Analytical model for BTEX natural attenuation in the presence of fuel ethanol and its anaerobic metabolite acetate

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1. ABSTRACT

Flow-through column studies were conducted to mimic the natural attenuation of ethanol and BTEX mixtures, and to consider potential inhibitory effects of ethanol and its anaerobic metabolite acetate on BTEX biodegradation. Results were analyzed using a one-dimensional analytical model that was developed using consecutive reaction differential equations based on first-order kinetics. Decrease in pH due to acetogenesis was also modeled, using charge balance equations under $CaCO_3$ dissolution conditions. Delay in BTEX removal was observed and simulated in the presence of ethanol and acetate. Acetate was the major volatile fatty acid intermediate produced during anaerobic ethanol biodegradation (accounting for about 58% of the volatile fatty acid mass) as suggested by the model data fit. Acetate accumulation (up to 1.1 g/L) near the source zone contributed to a pH decrease by almost one unit. The anaerobic degradation of ethanol (2 g/L influent concentration) at the source zone produced methane at concentrations exceeding its solubility (≅ 26 mg/L). Overall, this simple analytical model adequately described ethanol degradation, acetate accumulation and methane production patterns, suggesting that it could be used as a screening tool to simulate lag times in BTEX biodegradation, changes in groundwater pH and methane generation following ethanol-blended fuel releases.

Keywords: Acetate, BTEX, ethanol, natural attenuation, modeling, methane, pH

2. INTRODUCTION

Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene and the three isomers of xylene (BTEX) are ubiquitous groundwater pollutants commonly associated with releases of petroleum products. Benzene, which can cause leukemia, is the most toxic of the BTEX compounds with an EPA MCL (Maximum Contaminant Level) of 5 ppb (U.S. Environmental Protection Agency, 2009a). Thus, the presence of benzene often dictates the need for remedial action (Alvarez and Illman, 2006). The impacts of benzene in groundwater aquifers can be minimized by natural attenuation (Alvarez et al., 1991). However, some co-occurring substrates such as ethanol may hinder benzene biodegradation.

The use of ethanol in reformulated gasoline has seen a widespread increase in use throughout the United States to meet the requirements of the Clean Air Act (U.S. Environmental Protection Agency, 2009b) and the Energy Independence and Security Act (United States Congress Senate, 2007), as well as to substitute MTBE (methyl-tert-butyl ether - an oxygenating additive). E10 or E85 (10 and 85% ethanol vol/vol, respectively) are the most common blends currently in use in the United States (Yacobucci, 2007) and amount to 3.4% of the total transportation fuel consumption (U.S. Department of Energy, 2009). This change in energy policy has resulted in increased potential for ethanol to be present in groundwater contaminated with BTEX and other fuel constituents.

Ethanol and its biodegradation products (e.g., acetate) can hinder BTEX biodegradation by multiple mechanisms, including the accelerated depletion of electron acceptors (Corseuil et al., 1998; Da Silva and Alvarez, 2002; Cápiro et al., 2007) that could otherwise be available for BTEX bio-oxidation. The preferential degradation of these compounds (or other common substrates that are degraded through central metabolic pathways) appears to be related to

catabolite repression and metabolic flux dilution, which hinder the rate of BTEX degradation per unit cell (Lovanh et al., 2002; Lovanh and Alvarez, 2004; Da Silva and Alvarez, 2010). Acetate accumulation during the anaerobic degradation of ethanol can also make the fermentative biodegradation of BTEX endergonic, thus hindering the thermodynamic feasibility of anaerobic natural attenuation (Corseuil et al., 2011). Therefore the presence of ethanol and ethanol-derived acetate in groundwater can contribute to longer BTEX plumes (Ruiz-Aguilar et al., 2002; Corseuil et al., 2011) increasing the risk of human exposure (Powers, Rice, et al., 2001). These observations have been corroborated by laboratory studies (Corseuil et al., 1998; Da Silva and Alvarez, 2002; Cápiro et al., 2007, 2008), field research (Ruiz-Aguilar et al., 2002; Corseuil et al., 2011) and modeling studies (Heermann and Powers, 1998; Mcnab et al., 1999; Molson et al., 2002; Gomez et al., 2008; Gomez and Alvarez, 2009, 2010). Nevertheless, the intensity of these effects can be system specific, which underscores the need for simple models that facilitate preliminary risk assessment and evaluate the potential performance of natural attenuation.

In this paper, a simple one-dimensional fate and transport analytical model was developed to provide a natural attenuation screening tool that considers the inhibitory effects of ethanol and acetate on biodegradation. Flow-through aquifer columns simulating ethanol and BTEX natural attenuation were used to validate the model. Our approach is similar to the convective-dispersive transport model presented by Van Genuchten (1985) to describe sequential first order decay reactions for radionuclide and nitrification. We advance that work by simulating different processes of emerging relevance: (1) inhibition of BTEX biodegradation (increased lag time) by the presence of ethanol and its byproduct acetate; (2) methane formation from ethanol blend releases, which is important to assess explosion risk; and (3) pH decrease due to volatile fatty acids (VFA) accumulation, which could be an important stressor that hinders natural attenuation.

3. MATERIAL AND METHODS

3.1. Aquifer columns experiments

Three large glass columns (120-cm long, 5-cm diameter) were used to investigate natural attenuation of benzene, toluene, ethylbenzene, *o*-*m*-*p*-xylenes (BTEX) and ethanol, and their potential interactive effects (Da Silva and Alvarez, 2002). The columns were equipped with eight sampling ports (at 2.5, 7.6, 14, 20, 40, 60, 80, and 100 cm from the inlet), and packed with a non-contaminated aquifer material collected from the Northwest Terminal in Tigard, Oregon. Soil pH was 5.1, buffer index of 6.6 and organic matter of 1.2%.

Hydraulic characteristics of the experimental columns were determined using bromide tracer studies. Hydraulic parameters were estimated by fitting bromide tracer data to the one-dimensional (1-D) advection-dispersion equation (Domenico and Schwartz, 1997):

$$C = \left(\frac{Co}{2}\right) \operatorname{erfc}\left[\frac{\left(R_f x - \left(\frac{Q}{A\eta_e}\right)t\right)}{2\sqrt{DR_f t}}\right] + \exp\left[\frac{\left(\frac{Q}{A\eta_e}\right)x}{D}\right] \operatorname{erfc}\left[\frac{\left(R_f x + \left(\frac{Q}{A\eta_e}\right)t\right)}{2\sqrt{DR_f t}}\right] \quad (1)$$

Where *C* = effluent concentration; *Co* = influent concentration; *x* = column inlet distance; *t* = elapsed time; *Q* = flow rate (6.7 to 7.4 mL h^{-1}); *A* = cross sectional area; η_e = effective porosity (0.37); D = dispersion coefficient (5 cm^2 h^{-1}); R_f = retardation factor (R_f = 1 for bromide) and erfc = complementary error function. Approximately six days were required to displace one pore volume.

One column was fed BTEX at 10 mg L^{-1} total, to provide a baseline for the effect of ethanol on BTEX attenuation. A second column was fed BTEX plus ethanol (initially 2 g L^{-1}, then 1 g/L at the end of the experiments). A third, sterile control column was run to distinguish

biodegradation from potential abiotic losses (negative control). Sterile conditions were maintained by poisoning the synthetic groundwater with Kathon biocide (CG/ICP 23-25%; Sigma, St. Louis, MO) and later fed BTEX plus ethanol (2 g L^{-1}). Each of the columns was fed continuously with a carbonate-buffered synthetic groundwater with the following composition was (mg/L): nitrate (30); sulfate (150); carbonate (as $CaCO_3$) (1,000); Mg^{2+} (1.5); PO_4^{-3} (0.06) ; Ni(II), Cu(II), Zn(II), Co(II), and Mo(IV) (0.002 each) (von Gunten and Zobrist, 1993). This solution was and purged with 5% CO_2/ 95% air prior to entering the columns. All columns were operated in the dark at 22°C and fed continuously. A peristaltic pump (Master- flex Mod. 7519-15) was used to feed the mineral medium and a syringe pump (Harvard Apparatus Mod. 22) was used to feed the volatile organic compounds (i.e., BTEX and ethanol). The ratio of the peristaltic to syringe pump rates was set at 1:20.

Aqueous (1 mL) samples from the influent and side ports were collected with gas-tight syringes (Hamilton Co., Reno, Nev.). BTEX, ethanol and methane were analyzed using an HP 5890 gas chromatograph, as described by Alvarez et al. (1998). Bromide and acetate were analyzed in an Alcott 728 autosampler coupled to ion chromatograph interfaced to a conductivity detector (Dionex Inc., Sunnyvale, CA). The pH of the samples was measured using a Fisher Scientific AB15 pH meter.

3.2. Simulating acetogenesis and methanogenesis

An analytical model was developed to track ethanol degradation and the formation of its metabolites: acetate and methane. Predicting acetate and volatile-fatty acids-derived methane accumulation is relevant because: a) acetic acid produced during the fermentation of organic compounds can accumulate transiently in methanogenic environments and inhibit BTEX biodegradation (Cozzarelli et al., 1994; Corseuil et al., 2011); b) methane formation and

accumulation in confined aquifers can pose a potential explosion hazard (Coward and Jones, 1931; Marrin, 1989; Jones III and Agostino, 1998) and/ or serve as substrate for methanotrophic activity, thus consuming oxygen and enhancing benzene vapor intrusion pathways through the vadose zone (Ma et al., 2012). Furthermore, studies by (Jewell and Wilson, 2011) show that methane in soil gas has the potential to form flammable mixtures under sufficient aerobic conditions. Ethanol degradation, with the resulting acetate and methane production, was simulated using consecutive reaction differential equations (Schnoor, 1996) based on the following stoichiometry reactions (Powers, Hunt, et al., 2001):

$2\ CH_3CH_2OH + CO_2 \rightarrow 2\ CH_3COOH + CH_4$ k_1 (acetogens) (2)

$CH_3COOH \rightarrow 1.5\ CH_{4(g)} + 0.5\ CO_{2(g)}$ k_2 (methanogens) (3)

The following three differential equations, representing the rate of change of the concentration of species associated with specific degradation pathways, were used in the model:

$$d\frac{[EtOH]}{dt} = -k_1[EtOH]$$ Ethanol (EtOH) degradation rate (4)

$$d\frac{[HAc]}{dt} = +k_1[EtOH] - k_2[HAc]$$ Acetate (HAc) formation rate (5)

$$d\frac{[CH_4]}{dt} = +k_2[HAc]$$ Methane (CH_4) formation rate (6)

Where t is the travel time (distance/ seepage velocity). The solution for the third species, (i.e. methane) was obtained by using the mass balance principle:

$$[Total] = [EtOH] + [HAc] + [CH_4] \quad (7)$$

Where [*Total*] is the total moles of species. Finally, the solution for the third species (methane) was:

$$[CH_4] = [Total] - [EtOH]e^{-k_1 t} - [HAc]e^{-k_2 t} - \left[\left(\frac{EtOH}{k_2 - k_1}\right)k_1\left(e^{-k_1 t} - e^{-k_2 t}\right)\right] \quad (8)$$

The rate constants for ethanol degradation and methane production were estimated from concentration profiles along the column length, as the slope of natural logarithm (ln) of concentration versus travel time [i.e., distance/ pore velocity (x/v)] (Figure 1). The rate constants obtained for acetogens (k_1) and methanogens (k_2) were 0.205±0.009 h^{-1} and 0.023±0.014 h^{-1}, respectively. These values are in accordance with typical ethanol degradation rates under methanogenic conditions (0.02-0.1 d^{-1}) (Adair et al., 2003). Water-phase methane was calculated by equation 8 corrected for the stoichiometry of ethanol degradation and Henry's coefficient (K_H = 680 atm-L/mol at 25°C) (Metcalf & Eddy, 1991).

3.3. Simulating changes in groundwater pH

The accumulation of volatile fatty acids can decrease soil buffering capacity and contribute to lower groundwater pH values that can ultimately affect the biodegradation of target compounds. Changes in groundwater pH in the column system were simulated based on the dissolution of $CaCO_{3(s)}$, used as a natural buffer. Conditions within the columns were assumed as an open system with no calcium complexation and ionic strength effects at temperature of 25°C (Snoeyink and Jenkins, 1980).

At equilibrium with the atmosphere:

$$\left[H_2CO_3^*\right] = K_H P_{CO_2} \qquad (9)$$

Where $[H_2CO_3^*]$ is the carbonic acid concentration (mainly dissolved CO_2) as predicted by Henry´s law ($[H_2CO_3^*] = K_H \times PCO_2$, with $K_H = 10^{-1.5}$ mole/L-atm at 25°C). A typical PCO_2 value for limestone strata-groundwater systems (10^{-2} atm) was used (Snoeyink and Jenkins, 1980). Since soil microbial activity evolves significant CO_2, a higher PCO_2value (10^{-1} atm) was assumed for locations with high ethanol biodegradation activity (2.5 to 20 cm from the column inlet).

The mass balance is:

$$[Ca] = [Ca^{2+}] \quad (10)$$

$$[C_T, CO_3] = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}] \quad (11)$$

$$[C_T, HAc^-] = [H^+] + [Ac^-] \quad (12)$$

Where C_T,CO_3 is the concentration of total inorganic carbon species and C_T,HAc^- is the total acid acetic plus acetate concentration.

The charge balance is:

$$2[Ca^{2+}] + [H^+] = [OH^-] + [HCO_3^-] + 2[CO_3^{2-}] + [HAc^-] \quad (13)$$

From equation 11 and using α:

$$[H_2CO_3^*] = [10^{-3.5}] = \alpha_0 C_T, CO_3 \quad (14)$$

From the calcite solubility reaction:

$$CaCO_{3(s)} \rightarrow Ca^{2+} + CO_3^{2-}, K_{SO} = 10^{-8.3} \quad (15)$$

$$[Ca^{2+}][CO_3^{2-}] = K_{SO} = 10^{-8.3} \quad (16)$$

$$[Ca^{2+}] = \frac{K_{SO}}{CO_3^{2-}} = \frac{K_{SO}}{(\alpha_2 C_T, CO_3)} \quad (17)$$

Substituting $10^{-5}/\alpha_0$ for $C_{T,}CO_3$:

$$[Ca^{2+}] = \frac{K_{SO}\alpha_0}{(\alpha_2 10^{-5})}$$

(18)

Substituting into (13) yields:

$$\frac{2\alpha_0 K_{SO}}{\left(10^{-5}\alpha_2 + [H^+]\right)} = \frac{K_W}{\left[[H^+] + \alpha_1\left(\frac{10^{-5}}{\alpha_0}\right) + 2\alpha_2\left(\frac{10^{-5}}{\alpha_0}\right)\right]} + \alpha_1[C_T, Ac^-] \quad (19)$$

Where:

$$K_W = [H^+][OH^-] = 10^{-14} \quad (20)$$

$$\alpha_0 = \frac{[H^+]}{E} = \frac{[H_2CO_3^*]}{C_T, CO_3} \quad (21)$$

$$\alpha_1 = \frac{[H^+]Ka_1}{E} = \frac{[HCO_3^-]}{C_T, CO_3} \quad (22)$$

$$\alpha_2 = \frac{Ka_1 Ka_2}{E} = \frac{[CO_3^{2-}]}{C_T, CO_3} \quad (23)$$

$$Ka_1 = 10^{-6.3}; Ka_2 = 10^{-10.3}; E = [H^+]^2 + [H^+]Ka_1 + Ka_1 Ka_2 \quad (24)$$

$$\alpha_1 = \frac{Ka_1}{[H^+] + Ka_1} = \frac{[Ac^-]}{C_T, HAc} \quad (25)$$

$$Ka_1 = 10^{-4.7} \quad (26)$$

Therefore, the only unknown in the charge balance (Equation 13) is [H^+], which was determined using iterative methods to correspond to a pH of 7.3.

3.4. Analytical model limitations

The mathematical model presented in this manuscript was developed using an EXCEL spreadsheet using equations for ethanol degradation and byproducts formation (i.e., acetate and methane, reactions 4 to 8). The model represents a fast and simple tool for tracking acetate and methane, which are relevant to understand BTEX plume dynamics in sites impacted by ethanol-blend releases. The model output response to uncertainty (within 25% from the median) was

evaluated by performing Monte Carlo simulations (Figure 4). Random simulations (100 for each parameter (k_1[EtOH[and k_2[HAc]) were performed.

Although the analytical solution presented is fast and accurate, it has several limitations: (1) the analytical model considers a 1D contaminant plume with a continuous source of ethanol, and an acclimated microbial biomass. Acclimation time and changes in microbial populations can have a significant impact on degradation rates and is an important consideration when comparing field and modeling data, although in our case the simulations adequately characterized system behavior (Figure 3). (2) The model was simplified to ignore methane oxidation by methanotrophs that consume O_2. This can lead to an overestimation of methane present in the system. However, this error is expected to be small relative to model outputs, as methanotrophic activity is expected to be low within the anaerobic core of the plume. (3) The model uses an analytical solution instead of a computationally intensive numerical method. Although less flexible in regards to domain characteristics and heterogeneity is not considered, an analytical solution provides a fast and easy-to-use screening tool for the region of influence of ethanol and acetate. This information can be used to estimate the region where BTEX degradation is potentially hindered by the presence of ethanol or acetate at relatively high concentrations. (4) The model does not consider BTEX degradation kinetics directly. This simplifies the system of equations and facilitates obtaining a fast and accurate closed form solution. As a system of equations gets larger and model domain more complex, analytical solutions become less appropriate and harder to derive compared to numerical methods. (5) The model does not explicitly incorporate microbial growth or changes in microbial community structure; the analytical solution considers only first-order degradation rates for ethanol and acetate and methane generation rate, as shown in equations 4-7.

As is the case with most analytical models, one must fully justify that modeling assumptions accurately reflect site conditions, that site-specific data used in modeling are valid, and that literature values abstracted for input are realistic and applicable. Without such detailed justification, the accuracy of the simulations cannot be guaranteed.

4. RESULTS AND DISCUSSION

BTEX and ethanol volatilization losses in sterile (control) columns were minor (<8%; data not shown), indicating that biodegradation was the main contaminant removal and natural attenuation mechanism (Da Silva and Alvarez, 2002). Ethanol was preferentially degraded within 3 cm of the column inlet (Figure 1). These results corroborate previous reports that ethanol can be degraded faster than other gasoline constituents (Corseuil et al., 1998, 2011; Da Silva and Alvarez, 2002; Cápiro et al., 2007). The biodegradation of ethanol led to the formation and accumulation of acetate (Figure 1). Although not toxic, the presence of acetate as low as 0.3 mM (Rakoczy et al., 2011) could hinder the thermodynamic feasibility of BTEX degradation by syntrophic microorganisms typically associated with sulfate-reducing and methanogenic conditions (Ulrich and Edwards, 2003). Benzene and toluene degradation in our column experiments only occurred after the onset of acetate removal (Figure 1). Such inhibitory effects on BTEX biodegradation were previously demonstrated to also occur during the natural attenuation of BTEX and ethanol mixtures at field scale (Corseuil et al., 2011).Thus, estimating ethanol and acetate remediation time for gasohol contaminated sites is particularly important to predict BTEX natural attenuation lag and plume length, as significant BTEX biodegradation typically occurs after most of the ethanol and acetate have been depleted (Da Silva and Alvarez, 2002, 2004; Corseuil et al., 2011).

We developed a simple model to simulate the natural attenuation of ethanol-BTEX mixtures and facilitate preliminary risk assessment. First-order degradation rate constants obtained for ethanol from our columns experiments (0.19 ± 0.008 d^{-1}) (Figure 2) were within the typical values observed in the field 0.02-0.1 d^{-1} (Adair et al., 2003). It was considered that ethanol-derived acetate served as the dominant substrate for methanogens and that acetate produced from propionate or butyrate degradation was immediately consumed by methanogens (Öztürk, 1991). Thus, whereas other VFAs besides acetate [i.e., propionate (Wu et al., 1991; Wu and Hickey, 1996), formate (Wu et al., 1991) and/ or butyrate (Freitas et al., 2010)] could also be produced during anaerobic ethanol biodegradation, their production was not considered in this modeling effort for simplicity. Acetate accounted for approximately 58% of the total VFA produced as suggested by adjusting model fitting with the measured data ($R^2 = 0.84$) (Figure 3) and Monte Carlo analysis (Figure 4). This estimate is consistent with previous studies that showed approximately 50:50 (acetate:butyrate) production during anaerobic ethanol biodegradation in groundwater (Ma et al., 2011).

Acetogenesis during ethanol and BTEX degradation decreased the pH of the system by about 1 unit (7.3 to 6.4) even though the medium was well buffered with calcium carbonate ($CaCO_3 = 1$ g L^{-1}) (Figure 5). The model suggests that poorly buffered systems could experience a larger drop in pH, with the potential to affect bacterial metabolism and hinder the activity of ethanol and/or BTEX degraders. Simulation of ethanol-derived acetate accumulation can also help discern potential corrosive conditions in impacted subsurface infrastructures (Suflita et al., 2008; Duncan et al., 2009).

Ethanol-derived acetate served as substrate for methanogenesis, and methane accumulation was relatively well predicted in these aquifer columns using equations 2 to 8. The maximum

methane concentration in the water phase was close to its solubility limit of 0.09 mM. For 43 mM (~ 2 g L^{-1}) of ethanol added, an expected 65 mM (1.04 g L^{-1}) of total methane would be produced according to the theoretical stoichiometry (Figure 4). Because the columns are effectively an open system, the maximum water-fraction methane concentration should not exceed 1.6 mM (or 26 mg L^{-1} at 20° C and 1 atm) (Metcalf & Eddy, 1991) as shown by the plateau in simulated methane concentration unless there was an unexpected increase in the system pressure (e.g., clogging). Thus, an increase in the system pressure would raise the dissolved-methane concentration as determined by the partial pressure law for gases (i.e., PV = nRT where P= 1 atm, V = volume (L), R=0.082 atm L (mole K)$^{-1}$, n=methane concentration (M), and T=293°K). For example, considering the column void volume of 872 mL [i.e., 2,356 mL total volume × 0.37 effective porosity (η_e)], and the theoretical methane accumulation (65 mM or 1.04 g L^{-1}), the actual pressure inside the column would be equal to 1.63 atm (at 20°C). The resulting 9.3 pound per square inch (psi) pressure above atmospheric pressure of 14.7 psi (1 atm) suggests that methane accumulation has the potential to pose an explosion hazard if it migrates into enclosed spaces where ignitable conditions exist [i.e., the lower explosion limit (LEL) of methane in air is about 5% v/v (Jewell and Wilson 2011)].

5. CONCLUSIONS

The analytical models presented in this work predicted accurately ethanol degradation, acetate formation and consumption, and methane generation in flow-through aquifer columns simulating natural attenuation of a continuous release of ethanol-blended fuel. Changes in groundwater pH were also satisfactorily simulated by considering ethanol-derived acetate accumulation and PCO_2 increase linked to biological activities stimulated by ethanol. Future

development of the model is necessary to improve its capabilities. This includes: a) consideration of BTEX degradation kinetics and fate; b) model validation based on total ethanol-derived volatile fatty acids (and not just acetate); c) validation of the groundwater pH changes based on measured VFAs and PCO_2 concentrations; d) changes in methane accumulation rates based on the presence and activity of methanotrophs, and e) evaluation of the explosion potential associated with methane accumulation. Even at this early stage of development, however, the presented analytical model is relevant to guide future research, as foundation for more advanced computational models; and as a preliminary screening tool for risk assessment and characterization of sites impacted by ethanol blend releases.

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8. FIGURE CAPTIONS

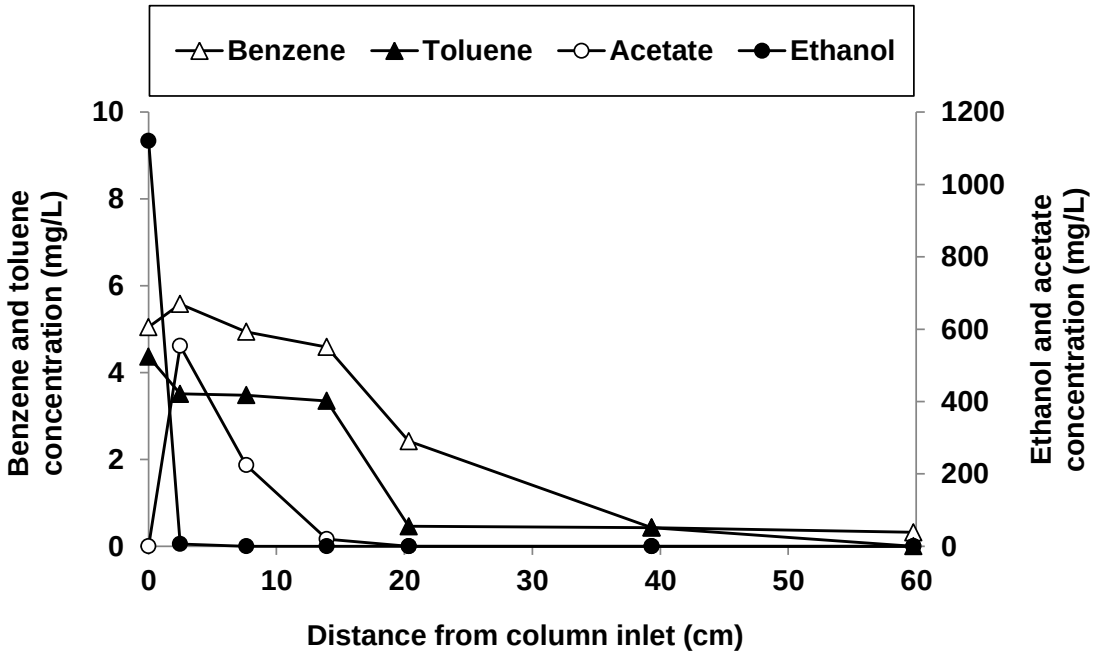
Figure 1. Measured ethanol, acetate, benzene and toluene concentrations profile. Significant benzene and toluene biodegradation occurred only after complete ethanol or acetate removal. Influent ethanol concentration was reduced from 2 to 1 g/L in this experiment to minimize acetate accumulation and demonstrate benzene and toluene degradation (Adapted from Da Silva and Alvarez, 2004).

Figure 2. Ln of ethanol concentration (C/C_0) versus distance (A). The slope multiplied by the pore velocity is k_1. Ln of methane concentration (C/C_0) versus distance (B). The slope multiplied by the pore velocity is k_2.

Figure 3. Ethanol degradation, acetate and methane production profiles in the column fed BTEX and ethanol after 550 days of operation. Dotted lines denote simulation curves determined by equations 2 to 10. Solid line represents methane model.

Figure 4. Ethanol, acetate and theoretical total volatile fatty acids (VFAs) concentration profile in the column fed BTEX and ethanol after 153 days of operation. Lines denote simulation curves determined by equations 2 to 10, and Monte Carlo analyses.

Figure 5. pH profile in the column fed ethanol after 550 days of operation. Solid line denotes pH simulation curve. Shadowed box represents locations where high ethanol and its metabolites biodegradation contributed to higher microbial activity and increased PCO_2 (10^{-1} atm).



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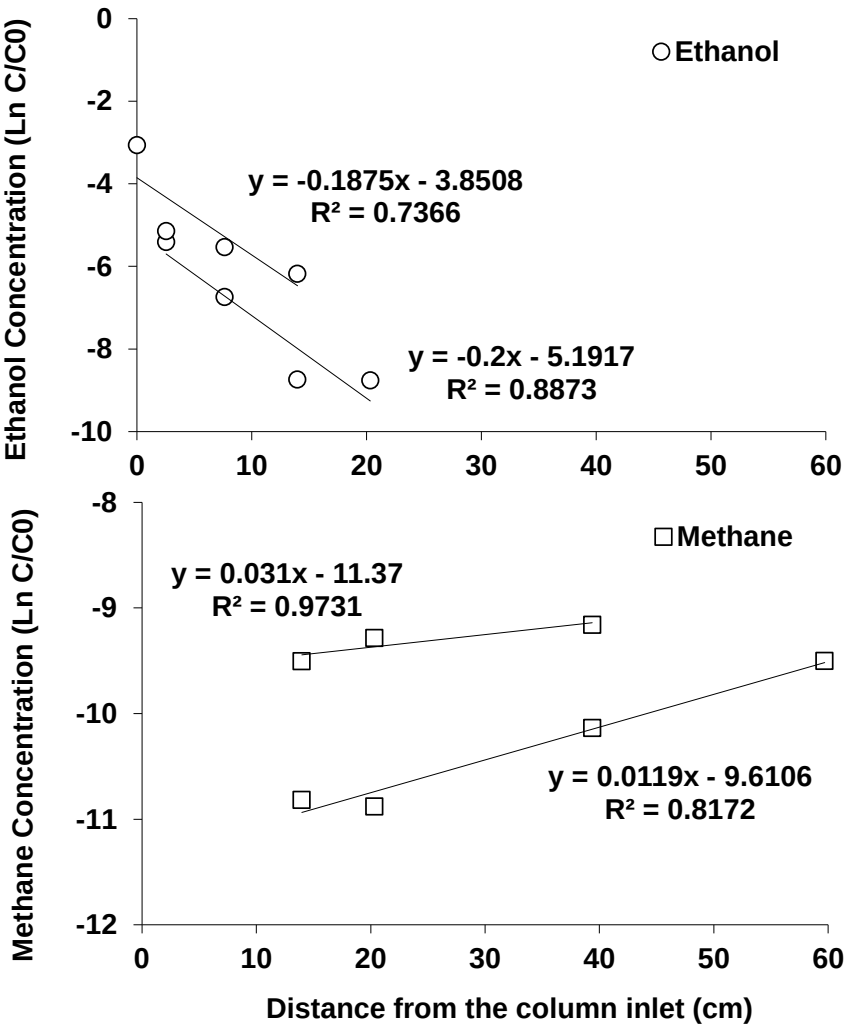

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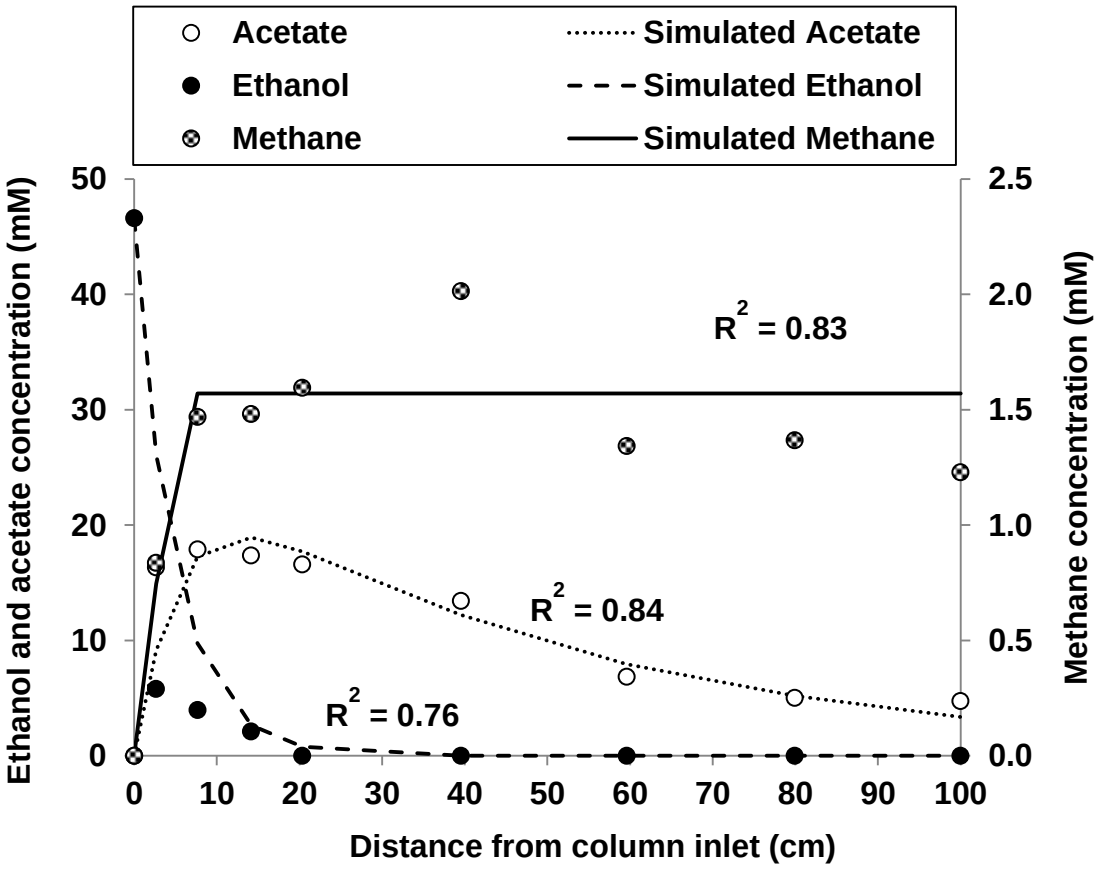

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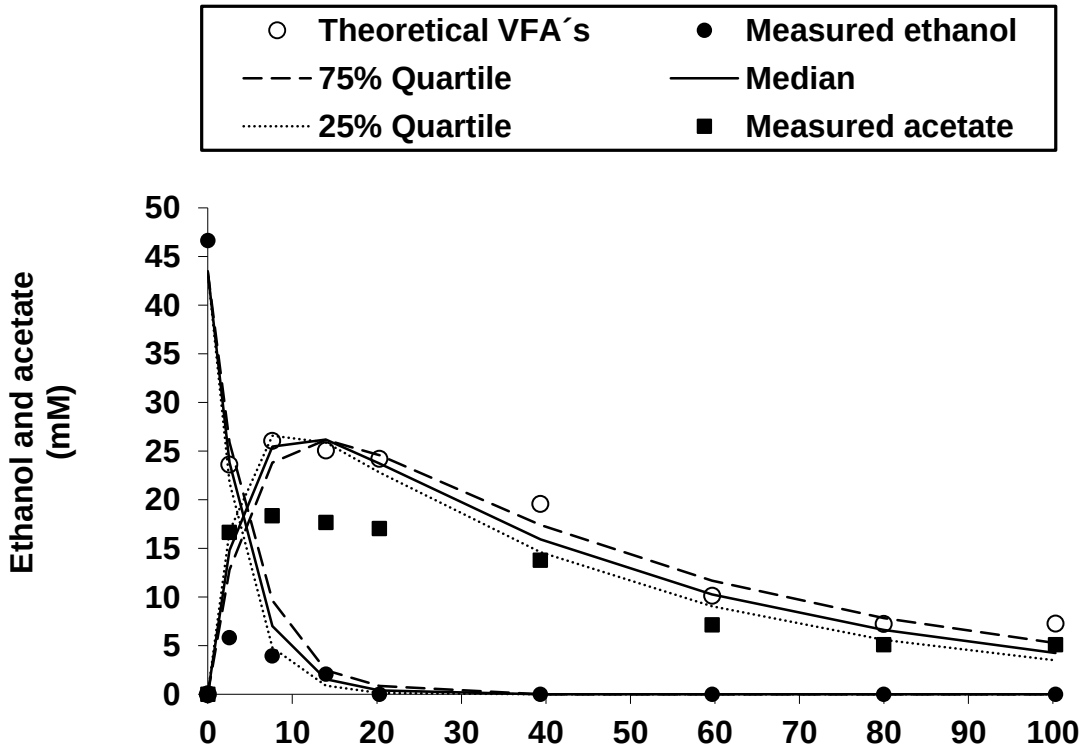

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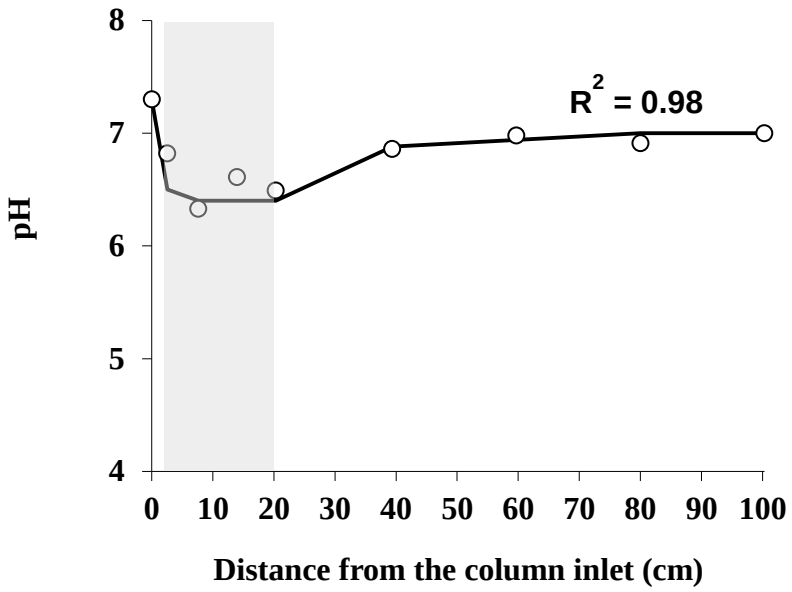

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