX)	3.64E-01	



Results	
Total Mass Dissolved	
Ethanol (Kg)	7.93
Benzene (Kg)	0.45
TEX (Kg)	5.63
Total Mass Volatilized	
Ethanol (Kg)	1.00
Benzene (Kg)	0.86
TEX (Kg)	12.32
Time to Depletion (< MCL)	
1525 Ethanol (years)	4.18
6095 Benzene (years)	16.70
TEX (years)	0.00
Depletion Rates	
Ethanol (Kg/y)	2.14
Benzene (Kg/y)	0.08
TEX (Kg/y)	#DIV/0!
Initial Fringe Concentrations	
Ethanol (mg/l)	3950.00
Benzene (mg/l)	37.85
BTEX (mg/l)	70.73
Initial Cell Concentrations	
Ethanol (mg/l)	1975.00
Benzene (mg/l)	18.93
BTEX (mg/l)	35.37

Soil Poperties	
Type	Fine Sand
Soil Grain Size (mm)	0.08
Sow (dyne/cm)	25.60
Porosity	0.300
Effective Porosity	0.27

Figure (c) – E85 Input Sheet

Source Zone Dissolution Spreadsheet

In Red - User Input Parameters

In Green - Model Outputs

Chemical Properties

	Fuel Alcohol		*Benzene*		*TEX*	
Density	0.79	g/cc	0.87	g/cc	0.86	g/cc
MW	46.07	g/mol	78.11	g/mol	101.51	g/mol
Water Diffusivity	1.26E-05	cm^2/s	9.81E-06	cm^2/s	8.33E-06	cm^2/s
Air Diffusivity	0.122	cm^2/s	0.091	cm^2/s	0.073	cm^2/s
Vapor Pressure	0.112	atm	0.135	atm	0.024	atm

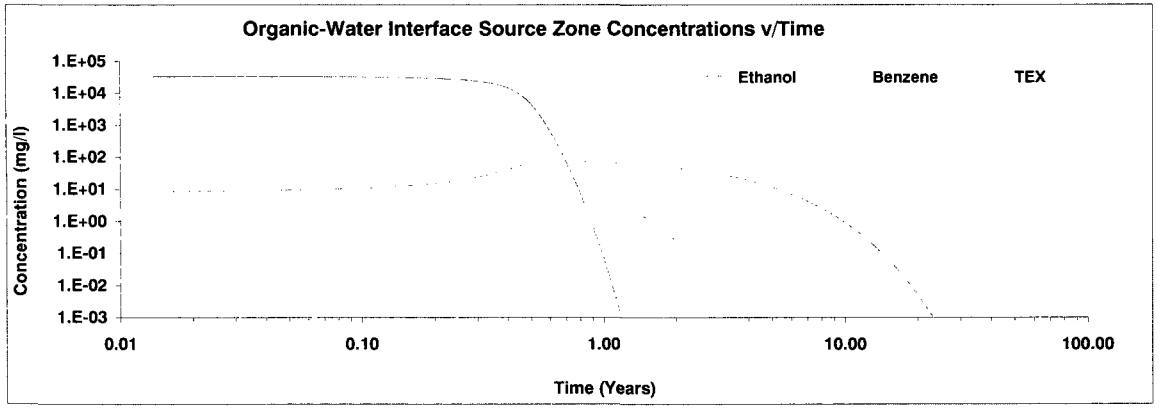
Fuel Alcohol Fraction in Organic Phase = 85.00%

Total Moles					*Molar Fraction*			
Fuel Alcohol	Benzene	TEX	Other	Total	Fuel Alcohol	Benzene	TEX	Other
14.5756	0.0247	0.2607	1.0884	15.9494	0.9139	0.0015	0.0163	0.0682

Spill Characteristics			Hydrology	
Depth (cm) (< max)	5.0	17.9	l (m)	0.669
Width (m)	4.0		Kh (m/d)	9.000
Length (m)	4.0		i	0.003
Total NAPL Mass (Kg)	82		q (m/d)	0.027
NAPL Density (g/cc)	0.73		v (m/d)	0.090
Volume (l)	419		Dt (days)	5.000
NAPL Vol (l)	113.10			

Benzene Linear/Log-linear Model	
Ci*W (Solubility) (Ben)	1780.00
γio (Activity) (Ben)	1.41
Ci*βγio (Ben)	4420.00
β (Ben)	0.27
Ci*cfio/xio (Ben)	963000.00
Ci*W (Solubility) (TEX)	282.00
γio (Activity) (TEX)	1.57
Ci*βγio (TEX)	761.00
β (TEX)	0.21
Ci*cfio/xio (TEX)	1156333.00

Volatilization	Activated?	1		
Theta_g	0.27		*Adimensional Henry's Constant*	
Theta_wr	0.030		Kh (Fuel Alcohol)	3.90E-04
Ca (mg/l)	0		Kh (Benzene)	2.96E-01
d (m)	9.144		Kh (TEX)	3.64E-01



Results	
Total Mass Dissolved	
Ethanol (Kg)	70.04
Benzene (Kg)	0.08
TEX (Kg)	0.94
Total Mass Volatilized	
Ethanol (Kg)	5.90
Benzene (Kg)	0.14
TEX (Kg)	2.05
Time to Depletion (< MCL)	
410 Ethanol (years)	1.12
1130 Benzene (years)	3.10
TEX (years)	9.79
Depletion Rates	
Ethanol (Kg/y)	67.61
Benzene (Kg/y)	0.07
TEX (Kg/y)	0.31
Initial Fringe Concentrations	
Ethanol (mg/l)	33575.00
Benzene (mg/l)	4.35
BTEX (mg/l)	8.28
Initial Cell Concentrations	
Ethanol (mg/l)	16787.50
Benzene (mg/l)	2.17
BTEX (mg/l)	4.14

Soil Poperties	
Type	Fine Sand
Soil Grain Size (mm)	0.08
Sow (dyne/cm)	25.60
Porosity	0.300
Effective Porosity	0.27

Figure (d) – E10 Input Sheet without volatilization

Source Zone Dissolution Spreadsheet

In Red - User Input Parameters

In Green - Model Outputs

Chemical Properties

	Fuel Alcohol		*Benzene*		*TEX*	
Density	0.79	g/cc	0.87	g/cc	0.86	g/cc
MW	46.07	g/mol	78.11	g/mol	101.51	g/mol
Water Diffusivity	1.26E-05	cm^2/s	9.81E-06	cm^2/s	8.33E-06	cm^2/s
Air Diffusivity	0.122	cm^2/s	0.091	cm^2/s	0.073	cm^2/s
Vapor Pressure	0.112	atm	0.135	atm	0.024	atm

Fuel Alcohol Fraction in Organic Phase = 10.00%

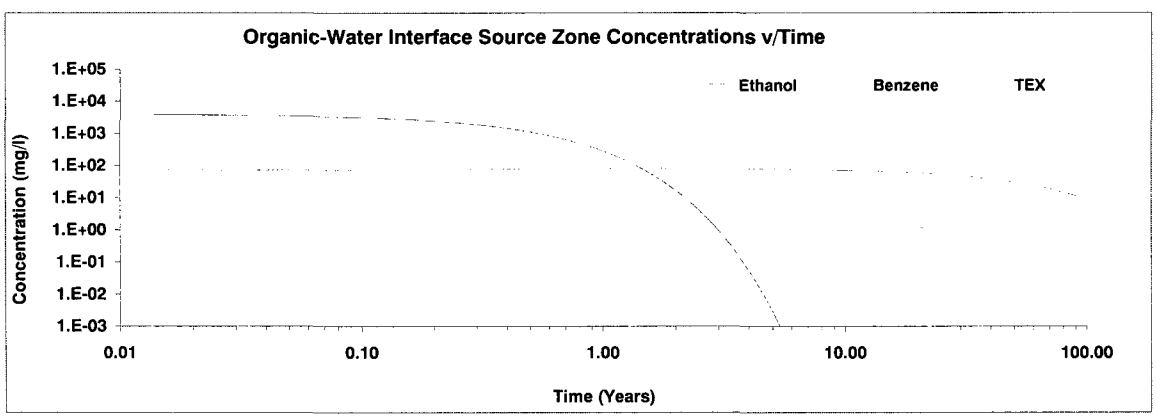
Total Moles					*Molar Fraction*			
Fuel Alcohol	Benzene	TEX	Other	Total	Fuel Alcohol	Benzene	TEX	Other
1.7148	0.1481	1.5641	6.5301	9.9571	0.1722	0.0149	0.1571	0.6558

Spill Characteristics		
Depth (cm) (< max)	5.0	17.9
Width (m)	4.0	
Length (m)	4.0	
Total NAPL Mass (Kg)	82	
NAPL Density (g/cc)	0.73	
Volume (l)	419	
NAPL Vol (l)	113.10	

Hydrology	
I (m)	0.669
Kh (m/d)	9.000
i	0.003
q (m/d)	0.027
v (m/d)	0.090
Dt (days)	5.000

Benzene Linear/Log-linear Model	
Ci*W (Solubility) (Ben)	1780.00
γio (Activity) (Ben)	1.41
Ci*βγio (Ben)	4420.00
β (Ben)	0.27
Ci*cfio/xio (Ben)	963000.00
Ci*W (Solubility) (TEX)	282.00
γio (Activity) (TEX)	1.57
Ci*βγio (TEX)	761.00
β (TEX)	0.21
Ci*cfio/xio (TEX)	1156333.00

Volatilization	Activated?	0	
Theta_g	0.27	*Adimensional Henry's Constant*	
Theta_wr	0.030	Kh (Fuel Alcohol)	3.90E-04
Ca (mg/l)	0	Kh (Benzene)	2.96E-01
d (m)	9.144	Kh (TEX)	3.64E-01



	Results	
	Total Mass Dissolved	
	Ethanol (Kg)	8.93
	Benzene (Kg)	1.31
	TEX (Kg)	16.46
	Total Mass Volatilized	
	Ethanol (Kg)	0.00
	Benzene (Kg)	0.00
	TEX (Kg)	0.00
	Time to Depletion (< MCL)	
1760	Ethanol (years)	4.82
17855	Benzene (years)	48.92
	TEX (years)	0.00
	Depletion Rates	
	Ethanol (Kg/y)	1.85
	Benzene (Kg/y)	0.03
	TEX (Kg/y)	#DIV/0!
	Initial Fringe Concentrations	
	Ethanol (mg/l)	3950.00
	Benzene (mg/l)	37.85
	BTEX (mg/l)	70.73
	Initial Cell Concentrations	
	Ethanol (mg/l)	1975.00
	Benzene (mg/l)	18.93
	BTEX (mg/l)	35.37

Soil Poperties	
Type	Fine Sand
Soil Grain Size (mm)	0.08
Sow (dyne/cm)	25.60
Porosity	0.300
Effective Porosity	0.27

Ethanol File			Benzene File			TEX File		
	0.0	1975.000		0.0	18.926		0.0	35.366
	5.0	1914.121		5.0	18.994		5.0	35.551
	10.0	1854.714		10.0	19.059		10.0	35.732
	15.0	1796.768		15.0	19.121		15.0	35.909
	20.0	1740.269		20.0	19.181		20.0	36.082
	25.0	1685.202		25.0	19.239		25.0	36.251
	30.0	1631.552		30.0	19.294		30.0	36.416
	35.0	1579.301		35.0	19.346		35.0	36.578
	40.0	1528.431		40.0	19.396		40.0	36.735
	45.0	1478.924		45.0	19.443		45.0	36.888
	50.0	1430.759		50.0	19.488		50.0	37.038
	55.0	1383.917		55.0	19.531		55.0	37.184
	60.0	1338.377		60.0	19.571		60.0	37.326
	65.0	1294.116		65.0	19.608		65.0	37.464
	70.0	1251.112		70.0	19.644		70.0	37.598
	75.0	1209.344		75.0	19.677		75.0	37.729
	80.0	1168.786		80.0	19.708		80.0	37.856
	85.0	1129.416		85.0	19.736		85.0	37.980
	90.0	1091.211		90.0	19.763		90.0	38.100
	95.0	1054.145		95.0	19.787		95.0	38.216
	100.0	1018.196		100.0	19.809		100.0	38.329
	105.0	983.338		105.0	19.830		105.0	38.438
	110.0	949.547		110.0	19.848		110.0	38.545
	115.0	916.798		115.0	19.864		115.0	38.648
	120.0	885.068		120.0	19.878		120.0	38.747
	125.0	854.332		125.0	19.891		125.0	38.844
	130.0	824.566		130.0	19.901		130.0	38.937
	135.0	795.745		135.0	19.910		135.0	39.028
	140.0	767.846		140.0	19.917		140.0	39.115
	145.0	740.844		145.0	19.922		145.0	39.199
	150.0	714.717		150.0	19.926		150.0	39.281
	155.0	689.441		155.0	19.928		155.0	39.360
	160.0	664.993		160.0	19.928		160.0	39.436
	165.0	641.350		165.0	19.927		165.0	39.510
	170.0	618.491		170.0	19.925		170.0	39.580
	175.0	596.392		175.0	19.921		175.0	39.649
	180.0	575.033		180.0	19.915		180.0	39.715
	185.0	554.391		185.0	19.909		185.0	39.778
	190.0	534.447		190.0	19.901		190.0	39.839
	195.0	515.179		195.0	19.891		195.0	39.898
	200.0	496.568		200.0	19.881		200.0	39.955
	205.0	478.593		205.0	19.869		205.0	40.010
	210.0	461.235		210.0	19.856		210.0	40.062
	215.0	444.476		215.0	19.842		215.0	40.112
	220.0	428.297		220.0	19.827		220.0	40.161
	225.0	412.680		225.0	19.811		225.0	40.207
	230.0	397.607		230.0	19.794		230.0	40.252
	235.0	383.061		235.0	19.776		235.0	40.295
	240.0	369.025		240.0	19.757		240.0	40.336
	245.0	355.483		245.0	19.737		245.0	40.375
	250.0	342.418		250.0	19.716		250.0	40.413

Figure (e) – 250 days raw data output from spreadsheet.

Appendix II – GSIM FORTRAN Source Code

Reactions file:

```
!
!     General Substrate Interaction Module for RT3D v2.0
!     Rice University
!     October 2009
!
      SUBROUTINE Rxns(NCOMP,nvrxndata,jmain,imain,kmain,y,dydt,
     + poros,rhob,reta,rc,nlay,nrow,ncol,vrc)
!
      INCLUDE 'PARAM.INC'
!
      PARAMETER (MAXNOB=MAXBIO-MAXBS+1)
      PARAMETER (MAXBEQ=MAXBIO+MAXBS*(MAXNOB+3))
!
      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
!
      COMMON DENBIO(MAXBIO)
      COMMON /BIOCALC/ BIOMIN(MAXBS)
      COMMON /BIODAT/ AKA(MAXMET),AKN(MAXMET,MAXNOB),AKS(MAXMET),
     + BRMAX(MAXMET),BRMAXB(MAXMET),BVOLMX,COSOL(maxbio),
     + BSIHB(MAXMET,MAXNOB),CBIOMN(MAXBIO),CMIN,Cfract(maxbio),
     + ENDOGB(MAXBS),FEA(MAXMET),FN(MAXMET,MAXNOB),TOX(maxbio),
     + FP(MAXMET,MAXNOB),FPABIO(MAXBIO,MAXBIO),DECAY(MAXBIO),
     + RCOL(MAXBS),YXS(MAXMET),ICSUB(MAXMET,MAXNOB),
     + IDMET(MAXBIO,MAXBIO,MAXBIO),IPABIO(MAXBIO,MAXBIO),
     + IDECAY(MAXBIO),NCOMPS(MAXMET),NIHB(MAXMET),NNUT(MAXMET),
     + NPABIO(MAXBIO),NPROD(MAXMET),NARTOT
      COMMON /BIOIDX/ IMSUB(MAXMET),IMEA(MAXMET),IMBS(MAXMET),
     + IHB(MAXMET,MAXNOB),IPR(MAXMET,MAXNOB),INUT(MAXMET,MAXNOB),
     + IKCB(MAXBIO),IBIOC(MAXBIO),IBS(MAXBS),IMSUB2(MAXMET),
     + IORG(maxbio),INCIHB(maxbio),IMFD(maxbio)
      COMMON /BIORD/ IBKIN,IBNONB,NBC,NBS,NBCNOB,NBIOEQ,NRLIM,NMET,
     + NBCAQ,NBCNAQ,IBINAQ,IBFNAQ,IBIAQ,NAPTOT
      COMMON zero, one
!
!     List of calling arguments
!     NCOMP - Total number of components
!     nvrxndata - Total number of variable reaction parameters to be
input via RCT file
!     J, I, K - node location (used if reaction parameters are
spatially variable)
!     y - Concentration value of all component at the node [array
variable y(NCOMP)]
!     dydt - Computed RHS of your differential equation [array variable
dydt(NCOMP)]
!     poros - porosity of the node
!     reta - Retardation factor [ignore dummy reta values of immobile
species]
!     rhob - bulk density of the node
!     rc - Stores spatially constant reaction parameters (can dimension
upto 100 values)
!     nlay, nrow, ncol - Grid size (used only for dimensioning
purposes)
!     vrc - Array variable that stores spatially variable reaction
parameters
```

```
!
      !MS$ATTRIBUTES DLLEXPORT :: rxns
      INTEGER ncol,nrow,nlay
      INTEGER NCOMP,nvrxndata,j,i,k,i2
      INTEGER, SAVE :: First_time=1
      DOUBLE PRECISION y,dydt,dydt2,poros,rhob,reta,reta2,bio_f,dydt3
      DOUBLE PRECISION rc,vrc,dydt_BIO,rmonodf,TOTBIO,dydt_EA,RMONOD2,
     + dydt_SUB,biosat,biosat2,ytemp,bio_grow,fbio(MAXBEQ),total_bio
      DIMENSION y(NCOMP),dydt(NCOMP),dydt2(NCOMP),rc(100),dydt3(NCOMP),
     + dydt_BIO(MAXMET),rmonodf(MAXMET),dydt_EA(MAXMET),reta2(NCOMP),
     + dydt_SUB(MAXMET),TOTBIO(NCOMP,NCOMP),ytemp(NCOMP),RMONOD2(NCOMP)
      DIMENSION vrc(ncol,nrow,nlay,nvrxndata),reta(1)
      DOUBLE PRECISION TOC,TOC2,fncihb(MAXBEQ),RBIOMB,RMONOD,MAX_BIO,
     + TOTAL_TIME,ELAPSED_TIME,TIME_STEP,VELOCITY,CHI,fmfd(MAXBEQ)
!
      IF (First_time .EQ. 1) THEN
       write(*,*)
       write(*,*) '************************************************'
       write(*,*) "General Substrate Interaction Module - Fast Version
0.9"
       write(*,*) "Release 13 Thursday 2009"
       write(*,*) '************************************************'
       call bioread(NCOMP)
!
!     reset First_time to skip this block later
!
       First_time = 0
      END IF
!
      TOTAL_TIME = 0.2
      TIME_STEP = TOTAL_TIME/3.1
      ELAPSED_TIME = 0
      VELOCITY = 0.027
      DO WHILE (ELAPSED_TIME .LT. TOTAL_TIME)
!     Assign or compute the values of new variables, if required*
!     Differential Reaction Equations*
!
      DO i=1,NCOMP
       dydt2(i)=0
       dydt3(i)=0
       dydt(i)=0
       fmfd(i)=1
       fncihb(i)=1
       fbio(i)=1
       reta2(i)=1
       RMONOD2(i)=0
      END DO
!
!     ABIOTIC REACTIONS AND ENDOGENEOUS DECAY
!
      IF (NARTOT.NE.0) THEN
       DO i = 1,NCOMP
        IF(DECAY(i).NE.0) THEN
         dydt2(i) = dydt2(i)-DECAY(i)*y(i)
         IF(NPABIO(i).NE.0) THEN
!
!     PRODUCT GENERATION FROM DECAY REACTIONS
```

```
!
          DO J=1,NPABIO(i)
           dydt2(IPABIO(i,j)) = dydt2(IPABIO(i,j)) +
     +       DECAY(i)*y(i)*FPABIO(i,j)
           END DO
         END IF
        END IF
        dydt3(i)=dydt2(i)
       END DO
      END IF
!
!     Skip biodegradation reactions if there is only abiotic DECAY
!
      IF(nmet.NE.0) THEN
!
!     ATTACHED BIOMASS BIODEGRADATION - NO MASS TRANSFER
!
!     THIS SECTION FOR BIODEGRADATION BY ATTACHED BIOMASS WHEN THERE IS
!     NO MASS TRANSFER RESISTANCE
!
!     Calculate TOC for MFD term
!
       TOC = 0
       DO i=1,NCOMP
        IF(iorg(i).NE.0) THEN
         TOC = TOC + y(i)*cfract(i)
        END IF
       END DO
!
       IF(TOC.EQ.0) TOC = 1
!
      DO i=(NBC-NBS+1),NBC
       IF(y(i).lt.cbiomn(i)) THEN
        y(i) = cbiomn(i)
       END IF
      END DO
!
!     Calculate biomass saturation for use in limiting biomass growth
!
       biosat = 0
       DO i=(NBC-NBS+1),NBC
        biosat = biosat + y(i)
       END DO
       biosat=biosat/(10**5)
!
!     CALCULATE BIODEGRADATION TERMS FOR EACH COMBINATION OF SUBSTRATE,
!     ELECTRON ACCEPTOR, AND BIOLOGICAL SPECIES.
!
       DO IMET=1,NMET
!
!     THE BIOLOGICAL RATE CONSTANTS AND THE ELECTRON ACCEPTOR HALF-
!     SATURATION COEFFICIENTS MUST BE READ INTO VARIABLES HERE SO THAT
THEY
!     DO NOT CHANGE WITH EACH LOOP SINCE THEY ARE MODIFIED BY
INHIBITION
!     TERMS.
!
```

```
        RBIOMB = BRMAXB(IMET)
        AKSC = AKS(IMET)
!
!     Modify biodegradaiton rate by f or f2 for each species
!     for MFD and non-competitive inhibition
!
!       fmfd(imet) = y(IMSUB2(IMET))*cfract(imsub(imet))/TOC
!
!       TOC2 = y(1)*cfract(1)
!     +  + y(IMSUB2(IMET))*cfract(imsub(imet))
!        IF(TOC2.EQ.0) TOC2 = 1
!
!       fncihb(imet) = y(IMSUB2(IMET))*cfract(imsub(imet))/TOC2
!
!        RBIOMB = BRMAXB(IMET)*
!     +  (fncihb(imet)**(incihb(IMSUB(IMET))))*
!     +  (fmfd(imet)**(imfd(IMSUB(IMET))))
!
      fbio(imet) = y(IMSUB2(IMET))*cfract(IMSUB2(IMET))/TOC
!
      RBIOMB = BRMAXB(IMET)*fbio(imet)**(incihb(IMSUB(IMET))
     +  + imfd(IMSUB(IMET)))
!
!     CALCULATE MODIFIED HALF-SATURATION CONSTANTS FOR EACH COMBINATION
OF
!     SUBSTRATE, ELECTRON ACCEPTOR AND BIOLOGICAL SPECIES FOR WHICH
THERE
!     IS SUBSTRATE COMPETITION.
!
        IF (NCOMPS(IMET).NE.0) THEN
         COMPKS = 0.
         DO INUM = 1,NCOMPS(IMET)
            COMPKS = COMPKS+y(ICSUB(IMET,INUM))/
     +       AKS(IDMET(ICSUB(IMET,INUM),IMEA(IMET),IMBS(IMET)))
         END DO
         AKSC = AKSC*(1+COMPKS)
        END IF
!
!     MODIFY MAXIMUM SUBSTRATE UTILIZATION RATE IF INHIBITED BY THE
!     SUBSTRATE OR ELECTRON ACCEPTOR (BIOMASS PHASE).
!
        IF (NIHB(IMET).NE.0) THEN
         DO I = 1,NIHB(IMET)
            IF (y(IHB(IMET,I)).GT.0) THEN
             RBIOMB = RBIOMB*BSIHB(IMET,I)/
     +        (BSIHB(IMET,I)+y(IHB(IMET,I)))
          END IF
         END DO
        END IF
!
!     Toxicological Inhibition
!
        DO I=1,NCOMP
         IF (TOX(I).GT.0) THEN
            RBIOMB = RBIOMB*(TOX(I)/(TOX(I)+y(I)))
         ENDIF
        END DO
```

```
!
!     MODIFY MAXIMUM SUBSTRATE UTILIZATION RATE IF INHIBITED BY
!     NUTRIENTS
!
         IF (NNUT(IMET).NE.0) THEN
          DO I = 1,NNUT(IMET)
             RBIOMB = RBIOMB*y(INUT(IMET,I))/(AKN(IMET,I)+
     +       y(INUT(IMET,I)))
          END DO
         END IF
!
!      CALCULATE THE MONOD/INHIBITION PORTION OF THE KINETIC EXPRESSION
!
         RMONOD = RBIOMB*y(IMBS(IMET))*
     +   y(IMSUB(IMET))/(AKSC+y(IMSUB(IMET)))*
     +   y(IMEA(IMET))/(AKA(IMET)+y(IMEA(IMET)))
!
!      CALCULATE THE DERIVATIVE TERM VALUES FOR THIS METABOLIC
COMBINATION
!
         DCBIOB = RMONOD/YXS(IMET)
         dydt2(IMSUB(IMET)) = dydt2(IMSUB(IMET))-DCBIOB
         dydt2(IMEA(IMET)) = dydt2(IMEA(IMET))-DCBIOB*FEA(IMET)
!
!      Calculate biological growth
!
         dydt2(IMBS(IMET)) = dydt2(IMBS(IMET))+
     +   RMONOD*(1-((biosat)/(bvolmx*poros)))
!
!      Backup of rates
         dydt_BIO(IMET) = RMONOD*(1-((biosat)/(bvolmx*poros)))
         dydt_SUB(IMET) = -(RMONOD/YXS(IMET))
         dydt_EA(IMET) = -(RMONOD/YXS(IMET))*FEA(IMET)
         RMONOD2(IMSUB(IMET))=RMONOD2(IMSUB(IMET))+dydt_BIO(IMET)
         RMONOD2(IMBS(IMET))=RMONOD2(IMBS(IMET))+dydt_SUB(IMET)
         RMONOD2(IMEA(IMET))=RMONOD2(IMEA(IMET))+dydt_EA(IMET)
!
!      PRODUCT GENERATION
!
         IF (NPROD(IMET).NE.0) THEN
          DO I = 1,NPROD(IMET)
             dydt2(IPR(IMET,I)) = dydt2(IPR(IMET,I))+DCBIOB*FP(IMET,I)
          END DO
         END IF
!
!      NUTRIENT CONSUMPTION
!
         IF (NNUT(IMET).NE.0) THEN
          DO I = 1,NNUT(IMET)
             dydt2(INUT(IMET,I)) = dydt2(INUT(IMET,I))-DCBIOB*FN(IMET,I)
          END DO
         END IF
        END DO
       END IF
!
!      Apply retardation factor to all reaction rate considering
cosolvency to benzene
```

```
!
      DO i=1,NCOMP
       IF (reta(i).GT.0) THEN
        reta2(i) = reta(i)
        IF((COSOL(i).GT.0).and.(i.GT.1)) THEN
         reta2(i) = ((reta2(i)-1)/
     +   (10**(COSOL(i)*(y(1)/1000000)/COSOL(1))))+1
        END IF
        dydt2(i)=dydt2(i)/reta2(i)
       END IF
      END DO
!
!
================================================================================
=========================================(1)
!     SUBSTRATE AND ELECTRON ACCEPTOR MASS BALANCE CHECK BLOCK
!
      bio_f=1
!
!     Check available substrate and electron acceptors for mass balance
      DO i=1,(NBC-NBS)
       IF ((y(i)+(dydt2(i)*TIME_STEP)).LT.0) THEN
        rmonodf(i)=ABS((((1-
VELOCITY*TIME_STEP)*y(i)*reta2(i)/TIME_STEP)
     +  - (dydt3(i)) ) / (RMONOD2(i)))
       ELSE
        rmonodf(i)=(1-VELOCITY*TIME_STEP)
       ENDIF
      END DO
!
!     Add original decay rates to new balanced Monod rates
      DO i=1,NCOMP
       dydt2(i)=dydt3(i)
      END DO
!
!     Choose the limiting factor from electron acceptors or substrates
and apply it
      DO i=1,NMET
       bio_f = MIN(rmonodf(IMSUB(i)), rmonodf(IMEA(i)))
!
       dydt2(IMBS(i)) = dydt2(IMBS(i))+(bio_f*dydt_BIO(i))
!
       dydt2(IMSUB(i)) = dydt2(IMSUB(i))+(bio_f*dydt_SUB(i))
!
       dydt2(IMEA(i)) = dydt2(IMEA(i))+(bio_f*dydt_EA(i))
!
      END DO
!
      DO i=1,NCOMP
        dydt2(i)=dydt2(i)/reta2(i)
      END DO
!
!     Recalculate final changes
      DO i=1,NCOMP
       y(i)=y(i)+(dydt2(i)*TIME_STEP)
       IF(y(i).lt.0) THEN
        y(i) = 0
```

```
        END IF
       END DO
!
       ELAPSED_TIME = ELAPSED_TIME+TIME_STEP
!
       IF (ELAPSED_TIME + TIME_STEP > TOTAL_TIME) THEN
        TIME_STEP = TOTAL_TIME - ELAPSED_TIME
       END IF
!
       END DO
!
       i=imain
       j=jmain
       k=kmain
!
       END
```

Input Read File:

```
!
!     General Substrate Interaction Module for RT3D
!     Rice University
!     October 2009
!
      SUBROUTINE BIOREAD(ncomp)
!     ------------------------------------------------------------
!     PURPOSE:  READ AND ECHO THE INPUT DATA FOR THE BIODEGRADATION
!               OPTION (IBIO=1)
!
!     ------------------------------------------------------------
!
      IMPLICIT DOUBLE PRECISION (A-H,O-Z)
!
      INCLUDE 'PARAM.INC'
!
      PARAMETER (MAXNOB=MAXBIO-MAXBS+1)
      PARAMETER (MAXBEQ=MAXBIO+MAXBS*(MAXNOB+3))
!
      COMMON DENBIO(MAXBIO)
      COMMON /BIOCALC/ BIOMIN(MAXBS)
      COMMON /BIODAT/ AKA(MAXMET),AKN(MAXMET,MAXNOB),AKS(MAXMET),
     +  BRMAX(MAXMET),BRMAXB(MAXMET),BVOLMX,COSOL(maxbio),
     +  BSIHB(MAXMET,MAXNOB),CBIOMN(MAXBIO),CMIN,Cfract(maxbio),
     +  ENDOGB(MAXBS),FEA(MAXMET),FN(MAXMET,MAXNOB),TOX(maxbio),
     +  FP(MAXMET,MAXNOB),FPABIO(MAXBIO,MAXBIO),decay(MAXBIO),
     +  RCOL(MAXBS),YXS(MAXMET),ICSUB(MAXMET,MAXNOB),
     +  IDMET(MAXBIO,MAXBIO,MAXBIO),IPABIO(MAXBIO,MAXBIO),
     +  Idecay(MAXBIO),NCOMPS(MAXMET),NIHB(MAXMET),NNUT(MAXMET),
     +  NPABIO(MAXBIO),NPROD(MAXMET),NARTOT
      COMMON /BIOIDX/ IMSUB(MAXMET),IMEA(MAXMET),IMBS(MAXMET),
     +  IHB(MAXMET,MAXNOB),IPR(MAXMET,MAXNOB),INUT(MAXMET,MAXNOB),
     +  IKCB(MAXBIO),IBIOC(MAXBIO),IBS(MAXBS),IMSUB2(MAXMET),
     +  IORG(maxbio),INCIHB(maxbio),IMFD(maxbio)
      COMMON /BIORD/ IBKIN,IBNONB,NBC,NBS,NBCNOB,NBIOEQ,NRLIM,NMET,
     +  NBCAQ,NBCNAQ,IBINAQ,IBFNAQ,IBIAQ,NAPTOT
      common zero, one
      DIMENSION ICOUNT(MAXBEQ)
      character(16) spname(maxbio),snamtmp
!
      OPEN (FILE='Bio.dat',UNIT=5,STATUS='OLD')
      OPEN (FILE='Bio_Echo.txt',UNIT=2,STATUS='unknown')
      write (*,*)
      write(*,*) "Entered BIOREAD subroutine"
      write(*,*)
      zero = 0.0d+0
      one = 1.0d+0
      READ (5,225)
      WRITE (2,230)
      READ (5,220)
      READ (5,*) BVOLMX
      write (2,301)
      write(2,300) BVOLMX
      READ (5,220)
      READ (5,*) NBC,NBS,NMET
      WRITE (2,299)
```

```
      WRITE (2,310) NBC,NBS,NMET
      READ (5,220)
!
!     CHECK DIMENSIONING IN SOURCE CODE
!
      IF ((NBC.GT.MAXBIO).OR.(NMET.GT.MAXMET).or.(NBS.GT.MAXBS)) THEN
        WRITE (2,*)
        WRITE (2,*) 'ERROR'
        WRITE (2,*)
        WRITE (2,*) 'SPECIFIED NUMBER OF BIODEGRADATION CONSTITUENTS,'
        WRITE (2,*) 'BIOLOGICAL SPECIES, OR METABOLIC COMBINATIONS'
        WRITE (2,*) 'EXCEEDS DIMENSIONS IN SOURCE CODE.'
        WRITE (2,*)
        write (2,*) 'MAXIMUM NUMBERS ARE:'
        write (2,*) 'BIODEGRADATION CONSTITUENTS = ',MAXBIO
        write (2,*) 'BIOLOGICAL SPECIES = ',MAXBS
        write (2,*) 'METABOLIC COMBINATIONS = ',MAXMET
        WRITE (2,*)
        WRITE (2,*) 'SOURCE CODE MUST BE RECOMPILED OR NUMBER OF'
        WRITE (2,*) 'SPECIES MUST BE REDUCED TO WITHIN THESE LIMITS.'
        WRITE (2,*)
        WRITE (2,*) 'FOR CODE RECOMPILATION, CONTACT:'
        WRITE (2,*) '  Groundwater Services, Inc.'
        WRITE (2,*) '  2211 Norfolk St., Suite 1000'
        WRITE (2,*) '  Houston, Texas 77098'
        WRITE (2,*) '  713-522-6300'
        WRITE (2,*) '  www.gsi-net.com'
        WRITE (2,*)
        WRITE (*,*)
        WRITE (*,*) 'ERROR'
        WRITE (*,*)
        WRITE (*,*) 'SPECIFIED NUMBER OF BIODEGRADATION CONSTITUENTS,'
        WRITE (*,*) 'BIOLOGICAL SPECIES, OR METABOLIC COMBINATIONS'
        WRITE (*,*) 'EXCEEDS DIMENSIONS IN SOURCE CODE.'
        WRITE (*,*)
        write (*,*) 'MAXIMUM NUMBERS ARE:'
        write (*,*) 'BIODEGRADATION CONSTITUENTS = ',MAXBIO
        write (*,*) 'BIOLOGICAL SPECIES = ',MAXBS
        write (*,*) 'METABOLIC COMBINATIONS = ',MAXMET
        WRITE (*,*)
        WRITE (*,*) 'SOURCE CODE MUST BE RECOMPILED OR NUMBER OF'
        WRITE (*,*) 'SPECIES MUST BE REDUCED TO WITHIN THESE LIMITS.'
        WRITE (*,*)
        WRITE (*,*) 'FOR CODE RECOMPILATION, CONTACT:'
        WRITE (*,*) '  Groundwater Services, Inc.'
        WRITE (*,*) '  2211 Norfolk St., Suite 1000'
        WRITE (*,*) '  Houston, Texas 77098'
        WRITE (*,*) '  713-522-6300'
        WRITE (*,*) '  www.gsi-net.com'
        WRITE (*,*)
        STOP
      ENDIF
!
!     Skip reading biological species parameters if there are none.
!
      if(nmet.NE.0) THEN
!
```

```
      DO i=1,NBS
        READ (5,*) KC,temp,temp1
        denbio(kc)=temp*1.0d+6
        cbiomn(kc)=temp1
        icount(i)=kc
      END DO
!
      READ (5,220)
      write (2,*) 'Biomass densities (mg/L)'
      write(2,*)
!
      DO i=1,nbs
        write(2,*) "Biomass ",icount(i)," = ",denbio(icount(i))
      END DO
!
      write (2,*)
      write (2,*) 'Minimum biomass concentration (mg/L)'
      write(2,*)
!
      DO i=1,nbs
       write(2,*) "Biomass ",icount(i)," = ",cbiomn(icount(i))
      END DO
!
      END IF
!
      BIOTIM = 0.0
      WRITE(2,*)
      WRITE(2,*) 'NUMBER OF BIODEGRADATION SPECIES = ',NBC
      WRITE(2,*) 'NUMBER OF BIOLOGICAL SPECIES =     ',NBS
      WRITE(2,*) 'NUMBER OF METABOLIC COMBINATIONS = ',NMET
      write(2,*)
!
!     INITIAL CONCENTRATIONS AND SPECIES INDENTIFICATION
!
      NBCNOB = 0
      NARTOT = 0
      NBTS = 0
      BTSAVG = 0.
      ITOTA = 0
!
      DO I = 1,ncomp
         NPABIO(I) = 0
         Idecay(I) = 0
         icount(i) = 0
         DO J = 1,ncomp
            IPABIO(I,J) = 0
            FPABIO(I,J) = 0.
         END DO
      END DO
      NBCNAQ = 0
      NBCAQ = 0
      NAPTOT = 0
      DO I = 1,NBC
         READ(5,*) KC,TEMP2,ITEMP3,itemp4,itemp5,
     +   itemp6,temp7,temp8,temp9,snamtmp
         icount(i)=kc
         decay(KC) = TEMP2
```

```
          NPABIO(KC) = ITEMP3
          iorg(kc)=itemp4
          incihb(kc)=itemp5
          imfd(kc)=itemp6
          cfract(kc) = temp7
          TOX(kc) = temp8
          COSOL(kc) = temp9
          spname(kc) = snamtmp
            IF (NPABIO(KC).NE.0) NAPTOT = NAPTOT+NPABIO(kc)
            IF(decay(KC).gt.0.) NARTOT = NARTOT+1
        END DO
!
!       CHECK DIMENSIONING IN SOURCE CODE
!
        IF (NBS.GT.MAXBS) THEN
          WRITE (2,*) 'CHECK DIMENSIONING OF MAXBS IN',
     +   ' PARAM.INC'
          STOP
        ENDIF
        WRITE (2,359)
        DO I=1,NBC
          WRITE (2,370) icount(i),decay(icount(i)),NPABIO(icount(i)),
     +     iorg(icount(i)),incihb(icount(i)),imfd(icount(i)),
     +     Cfract(icount(i)),TOX(icount(i)),COSOL(icount(i)),
     +     SPNAME(icount(i))
        END DO
!
!       INITIALIZE METABOLIC COMB. IDENTIFIER TO 0 FOR ALL COMBINATIONS
!
        DO I=1,MAXBIO
          DO J=1,MAXBIO
            DO L=1,MAXBIO
              IDMET(I,J,L)=0
              END DO
          END DO
        END DO
!
        DO IMET = 1,NMET
          BRMAX(IMET) = 0.
          BRMAXB(IMET) = 0.
          NCOMPS(IMET) = 0
          YXS(IMET) = 0.
          AKS(IMET) = 0.
          AKA(IMET) = 0.
          FEA(IMET) = 0.
          NIHB(IMET) = 0
          NPROD(IMET) = 0
          NNUT(IMET) = 0
          DO J = 1,ncomp
            FP(IMET,J) = 0.
          END DO
          DO J = 1,ncomp
            FN(IMET,J) = 0.
          END DO
          DO J = 1,ncomp
            BSIHB(IMET,J)=0.
          END DO
```

```
          DO I=1,ncomp
            ICSUB(IMET,I)=0
          END DO
        END DO
!
!       METABOLIC COMBINATION INFORMATION
!
!       Skip if all abiotic reactions
!
        if(nmet.NE.0) THEN
!
        READ (5,220)
        DO IMET=1,NMET
          READ (5,*) J,J2,K,L,BRMAXB(IMET),YXS(IMET),
     +     AKS(IMET),AKA(IMET),FEA(IMET)
          IMSUB(IMET)=J
            IMSUB2(IMET)=J2
          IMEA(IMET)=K
          IMBS(IMET)=L
        IDMET(J,K,L)=IMET
        END DO
        WRITE (2,322)
        WRITE (2,319)
        DO IMET = 1,NMET
        WRITE (2,320) IMSUB(IMET),IMSUB2(IMET),IMEA(IMET),IMBS(IMET),
     +  BRMAXB(IMET),YXS(IMET),AKS(IMET),
     +  AKA(IMET),FEA(IMET)
        END DO
!
!       FLAGS FOR COMPETITION, INHIBITION, PRODUCT GENERATION, NUTRIENTS,
!       COMETABOLISM.
!
        ITOTB = 0
        READ (5,220)
        DO I=1,NMET
          READ (5,*) J,K,L,ITEMP1,ITEMP2,ITEMP3,ITEMP4
          IMET=IDMET(J,K,L)
!
!       PRINT WARNING IF METABOLIC COMBINATION IS INVALID
!
        IF(IMET.EQ.0) THEN
          WRITE(2,*)
          WRITE(2,*) 'PROGRAM STOPPED.'
          WRITE(2,*) 'CHECK METABOLIC COMBINATIONS IN THE METABOLIC',
     +     ' FLAGS SECTION'
          STOP
        ENDIF
          NCOMPS(IMET)=ITEMP1
          NIHB(IMET)=ITEMP2
          NPROD(IMET)=ITEMP3
            ITOTB=ITOTB+NPROD(IMET)
          NNUT(IMET)=ITEMP4
        END DO
!
        WRITE (2,323)
        WRITE (2,324)
        DO IMET = 1,NMET
```

```
         WRITE (2,325) IMSUB(IMET),IMEA(IMET),
     +     IMBS(IMET),NCOMPS(IMET),NIHB(IMET),NPROD(IMET),
     +     NNUT(IMET)
          END DO
!
!      SUBSTRATE COMPETITION PARAMETERS
!
          ITOT = 0
          DO IMET = 1,NMET
             ITOT = ITOT+NCOMPS(IMET)
          END DO
!
          IF(ITOT.NE.0) THEN
!
!      REMINDER ABOUT ORDER OF INFO. IN THIS SECTION.
!
          WRITE(2,*)
          WRITE(2,*) '!!!REMINDER - METABOLIC COMBINATIONS FOR',
     +   ' SUBSTRATE COMPETITION ENTERED IN THE SECTION BELOW'
          WRITE(2,*) 'MUST BE LISTED IN THE SAME ORDER AS IN',
     +   ' THE METABOLIC COMBINATION MONOD PARAM. SECTION ABOVE'
          WRITE(2,*)
          WRITE(2,*) 'ALSO - COMPETING SUBSTRATES MUST BE BIODEGRADED',
     +   ' BY THE SAME '
          WRITE(2,*) 'BIOLOGICAL SPECIES USING THE SAME ELECTRON',
     +   ' ACCEPTOR.'
          DO IMET=1,NMET
            ICOUNT(IMET)=0
          END DO
!     NOTE: MUST BE ENTERED IN SAME ORDER AS METABOLIC COMBINATION INFO.
          READ (5,220)
          DO I=1,NMET
            IF(NCOMPS(I).NE.0) THEN
            READ (5,*) J,K,L,(ICOUNT(M),M=1,NCOMPS(I))
            IMET=IDMET(J,K,L)
!
!      PRINT WARNING IF METABOLIC COMBINATION IS INVALID
!
          IF(IMET.EQ.0) THEN
            WRITE(2,*)
            WRITE(2,*) 'PROGRAM STOPPED.'
            WRITE(2,*) 'CHECK METABOLIC COMBINATIONS IN THE SUBSTRATE',
     +      ' COMPETITION SECTION'
            STOP
          ENDIF
!
              DO INUM=1,NCOMPS(IMET)
                ICSUB(IMET,INUM)=ICOUNT(INUM)
                END DO
                END IF
          END DO
            WRITE (2,351)
          WRITE (2,349)
          DO IMET=1,NMET
             IF (NCOMPS(IMET).NE.0) THEN
             WRITE (2,350) IMSUB(IMET),IMEA(IMET),IMBS(IMET),
     +       (ICSUB(IMET,INUM),INUM=1,NCOMPS(IMET))
```

```
            END IF
          END DO
          END IF
!
!         INHIBITION CONSTANTS
!
          ITOT = 0
          DO IMET = 1,NMET
             ITOT = ITOT+NIHB(IMET)
          END DO
          IF(ITOT.NE.0) THEN
          DO IMET=1,NMET
            ICOUNT(IMET)=0
          END DO
          READ (5,220)
          DO I=1,ITOT
            READ (5,*) J,K,L,M,TEMP
            IMET=IDMET(J,K,L)
!
!         PRINT WARNING IF METABOLIC COMBINATION IS INVALID
!
          IF(IMET.EQ.0) THEN
            WRITE(2,*)
            WRITE(2,*) 'PROGRAM STOPPED.'
            WRITE(2,*) 'CHECK METABOLIC COMBINATIONS IN THE INHIBITION',
     +      ' SECTION'
            STOP
          ENDIF
!
            ICOUNT(IMET)=ICOUNT(IMET)+1
            IHB(IMET,ICOUNT(IMET))=M
            BSIHB(IMET,ICOUNT(IMET))=TEMP
          END DO
!
          WRITE (2,345)
          WRITE (2,339)
          DO IMET=1,NMET
            IF (NIHB(IMET).NE.0) THEN
              DO I=1,NIHB(IMET)
                WRITE (2,340) IMSUB(IMET),IMEA(IMET),
     +            IMBS(IMET),IHB(IMET,I),BSIHB(IMET,I)
              END DO
            END IF
          END DO
          END IF
!
!         PRODUCT GENERATION
!
          END IF
!
          IF(ITOTB.NE.0) THEN
          DO IMET=1,NMET
            ICOUNT(IMET)=0
          END DO
          IF(ITOTB.NE.0) THEN
!
!         READ INFORMATION FOR PRODUCTS OF BIOLOGICAL REACTIONS
```

```
!
      WRITE (2,365)
      WRITE (2,369)
      READ (5,220)
      DO I=1,ITOTB
        READ (5,*) J,K,L,M,TEMP
        IMET=IDMET(J,K,L)
!
!     CHECK VALIDITY OF METABOLIC COMBINATION
!
      IF(IMET.EQ.0) THEN
        WRITE(2,*)
        WRITE(2,*) 'CHECK METABOLIC COMBINATIONS IN THE SECTION ABOVE'
        STOP
      ENDIF
        ICOUNT(IMET)=ICOUNT(IMET)+1
        IPR(IMET,ICOUNT(IMET))=M
        FP(IMET,ICOUNT(IMET))=TEMP
      END DO
      END IF
      DO IMET=1,NMET
         IF (NPROD(IMET).NE.0) THEN
         DO I=1,NPROD(IMET)
           WRITE (2,380) IMSUB(IMET),IMEA(IMET),
     +       IMBS(IMET),IPR(IMET,I),
     +       FP(IMET,I)
         END DO
         END IF
      END DO
      IF(NAPTOT.NE.0) THEN
!
!     READ INFORMATION FOR PRODUCTS OF ABIOTIC REACTIONS
!
      READ (5,220)
      DO I=1,ncomp
        ICOUNT(I)=0
      END DO
      DO I=1,NAPTOT
        READ(5,*) J,K,TEMP
        ICOUNT(J)=ICOUNT(J)+1
        IPABIO(J,ICOUNT(J))=K
        FPABIO(J,ICOUNT(J))=TEMP
      END DO
      END IF
        WRITE (2,366)
        WRITE (2,367)
      DO I=1,ncomp
        IF(NPABIO(I).NE.0) THEN
        DO J=1,NPABIO(I)
          WRITE (2,368) I,IPABIO(I,J),FPABIO(I,J)
        END DO
        END IF
      END DO
      END IF
!
!     NUTRIENT LIMITATIONS
!
```

```
      ITOT = 0
      DO IMET = 1,NMET
         ITOT = ITOT+NNUT(IMET)
      END DO
      IF(ITOT.NE.0) THEN
      DO IMET = 1,NMET
         ICOUNT(IMET) = 0
      END DO
      READ (5,220)
      DO I = 1,ITOT
         READ (5,*) J,K,L,M,TEMP1,TEMP2
         IMET = IDMET(J,K,L)
!
!     PRINT WARNING IF METABOLIC COMBINATION IS INVALID
!
         IF (IMET.EQ.0) THEN
            WRITE(2,*)
            WRITE(2,*) 'PROGRAM STOPPED.'
            WRITE(2,*) 'CHECK METABOLIC COMBINATIONS IN THE NUTRIENT',
     +           ' LIMITATIONS SECTION'
            STOP
         ENDIF
         ICOUNT(IMET)=ICOUNT(IMET)+1
         INUT(IMET,ICOUNT(IMET))=M
         AKN(IMET,ICOUNT(IMET))=TEMP1
         FN(IMET,ICOUNT(IMET))=TEMP2
      END DO
      WRITE (2,385)
      WRITE (2,379)
      DO IMET = 1,NMET
        IF (NNUT(IMET).NE.0) THEN
           DO I = 1,NNUT(IMET)
             WRITE (2,340) IMSUB(IMET),IMEA(IMET),
     +             IMBS(IMET),INUT(IMET,I),
     +             AKN(IMET,I),FN(IMET,I)
           END DO
        END IF
      END DO
      END IF
      WRITE (2,360)
      write(*,*)
      write(*,*) "Exited BIOREAD subroutine"
      write (*,*)
!
220   FORMAT (//)
225   FORMAT (/////)
230   FORMAT (//'******************************************************'
     +   //'BIOLOGICAL DATA:'//)
300   FORMAT (/1X,'BVOLMX = ',T10,E15.5/)
301   format ('MAXIMUM FRACTION OF PORE SPACE OCCUPIABLE BY BIOMASS')
299   FORMAT(/'NUMBER OF BIODEGRADATION SPECIES, BIOLOGICAL SPECIES',
     +   ' NUMBER OF METABOLIC COMBINATIONS'/)
310   FORMAT(1X,'NBC= ',T10,I3/1X,'NBS= ',T10,I3/1X,'NMET= ',T10,I3/)
319   FORMAT (1X,/3X,'ISUB',T10,'ISUB2',T20,'IEA',T24,'IBS',T36,
     +   'BRMAXB',T48,'YXS',T60,'AKS',T72,'AKA',T84,'FEA'/)
320   FORMAT (1X,T5,I2,T10,I2,T20,I2,T24,I2,T36,E9.3,T48,E9.3,T60,
     +   E9.3,T72,E9.3,T84,E9.3)
```

```
321   FORMAT(/'BIOLOGICAL SPECIES PROPERTIES')
322   FORMAT(/'METABOLIC COMBINATION MONOD PARAMETERS')
323   FORMAT(/'METABOLIC COMBINATION KINETICS FLAGS')
324   FORMAT (1X,/3X,'ISUB',2X,'IEA',2X,'IBS',T20,'NCOMPS',T28,
     +   'NIHB',T36,'NPROD',T44,'NNUT',T52/)
325   FORMAT (1X,(T2,3I5,T19,I4,T27,I4,T35,I4,T43,I4,T51,I4))
330   FORMAT (1X,(T2,I5,8(3X,E9.3),2x,I3))
339   FORMAT (1X,/T5,'ISUB',T10,'IEA',T15,'IBS',T20,'IHB',
     +   T30,'BSIHB'/)
340   FORMAT (1X,(T3,4I5,3X,2(E9.3,8X)))
345   FORMAT(/'INHIBITING SPECIES AND INHIBITION CONSTANTS')
349   FORMAT (1X,/T4,'ISUB',T9,'IEA',T14,'IBS',T19,
     +   10X,'COMPONENT NUMBERS OF COMPETITIVE SUBSTRATES'/)
350   FORMAT (1X,(T2,3I5,10X,10I5))
351   FORMAT(/'COMPETING SUBSTRATES')
352   FORMAT (1X,'NBC= ',T20,I3/1X,'NBCNOB= ',T20,I3/
     +   1X,'IBNONB= ',T20,I3/)
355   FORMAT (1X,'RT3D COMPONENT INDEX',T35,'BIOD. COMP. INDEX'/)
356   FORMAT(1X,T14,I3,T40,I3)
359   FORMAT (1X,/'ABIOTIC DECAY AND REACTION DEFINITION FLAGS:'
     +   //'COMPONENT INDEX',T18,'ABIOTIC_DECAY_K',T36,
     +   'ABIOTIC_PRODUCTS',T56,'IORG',
     +   T64,'INCIHB',t73,'IMFD',t80,'CFRACT',t92,'TOX',t102,
     +   'COSOL', t114,'NAME'/)
365   FORMAT (/'BIODEGRADATION PRODUCTS AND STOICH. RATIO')
366   FORMAT (/'ABIOTIC PRODUCTS')
367   FORMAT (1X,/T5,' KC ',T10,'IPR',T15,'FPABIO'/)
368   FORMAT (1X,T6,I2,T10,I2,T14,E9.3)
370   FORMAT (1X,T5,I2,T20,E9.3,T42,I3,T56,I3,T65,i2,t73,i2,
     +  t77,E12.6,t88,E12.3,t98,E12.3,t114,A16)
369   FORMAT (1X,/T5,'ISUB',T10,'IEA',T15,'IBS',T20,'IPR',T29,'FP'/)
379   FORMAT (1X,/T5,'ISUB',T10,'IEA',T15,'IBS',T20,
     +   'INUT',T29,'AKN',T43,'FN'/)
380   FORMAT (1X,(T3,4I5,3X,2(E9.3,3X)))
385   FORMAT (/'NUTRIENT LIMITATION PARAMETERS')
389   FORMAT (1X,/T5,'ISUB',T10,'IEA',T15,'IBS',T25,
     +   'TC',T33,'IRLIM'/)
390   FORMAT (1X,(T3,3I5,3X,E9.3,3X,I3))
395   FORMAT (1X,T2,4I5,4(3X,E9.3))
360   FORMAT (1X,//'END OF BIOLOGICAL DATA',/

     +  '*****************************************************'/)
      RETURN
      END
```

Appendix III – Directory of electronic resources

Electronic media included with this thesis:

- **Source Zone Dissolution Spreadsheet**

  \GSIM Data\Souce_Zone.xls (Source Zone EXCEL file)

- **MODEL open source files**

  \GSIM Data\RT3D.zip (RT3D model Files)

  \GSIM Data\MODFLOW.zip (MODFLOW model Files)

  \GSIM Data\LNAST.zip (LNAST model files)

  \GSIM Data\HYDRO.zip (HYDRO_GEN model files)

- **GSIM Files**

  \GSIM Data\GSIM\Interface.zip (GSIM Visual Basic Files)

  \GSIM Data\GSIM\Rxns.zip (Rxns.dll versions)

- **Documentation**

  \GSIM Data\Manuals\GSIM Tutorial.pdf (GSIM module tutorial)

  \GSIM Data\Manuals\RT3D Manual.pdf (RT3D user manual)

  \GSIM Data\Manuals\MODFLOW Manual.pdf (MODFLOW user manual)

  \GSIM Data\Manuals\HYDRO_GEN Manual.pdf (HYDRO_GEN user manual)

  \GSIM Data\Manuals\Thesis Diego Gomez.pdf (This document)

- **Simulation Data**

\GSIM Data\DATA\C4.zip	(GMS simulation files chapter 4)
\GSIM Data\DATA\C5.zip	(GMS simulation files chapter 5)
\GSIM Data\DATA\C6.zip	(GMS simulation files chapter 6)
\GSIM Data\DATA\C7.zip	(GMS simulation files chapter 7)
\GSIM Data\DATA\C8.zip	(GMS simulation files chapter 8)

Appendix IV – GSIM Equations Spreadsheet Validation

A batch spreadsheet with the equations involved in the GSIM module was setup to calculate changes in substrate, electron acceptor and microbial populations without groundwater flow. Results from this spreadsheet were compared to simulations using GSIM/RT3D model.

Figure (a) shows comparison for Benzene, Oxygen and microbial populations for regular gasoline degradation.

Figure (b) shows comparison for Benzene, Oxygen, Ethanol and microbial populations for 10% ethanol gasoline blend degradation.

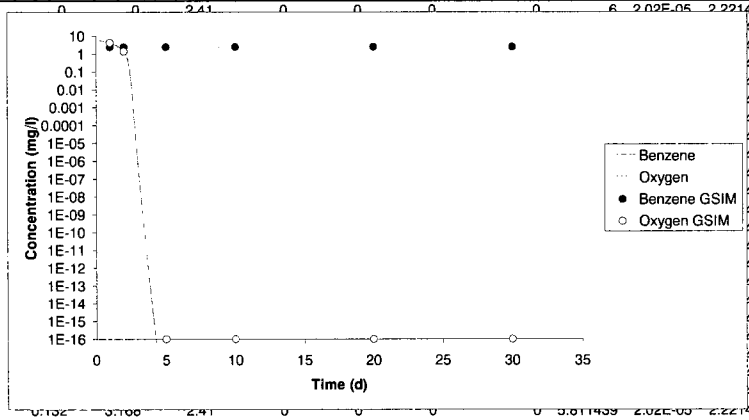
Benzene Baseline Validation

All units are mg, d, L.	IO	0.1	dt	0.006	Xaer_min	0.01
	n	0.3			Xan_min	0.001

Cells highlighted in yellow need to be named.

	Parameter																
Species	C_0	ui_Aer	ui_An	Yl_Aer	Yl_an	Kl_Aer	Kl_An	kl_ablo	El_Aer	El_An	b_Aer	b_An	Rho	MW	fOC	k_l_aer	k_l_an
B	2.41	1.83	0.18	0.39	0.12	3.53	11.48	0	2	0				78.11	0.921777	1.3292656	0.130662
T	0	8.4	0.31	1.18	0.33	0.013	1	0	0	0				92.15	0.9115572	547.58801	0.939394
EB	0	8.4	0.31	1.18	0.33	0.013	1	0	0	0				106.16	0.9042954	547.58801	0.939394
Z	0	8.4	0.31	1.18	0.33	0.013	1	0	0	0				106.2	0.9039548	547.58801	0.939394
Eth	0	11.04	1.1	0.93	0.04	12.75	0.9	0	1	0				46.06	0.5210595	0.9310563	30.55556
O	6					0.21											
N	2.02E-05						0.00E+00										
Xaer	0.376										0.2		1.00E+05				
Xan	0.0376											0.03	1.00E+05				

time (days)	time (hrs)	Substrate conc (mg/L) B	T	EB	Z	Eth	EA conc. (mg/L) O	N	Mass substrate as TOC (mgC/L) Bc	Tc	EBc	Zc	Ethc	Total Concentr Carbon Mass Fractions ST	fB	fT	fEB
0	0	2.41	0	0	0	0	6	2.02E-05	2.221482525	0	0	0	0	2.221482525	1	0	0
0.138	3.312	2.41	0	0	0	0	5.802565	2.02E-05	2.221482525	0	0	0	0	2.221482525	1	0	0



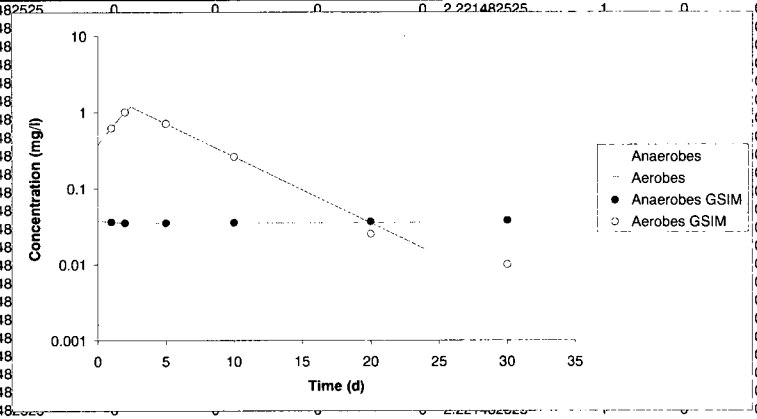


Figure (a) – Regular gasoline degradation spreadsheet

Figure (b) – Regular gasoline degradation spreadsheet

Benzene/Ethanol validation

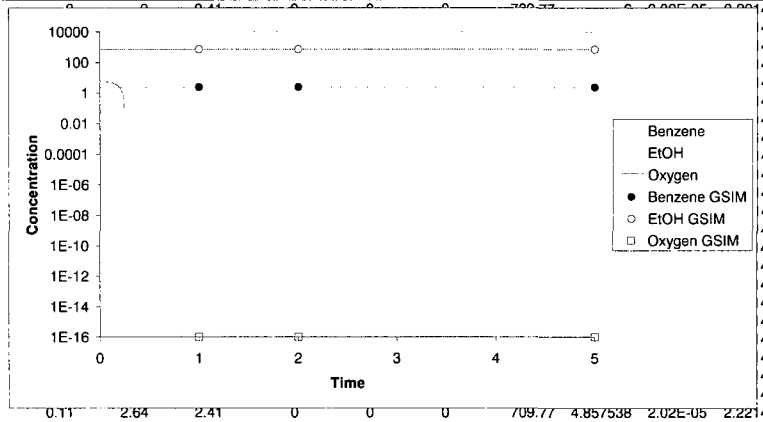
All units are mg, d, L.

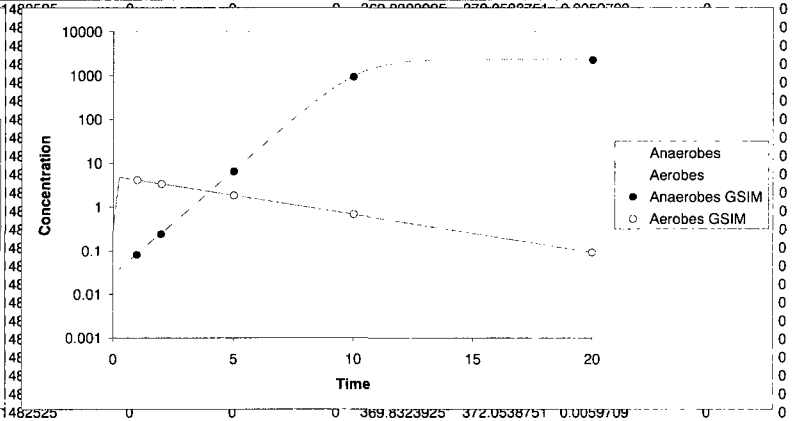
IO	0.1	dt	0.005	Xaer_min	0.01
n	0.3			Xan_min	0.001

Cells highlighted in yellow need to be named.

	Parameter																
Species	C_0	ul_Aer	ul_An	Yl_Aer	Yl_an	Kl_Aer	Kl_An	kl_abio	El_Aer	El_An	b_Aer	b_An	Rho	MW	fOC	k_l_aer	k_l_an
B	2.41	1.83	0.18	0.39	0.12	3.53	11.48	0	2	0				78.11	0.921777	1.3292656	0.130662
T	0	8.4	0.31	1.18	0.33	0.013	1	0	0	0				92.15	0.9115572	547.58801	0.939394
EB	0	8.4	0.31	1.18	0.33	0.013	1	0	0	0				106.16	0.9042954	547.58801	0.939394
Z	0	8.4	0.31	1.18	0.33	0.013	1	0	0	0				106.2	0.9039548	547.58801	0.939394
Eth	709.77	11.04	1.1	0.93	0.04	12.75	0.9	0	1	0				46.06	0.5210595	0.9310563	30.55556
O	6					0.21											
N	2.02E-05						0.00E+00										
Xaer	0.376										0.2		1.00E+05				
Xan	0.0376											0.03	1.00E+05				

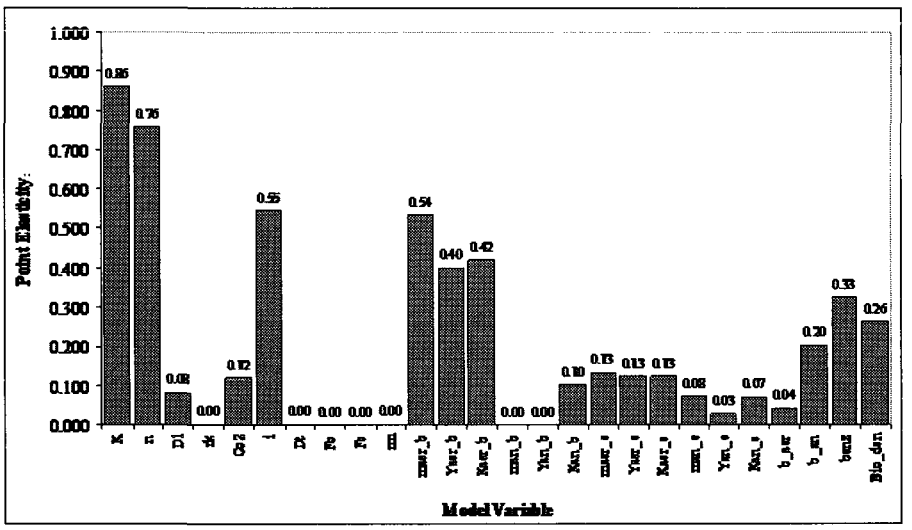
time (days)	time (hrs)	Substrate conc (mg/L)					EA conc. (mg/L)		Mass substrate as TOC (mgC/L)					Total Concentn Carbon Mass Fractions			
		B	T	EB	Z	Eth	O	N	Bc	Tc	EBc	Zc	Ethc	ST	fB	fT	fEB
0.11	2.64	2.41	0	0	0	709.77	4.857538	2.02E-05	2.221482525	0	0	0	369.8323925	372.0538751	0.0059709		0





Appendix V – Elasticity Sensitivity Analysis

Figure (a) shows the detailed results of the elasticity sensitivity analysis performed on the GSIM model.



K	Hydraulic Conductivity (m/d)
n	Aquifer Porosity (v/v)
Dl	Longitudinal Dispersivity (m)
rh	Bulk Density (mg/m³)
Co2	Oxygen Initial Concentration (mg/l)
i	Hydraulic gradient (m/m)
Dt	Transport step size (d)
Fo	Fraction of soil organic matter (g/g)
Fc	Fraction of microbial available pore space g/g)
xm	Microbial Density (g/ml)
maer_b	Benzene Aerobic Specific Growth Rate (1/d)
Yaer_b	Benzene Aerobic Biomass Yield (g/g)
Kaer_b	Benzene Aerobic Half-Saturation Coefficient (mg/l)
man_b	Benzene Anaerobic Specific Growth Rate (1/d)
Yan_b	Benzene Anaerobic Biomass Yield (g/g)
Kan_b	Benzene Anaerobic Half Saturation Coefficient (mg/l)
maer_e	Ethanol Aerobic Specific Growth Rate (1/d)
Yaer_e	Ethanol Aerobic Biomass Yield (g/g)
Kaer_e	Ethanol Aerobic Half-Saturation Coefficient (mg/l)
man_e	Ethanol Anaerobic Specific Growth Rate (1/d)
Yan_e	Ethanol Anaerobic Biomass Yield (g/g)
Kan_e	Ethanol Anaerobic Half-Saturation Coefficient (mg/l)
b_aer	Aerobic decay rate (1/d)
b_an	Anaerobic decay rate (1/d)
Benz	Benzene Concentration (mg/l)
Bio_den	Biofilm Density (kg/m³)

Appendix VII – Multilinear Regression Sensitivity Analysis

A Latin hypercube sample of 100 vectors, each of 17 variables following uniform distributions, was generated with the command:

```
L = lhsdesign(100,17)
```

With the result (L) shown on figure (a). Based on the ranges and values of each the variables included (Table 1), matrix L was used to generate matrix INPUTS (Figure b).

GSIM/RT3D was run using the degradation kinetics included in each of the 100 vectors (rows in the matrix M). The resulting centerline benzene plume lengths (to the 5 ppb counter line) were put as a 1 column matrix, OUTPUTS (Figure c).

Stepwise multilinear regression analysis of this data was done with the command:

```
b = stepwisefit(INPUTS,OUTPUTS)
```

The outputs of the command including the calculated coefficient, standard error, status, and p-value, are:

```
Initial columns included:  none
Step 1, added column 5, p=0.000629594
Step 2, added column 17, p=0.000167074
Step 3, added column 4, p=0.000438639
Step 4, added column 6, p=4.76545e-005
Step 5, added column 13, p=7.32946e-006
Final columns included:  4 5 6 13 17
    'Coeff'      'Std.Err.'    'Status'    'P'
    [-1.0703]    [  2.5913]    'Out'       [     0.6805]
    [ 1.6707]    [  2.1430]    'Out'       [     0.4376]
    [-0.5255]    [  0.3508]    'Out'       [     0.1376]
```

```
[-0.5068]    [  0.1030]    'In'        [3.6483e-006]
[ 9.9475]    [  2.0749]    'In'        [6.1053e-006]
[ 0.0522]    [  0.0105]    'In'        [3.2318e-006]
[ 0.0253]    [  0.1052]    'Out'       [     0.8106]
[ 1.4141]    [  2.1144]    'Out'       [     0.5053]
[ 0.0116]    [  0.0105]    'Out'       [     0.2715]
[ 0.0183]    [  0.1091]    'Out'       [     0.8670]
[ 3.9836]    [  2.1495]    'Out'       [     0.0670]
[ 0.0058]    [  0.0105]    'Out'       [     0.5843]
[-0.4911]    [  0.1034]    'In'        [7.3295e-006]
[ 1.0465]    [  2.1257]    'Out'       [     0.6237]
[ 0.0184]    [  0.0106]    'Out'       [     0.0876]
[-3.5343]    [  4.2254]    'Out'       [     0.4051]
[22.6102]    [  4.1673]    'In'        [4.4776e-007]
```

Table 1 – Degradation kinetic variables and ranges.

Variable	Description	Units	Base Value	Lower Range	Upper Range
γ	Microbial Pore Space Availability	m3/m3	0.2	0.8	0
K_O	Oxygen Half-Saturation Coefficient	mg/l	0.21	1	0.001
$I_{an,O}$	Anaerobic Inhibition due to O2	mg/l	0.1	1E+01	1E-05
$\mu_{mB,Aer}$	Benzene Aerobic Specific Growth Rate	1/d	3.24	20	0
$Y_{B,Aer}$	Benzene Aerobic Biomass Yield	g/g	0.39	1	0
$K_{B,Aer}$	Benzene Aerobic Half-aturation Coefficient	mg/l	7.6	200	0
$\mu_{mB,An}$	Benzene Anaerobic Specific Growth Rate	1/d	0.3	20	0
$Y_{B,An}$	Benzene Anaerobic Biomass Yield	g/g	0.05	1	0
$K_{B,An}$	Benzene Anaerobic Half-aturation Coefficient	mg/l	21	200	0
$\mu_{mE,Aer}$	Ethanol Aerobic Specific Growth Rate	1/d	11.04	20	0
$Y_{E,Aer}$	Ethanol Aerobic Biomass Yield	g/g	0.5	1	0
$K_{E,Aer}$	Ethanol Aerobic Half-aturation Coefficient	mg/l	63.09	200	0
$\mu_{mE,An}$	Ethanol Anaerobic Specific Growth Rate	1/d	1.1	20	0
$Y_{E,An}$	Ethanol Anaerobic Biomass Yield	g/g	0.07	1	0
$K_{E,An}$	Ethanol Anaerobic Half-aturation Coefficient	mg/l	78.86	200	0
b_{Aer}	Aerobic Microbial Population Decay Rate	1/d	0.2	0.5	0
b_{An}	Anaerobic Microbial Population Decay Rate	1/d	0.03	0.5	0

0.698	0.327	0.570	0.194	0.142	0.142	0.458	0.207	0.300	0.056	0.873	0.851	0.049	0.266	0.394	0.668	0.995
0.366	0.453	0.603	0.404	0.245	0.287	0.515	0.765	0.568	0.493	0.315	0.810	0.553	0.450	0.269	0.575	0.610
0.991	0.244	0.301	0.843	0.365	0.845	0.013	0.364	0.807	0.538	0.700	0.762	0.767	0.464	0.652	0.487	0.273
0.758	0.774	0.916	0.809	0.028	0.819	0.482	0.192	0.123	0.278	0.346	0.398	0.225	0.905	0.244	0.785	0.543
0.663	0.397	0.235	0.810	0.518	0.707	0.193	0.446	0.932	0.300	0.284	0.189	0.625	0.833	0.966	0.103	0.609
0.412	0.718	0.409	0.549	0.411	0.866	0.506	0.861	0.763	0.750	0.751	0.481	0.115	0.428	0.704	0.178	0.874
0.157	0.728	0.019	0.237	0.271	0.119	0.835	0.299	0.915	0.821	0.505	0.915	0.806	0.222	0.695	0.258	0.558
0.217	0.559	0.545	0.655	0.014	0.166	0.607	0.756	0.622	0.567	0.369	0.155	0.750	0.545	0.764	0.061	0.930
0.050	0.581	0.481	0.622	0.564	0.254	0.050	0.174	0.664	0.261	0.592	0.029	0.724	0.033	0.054	0.694	0.250
0.818	0.781	0.986	0.368	0.284	0.530	0.336	0.491	0.333	0.675	0.166	0.899	0.919	0.145	0.221	0.204	0.216
0.116	0.923	0.367	0.170	0.977	0.909	0.067	0.123	0.894	0.331	0.676	0.378	0.418	0.664	0.539	0.514	0.430
0.173	0.988	0.288	0.561	0.681	0.580	0.403	0.348	0.237	0.517	0.219	0.227	0.666	0.521	0.814	0.856	0.846
0.066	0.014	0.071	0.146	0.390	0.378	0.155	0.793	0.089	0.611	0.188	0.904	0.645	0.988	0.994	0.192	0.388
0.823	0.047	0.157	0.929	0.468	0.699	0.412	0.423	0.285	0.705	0.476	0.102	0.351	0.714	0.596	0.709	0.137
0.190	0.965	0.931	0.762	0.925	0.065	0.267	0.519	0.505	0.645	0.764	0.281	0.907	0.968	0.018	0.376	0.727
0.233	0.478	0.781	0.941	0.889	0.318	0.352	0.019	0.138	0.217	0.413	0.885	0.854	0.912	0.874	0.294	0.868
0.783	0.628	0.847	0.572	0.440	0.580	0.763	0.165	0.544	0.954	0.577	0.361	0.200	0.763	0.759	0.803	0.209
0.748	0.610	0.827	0.271	0.847	0.174	0.814	0.104	0.257	0.460	0.143	0.792	0.531	0.113	0.523	0.434	0.631
0.241	0.497	0.325	0.203	0.641	0.275	0.997	0.749	0.969	0.426	0.608	0.779	0.455	0.475	0.743	0.366	0.466
0.452	0.575	0.714	0.313	0.953	0.922	0.899	0.504	0.317	0.471	0.274	0.336	0.963	0.007	0.609	0.827	0.810
0.639	0.467	0.414	0.386	0.374	0.916	0.278	0.717	0.392	0.123	0.985	0.198	0.021	0.738	0.434	0.083	0.296
0.547	0.842	0.739	0.995	0.209	0.223	0.281	0.610	0.876	0.105	0.437	0.325	0.231	0.864	0.232	0.072	0.315
0.954	0.619	0.794	0.135	0.808	0.329	0.948	0.146	0.308	0.696	0.333	0.170	0.987	0.942	0.175	0.091	0.238
0.905	0.239	0.765	0.894	0.937	0.399	0.720	0.488	0.695	0.856	0.533	0.302	0.833	0.374	0.328	0.603	0.155
0.770	0.751	0.657	0.263	0.756	0.188	0.082	0.988	0.514	0.118	0.884	0.114	0.056	0.565	0.331	0.967	0.940
0.082	0.141	0.160	0.935	0.059	0.207	0.539	0.413	0.047	0.376	0.773	0.625	0.032	0.701	0.400	0.506	0.660
0.358	0.338	0.908	0.477	0.213	0.802	0.182	0.873	0.260	0.018	0.641	0.759	0.890	0.236	0.546	0.305	0.740
0.139	0.175	0.528	0.986	0.732	0.683	0.883	0.065	0.451	0.082	0.997	0.477	0.519	0.972	0.881	0.001	0.505
0.092	0.484	0.110	0.047	0.253	0.541	0.906	0.185	0.711	0.687	0.178	0.560	0.788	0.026	0.063	0.863	0.752
0.293	0.704	0.043	0.667	0.813	0.643	0.696	0.780	0.980	0.934	0.937	0.965	0.247	0.252	0.130	0.952	0.268
0.981	0.954	0.625	0.006	0.133	0.300	0.048	0.321	0.680	0.447	0.919	0.205	0.477	0.516	0.492	0.983	0.681
0.506	0.305	0.145	0.309	0.900	0.351	0.972	0.477	0.815	0.962	0.898	0.927	0.177	0.576	0.306	0.909	0.784
0.187	0.381	0.107	0.499	0.224	0.871	0.684	0.614	0.213	0.488	0.252	0.357	0.003	0.291	0.915	0.129	0.102
0.803	0.660	0.473	0.171	0.073	0.049	0.091	0.832	0.161	0.810	0.298	0.731	0.957	0.999	0.834	0.478	0.970
0.976	0.748	0.991	0.694	0.480	0.389	0.342	0.271	0.199	0.656	0.469	0.542	0.501	0.485	0.972	0.395	0.703
0.575	0.364	0.456	0.529	0.825	0.946	0.792	0.682	0.497	0.319	0.968	0.052	0.064	0.082	0.161	0.839	0.057
0.968	0.273	0.872	0.824	0.712	0.129	0.244	0.786	0.637	0.070	0.511	0.551	0.408	0.756	0.793	0.244	0.913
0.910	0.565	0.564	0.115	0.869	0.755	0.033	0.232	0.078	0.626	0.637	0.722	0.711	0.275	0.614	0.873	0.957
0.594	0.350	0.379	0.351	0.356	0.053	0.120	0.570	0.374	0.381	0.085	0.403	0.494	0.345	0.200	0.546	0.419
0.409	0.510	0.346	0.121	0.455	0.964	0.400	0.908	0.975	0.138	0.560	0.320	0.949	0.309	0.779	0.753	0.526
0.705	0.062	0.217	0.507	0.542	0.669	0.738	0.993	0.687	0.079	0.245	0.656	0.307	0.127	0.458	0.686	0.750
0.288	0.933	0.617	0.460	0.151	0.106	0.107	0.844	0.821	0.789	0.094	0.451	0.146	0.586	0.102	0.942	0.070
0.371	0.228	0.275	0.903	0.775	0.459	0.548	0.151	0.060	0.168	0.858	0.385	0.824	0.318	0.565	0.780	0.826
0.831	0.542	0.084	0.589	0.345	0.562	0.135	0.695	0.438	0.225	0.626	0.036	0.153	0.054	0.091	0.352	0.191
0.264	0.799	0.421	0.876	0.665	0.650	0.668	0.351	0.038	0.555	0.135	0.594	0.845	0.688	0.185	0.732	0.761
0.147	0.434	0.340	0.796	0.181	0.304	0.591	0.669	0.405	0.604	0.715	0.689	0.797	0.639	0.480	0.939	0.777
0.880	0.666	0.643	0.010	0.989	0.737	0.646	0.808	0.557	0.236	0.371	0.956	0.442	0.249	0.508	0.150	0.456
0.013	0.120	0.598	0.072	0.768	0.411	0.829	0.881	0.887	0.396	0.303	0.822	0.730	0.742	0.153	0.886	0.475
0.848	0.059	0.922	0.294	0.692	0.791	0.570	0.077	0.360	0.742	0.823	0.934	0.815	0.363	0.074	0.225	0.394
0.528	0.104	0.533	0.604	0.333	0.749	0.117	0.678	0.864	0.026	0.928	0.499	0.318	0.444	0.715	0.115	0.899
0.443	0.871	0.668	0.089	0.083	0.953	0.730	0.467	0.222	0.544	0.229	0.648	0.523	0.405	0.983	0.317	0.697
0.474	0.804	0.057	0.673	0.493	0.092	0.849	0.455	0.027	0.526	0.807	0.691	0.468	0.660	0.671	0.713	0.982
0.350	0.421	0.030	0.740	0.996	0.369	0.436	0.389	0.928	0.005	0.050	0.252	0.213	0.884	0.198	0.900	0.975
0.534	0.763	0.132	0.374	0.400	0.005	0.004	0.283	0.743	0.416	0.585	0.132	0.934	0.620	0.808	0.405	0.117
0.330	0.856	0.182	0.240	0.161	0.503	0.028	0.584	0.729	0.289	0.866	0.295	0.337	0.692	0.372	0.268	0.404
0.323	0.086	0.586	0.917	0.800	0.989	0.315	0.963	0.794	0.298	0.101	0.243	0.900	0.606	0.727	0.742	0.490
0.850	0.731	0.513	0.397	0.171	0.021	0.954	0.544	0.013	0.360	0.943	0.266	0.438	0.212	0.643	0.132	0.088
0.551	0.836	0.756	0.740	0.007	0.529	0.441	0.956	0.641	0.325	0.559	0.213	0.373	0.071	0.024	0.388	0.714
0.684	0.190	0.633	0.323	0.788	0.463	0.178	0.055	0.482	0.595	0.027	0.863	0.657	0.152	0.271	0.492	0.363
0.733	0.169	0.313	0.868	0.946	0.832	0.366	0.627	0.599	0.735	0.014	0.509	0.775	0.327	0.864	0.817	0.143
0.162	0.643	0.851	0.054	0.604	0.715	0.215	0.898	0.602	0.260	0.125	0.072	0.162	0.853	0.682	0.633	0.901
0.463	0.681	0.895	0.346	0.596	0.034	0.421	0.331	0.176	0.712	0.848	0.047	0.867	0.439	0.282	0.145	0.230
0.648	0.092	0.696	0.520	0.444	0.761	0.707	0.085	0.856	0.193	0.488	0.749	0.608	0.182	0.577	0.913	0.009
0.439	0.975	0.509	0.091	0.485	0.210	0.757	0.654	0.271	0.364	0.158	0.121	0.384	0.814	0.352	0.528	0.835
0.677	0.207	0.946	0.958	0.124	0.599	0.143	0.213	0.415	0.633	0.060	0.574	0.638	0.727	0.128	0.612	0.646
0.022	0.005	0.672	0.430	0.581	0.607	0.304	0.312	0.158	0.206	0.235	0.674	0.108	0.679	0.031	0.272	0.175
0.610	0.673	0.806	0.969	0.705	0.850	0.982	0.252	0.247	0.798	0.070	0.239	0.687	0.558	0.944	0.218	0.183
0.618	0.882	0.244	0.707	0.650	0.887	0.744	0.701	0.059	0.044	0.908	0.978	0.083	0.289	0.586	0.013	0.857
0.940	0.500	0.889	0.456	0.116	0.159	0.471	0.043	0.789	0.032	0.830	0.830	0.546	0.108	0.627	0.324	0.090
0.943	0.893	0.229	0.221	0.837	0.726	0.165	0.523	0.358	0.994	0.262	0.421	0.692	0.418	0.259	0.800	0.044
0.481	0.825	0.120	0.190	0.295	0.897	0.492	0.228	0.654	0.723	0.118	0.341	0.925	0.957	0.857	0.595	0.011
0.312	0.265	0.383	0.636	0.262	0.974	0.630	0.431	0.103	0.943	0.682	0.943	0.871	0.043	0.467	0.996	0.514
0.004	0.216	0.444	0.158	0.904	0.436	0.775	0.035	0.342	0.864	0.196	0.412	0.264	0.619	0.212	0.539	0.329
0.397	0.525	0.061	0.420	0.103	0.084	0.225	0.921	0.115	0.975	0.055	0.001	0.672	0.925	0.040	0.424	0.486
0.561	0.913	0.267	0.711	0.639	0.245	0.851	0.555	0.330	0.099	0.358	0.148	0.280	0.599	0.089	0.041	0.425
0.072	0.692	0.433	0.770	0.310	0.403	0.293	0.859	0.444	0.890	0.403	0.713	0.396	0.775	0.510	0.620	0.580
0.121	0.023	0.749	0.434	0.092	0.931	0.926	0.131	0.577	0.504	0.452	0.638	0.181	0.785	0.731	0.285	0.594
0.711	0.991	0.869	0.286	0.970	0.477	0.961	0.823	0.582	0.846	0.669	0.273	0.587	0.392	0.314	0.162	0.360
0.922	0.596	0.461	0.031	0.559	0.425	0.553	0.942	0.831	0.761	0.960	0.433	0.017	0.195	0.009	0.730	0.932
0.874	0.813	0.491	0.554	0.729	0.483	0.389	0.118	0.846	0.879	0.654	0.176	0.365	0.203	0.663	0.849	0.337
0.383	0.401	0.200	0.615	0.917	0.072	0.231	0.399	0.470	0.834	0.733	0.841	0.297	0.500	0.149	0.460	0.627
0.037	0.193	0.811	0.851	0.528	0.493	0.078	0.592	0.462	0.248	0.792	0.872	0.128	0.063	0.555	0.342	0.025
0.253	0.072	0.299	0.688	0.039	0.772	0.205	0.001	0.734	0.158	0.033	0.538	0.704	0.804	0.900	0.927	0.804
0.721	0.904	0.008	0.532	0.400	0.131	0.676	0.936	0.532	0.771	0.815	0.096	0.254	0.850	0.479	0.051	0.344
0.630	0.129	0.962	0.591	0.193	0.337	0.785	0.535	0.900	0.989	0.396	0.669	0.290	0.494	0.829	0.231	0.884
0.307	0.534	0.975	0.442	0.503	0.998	0.530	0.976	0.948	0.892	0.521	0.988	0.994	0.335	0.429	0.021	0.250
0.428	0.258	0.772	0.252	0.328	0.447	0.328	0.249	0.616	0.187	0.007	0.584	0.613	0.178	0.847	0.035	0.076
0.659	0.155	0.254	0.884	0.532	0.671	0.652	0.372	0.756	0.149	0.725	0.527	0.349	0.531	0.362	0.588	0.288
0.866	0.130	0.192	0.481	0.742	0.786	0.865	0.308	0.380	0.456	0.444	0.992	0.191	0.388	0.110	0.419	0.303
0.769	0.319	0.351	0.730	0.628	0.625	0.253	0.025	0.009	0.901	0.327	0.813	0.973	0.875	0.446	0.465	0.564
0.101	0.630	0.683	0.648	0.306	0.196	0.803	0.097	0.522	0.176	0.490	0.520	0.135	0.160	0.381	0.189	0.536
0.225	0.341	0.953	0.101	0.576	0.017	0.582	0.409	0.185	0.661	0.745	0.012	0.322	0.824	0.954	0.641	0.671
0.205	0.299	0.557	0.214	0.062	0.233	0.566	0.634	0.144	0.402	0.611	0.608	0.489	0.647	0.939	0.568	0.164
0.270	0.282	0.399	0.335	0.676	0.553	0.618	0.270	0.952	0.814	0.785	0.784	0.077	0.932	0.295	0.656	0.378
0.892	0.417	0.022	0.024	0.614	0.345	0.373	0.648	0.771	0.581	0.973	0.060	0.578	0.791	0.909	0.553	0.576
0.795	0.950	0.093	0.785	0.046	0.266	0.463	0.910	0.201	0.347	0.546	0.090	0.092	0.136	0.790	0.336	0.796
0.587	0.446	0.833	0.834	0.420	0.512	0.911	0.733	0.090	0.436	0.421	0.706	0.598	0.891	0.928	0.672	0.445
0.512	0.034	0.178	0.979	0.873	0.618	0.874	0.579	0.991	0.923	0.693	0.446	0.421	0.093	0.638	0.971	0.666
0.049	0.376	0.721	0.068	0.232	0.826	0.935	0.729	0.706	0.918	0.201	0.469	0.740	0.011	0.344	0.442	0.121
0.498	0.865	0.705	0.760	0.859	0.634	0.624	0.812	0.422	0.577	0.388	0.615	0.564	0.353	0.416	0.763	0.031

Figure (a) – Latin Hypercube Matrix L

0.56	0.3278	0.026308	3.89	0.14	28.34	9.16	0.21	59.99	1.12	0.87	170.13	0.98	0.27	78.82	0.33	0.50
0.29	0.4532	0.041389	8.07	0.25	57.37	10.31	0.77	113.57	9.87	0.31	161.95	11.05	0.45	53.78	0.29	0.31
0.79	0.2451	0.000642	16.86	0.36	168.92	0.25	0.36	161.45	10.76	0.70	152.43	15.33	0.46	130.43	0.24	0.14
0.61	0.7740	3.121824	16.19	0.03	163.87	9.64	0.19	24.59	5.57	0.35	79.69	4.51	0.91	48.77	0.39	0.27
0.53	0.3980	0.000258	16.20	0.52	141.46	3.86	0.45	186.39	6.01	0.28	37.85	12.50	0.83	193.29	0.05	0.30
0.33	0.7186	0.002831	10.99	0.41	173.29	10.13	0.86	152.68	15.00	0.75	96.29	2.30	0.43	140.75	0.09	0.44
0.13	0.7279	0.000013	4.74	0.27	23.89	16.69	0.30	183.06	16.41	0.50	182.92	16.12	0.22	139.05	0.13	0.28
0.17	0.5595	0.018668	13.11	0.01	33.11	12.14	0.76	124.47	11.34	0.37	30.96	15.00	0.55	152.75	0.03	0.46
0.04	0.5819	0.007659	12.44	0.56	50.88	1.00	0.17	132.82	5.21	0.59	5.72	14.47	0.03	10.72	0.35	0.13
0.65	0.7814	8.187918	7.36	0.28	106.06	6.71	0.49	66.51	13.49	0.17	179.87	18.39	0.14	44.22	0.10	0.11
0.09	0.9232	0.001590	3.39	0.98	181.82	1.34	0.12	178.77	6.63	0.68	75.58	8.36	0.66	107.85	0.26	0.22
0.14	0.9876	0.000531	11.21	0.68	115.97	8.06	0.35	47.43	10.35	0.22	45.31	13.32	0.52	162.70	0.43	0.42
0.05	0.0148	0.000027	2.92	0.39	75.54	3.11	0.79	17.75	12.21	0.19	180.86	12.90	0.99	198.76	0.10	0.19
0.66	0.0478	0.000087	18.57	0.47	139.87	8.25	0.42	57.04	14.10	0.48	20.33	7.02	0.71	119.14	0.35	0.07
0.15	0.9655	3.871347	15.23	0.92	12.99	5.34	0.52	100.99	12.90	0.76	56.10	18.14	0.97	3.59	0.19	0.36
0.19	0.4789	0.483268	18.83	0.89	63.53	7.05	0.02	27.55	4.35	0.41	176.91	17.08	0.91	174.72	0.15	0.43
0.63	0.6281	1.201580	11.45	0.44	116.03	15.26	0.16	108.84	19.08	0.58	72.21	4.00	0.76	151.89	0.40	0.10
0.60	0.6100	0.919877	5.42	0.85	34.79	16.28	0.10	51.48	9.21	0.14	158.39	10.61	0.11	104.56	0.22	0.32
0.19	0.4975	0.000888	4.06	0.64	55.04	19.94	0.75	193.90	8.51	0.61	155.71	9.09	0.48	148.62	0.18	0.23
0.36	0.5757	0.192342	6.27	0.95	184.40	17.98	0.50	63.44	9.43	0.27	67.18	19.25	0.01	121.88	0.41	0.41
0.51	0.4671	0.003063	7.71	0.37	183.26	5.56	0.72	78.47	2.46	0.99	39.65	0.42	0.74	86.75	0.04	0.15
0.44	0.8419	0.270739	19.90	0.21	44.70	5.63	0.61	175.27	2.10	0.44	64.97	4.61	0.86	46.43	0.04	0.16
0.76	0.6190	0.583417	2.71	0.81	65.85	18.96	0.15	61.66	13.92	0.33	33.95	19.74	0.94	35.10	0.05	0.12
0.72	0.2399	0.390508	17.88	0.94	79.75	14.39	0.49	139.01	17.12	0.53	60.43	16.65	0.37	65.68	0.30	0.08
0.62	0.7512	0.087865	5.26	0.76	37.64	1.63	0.99	102.72	2.37	0.88	22.72	1.12	0.56	66.15	0.48	0.47
0.07	0.1418	0.000091	18.70	0.06	41.40	10.78	0.41	9.32	7.52	0.77	124.94	0.64	0.70	80.10	0.25	0.33
0.29	0.3383	2.800402	9.54	0.21	160.35	3.65	0.87	52.01	0.35	0.64	151.73	17.80	0.24	109.23	0.15	0.37
0.11	0.1757	0.014622	19.72	0.73	136.62	17.65	0.06	90.23	1.64	1.00	95.45	10.38	0.97	176.26	0.00	0.25
0.07	0.4850	0.000046	0.93	0.25	108.11	18.11	0.19	142.11	13.74	0.18	112.07	15.75	0.03	12.52	0.43	0.38
0.23	0.7039	0.000018	13.35	0.81	128.54	13.93	0.78	196.10	18.69	0.94	192.96	4.94	0.25	26.08	0.48	0.13
0.78	0.9543	0.056144	0.11	0.13	59.92	0.96	0.32	135.95	8.94	0.92	41.00	9.54	0.52	98.33	0.49	0.34
0.40	0.3054	0.000075	6.18	0.90	70.11	19.43	0.48	163.08	19.24	0.90	185.43	3.53	0.58	61.20	0.45	0.39
0.15	0.3814	0.000044	9.97	0.22	174.11	13.69	0.61	42.58	9.76	0.25	71.36	0.06	0.29	182.99	0.06	0.05
0.64	0.6602	0.006874	3.43	0.07	9.82	1.82	0.83	32.24	16.20	0.30	146.15	19.14	1.00	166.71	0.24	0.48
0.78	0.7482	8.820898	13.89	0.48	77.73	6.85	0.27	39.74	13.12	0.47	108.37	10.02	0.49	194.45	0.20	0.35
0.46	0.3648	0.005412	10.58	0.82	189.27	15.84	0.68	99.33	6.37	0.97	10.34	1.28	0.08	32.17	0.42	0.03
0.77	0.2733	1.712113	16.47	0.71	25.73	4.88	0.79	127.43	1.39	0.51	110.15	8.16	0.76	158.59	0.12	0.46
0.73	0.5652	0.024317	2.30	0.87	151.00	0.66	0.23	15.54	12.52	0.64	144.47	14.21	0.27	122.75	0.44	0.48
0.48	0.3511	0.001878	7.03	0.36	10.57	2.40	0.57	74.89	7.63	0.08	80.58	9.88	0.35	40.06	0.27	0.21
0.33	0.5106	0.001190	2.42	0.45	192.75	8.00	0.91	195.06	2.76	0.56	63.98	18.97	0.31	155.84	0.38	0.26
0.56	0.0630	0.000199	10.13	0.54	133.78	14.75	0.99	137.47	1.58	0.24	131.25	6.13	0.13	91.61	0.34	0.37
0.23	0.9328	0.050635	9.21	0.15	21.27	2.13	0.84	164.13	15.77	0.09	90.12	2.93	0.59	20.49	0.47	0.03
0.30	0.2292	0.000449	18.05	0.77	91.80	10.95	0.15	12.06	3.37	0.86	76.98	16.49	0.32	112.94	0.39	0.41
0.66	0.5420	0.000032	11.78	0.35	112.37	2.71	0.70	87.57	4.50	0.63	7.25	3.06	0.05	18.20	0.18	0.10
0.21	0.7997	0.003379	17.53	0.66	130.00	13.37	0.35	7.68	11.10	0.13	118.84	16.91	0.69	37.03	0.37	0.38
0.12	0.4341	0.001092	15.92	0.18	60.72	11.81	0.67	81.09	12.09	0.72	137.85	15.94	0.64	96.05	0.47	0.39
0.70	0.6659	0.071772	0.20	0.99	147.39	12.93	0.81	111.49	4.71	0.37	191.15	8.85	0.25	101.55	0.08	0.23
0.01	0.1207	0.038558	1.44	0.77	82.29	16.58	0.88	177.47	7.92	0.30	164.49	14.60	0.74	30.57	0.44	0.24
0.68	0.0595	3.413072	5.89	0.69	158.13	11.41	0.08	72.02	14.84	0.82	186.89	16.31	0.36	14.77	0.11	0.20
0.42	0.1047	0.015824	12.07	0.33	149.87	2.35	0.68	172.88	0.52	0.93	99.71	6.36	0.44	142.97	0.06	0.45
0.35	0.8708	0.102387	1.78	0.08	190.54	14.59	0.47	44.30	10.88	0.23	129.61	10.47	0.40	196.66	0.16	0.35
0.38	0.8038	0.000022	13.47	0.49	18.39	16.98	0.45	5.35	10.53	0.81	138.11	9.37	0.66	134.16	0.36	0.49
0.28	0.4214	0.000015	14.79	1.00	73.84	8.71	0.39	185.53	0.11	0.05	50.45	4.26	0.88	39.67	0.45	0.49
0.43	0.7628	0.000062	7.49	0.40	1.06	0.08	0.28	148.55	8.32	0.58	26.44	18.68	0.62	161.53	0.20	0.06
0.26	0.8564	0.000123	4.80	0.16	100.62	0.57	0.58	145.72	5.77	0.87	58.90	6.74	0.69	74.43	0.13	0.20
0.26	0.0873	0.032947	18.35	0.80	197.83	6.31	0.96	158.82	5.97	0.10	48.62	17.99	0.61	145.46	0.37	0.25
0.68	0.7310	0.012006	7.93	0.17	4.11	19.09	0.54	2.63	7.20	0.94	53.17	8.75	0.21	128.56	0.07	0.04
0.44	0.8361	0.342058	14.80	0.01	105.76	8.83	0.96	128.12	6.51	0.56	42.58	7.47	0.07	4.84	0.19	0.36
0.55	0.1906	0.063207	6.47	0.79	92.59	3.55	0.05	96.42	11.89	0.03	172.67	13.15	0.15	54.21	0.25	0.18
0.59	0.1700	0.000757	17.36	0.95	166.38	7.33	0.63	119.71	14.69	0.01	101.89	15.51	0.33	172.77	0.41	0.07
0.13	0.6433	1.268092	1.09	0.60	142.96	4.31	0.90	120.48	5.19	0.13	14.42	3.24	0.85	136.33	0.32	0.45
0.37	0.6813	2.342142	6.93	0.60	6.88	8.42	0.33	35.19	14.24	0.85	9.42	17.34	0.44	56.47	0.07	0.11
0.52	0.0926	0.149075	10.39	0.44	152.16	14.14	0.09	171.28	3.87	0.49	149.78	12.16	0.18	115.42	0.46	0.00
0.35	0.9752	0.011330	1.82	0.48	42.07	15.14	0.65	54.20	7.28	0.16	24.23	7.69	0.81	70.46	0.26	0.42
0.54	0.2080	4.711164	19.15	0.12	119.89	2.86	0.21	83.09	12.66	0.06	114.84	12.76	0.73	25.60	0.31	0.32
0.02	0.0063	0.107394	8.59	0.58	121.41	6.09	0.31	31.67	4.12	0.23	134.75	2.16	0.68	6.27	0.14	0.09
0.49	0.6733	0.684265	19.37	0.70	170.03	19.64	0.25	49.31	15.96	0.07	47.77	13.74	0.56	188.80	0.11	0.09
0.49	0.8825	0.000291	14.15	0.65	177.42	14.89	0.70	11.90	0.87	0.91	195.53	1.67	0.29	117.13	0.01	0.43
0.75	0.5006	2.161719	9.12	0.12	31.85	9.43	0.04	157.81	0.64	0.83	166.01	10.93	0.11	125.31	0.16	0.05
0.75	0.8929	0.000236	4.41	0.84	145.28	3.31	0.52	71.67	19.89	0.26	84.29	13.83	0.42	51.89	0.40	0.02
0.39	0.8256	0.000053	3.79	0.29	179.34	9.84	0.23	130.74	14.46	0.12	68.18	18.50	0.96	171.36	0.30	0.01
0.25	0.2656	0.001999	12.72	0.26	194.74	12.60	0.43	20.51	18.86	0.68	188.66	17.43	0.04	93.39	0.50	0.26
0.00	0.2172	0.004590	3.16	0.90	87.17	15.49	0.04	68.44	17.27	0.20	82.44	5.28	0.62	42.46	0.27	0.16
0.32	0.5256	0.000023	8.39	0.10	16.84	4.51	0.92	22.96	19.49	0.05	0.18	13.43	0.93	8.06	0.21	0.24
0.45	0.9130	0.000401	14.22	0.64	49.04	17.03	0.55	65.96	1.97	0.36	29.60	5.59	0.60	17.80	0.02	0.21
0.06	0.6921	0.003981	15.41	0.31	80.59	5.85	0.86	88.80	17.80	0.40	142.57	7.91	0.77	102.09	0.31	0.29
0.10	0.0236	0.312982	8.68	0.09	186.15	18.53	0.13	115.32	10.09	0.45	127.57	3.62	0.78	146.24	0.14	0.30
0.57	0.9905	1.647054	5.73	0.97	95.39	19.21	0.82	116.43	16.92	0.67	54.58	11.74	0.39	62.88	0.08	0.18
0.74	0.5969	0.005861	0.62	0.56	84.93	11.06	0.94	166.21	15.22	0.96	86.54	0.33	0.20	1.71	0.36	0.47
0.70	0.8135	0.008845	11.07	0.73	96.65	7.79	0.12	169.15	17.58	0.65	35.11	7.30	0.20	132.55	0.42	0.17
0.31	0.4017	0.000159	12.31	0.92	14.37	4.62	0.40	94.00	16.69	0.73	168.27	5.94	0.50	29.81	0.23	0.31
0.03	0.1939	0.734696	17.02	0.53	98.58	1.56	0.59	92.34	4.95	0.79	174.46	2.56	0.06	110.95	0.17	0.01
0.20	0.0733	0.000620	13.76	0.04	154.45	4.11	0.00	146.74	3.16	0.03	107.61	14.07	0.80	179.96	0.46	0.40
0.58	0.9046	0.000011	10.64	0.40	26.28	13.53	0.94	106.42	15.41	0.82	19.14	5.07	0.85	95.76	0.03	0.17
0.50	0.1302	5.904628	11.82	0.19	67.45	15.70	0.53	180.03	19.78	0.40	133.85	5.79	0.49	165.80	0.12	0.44
0.25	0.5347	7.059843	8.84	0.50	199.64	10.60	0.98	189.64	17.84	0.52	197.65	19.88	0.34	85.71	0.01	0.12
0.34	0.2587	0.429010	5.04	0.33	89.30	6.57	0.25	123.13	3.73	0.01	116.83	12.27	0.18	169.36	0.02	0.04
0.53	0.1557	0.000334	17.68	0.53	134.15	13.04	0.37	151.17	2.98	0.72	105.35	6.99	0.53	72.40	0.29	0.14
0.69	0.1314	0.000143	9.62	0.74	157.24	17.30	0.31	76.07	9.13	0.44	198.35	3.82	0.39	22.05	0.21	0.15
0.62	0.3195	0.001269	14.59	0.63	124.98	5.06	0.03	1.78	18.03	0.33	162.51	19.46	0.88	89.27	0.23	0.28
0.08	0.6307	0.125938	12.95	0.31	39.17	16.06	0.10	104.38	3.53	0.49	103.98	2.70	0.16	76.18	0.09	0.27
0.18	0.3421	5.197870	2.01	0.58	3.42	11.64	0.41	37.08	13.21	0.75	2.46	6.44	0.82	190.72	0.32	0.34
0.16	0.3001	0.022127	4.27	0.06	46.55	11.33	0.63	28.75	8.05	0.61	121.68	9.79	0.65	187.83	0.28	0.08
0.22	0.2826	0.002479	6.70	0.68	110.54	12.36	0.27	190.43	16.27	0.79	156.83	1.55	0.93	58.95	0.33	0.19
0.71	0.4178	0.000014	0.48	0.61	69.06	7.47	0.65	154.15	11.62	0.97	12.03	11.55	0.79	181.74	0.28	0.29
0.64	0.9499	0.000036	15.70	0.05	53.28	9.27	0.91	40.14	6.93	0.55	17.98	1.84	0.14	157.90	0.17	0.40
0.47	0.4470	0.995137	16.68	0.42	102.32	18.21	0.73	18.02	8.72	0.42	141.14	11.96	0.89	185.56	0.34	0.22
0.41	0.0346	0.000117	19.58	0.87	123.68	17.48	0.58	198.16	18.46	0.69	89.10	8.43	0.09	127.57	0.49	0.33
0.04	0.3770	0.211297	1.35	0.23	165.25	18.69	0.73	141.14	18.35	0.20	93.86	14.80	0.01	68.86	0.22	0.06
0.40	0.8650	0.170578	15.20	0.86	126.76	12.48	0.81	84.44	11.54	0.39	123.02	11.29	0.35	83.14	0.38	0.02

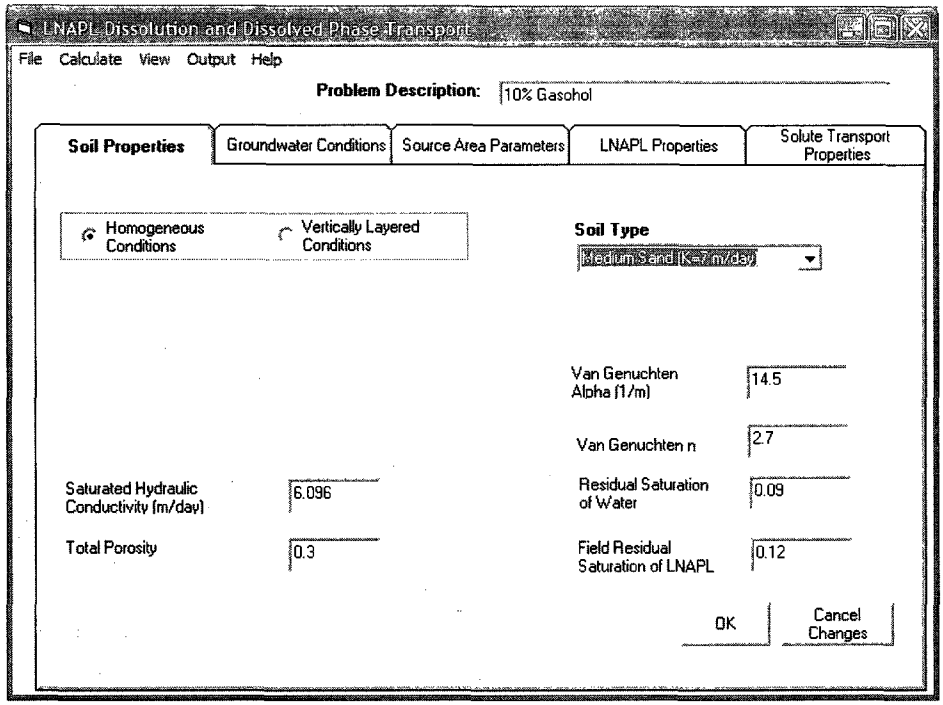
Figure (b) – Latin Hypercube Sample Matrix INPUT

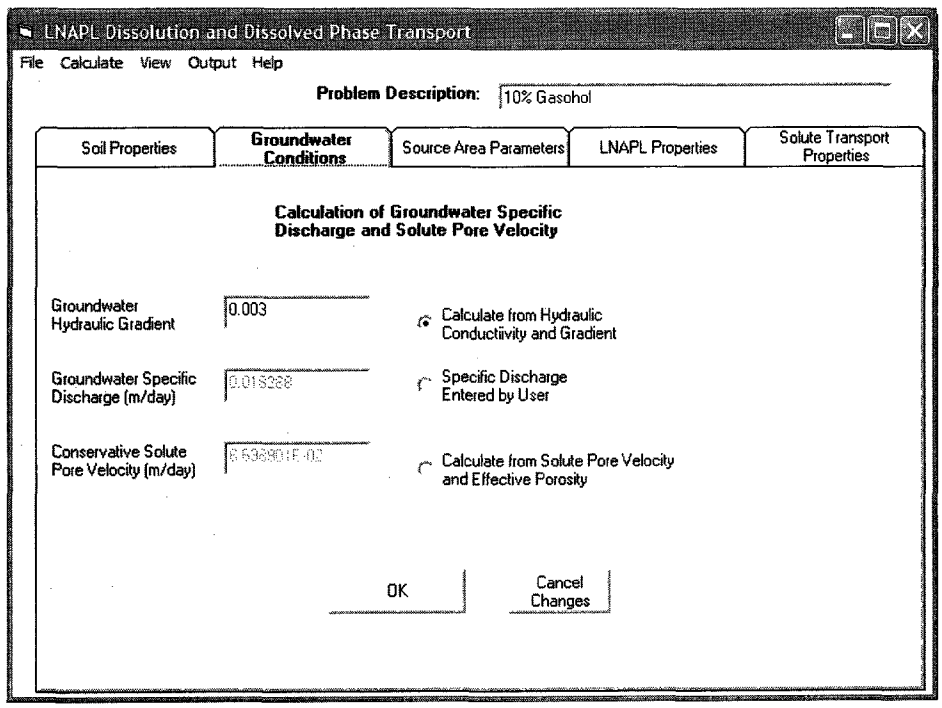
51.10
33.90
42.80
31.70
45.80
50.50
43.50
33.70
34.20
31.50
50.90
46.40
43.50
29.40
29.90
42.40
39.10
39.00
47.30
46.60
51.10
34.20
41.20
29.00
50.70
50.50
39.40
43.00
49.60
47.50
50.70
47.50
51.10
35.80
42.50
44.10
46.90
50.70
30.10
50.70
47.10
27.20
43.90
39.20
37.80
38.50
50.70
47.10
41.70
50.70
45.90
47.30
51.10
32.00
47.30
42.10
19.20
24.00
41.60
39.60
50.90
22.20
30.50
45.90
27.60
34.90
30.80
51.10
23.80
50.20
47.80
38.50
51.10
22.40
39.60
41.90
45.70
43.30
51.10
43.30
39.20
34.60
11.60
41.70
40.10
41.20
31.70
45.70
46.90
38.50
42.30
39.40
27.90
47.50
50.70
46.60
36.70
43.00
43.50
36.90

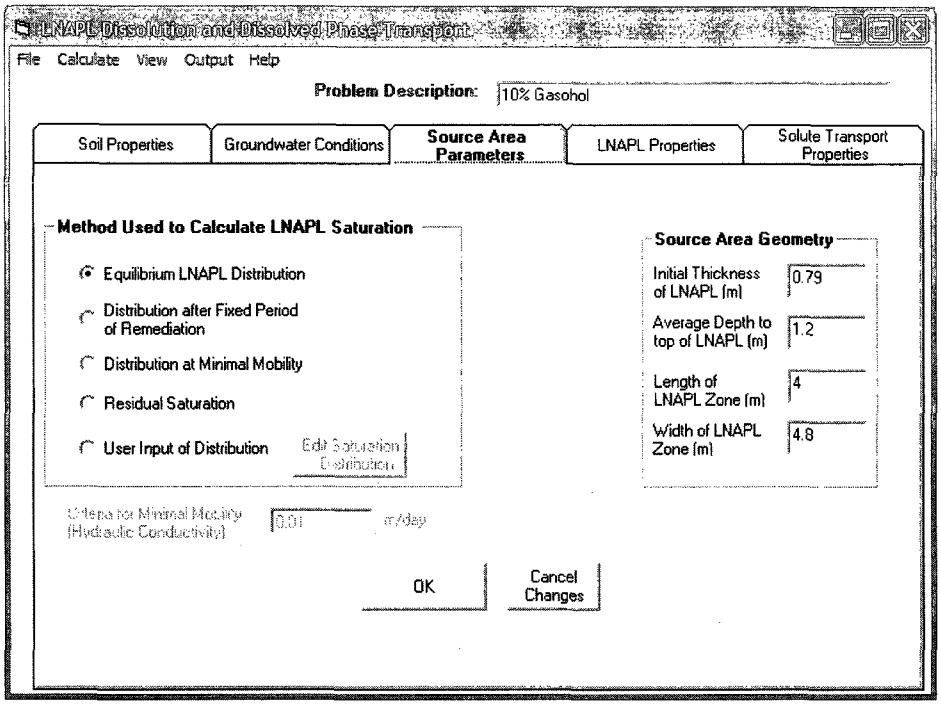
Figure (c) – GSIM/RT3D benzene centerline plume length (m) matrix OUTPUTS

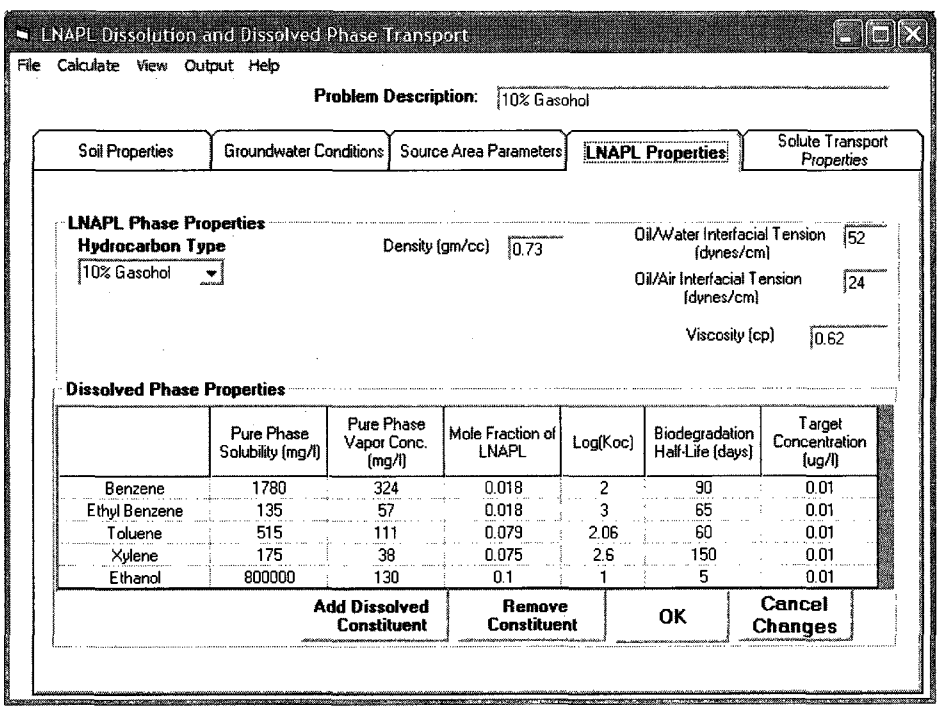
Appendix VIII – LNAST source zone

- **Dialogue options for setting up the source zone dissolution scenario using the LNAST model:**

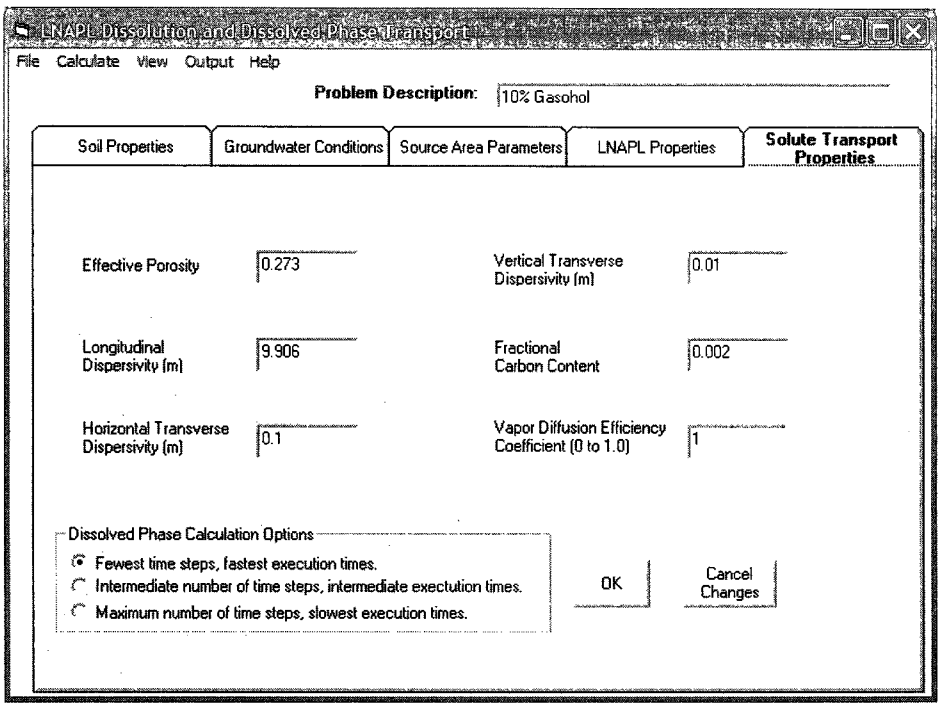


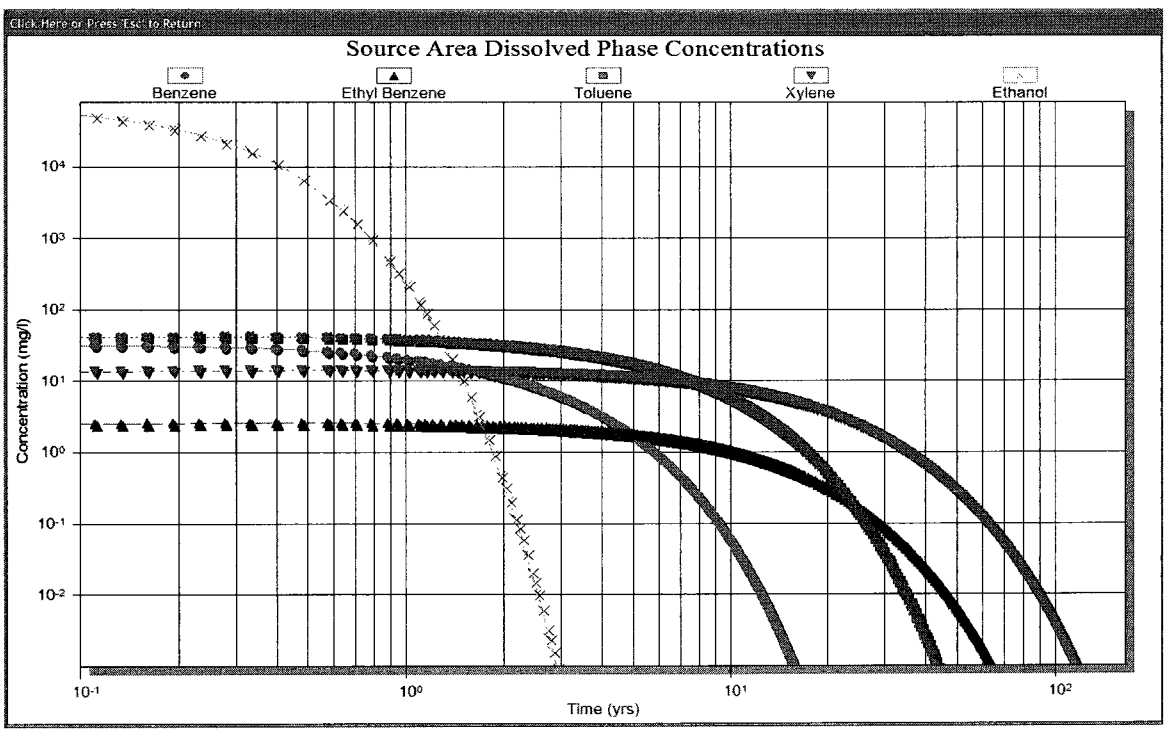






	Pure Phase Solubility (mg/l)	Pure Phase Vapor Conc. (mg/l)	Mole Fraction of LNAPL	Log(Koc)	Biodegradation Half-Life (days)	Target Concentration (ug/l)
Benzene	1780	324	0.018	2	90	0.01
Ethyl Benzene	135	57	0.018	3	65	0.01
Toluene	515	111	0.079	2.06	60	0.01
Xylene	175	38	0.075	2.6	150	0.01
Ethanol	800000	130	0.1	1	5	0.01


LNAPL Dissolution and Dissolved Phase Transport
File Calculate View Output Help
Problem Description: 10% Gasohol
Soil Properties
Groundwater Conditions
Source Area Parameters
LNAPL Properties
Solute Transport Properties
Effective Porosity 0.273
Vertical Transverse Dispersivity (m) 0.01
Longitudinal Dispersivity (m) 9.906
Fractional Carbon Content 0.002
Horizontal Transverse Dispersivity (m) 0.1
Vapor Diffusion Efficiency Coefficient (0 to 1.0) 1
Dissolved Phase Calculation Options
Fewest time steps, fastest execution times.
Intermediate number of time steps, intermediate exectution times.
Maximum number of time steps, slowest execution times.
OK
Cancel Changes


Click Here or Press Esc to Return
Source Area Dissolved Phase Concentrations
Benzene
Ethyl Benzene
Toluene
Xylene
Ethanol
Concentration (mg/l)
Time (yrs)

Appendix VIII –Model User Tutorial for Visual Basic Platform

General Substrate Interaction Module (GSIM) Interface

Tutorial and Reference Guide

December 4, 2009

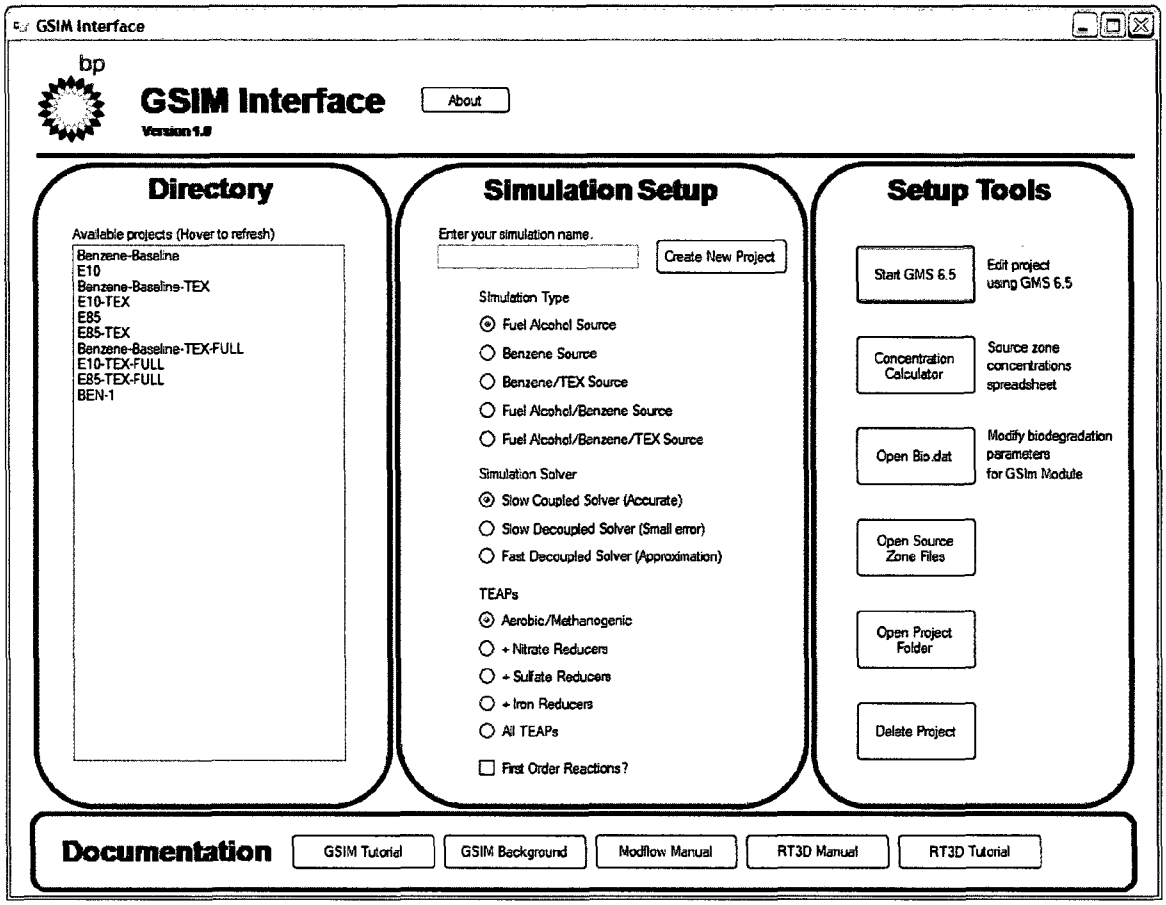
Before you start

The GSIM Interface is a visual basic software that integrates the tools required to evaluate the impact of a fuel alcohol on the biodegradation of groundwater dissolved contaminants. The interface provides easy access to GMS 6.5, pre-prepared simulation scenarios, GSIM modules, contaminant data, source zone concentration calculations and project file management tools.

The software is designed around the General Substrate Interaction Module (GSIM); a custom reaction module software developed for use with the RT3D reactive transport package. GSIM handles biodegradation kinetics and substrate interactions between multiple of dissolved contaminants in groundwater, one alcohol present in the water phase, and any number of microbial populations.

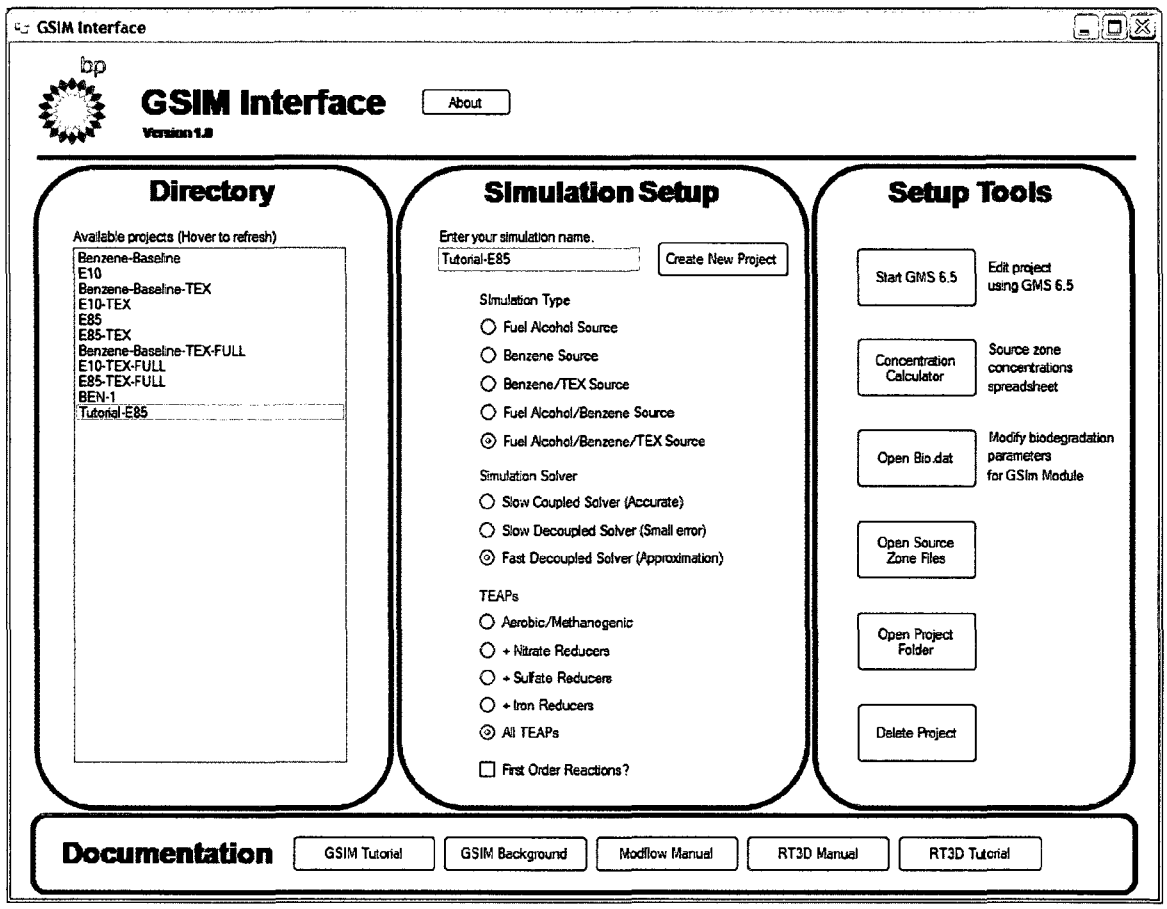
This tutorial guides through the creation, setup, execution and analysis of a 16-year simulation of the fate and transport of a E85 (85% Ethanol) release, considering BTEX degradation under several electron acceptor conditions. This example will illustrate the use of the different tools and how to make changes for different scenarios.

When opening the GSIM Interface, the main window pops up. The directory on the left of the application will list all available projects in the (C:\Program Files\GMS 6.5\GSIM\Projects) folder, from where you select the currently active one. The central section, simulation setup, allows the creation of a new project under several different scenario conditions. The rightmost pane contains the tools to work with the selected project. Finally, the bottom pane, documentation, contains links to user manuals and this guide.



Creating a new project

Start by entering the name of your simulation in the text input space in the middle pane. For this tutorial, we will use "Tutorial-E85". Please note that this field does not accept spaces or symbols reserved by the windows file system like " \". Once you have entered the name, select the desired options using the radio buttons. We will select "Fuel Alcohol/Benzene/TEX Source", "Fast Decoupled Solver (Approximation)" and "All TEAPs". Then, click on <Create New Project>. A message window will notify of the project creation. By moving the mouse over the directory list, we will refresh it, showing our newly created project at the bottom. The whole window should look like this:



Alternative project creation options:

- Simulation Type: Choose the type of LNAPL composition you will use. The advantage of simpler sources is a faster simulation time. Also, consider that sources with TEX have additional substrates that increase the electron acceptor demand of the system slightly and thus result in longer overall contaminant plumes.

- Simulation Solver: This defines the speed and accuracy of the GSIM module. *Slow Coupled Solver is the most accurate*; it calculates the rate of change of contaminant species and biological populations and then passes these parameters to RT3D to solve the reactive transport equation. It requires small time steps (default 0.01 days), which can be modified by the user. *Slow Decoupled Solver* is faster and introduces a small error by decoupling the reactive transport equation. GSIM solves the degradation rates explicitly and RT3D solves only transport processes. The simulations default to 0.01 days time step, which –cannot- be changed by the user in RT3D, as it would result in numerical errors. Finally, *Fast Decoupled Solver* provides an estimation (with a 1-5% numerical error) using a decoupled reactive transport equation with a large simulation time. This is the fastest simulation method with a fixed time step of 0.2 days (not changeable in RT3D). For a 30 year simulations, the solvers results in approximately 2 days, 12 hours and 3 hours simulation times respectively. It is recommended to use the fast solver to setup and test scenarios, and then use a more accurate solver for the final simulations as needed.

- TEAPs: This options allow the user to pick what terminal electron acceptor processes to use in the simulations. All simulations consider aerobic, then switching to methanogenic conditions as the system becomes anaerobic. Additional anerobic electron acceptors include nitrate, sulfate and iron.

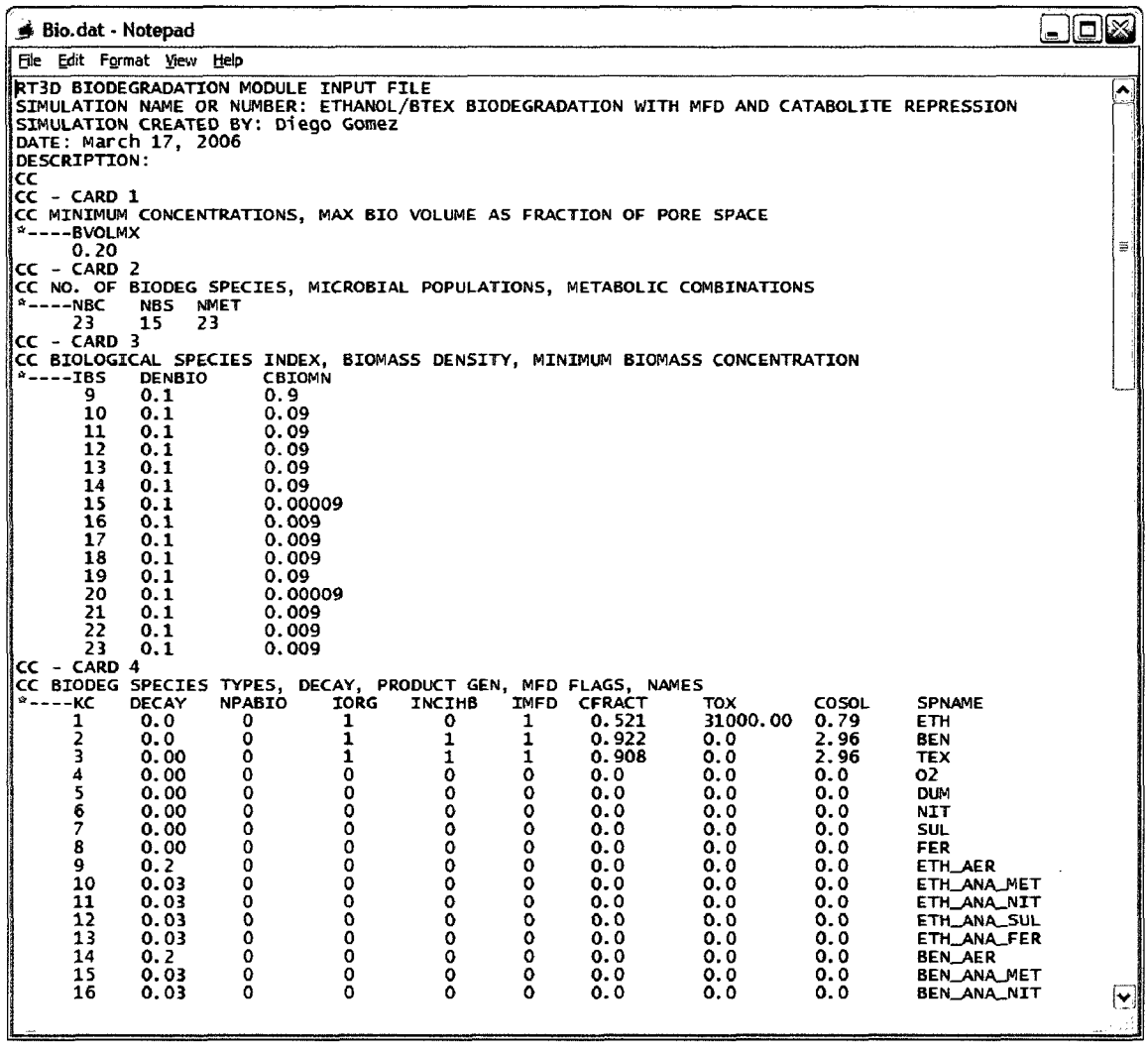
- First Order Reactions: Tick this if you want to use first order reaction rates instead of full MONOD kinetics. This will assume no microbial population changes. It is not recommended to use this option for simulations that evaluate the impact of ethanol on BTEX degradation, as fortuitous growth, metabolic flux dilution and catabolite repression cannot be properly implemented in the absence of microbial populations.

Managing Projects

The Setup tools section provides two tools that can help manage your projects better. The 'Open Project Folder' lets you browse the contents of the RT3D folder of your project. From there you can edit files and backup the bio.dat file if required. The 'Delete project' button will let you remove a project from the directory and will permanently delete all files related to that project from the hard drive. It is recommended you backup your project files often, as setup can be time consuming and on certain occasions the files can become corrupted.

GSIM Inputs : the bio.dat file

The GSIM module requires several inputs related to biodegradation, cosolvency and toxicity. These parameters are grouped in a single text file that describes the biological processes called 'bio.dat'. The 'Open Bio.dat' button on the Setup tools will open the file associated with the selected project in the directory, using notepad for editing. If we select 'Tutorial-E85' in the directory and press this button, we will see the following window:



Bio.dat - Notepad

File Edit Format View Help

```
RT3D BIODEGRADATION MODULE INPUT FILE
SIMULATION NAME OR NUMBER: ETHANOL/BTEX BIODEGRADATION WITH MFD AND CATABOLITE REPRESSION
SIMULATION CREATED BY: Diego Gomez
DATE: March 17, 2006
DESCRIPTION:
CC
CC - CARD 1
CC MINIMUM CONCENTRATIONS, MAX BIO VOLUME AS FRACTION OF PORE SPACE
*----BVOLMX
     0.20
CC - CARD 2
CC NO. OF BIODEG SPECIES, MICROBIAL POPULATIONS, METABOLIC COMBINATIONS
*----NBC    NBS   NMET
     23     15    23
CC - CARD 3
CC BIOLOGICAL SPECIES INDEX, BIOMASS DENSITY, MINIMUM BIOMASS CONCENTRATION
*----IBS    DENBIO      CBIOMN
      9     0.1         0.9
     10     0.1         0.09
     11     0.1         0.09
     12     0.1         0.09
     13     0.1         0.09
     14     0.1         0.09
     15     0.1         0.00009
     16     0.1         0.009
     17     0.1         0.009
     18     0.1         0.009
     19     0.1         0.09
     20     0.1         0.00009
     21     0.1         0.009
     22     0.1         0.009
     23     0.1         0.009
CC - CARD 4
CC BIODEG SPECIES TYPES, DECAY, PRODUCT GEN, MFD FLAGS, NAMES
*----KC    DECAY    NPABIO    IORG    INCIHB    IMFD    CFRACT      TOX         COSOL    SPNAME
     1     0.0      0         1       0         1       0.521       31000.00    0.79     ETH
     2     0.0      0         1       1         1       0.922       0.0         2.96     BEN
     3     0.00     0         1       1         1       0.908       0.0         2.96     TEX
     4     0.00     0         0       0         0       0.0         0.0         0.0      O2
     5     0.00     0         0       0         0       0.0         0.0         0.0      DUM
     6     0.00     0         0       0         0       0.0         0.0         0.0      NIT
     7     0.00     0         0       0         0       0.0         0.0         0.0      SUL
     8     0.00     0         0       0         0       0.0         0.0         0.0      FER
     9     0.2      0         0       0         0       0.0         0.0         0.0      ETH_AER
    10     0.03     0         0       0         0       0.0         0.0         0.0      ETH_ANA_MET
    11     0.03     0         0       0         0       0.0         0.0         0.0      ETH_ANA_NIT
    12     0.03     0         0       0         0       0.0         0.0         0.0      ETH_ANA_SUL
    13     0.03     0         0       0         0       0.0         0.0         0.0      ETH_ANA_FER
    14     0.2      0         0       0         0       0.0         0.0         0.0      BEN_AER
    15     0.03     0         0       0         0       0.0         0.0         0.0      BEN_ANA_MET
    16     0.03     0         0       0         0       0.0         0.0         0.0      BEN_ANA_NIT
```

This file contains all the inputs required for GSIM in text format, organized in seven different sections, or CARDS. Some of the features of the GSIM module that appear on the bio.dat cards have been disabled due to compatibility with the cosolvency

and substrate interaction processes incorporated into it; these parameters will appear as <unused>. Inputs required in each section are (Units of m, d and mg/l):

CARD1

BVOLMAX: Fraction of pore space available for microbial biofilm growth. Default: 0.2 [vol/vol], Range: 0 – 0.5.

```
CC - CARD 1
CC MINIMUM CONCENTRATIONS, MAX BIO VOLUME AS FRACTION OF PORE SPACE
*-----BVOLMX
      0.20
```

CARD2

NBC: Total number of species in the model, including susbtrates, electron acceptors and microbial populations. Default: 23, Range: 1-50.

NBS: Number of microbial populations. Default: 15, Range: Has to be smaller than NBC.

NMET: Number of metabolic combinations to be considered during the simulation (e.g., aerobic degradation of benzene would be 1). Default: 23, Range: Limited only by simulation speed.

```
CC - CARD 2
CC NO. OF BIODEG SPECIES, MICROBIAL POPULATIONS, METABOLIC COMBINATIONS
*-----NBC    NBS  NMET
      23     15   9
```

CARD3

IBS: Numerical index of microbial populations.

DENBIO: Density of biofims in [10^{-5} mg/l]. Default: 0.1.

CBIOMN: Minimum background concentration of microbial populations [mg/l]. Default: variable, 0.001 to 1.

```
CC - CARD 3
CC BIOLOGICAL SPECIES INDEX, BIOMASS DENSITY, MINIMUM BIOMASS CONCENTRATION
*-----IBS    DENBIO      CBIOMN
        9    0.1         0.9
       10    0.1         0.09
       11    0.1         0.09
       12    0.1         0.09
       13    0.1         0.09
       14    0.1         0.09
       15    0.1         0.00009
       16    0.1         0.009
       17    0.1         0.009
       18    0.1         0.009
       19    0.1         0.09
       20    0.1         0.00009
       21    0.1         0.009
       22    0.1         0.009
       23    0.1         0.009
```

CARD4

This section defines all the chemical species involved in the simulation and some of their properties.

KC: Numerical index of chemical species and populations involved in the simulation.

DECAY: Decay rate [1/d] of species. For microbial populations this is their death rate. Default: 0.2 aerobic microbial populations, 0.03 anaerobic microbial populations.

NPABIO: <Unused>

IORG: Flag [1 for on or 0 for off] indicating if this species is an organic contaminant acting as a substrate for microbial populations.

INCIHB: Catabolite repression flag [1 for on or 0 for off].

IMFD: Metabolic flux dilution flag [1 for on or 0 for off].

CFRACT: Carbon fraction of the species. Default: Variable, 0.5-0.99, Range: 0 to 1.

TOX: MC50 Toxicity of the species [mg/l]. Default: Variable, 2,000-40,000.

COSOL: Cosolvency power of the fuel alcohol on the associated chemical species. This value should be the same for all organic contaminants unless they have special or unusual interactions with the cosolvent. In row 1 of this card, this value should be set to the alcohol density (The cosolvent should always be species 1 in the list). Default: 2.96.

SPNAME: Name of the chemical species or microbial population for reference.

CC - CARD 4
CC BIODEG SPECIES TYPES, DECAY, PRODUCT GEN, MFD FLAGS, NAMES

*----KC	DECAY	NPABIO	IORG	INCIHB	IMFD	CFRACT	TOX	COSOL	SPNAME
1	0.0	0	1	0	1	0.521	31000.00	0.79	ETH
2	0.0	0	1	1	1	0.922	0.0	2.96	BEN
3	0.00	0	1	1	1	0.908	0.0	2.96	TEX
4	0.00	0	0	0	0	0.0	0.0	0.0	O2
5	0.00	0	0	0	0	0.0	0.0	0.0	DUM
6	0.00	0	0	0	0	0.0	0.0	0.0	NIT
7	0.00	0	0	0	0	0.0	0.0	0.0	SUL
8	0.00	0	0	0	0	0.0	0.0	0.0	FER
9	0.2	0	0	0	0	0.0	0.0	0.0	ETH_AER
10	0.03	0	0	0	0	0.0	0.0	0.0	ETH_ANA_MET
11	0.03	0	0	0	0	0.0	0.0	0.0	ETH_ANA_NIT
12	0.03	0	0	0	0	0.0	0.0	0.0	ETH_ANA_SUL
13	0.03	0	0	0	0	0.0	0.0	0.0	ETH_ANA_FER
14	0.2	0	0	0	0	0.0	0.0	0.0	BEN_AER
15	0.03	0	0	0	0	0.0	0.0	0.0	BEN_ANA_MET
16	0.03	0	0	0	0	0.0	0.0	0.0	BEN_ANA_NIT
17	0.03	0	0	0	0	0.0	0.0	0.0	BEN_ANA_SUL
18	0.03	0	0	0	0	0.0	0.0	0.0	BEN_ANA_FER
19	0.2	0	0	0	0	0.0	0.0	0.0	TEX_AER
20	0.03	0	0	0	0	0.0	0.0	0.0	TEX_ANA_MET
21	0.03	0	0	0	0	0.0	0.0	0.0	TEX_ANA_NIT
22	0.03	0	0	0	0	0.0	0.0	0.0	TEX_ANA_SUL
23	0.03	0	0	0	0	0.0	0.0	0.0	TEX_ANA_FER

CARD5

This card defines the metabolic combinations used in the model, and their associated Monod biokinetic parameters. The number of combinations defined must match NMET.

ISUB: Numerical index indicating the chemical species acting as a substrate for this metabolic combination.

ISUB2: Original substrate associated to the microbial population of the metabolic combination. These values are the same to ISUB for all cases, except for metabolic combinations used to represent fortuitous growth.

IEA: Numerical index indicating the chemical species acting as electron acceptor for this metabolic combination.

IBS: Numerical index indicating the microbial populations for this metabolic combination.

BRMAXB: Maximum specific growth rate associated to this metabolic combination [1/d]. Default: Variable, 0.21 – 11.04.

YXS: Biomass yield associated to this metabolic combination [mg/mg]. Default: Variable, 0.07 – 0.5.

AKS: Substrate half saturation coefficient for this metabolic combination [mg/l]. Default: Variable, 0.26 – 63.09.

AKA: Electron acceptor half saturation coefficient for this metabolic combination [mg/l]. Default: Variable, 0.21- 6.628.

FEA: Stoichiometric electron acceptor utilization per substrate degraded [mg/mg]. Default: Variable, 0 - 28.05.

CC - CARD 5
CC METABOLIC COMBINATION INFORMATION AND MONOD PARAMETERS

*----ISUB	ISUB2	IEA	IBS	BRMAXB	YXS	AKS	AKA	FEA
1	1	4	9	11.04	0.50	63.09	0.210	0.63
1	1	7	12	0.21	0.18	11.43	6.628	2.74
1	1	5	10	1.10	0.07	78.86	0.000	0.00
1	2	4	14	11.04	0.50	88.36	0.210	0.63
1	2	7	17	0.21	0.18	16.01	6.628	2.74
1	2	5	15	1.10	0.07	110.44	0.000	0.00
2	2	4	14	3.24	0.39	7.63	0.210	1.24
2	2	7	17	1.25	0.43	1.80	6.628	3.69
2	2	5	15	0.30	0.05	21.58	0.000	0.00

CARD6

This section defines which metabolic combinations are inhibited by the presence of certain chemical species in the system.

ISUB: Numerical index indicating the chemical species acting as a substrate for this metabolic combination.

IEA: Numerical index indicating the chemical species acting as electron acceptor for this metabolic combination.

IBS: Numerical index indicating the microbial populations for this metabolic combination.

COMPBIO: <Unused>

NIHB: Number of inhibiting species acting on this metabolic combination. (ie, oxygen inhibiting anaerobic degradation).

NPROD: <Unused>

NNUT: <Unused>

```
CC - CARD 6
CC FLAGS FOR COMPETITION, INHIBITION, PRODUCT GEN., NUTRIENT LIMITATIONS
*-----ISUB IEA  IBS  COMPBIO  NIHB  NPROD  NNUT
      1    4   9      0       0      0      0
      1    7   12     0       1      0      0
      1    5   10     0       2      0      0
      1    4   14     0       0      0      0
      1    7   17     0       1      0      0
      1    5   15     0       2      0      0
      2    4   14     0       0      0      0
      2    7   17     0       1      0      0
      2    5   15     0       2      0      0
```

CARD7

This section defines the inhibition factors for all the inhibited metabolic combinations defined in section 6.

ISUB: Numerical index indicating the chemical species acting as a substrate for this metabolic combination.

IEA: Numerical index indicating the chemical species acting as electron acceptor for this metabolic combination.

IBS: Numerical index indicating the microbial populations for this metabolic combination.

IHB: Numerical index indicating the chemical specie acting as inhibitor for this metabolic combination.

BIHB: Inhibiting factor. Default: 0.1- 0.48.

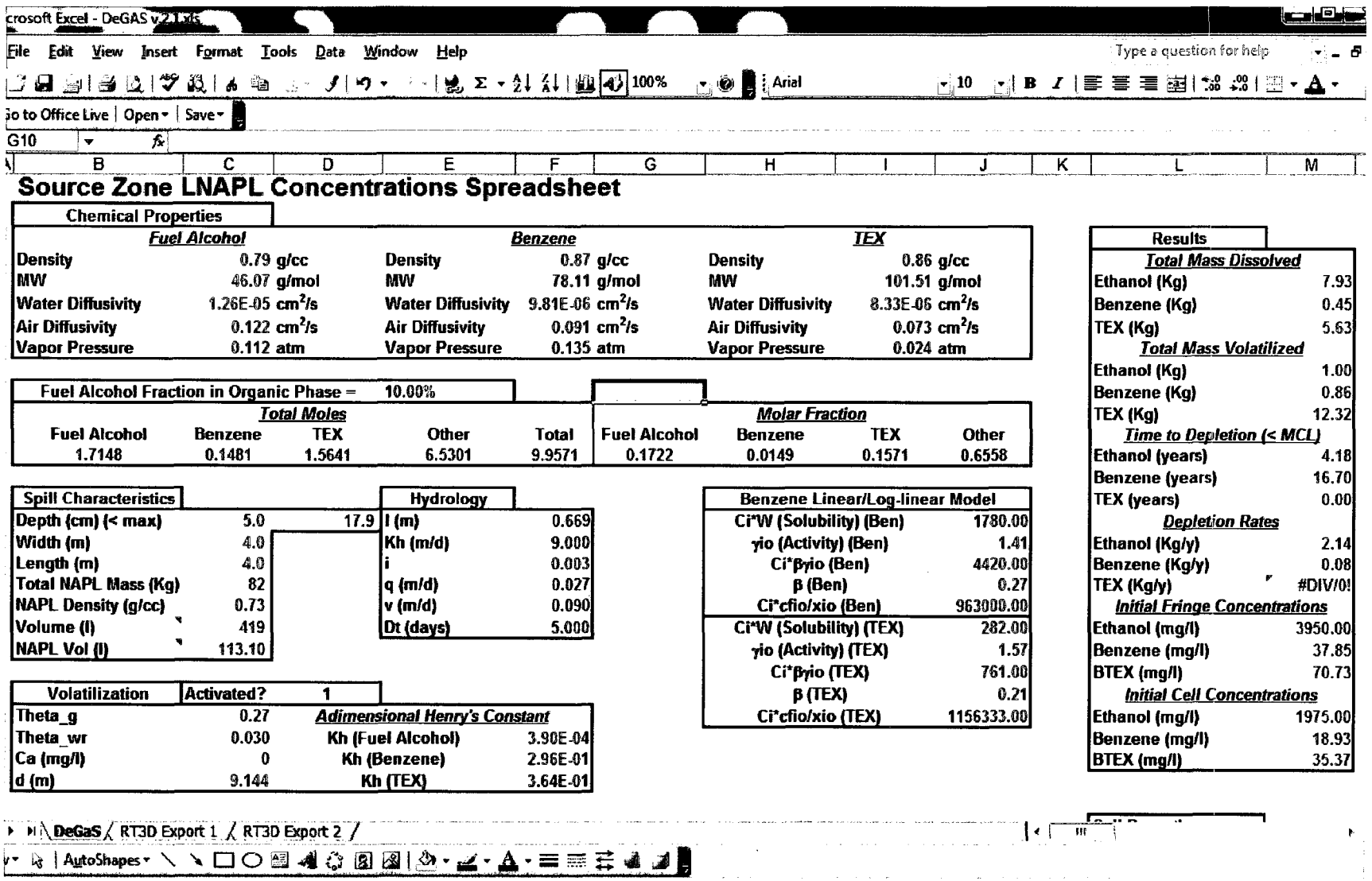
It is very important that the number (index) of species, microbial populations and electron acceptors match with those defined in RT3D. For example, if Benzene is number 2 in the

bio.dat file, then it has to be defined as number 2 in the RT3D list. Also, Indexes should correctly match between cards. Any errors on these indexes will cause the model to crash.

```
CC - Card 7
CC Substrate or Electron Acceptor Inhibition
*----ISUB IEA  IBS   IHB  BIHB
     1    7    12    4    0.100
     1    5    10    4    0.100
     1    5    10    7    0.480
     1    7    17    4    0.100
     1    5    15    4    0.100
     1    5    15    7    0.480
     2    7    17    4    0.100
     2    5    15    4    0.100
     2    5    15    7    0.480
```

Modifying Source Zone Concentrations

RT3D can handle several types of source zones, including constant concentration, mass loading, transient time series, etc. By default, the GSIM Interface creates a project that uses a transient time series of concentration for the different species involved (Alcohol, benzene, toluene, ethylbenzene and xylene), in a 4 by 4 meter area, resulting from a dissolving 30 gallons of LNAPL.


crosoft Excel - DeGAS v2.1.xls
File Edit View Insert Format Tools Data Window Help
Type a question for help
Arial 10
Go to Office Live | Open | Save
G10
B C D E F G H I J K L M
Source Zone LNAPL Concentrations Spreadsheet
Chemical Properties
Fuel Alcohol
Density 0.79 g/cc
MW 46.07 g/mol
Water Diffusivity 1.26E-05 cm²/s
Air Diffusivity 0.122 cm²/s
Vapor Pressure 0.112 atm
Benzene
Density 0.87 g/cc
MW 78.11 g/mol
Water Diffusivity 9.81E-06 cm²/s
Air Diffusivity 0.091 cm²/s
Vapor Pressure 0.135 atm
TEX
Density 0.86 g/cc
MW 101.51 g/mol
Water Diffusivity 8.33E-06 cm²/s
Air Diffusivity 0.073 cm²/s
Vapor Pressure 0.024 atm
Results
Total Mass Dissolved
Ethanol (Kg) 7.93
Benzene (Kg) 0.45
TEX (Kg) 5.63
Total Mass Volatilized
Ethanol (Kg) 1.00
Benzene (Kg) 0.86
TEX (Kg) 12.32
Time to Depletion (< MCL)
Ethanol (years) 4.18
Benzene (years) 16.70
TEX (years) 0.00
Depletion Rates
Ethanol (Kg/y) 2.14
Benzene (Kg/y) 0.08
TEX (Kg/y) #DIV/0!
Initial Fringe Concentrations
Ethanol (mg/l) 3950.00
Benzene (mg/l) 37.85
BTEX (mg/l) 70.73
Initial Cell Concentrations
Ethanol (mg/l) 1975.00
Benzene (mg/l) 18.93
BTEX (mg/l) 35.37
Fuel Alcohol Fraction in Organic Phase = 10.00%
Total Moles
Fuel Alcohol 1.7148
Benzene 0.1481
TEX 1.5641
Other 6.5301
Total 9.9571
Molar Fraction
Fuel Alcohol 0.1722
Benzene 0.0149
TEX 0.1571
Other 0.6558
Spill Characteristics
Depth (cm) (< max) 5.0 17.9
Width (m) 4.0
Length (m) 4.0
Total NAPL Mass (Kg) 82
NAPL Density (g/cc) 0.73
Volume (l) 419
NAPL Vol (l) 113.10
Hydrology
I (m) 0.669
Kh (m/d) 9.000
i 0.003
q (m/d) 0.027
v (m/d) 0.090
Dt (days) 5.000
Benzene Linear/Log-linear Model
Ci*W (Solubility) (Ben) 1780.00
γio (Activity) (Ben) 1.41
Ci*βγio (Ben) 4420.00
β (Ben) 0.27
Ci*cfio/xio (Ben) 963000.00
Ci*W (Solubility) (TEX) 282.00
γio (Activity) (TEX) 1.57
Ci*βγio (TEX) 761.00
β (TEX) 0.21
Ci*cfio/xio (TEX) 1156333.00
Volatilization Activated? 1
Theta_g 0.27
Theta_wr 0.030
Ca (mg/l) 0
d (m) 9.144
Adimensional Henry's Constant
Kh (Fuel Alcohol) 3.90E-04
Kh (Benzene) 2.96E-01
Kh (TEX) 3.64E-01
DeGaS RT3D Export 1 RT3D Export 2
AutoShapes

The volume, % of alcohol, and characteristics of the blend can be modified in a spreadsheet to calculate different source zone scenarios. Pressing the "Concentration Calculator" button on the main window, will open this spreadsheet. Each project contains a copy of this file, so it can be modified freely.

In this spreadsheet, values in red are inputs required by the user and values in green are parameters calculated by the spreadsheet. Parameters required are:

Chemicals Properties: Density, molecular weight, water and air diffusivity and vapor pressure of the fuel alcohol, benzene and TEX (average). In most cases, you only have to modify the fuel alcohols parameters to match your blend (e.g., methanol, butanol, etc). By default, ethanol data is presented.

Fuel Alcohol Fraction in Organic Phase: Only parameter required is the percent of fuel alcohols as volume in your blend.

Spill Characteristics: Data regarding the size and volume of your LNAPL. Width and length of the LNAPL lens and also the depth (thickness). This value has to be smaller than the limit (in green), to be consistent with viscosity.

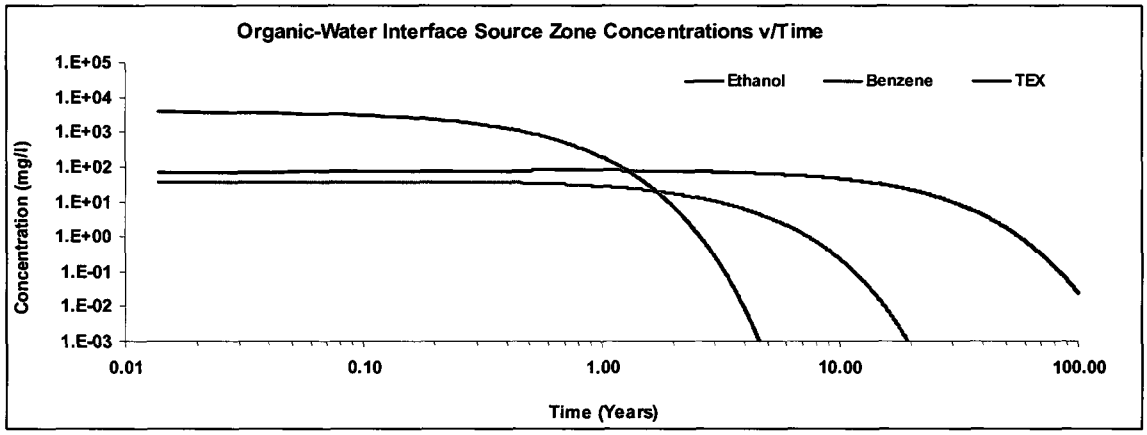
Hydrology: Site characteristics. Hydraulic conductivity, hydraulic gradient and the time step size. Time step size should be a multiple of the time step you use in RT3D.

Benzene Linear/Log-Linear Model: Data required for the Heermann and Powers cosolvency model. Current data is for benzene and TEX in the presence of ethanol. Other alcohols are assumed to follow a similar pattern, unless data is obtained.

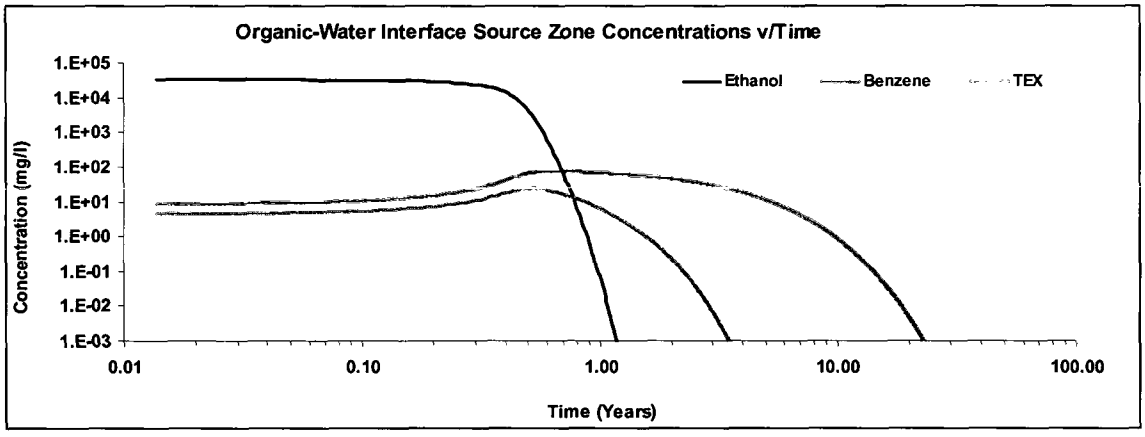
Volatilization: Can be activated or deactivated with a flag (1 or 0). Requires depth to aquifer, and the adimensional henry's constant for the organic species.

Soil Properties: soil grain size, surface tension of LNAPL, soil porosity and effective porosity.

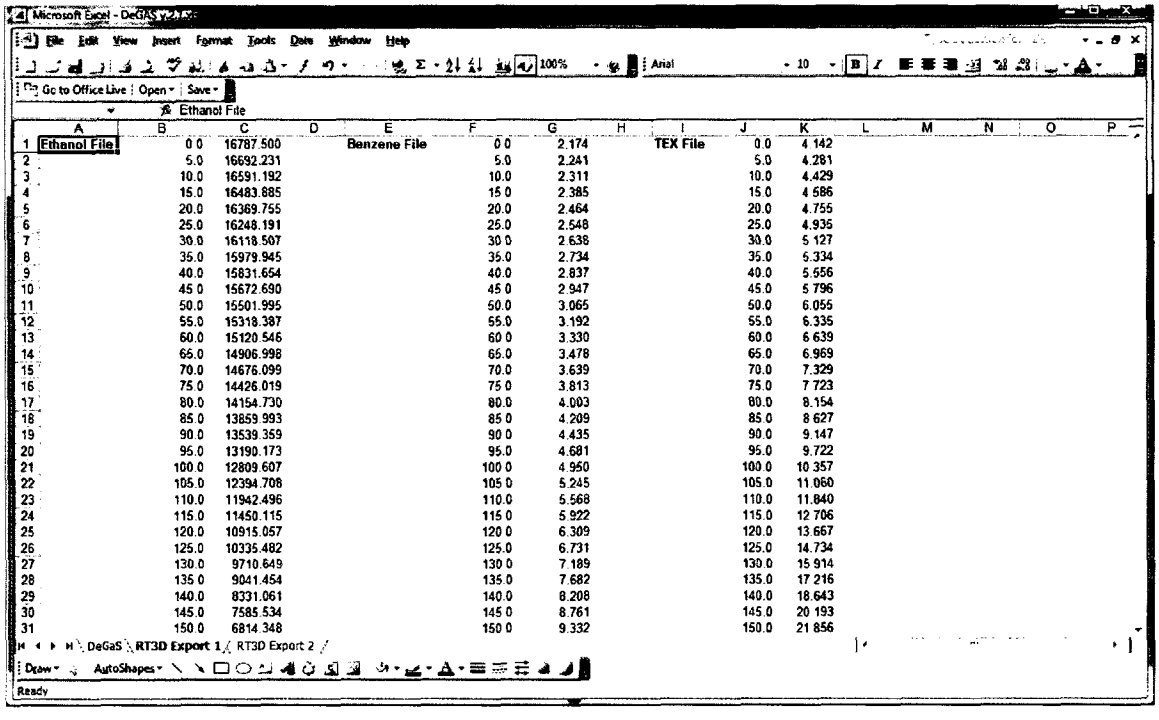
The resulting concentration over time for E10 with volatilization would be:



We will modify this for our E85 example. First, let's change the values in the spreadsheet to match E85 (85% Fuel Alcohol fraction in organic phase). This results in the following concentration curves:

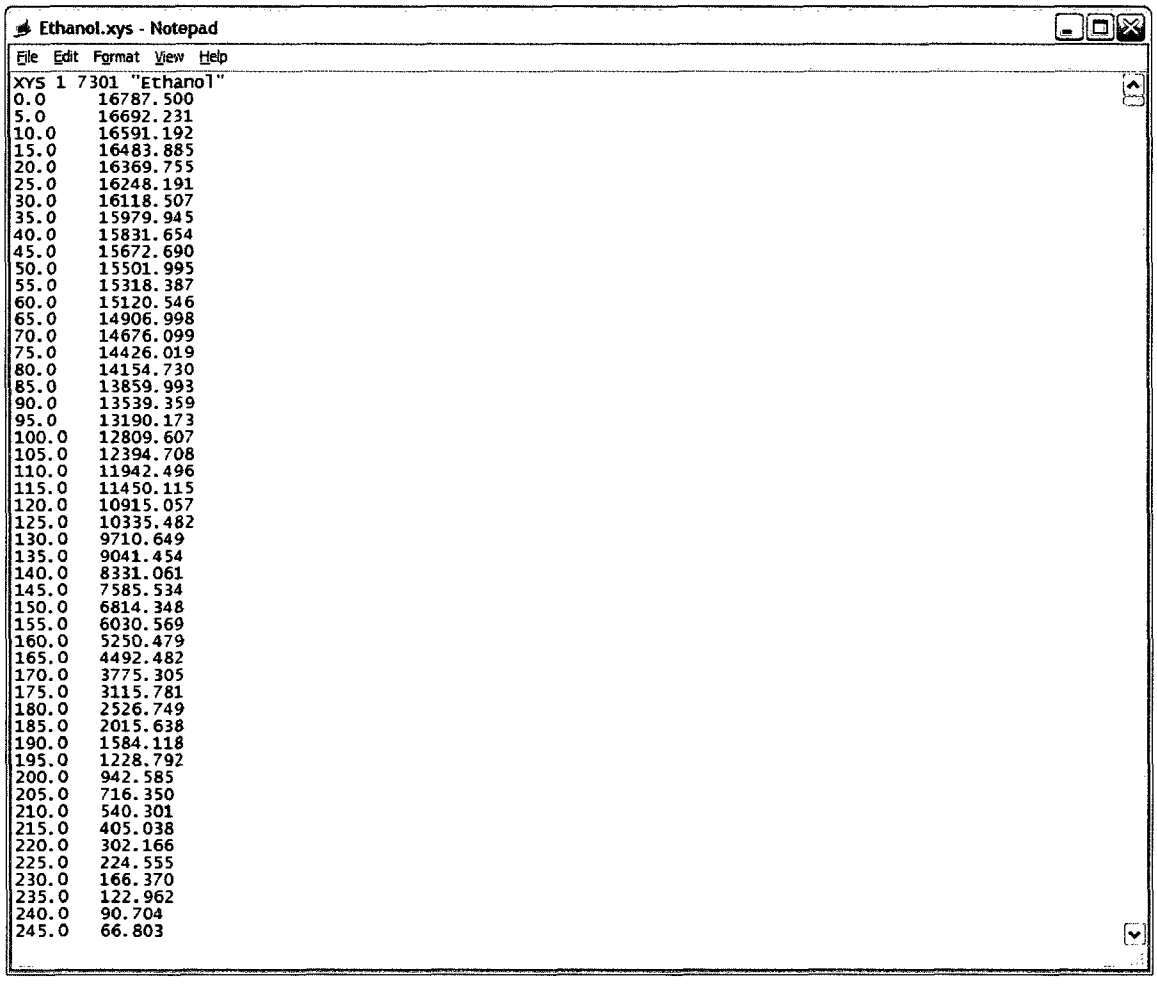


We now need to export these time series so that they can be usable in RT3D. The spreadsheet has two additional worksheets (tabs): RT3D Export 1 and RT3D Export 2. The first one is the average concentration for a cell assuming a mixing depth of ~3-2 meters and linear distribution. The second one is the concentration values on the boundary between water and LNAPL. We will use the first option.



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Ethanol File	0.0	16787.500		Benzene File	0.0	2.174		TEX File	0.0	4.142					
2		5.0	16692.231			5.0	2.241			5.0	4.281					
3		10.0	16591.192			10.0	2.311			10.0	4.429					
4		15.0	16483.885			15.0	2.385			15.0	4.586					
5		20.0	16369.755			20.0	2.464			20.0	4.755					
6		25.0	16248.191			25.0	2.548			25.0	4.935					
7		30.0	16118.507			30.0	2.638			30.0	5.127					
8		35.0	15979.945			35.0	2.734			35.0	5.334					
9		40.0	15831.654			40.0	2.837			40.0	5.556					
10		45.0	15672.690			45.0	2.947			45.0	5.796					
11		50.0	15501.995			50.0	3.065			50.0	6.055					
12		55.0	15318.387			55.0	3.192			55.0	6.335					
13		60.0	15120.546			60.0	3.330			60.0	6.639					
14		65.0	14906.998			65.0	3.478			65.0	6.969					
15		70.0	14676.099			70.0	3.639			70.0	7.329					
16		75.0	14426.019			75.0	3.813			75.0	7.723					
17		80.0	14154.730			80.0	4.003			80.0	8.154					
18		85.0	13859.993			85.0	4.209			85.0	8.627					
19		90.0	13539.359			90.0	4.435			90.0	9.147					
20		95.0	13190.173			95.0	4.681			95.0	9.722					
21		100.0	12809.607			100.0	4.950			100.0	10.357					
22		105.0	12394.708			105.0	5.245			105.0	11.060					
23		110.0	11942.496			110.0	5.568			110.0	11.840					
24		115.0	11450.115			115.0	5.922			115.0	12.706					
25		120.0	10915.057			120.0	6.309			120.0	13.667					
26		125.0	10335.482			125.0	6.731			125.0	14.734					
27		130.0	9710.649			130.0	7.189			130.0	15.914					
28		135.0	9041.454			135.0	7.682			135.0	17.216					
29		140.0	8331.061			140.0	8.208			140.0	18.643					
30		145.0	7585.534			145.0	8.761			145.0	20.193					
31		150.0	6814.348			150.0	9.332			150.0	21.856					

We now press the "Open Source Zone Files" button. This operation will open 3 text windows showing the files benzene.xys, ethanol.xys and TEX.xys. These files are in the format required to import into RT3D. Now, we select the two columns for a given organic species (for example, columns B and C for ethanol), copy these values (control-c), the paste them on top of the time series values in the corresponding opened file (ethanol.xys). Make sure there are no extra empty lines at the end of data, expect for 1 <return>. The ethanol.xys file should look like this:

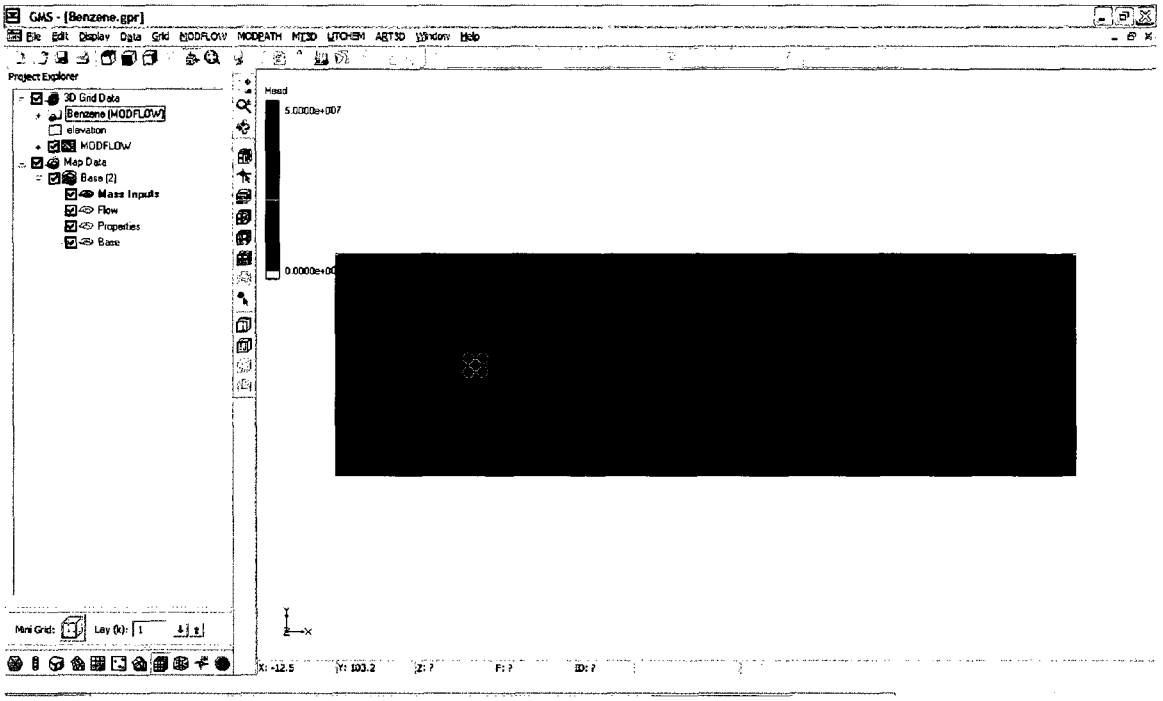


Note the third number in the first line: 7301. This indicates the number of data points in the file and should match the number of rows of the time series. Repeat this for

the other two files and save. Our new concentrations are now ready to be used in RT3D. We will see how to import them next, when using the GMS interface.

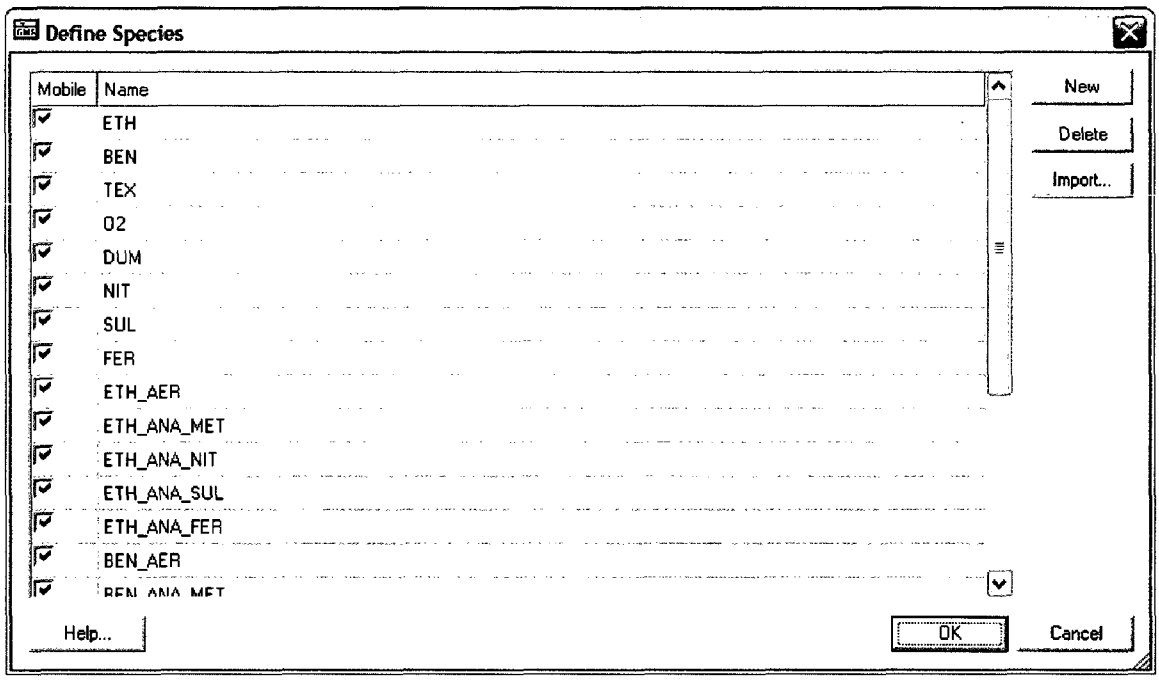
Running the model

Start by running the groundwater modeling system program (GMS 6.5), that should be installed the computer. The "Start GMS 6.5" button provides a quick access to it. The hardware USB lock should be present in the computer for this software to work correctly. Now, select File->Open, and navigate to "C:\Program Files\GMS 6.5\GSIM\Projects" where you will see the available saved folders. Double click on "Tutorial-E85" and then on the Benzene.grp file. This will open the project. There will be two error messages at this point, due to limitations on the amount of chemical species built into GMS. However, RT3D can run fine with the 23 species defined as long as we rebuild the boundary conditions every time we open the project, so ignore the messages. The opened project should look like this:



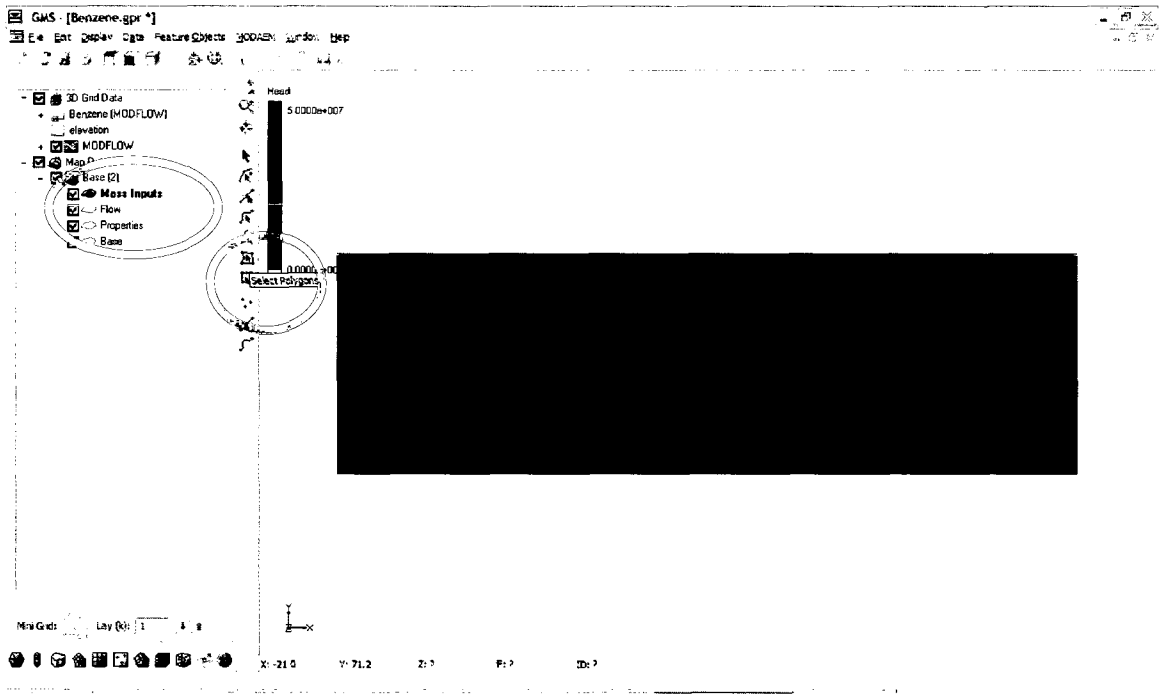
Once the project is open, you can run RT3D right away, by selecting MT3D->Run RT3D…; however, we want to do some changes first and add our new source zone concentrations.

To rebuild the boundary conditions, first define what species are immobile. On the left side of the screen you will see a list called "Project Explorer" that shows all of the elements present in the GMS file. Under Map Data, select the object that says Base(2), then right click on it. On the menu that appears select properties. Click on the button Define Species. The following window should appear:

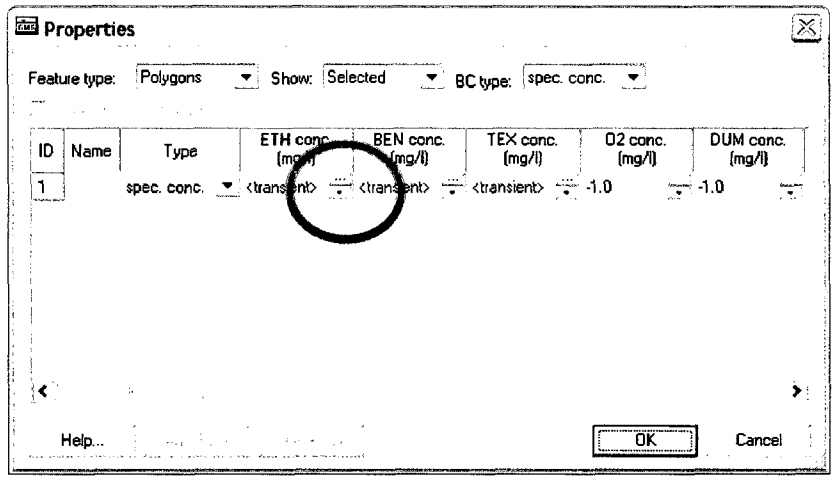


This is the list of the 23 chemical species used by the model (should match the list present in the bio.dat file from GSIM). Uncheck all the immovable species starting from bottom to top (this order is important). Uncheck all the biological species AND Iron, as we assume it is not dissolved in groundwater. Then click OK on both the opened windows.

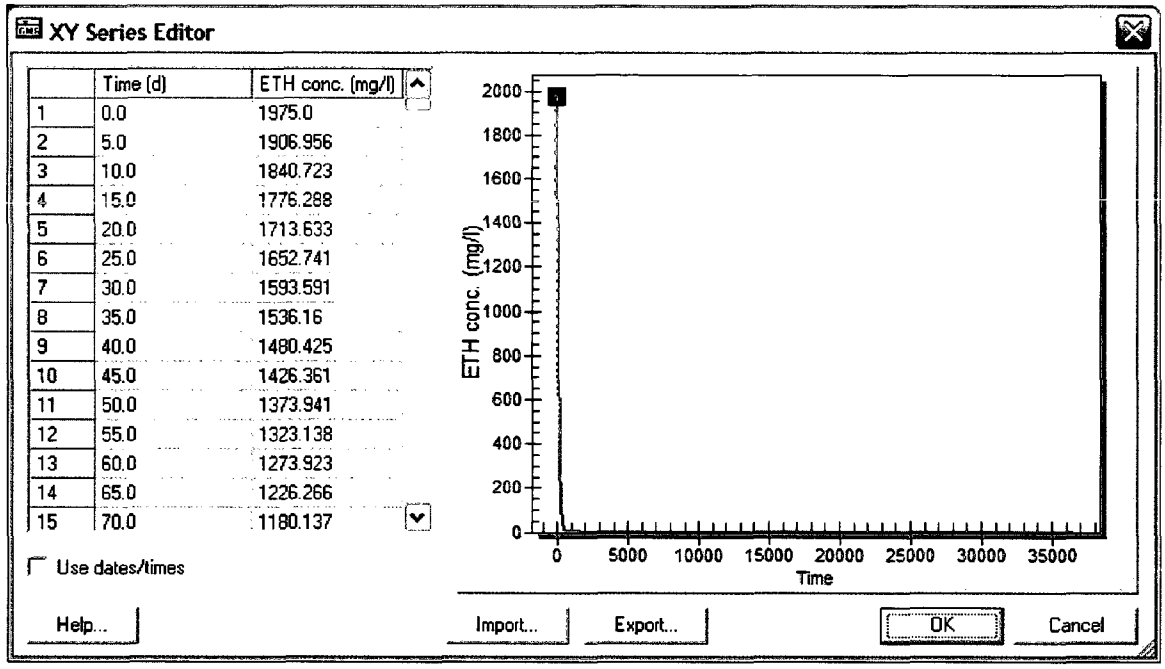
Now, select the object "Mass Inputs" and then the tool Select Polygons. With this tool, double click on the polygonal area within the model domain. This area defines where our LNAPL spill occurs from a top down point of view. You can change the shape if desired, but remember that the total LNAPL mass might change and thus concentrations should be recalculated using the concentration spreadsheet.



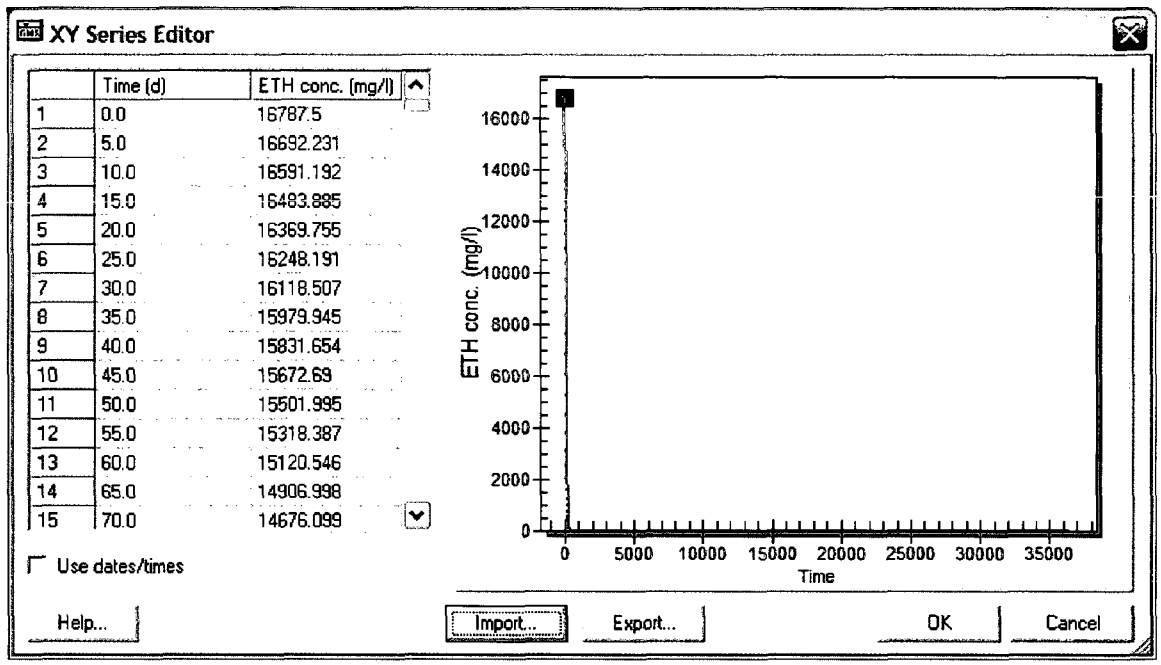
Double clicking will bring up the Properties window for our source zone polygon:



As you can see, ETH (ethanol or other alcohols), BEN (benzene) and TEX (toluene, ethylbenzene and xylene) concentrations are defined here. Click on the small "..." Button next to <transient> for each of the 3 organics to import our .XYS files. For ethanol, the following dialogue will pop:



Which shows the default concentrations for E10 (10% ethanol) every 5 days. Click on "Import..." and within the new dialogue, navigate to "C:\Program Files\GMS 6.5\GSIM\Projects\Tutorial-E85\Benzene_RT3D" and select the "Ethanol.xys" file. Click Open, and the new concentrations will be imported like so:

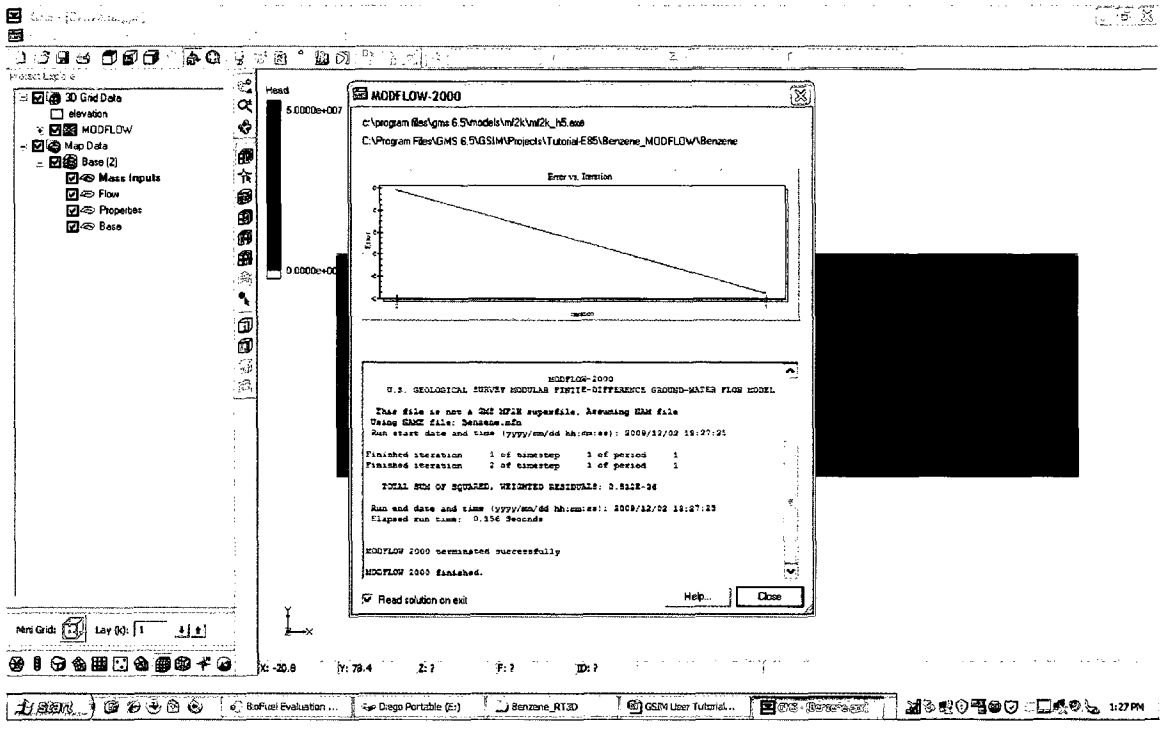


As expected. Ethanol concentrations are much higher for E85 than E10. Click OK and repeat the same procedure for benzene and TEX. Finally close the Properties window with OK. The last step is to assign the new boundary conditions to the RT3D model. For this, first save the project by clicking the save button on the toolbar, then click on the menu "Feature Objects" -> "Map -> MODFLOW", and "Feature Objects" -> "Map -> Modflow-> RT3D". A question will pop on each case, select "All applicable coverages" and OK. Save your project.

At this point you can change any of the other properties in the project, that are grouped on the elements of the project explorer of GMS, like Flow, Base properties (hydraulic conductivity, etc) and others.

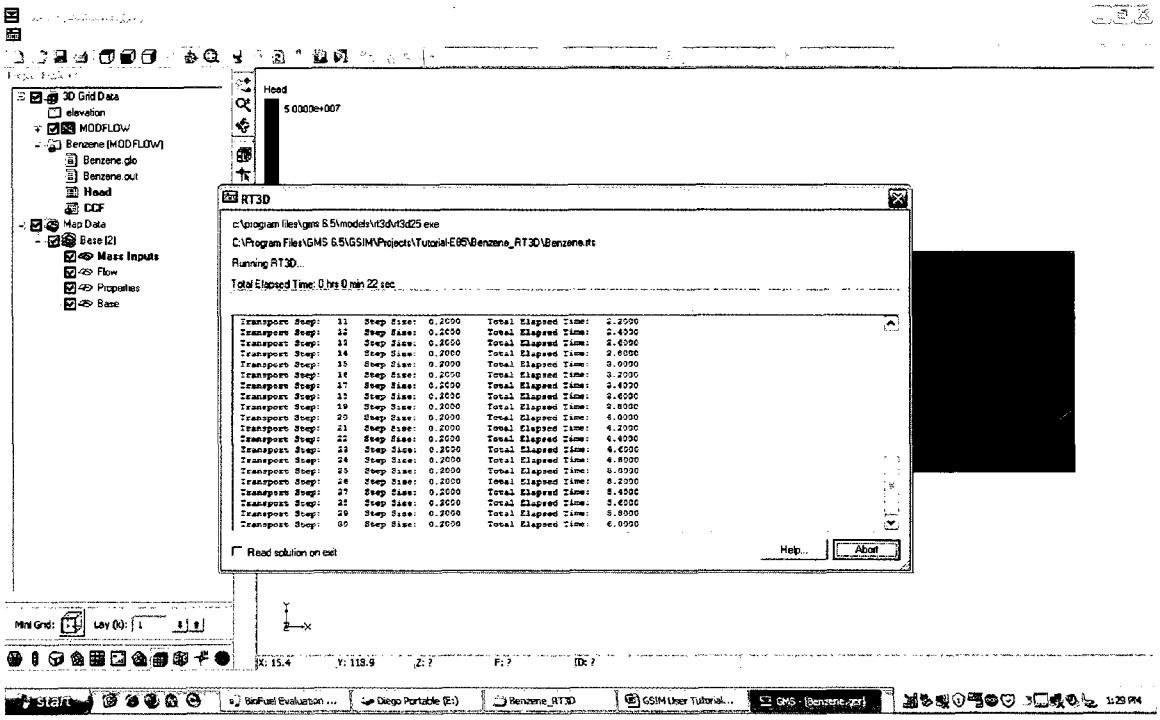
You can also create your own project with a new Grid following the GMS/RT3D tutorials. As long as your list of chemical species defined in RT3D matches those defined in the bio.dat file, and you tell RT3D to use the custom reaction module, then you can use GSIM with your new setup.

Now that all parameters are defined, click on the "3D Grid Data" element on the Project Explorer, save, and then select the menu "MODFLOW" -> "Run MODFLOW".



This popup should appear if everything went smoothly, indicating that MODFLOW has been successfully completed. Once you close the project, the groundwater head results for our domain will be imported and be ready to use with RT3D.

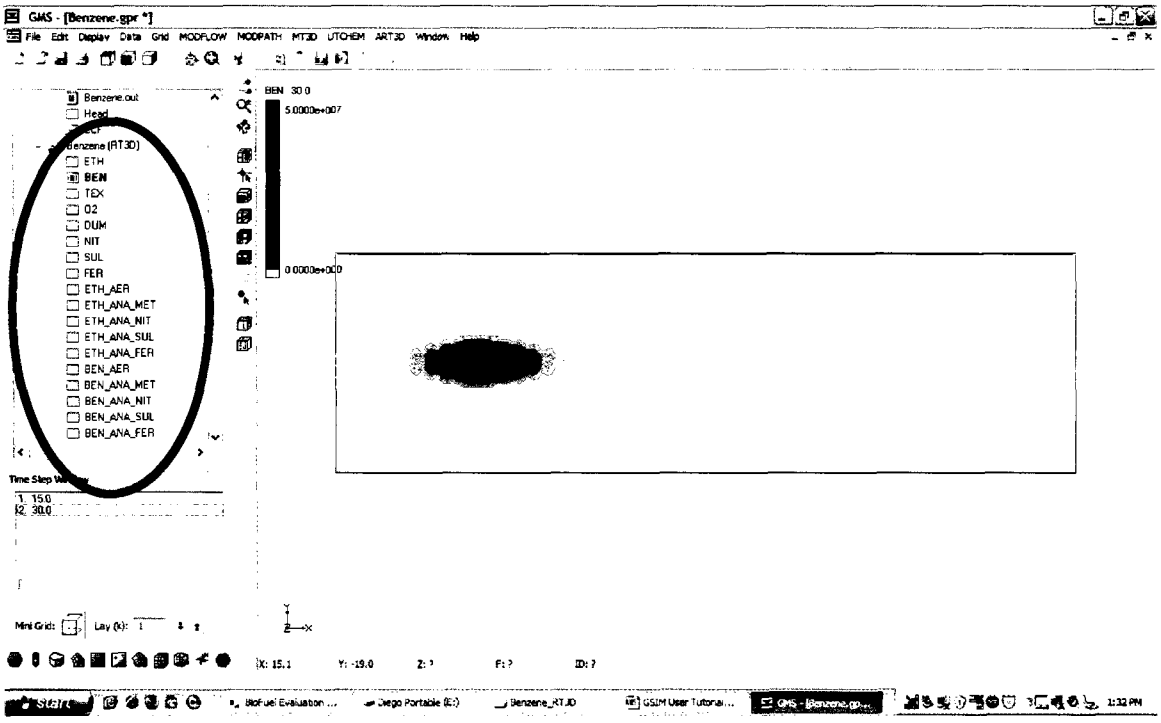
Click save. Note that it is important to click the save button often, ideally after each important operation. Now select the menu "MT3D" -> "Run RT3D…"



You will see a popup with RT3D progress and time to completion. In this case, with the fast solver, it should take about 2 hours. You can also check that you are running the correct RT3D file "C:\Program Files\GMS 6.5\GSIM\Projects\Tutorial-E85\Benzene_RT3D\Benzene.rts". Click OK to read the results once RT3D is finished.

Reading the results

Finally, we want to see the results. After completing RT3D you should see a list of new elements on the Project Explorer, including all our 23 chemical species.



Select BEN, and the benzene plume concentrations will be displayed on the main window. The default display is a range of concentrations down to 5 ppb, the current MCL for benzene. You can select a different time step to see the evolution of the plume. You can also use all of the common visual tools included in GMS, like creating animations of the plumes with time clock, changing the colors, etc.