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Reactive transport and stable isotope fractionation of volatilized petroleum hydrocarbons in porous media: Experimental and numerical analysis

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Table of Contents

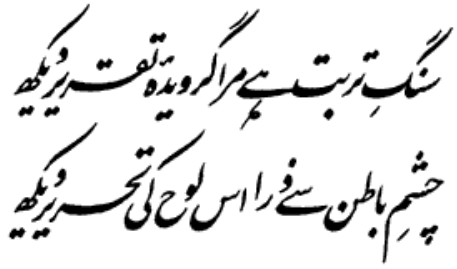
"No experiments! Dedicated to Imran Khan,

my Kaptaan"

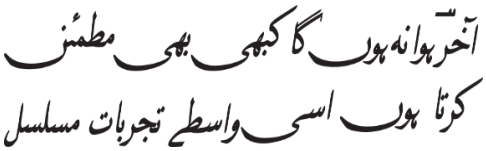
Stop acting so small. You are

the universe in ecstatic motion.

Rumi



Iqbal



Akhir

To my life-coach, my eternal cheerleader,

my late father Haji Ahmed Ali:

because I owe it all to you.

It was your dream to call me doctor.

I am sure you are witnessing this

from the heavens above.

Thanks for everything Dad!

Chapter 1

"One never notices what has been done; one can only see what remains to be done ."

Marie Curie (1867 - 1934)

Introduction

1.1 Background

With increasing human population, the demand of water is also increasing. Groundwater being cost effective in terms of transportation and filtration is the major source of drinking water. Accidental release of petroleum hydrocarbons due to leakage, spills, improper disposal etc. has deteriorated soil and groundwater at large scale in the world (Clement et al., 2000; Lundegard & Johnson, 2004). The contamination of groundwater by hydrocarbon pollutants is a major problem in many countries (Bear & Cheng, 2010; Schwarzenbach et al., 2010; Travis & Doty, 1990). However, microbial organisms are able to disintegrate hydrocarbons from complex into simpler molecules through metabolic or enzymatic action by a well-studied process known as biodegradation. Due to the buffer capacity of soil, where microbes act as a bio-filter the negative effects of contaminants are minimised. Harmful chemicals are converted into less harmful by-products either by the adsorption to the soil particles or by the microbial activity. However, due to disproportionate contaminant loading, soil and groundwater contamination is recognized as one of the major threats in recent years. Under normal circumstances the subsurface environment exhibits a natural balance, however, human activities including the land exploitation has resulted in disturbing this balance. In order to get this balance back in terms of drinking water, active restoration efforts are required. According to Swartjes (2011) remediation strategies are increasingly based on a risk approach and aim at a "fitness-for-use" specific for a specific location. Modern attenuation work is mainly focused on cost-effective and energy-efficient methods in passive environmental remediation (Reid & Jaffe, 2012). One major challenge is the quantification of the subsurface processes. Many studies have discussed the impacts of various chemicals and their restoration strategies. In this thesis volatile organic compounds (VOCs) have been elaborated, which are among the highest priority pollutants as they contribute to groundwater contamination through their use as petroleum additives, synthetic intermediates and as organic solvents. They are generally well soluble in water and easily migrate with the flow of groundwater, as a result end up in large plumes of contaminated groundwater and contaminated soil in vapor-phase in the subsequent unsaturated zone above. Aerobic biodegradation of petroleum hydrocarbons in the vadose zone has been well established (Holden & Fierer, 2005; Kurt & Spain, 2013) and most of the microbial activity in nature takes place at interfaces where polluted groundwater interacts with the overlying vadose zone as discussed by Kurt & Spain (2013).

Diffusion of VOCs in the gas phase is much faster than in the water phase and in unsaturated systems, this diffusive migration can lead to above ground emissions, imposing potential health risks. Recent studies have shown that microbial activity can respond to and reduce their gas phase concentrations (De Biase et al., 2011;

Hanzel et al., 2012). Therefore, outgassing of vapor phase VOCs from the vadose zone is subjected to biodegradation, and has gained attention as a new approach in bioremediation of these chemicals. Various processes e.g. volatilization, sorption, and dilution practically relocate the contaminant between different phases (Thullner, Stefanakis, & Dehestani, 2018), and in addition, mechanisms such as diffusive transport and biodegradation can lead to differential amounts of naturally occurring stable isotopes in the residual concentrations. This enrichment is quantified by the ratio of heavy to light isotopic changes, and is known as isotope fractionation. Gas permeability is an important parameter to describe processes involving gas flow and vapor transport in an unsaturated zone (Baehr & Hult, 1991; You et al., 2011). Reactive transport within contaminant plumes has been extensively studied, and compound-specific stable isotope fractionation involved in the degradation pathways have been used to determine hydrocarbon biodegradation (Alfreider & Vogt, 2007; Amos et al., 2011; Vieth et al., 2005). However, the vertical mass transfer in the gas phase and hydrogen isotope fractionation at the redox interfaces are also poorly understood. As well as, depending on the concentration, gasphase VOCs are metabolized at short length scales (Hanzel et al., 2012; Kurt & Spain, 2013). Therefore, the objective of this research is to bridge approaches based on diffusion driven gasphase transport, and to quantify the relative contributions of individual subsurface mechanisms e.g. volatilization, biodegradation etc. In addition, there is a need for a better understanding of the processes controlling the transport and biodegradation of VOCs in the unsaturated zone. Therefore, we hypothesized that aerobic degradation in the vadose zone has a sufficient capacity to protect the overlying environment from volatilized groundwater contaminants.

1.2 Volatile organic compounds

1.2.1 Significance

Hydrocarbon contamination is a common problem that affects groundwater and surface water quality in many regions across the globe (Thullner et al., 2018). They enter the environment mainly through processes related to gasoline and petroleum, as well as many other industrial and commercial activities (Andreoni & Gianfreda, 2007). Volatile organic compounds (VOCs) are hydrocarbons that tend to volatilize at atmospheric pressure. By definition, these compounds easily evaporate under normal temperature and pressure conditions (USEPA, 2018). Benzene, toluene, ethylbenzene and isomers of xylene, known as BTEX, are the most common volatile subsurface contaminants and make up approximately 11% of the petroleum products (Serrano & Gallego, 2004). Contamination by VOCs significantly impacts human and environmental health. Chronic exposure can lead to respiratory and other health issues. For example, prolonged exposure to toluene can damage central nervous system in humans (ATSDR, 1994). Therefore, fate and transport of VOCs has been extensively studied in saturated systems. More recently constructed wetlands (CWs) and vertical flow filters (VFFs) are characterized as a sustainable technological method of remediation of water contaminated with hydrocarbons (Thullner et al., 2018). VFFs may facilitate the vertical migration of chemicals and oxygen through a porous medium (soil), and due to the presence of microbes act as barrier to the emanating vapor phase VOCs. An advantage of VFFs is their high oxygen transfer capacity to efficiently remove contaminants in a relatively small area (Cooper, 1999; Kayser & Kunst, 2005) and recently have been applied to treat water containing VOCs (Eke & Scholz, 2008; Scholz, 2010; Tang et al., 2009; Wallace & Kadlec, 2005). Groundwater heavily contaminated with BTEX was investigated in a bioremediation facility in Leuna, Germany (**Figure 1.1**). The site used to be a refinery and an industrial area

for about 100 years. It was heavily impacted by the World War II, which lead to the leakage of chemicals and oil. More details of the site are mentioned in (De Biase et al., 2011; Martienssen et al., 2006). Concentration of many VOCs by far exceeds the World Health Organisation - WHO and German standards. For example concentration of benzene (C6H6) a known carcinogen, in this site was exceeding 10 mg/L (De Biase et al., 2011), while WHO suggests a drinking water threshold concentration of 10 µg/L (WHO, 2003b). At Leuna, chemicals were loaded heavily which in turn consumed available oxygen as the groundwater shows O_2 concentration of 0.1 mg/L, leading chemical species to be in reduced state (De Biase et al., 2011). Most of the contaminants found at Leuna are biodegradable however, anoxic conditions lead to very slow in situ rates of biodegradation under anaerobic conditions (Wiedemeier et al., 1999). Biodegradation therefore can potentially be accelerated by providing oxic conditions to the existing microbial communities.

The Helmholtz Centre for Environmental Research – UFZ in the scope of SAFIRA II, compartment transfer project, tested different types of CWs in Leuna at pilot scale, one such pilot system which showed huge potential to remove gasphase VOCs was VFFs. The pilot scale VFFs exhibited a very good removal performance for the used groundwater VOCs (De Biase et al., 2011; van Afferden et al., 2011) and it was hypothesized that the uppermost layer of the systems act as efficient barrier of vapor-phase VOCs (De Biase et al., 2013). This led us to study processes in the top layer of the VFF (30 cm), where oxygen does not seem to be a limiting factor, (**Figure 1.2**). However, both oxygen and chemicals were in gaseous form in this top layer of the filter suggesting that the degrading microbes were still able to stop the contaminant flux out of this region.

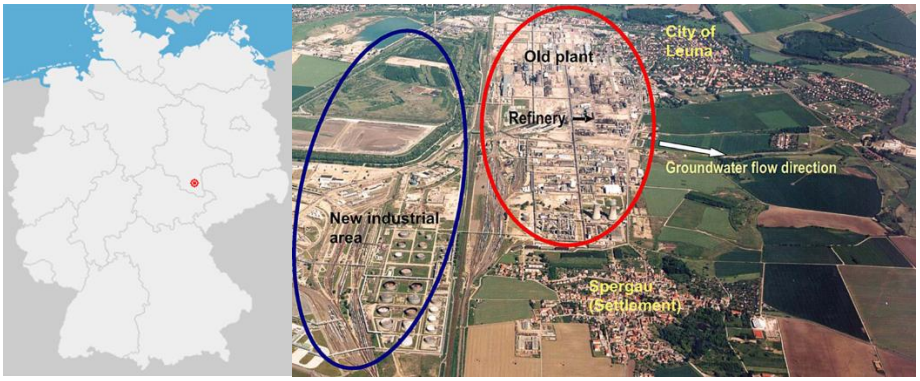


Figure 1.1: Aerial photo of Leuna taken from Martienssen et al. (2006).

1.3 Fate and transport of VOCs

1.3.1 Governing subsurface processes

In the unsaturated subsurface, contaminants will partition into different soil phases: solid, liquid, and gasphase (Mayer & Hassanizadeh, 2005). The abiotic solid matrix, microbes and the presence of water and oxygen, makes the unsaturated zone highly complex. The processes controlling and regulating this zone make it further intricate. These processes are not only highly interlinked, but also act in parallel, making the

identification and quantification of the key processes controlling the system dynamics difficult. The major subsurface processes governing the transport of VOCs through the subsurface include diffusion, advection, volatilization, biodegradation and sorption. In the absence of water movement, gas phase diffusion is the dominant transport process in many systems. Relevant processes in the scope of this thesis are illustrated in **Figure 1.3** and are briefly described below.

Diffusion

The process driven by the concentration gradient from higher concentration (source) to the low concentrations (environment) is called diffusion and is described mathematically by Fick's law. Diffusion takes place in the gas phase as well as in the liquid phase. However, the rate at which molecules mix in gas phase is many orders of magnitude higher than in the liquid phase. Hence, diffusion is a relevant process for the migration of VOCs, which exist in significant amounts in gaseous form at room temperature.

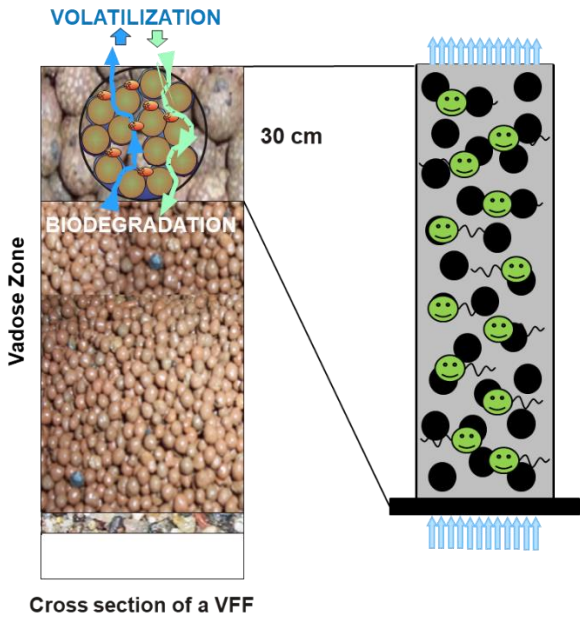


Figure 1.2: Schematic cross section of a vertical flow filter (left) conceived from De Biase et al. (2011) and schematic illustration of the column reactor (right).

Volatilization

Evaporation of hydrocarbons from liquid phase to gasphase is known as volatilization. At equilibrium, the ratio between the liquid phase and the vapor-phase is temperature and pressure dependent and is given by the dimensionless Henry's constant - Hc. The time needed for reaching such equilibrium and thus the kinetics controlling volatilization (and VOC dissolution as the inverse process) depends among others on the size of the interphase between gas and aqueous phase and the mobility of the involved molecules as described by

the boundary layer theory (Schwarzenbach et al., 2003). Volatilization can thus leads to the release of toxic chemicals into the environment.

Biodegradation

Many bacteria are capable of using VOCs as carbon and energy source (Attaway & Schmidt, 2002; Lee & Lee, 2001; Prenafeta-Boldú et al., 2004), and microbes are able to degrade chemicals that may appear to be harmful for man and environment. Biodegradation of VOCs in the unsaturated subsurface has been observed for different laboratory and field conditions (Bouchard et al., 2008b; De Biase et al., 2011; van Afferden et al., 2011), which indicates that natural attenuation may be a feasible remediation option for VOCs in the unsaturated subsurface. And effectively it is the only process that transforms these VOCs to less harmful products (Alexander, 1999). It is an important removal process of VOCs in natural systems (Hohener et al., 2003; Hunkeler et al., 2002; Martienssen et al., 2006) and in constructed wetlands (Eke & Scholz, 2008; Tang et al., 2009).

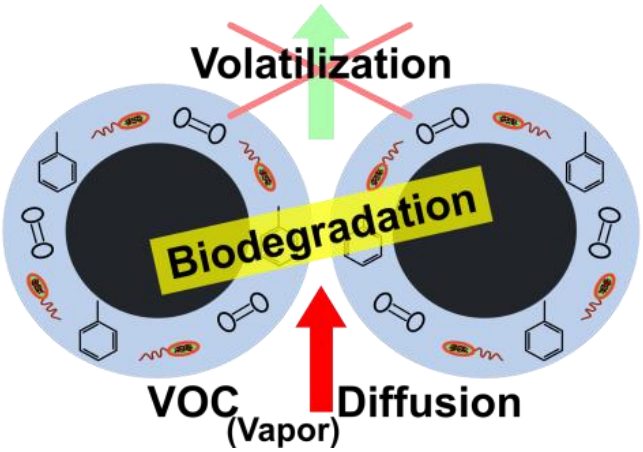


Figure 1.3: Illustration of the major removal processes of VOCs in porous media

However, biodegradation is controlled by a combination of factors such as the microbial species, redox conditions, pH, moisture, temperature and nutrient availability (Van der Zaan, 2010). Under aerobic conditions VOCs are biodegraded by means of oxygenase catalysis reactions, which requires molecular oxygen for the hydroxylation of the aromatic ring, or an alkyl substitution (Kim & Jaffe, 2007), and for example, in case of toluene the enzyme is oxygen monooxygenase. Complete aerobic mineralization of VOCs (i.e. their transformation into carbon dioxide and water) requires large amounts of oxygen and for example approximately 3.1 grams of oxygen are required per gram of toluene in stoichiometric terms. Also under anaerobic conditions, many VOCs such as toluene can be degraded by denitrifying and sulfate reducing bacteria (Chakraborty & Coates, 2004) but degradation rates are much slower than under aerobic conditions. This means that the efficiency of bioremediation of VOCs relies on the supply of sufficient amounts of oxygen.

1.4 Assessment of Removal Processes

Assessment of microbial VOC degradation processes in the vadose zone has been the subject of many studies in the recent past. The contribution of individual processes to the overall observed VOC removal has not been extensively studied, mainly because of the traditional VFF considers the system as a black box where the performance only focuses on the in- and outflow concentrations of the contaminants (Langergraber, 2008; Woźniak et al., 2007). This in turn does not allow distinguishing individual removal processes, and it is further challenging due to the occurrence of these processes simultaneously. Further to add, the rapid biological, physical and chemical changes in the subsurface environment and the dynamics of the vapor-phase VOCs add up to the complexity of the entire system. Besides the detailed analysis of concentration profiles (Pasteris et al., 2002), a semi-quantitative way to investigate biodegradation is the quantification of microbial abundance together with the estimation of aerobic degradation kinetic parameters (Deeb et al., 2000; Hers et al., 2000; Holden & Fierer, 2005; Kristensen et al., 2010; Schirmer et al., 2003). Considerable investigation of removal processes in the subsurface implies further investigation at intermediate to small scale (Rivett et al., 2011). As the above quantification methods may not be sufficient to assess subsurface processes, additional approaches for example, compound-specific stable isotope analysis - CSIA and reactive transport modelling – RTM need to be combined with concentration data to assess biodegradation.

1.4.1 CSIA as a tool in bioremediation

Each element in a compound has a distinct isotopic ratio between the light isotopes and the heavier isotopes, which can change in a systematic way during biodegradation. These changes are usually very small, and the analytical method to measure these changes is known as CSIA. In situ biodegradation can be assessed by CSIA (Meckenstock et al., 2004). In order to differentiate biodegradation from other processes, CSIA is widely accepted as a monitoring strategy and as a powerful tool in studying the fate of contaminants in groundwater systems (Elsner, 2010; Meckenstock et al., 2004; Schmidt et al., 2004; Thullner et al., 2012). Especially for a quantitative analysis of biodegradation using CSIA, the contributions from mixing (Druhan & Maher, 2017; Fischer et al., 2007; Thullner et al., 2012), sorption (Harrington et al., 1999; Kopinke et al., 2005; Schüth et al., 2003), small-scale mass transfer (Heße et al., 2014; Thullner et al., 2013) or dispersion (Abe & Hunkeler, 2006; Eckert et al., 2012; Rolle et al., 2010; Thullner et al., 2012) have to be either negligible or their influence has to be adequately considered. If these assumptions are fulfilled, the analytical Rayleigh model (Mariotti et al., 1981; Rayleigh, 1896) is frequently used to deduce the extent of biodegradation from the degree of isotopic enrichment (Hunkeler et al., 2008; Richnow et al., 2003) in groundwater systems with advection-dominated transport.

Diffusive transport can contribute to stable isotope fractionation (Bouchard et al., 2008b; Jin et al., 2014; Rolle & Jin, 2017). Furthermore, even if diffusive transport is not leading to any fractionation effects, diffusive mixing along concentration gradients reduces the changes in the stable isotope signatures caused by biodegradation. As a result, it has been adjudged that the standard Rayleigh model based approach of stable isotope fractionation is not applicable for diffusion dominated transport systems (Bouchard et al., 2008b). This means that, for diffusion-dominated transport systems, CSIA could only be used as qualitative indicator of biodegradation.

1.4.2 Assessment using numerical model simulations

Reactive transport models are powerful means to address the interaction of the processes and their impact on the fate of bioreactive compounds (Barry et al., 2002; Brun & Engesgaard, 2002; Murphy & Ginn, 2000; Thullner et al., 2007) and have shown potential for the analysis of VOC biodegradation in unsaturated systems as well (De Biase et al., 2013; Molins et al., 2010). Many models have been applied for simulating VOCs transport (Johnson & Ettinger, 1991) and biodegradation (DeVaull, 2007; Picone et al., 2012) in the unsaturated zone. However, in order to numerically simulate the removal of VOCs from VFF or laboratory setups, all potentially relevant processes have to be handled in the applied model. In recent years, reactive transport modeling concepts have been expanded to consider processes relevant for stable isotope fractionation (Alvarez-Zaldívar et al., 2016; Bouchard et al., 2008b; Centler et al., 2013; Druhan et al., 2014; Eckert et al., 2013; Heße et al., 2014; Hunkeler et al., 2009; Prommer et al., 2009; Thullner et al., 2008; van Breukelen et al., 2004). This provides an approach to extricate the potential influence of different processes on stable isotope fractionation effects experimentally observed in subsurface environment.

1.5 Thesis outline

Subsurface VOCs and their transport and biotransformation are addressed in this thesis. The aim was to fill the knowledge gap and improve the understanding of dynamics of vapor-phase VOCs in the unsaturated zone. The thesis presents results of *laboratory experiments* (column reactor setups and multi-phase batch systems) investigating gasphase transport, phase exchange, biodegradation, and stable isotope fractionation of VOCs; and *numerical model simulations* providing a theoretical explanation of the observed phenomena.

Experimental setups consisting of vertical column reactors were used to study biodegradation of gasphase VOCs, their transport in the subsurface and the interrelation of relevant processes in **Chapter-2**.

To address the fractionation effects observed in the column systems mentioned above and to understand the interrelation of subsurface processes, a combined approach of experiments, stable isotope fractionation and modelling is presented in **Chapter-3.** Parameters obtained by a series of lab experiments and verification batch experiments were used in a numerical model and are compared with analytically derived predictions.

The insight into the processes of phase exchange between vapor-phase and liquid phase and its impacts on the observed isotope fractionation effects are described in **Chapter-4**. Different sets of batch type reactors were used, which allowed simultaneous gas and liquid samples to study the phase exchange dynamics of VOCs.

Chapter-5 deals with the VOC toxicity and its limiting effects on the biochemical activity of the microbes. The qualitative and quantitative effects of variable VOC concentrations have shown varied isotope signatures, and it is the subject of investigation in this chapter. Bioavailability and toxicity were taken into account in this chapter.

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Chapter 2

"Nature may reach the same result in many ways."
Nikola Tesla (1856 - 1943)

Biodegradation of vapor-phase toluene in unsaturated porous media: column experiments

Abstract

Biodegradation of organic chemicals in the vapor phase of soils and vertical flow filters has gained attention as promising approach to clean up volatile organic contaminants (VOC). The drivers of VOC biodegradation in unsaturated systems however still remain poorly understood. Here, we analyzed the processes controlling aerobic VOC biodegradation in a laboratory setup mimicking the unsaturated zone above a shallow aquifer. The setup allowed for diffusive vapor-phase transport and biodegradation of three VOCs: non-deuterated and deuterated toluene as two compounds of highly differing biodegradability but (nearly) identical physical and chemical properties, and MTBE as (at the applied experimental conditions) non-biodegradable tracer and internal control. Our results showed for toluene an effective microbial degradation within centimeter VOC transport distances despite high gasphase diffusivity. Degradation rates were controlled by the reactivity of the compounds while oxic conditions were found everywhere in the system. This confirms hypotheses that vadose zone biodegradation rates can be extremely high and are able to prevent the outgassing of VOC to the atmosphere within a centimeter range if compound properties and site conditions allow for sufficiently high degradation rates.

Keywords: Toluene, VOC, biodegradation, gas phase, vertical flow filters, isotopically labeled compound.

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2.1 Introduction

Volatile organic compounds (VOC) significantly contribute to groundwater contamination. Due to their high vapor pressure and high air to liquid partition ratios, VOC are prone to enter the soil air and gasphase transport of VOCs in the vadose zone has been identified as an important mechanism contributing to the spreading of contaminants (Mendoza & Frind, 1990; Molins et al., 2010). Diffusion coefficients of VOC in the gas phase are up to 4 orders of magnitude greater than those in the aqueous phase. Hence the presence of a continuous air phase causes diffusive fluxes in the unsaturated zone to be significantly greater than those in the saturated zone (Rivett et al., 2011). In unsaturated subsurface environments diffusive migration hence may lead to above ground emissions or vapor intrusion into buildings, and may impose significant risks to environmental and human health (Luo et al., 2013). In turn, the capillary fringe, or more generally the interface between groundwater and the vadose zone, is seen as a hotspot of microbial activity (Kurt & Spain, 2013). This holds especially true when diffusion of oxygen from the surface and of volatilized groundwater VOC coincide and support metabolic activity of microorganisms as described in Kurt & Spain (2013). The groundwater-vadose zone environment thus represents an interface characterized by steep redox gradients, high availability of electron donors and acceptors and concomitant high microbial activity (Winderl et al., 2008). Similar phenomena can be observed at the interface between contaminated and clean groundwater (Bauer et al., 2008) and in contaminated meromictic lakes (Wick et al., 2000). The groundwater-vadose zone therefore likely promotes the biodegradation of VOC and limits above ground emissions of VOC.

Up to now, biotechnological approaches to degrade vapor-phase VOC emerging from the subsurface are scarce. However, few existing field studies (e.g., van Afferden et al., 2011) indicate a high potential of microbial attenuation in the vadose zone as a cost-effective and energy-efficient method for the clean-up of VOC in the vadose zone and other unsaturated porous media. This is supported by laboratory studies showing that vapor-phase petroleum hydrocarbons are biodegraded across relatively short meter-scale distances (Abreu et al., 2006; Davis et al., 2009; Fisher et al., 1993, 94; Holden & Fierer, 2005; Kurt & Spain, 2013; Luo et al., 2013; Luo et al., 2009; Patterson & Davis, 2009). More recent studies indicate that even lower, centimeter to decimeter-scale distances might be sufficient for the removal of vapor-phase hydrocarbons (De Biase et al., 2011; Hanzel et al., 2012). However, a rigorous experimental analysis of the processes controlling the diffusive transport and biodegradation of volatile organic contaminants in these systems is missing. In the existing studies such abiotic factors have varying potential to influence the observed VOC removal, or the studies emphasize the importance of gasphase diffusive transport for the degradation of VOC in unsaturated porous media but lack a spatially resolved analysis of VOC reactive transport (e.g., Kristensen et al., 2012). For instance, the presence of organic phases can promote VOC removal by retarding the spreading of the sorbing compounds and by improving the gasphase to water-phase mass transfer of hydrophobic compounds (e.g., Béchohra et al., 2015). Numerical model simulations of De Biase et al. (2013) indicate that biodegradation rates need to be very high to avoid undesired VOC emissions, but cannot provide a direct evidence if such high rates can be achieved in a highly unsaturated system with diffusion-driven transport in the vapor phase. These previous findings raise the questions if indeed such high rates can be achieved in unsaturated porous media and if conditions favoring high diffusive VOC fluxes allow for VOC removal due to biodegradation across centimeter to decimeter-scale distances also in absence of abiotic retardations.

The objective of this study is the experimental investigation of the main processes and parameters controlling the fate of VOC in unsaturated porous media. Particular focus is on the removal capacity of such an

unsaturated system for VOC emerging from contaminated groundwater and on the ability of the system to act as a barrier for diffusion-driven VOC emissions to the atmosphere. For this purpose a controlled laboratory reactor set-up was selected which facilitates high diffusive transport rates with only minimal restriction by a residual water phase and no organic phase retarding the spreading of the VOC. The set-up allows monitoring VOC concentration profiles at the centimeter-scale and thus enables to assess also VOC degradation at high spatial resolution. To distinguish between biodegradation and potentially remaining abiotic removal processes the fate of three different VOCs was investigated: two isotopomers of a reactive model compound (toluene (h-toluene) and per-deuterated toluene (d-toluene)) which exhibit nearly identical physical properties but highly different biodegradability, and a non-reactive VOC (MTBE). Furthermore, we have adopted a novel approach to analyze VOCs in gasphase and liquid phase at the same time, which allows checking the assumption of instantaneous equilibrium with respect to Henry's law. The results are relevant for bioremediation of areas of high VOC concentrations which could lead to atmospheric emissions or vapor intrusion to the buildings through basement and ultimately to human health impacts.

2.2 Experimental Procedures

Reactor design and operation

2.2.1 Reactor design

Vertical chromoflax glass columns (l = 35 cm, i.d. = 4.1 cm; cf. Figure 1) packed with agar-covered (approximate agar layer thickness of 60 μm) glass beads (d = 2.9 − 3.5 mm) were used to study the spatiotemporal concentration of the VOC in a water-unsaturated fixed-bed reactor (tortuosity, τ = 0.5, derived from measured drainage curves using standard soil physical procedures; porosity, ϕ = 0.39). Sampling ports allowed the sampling of VOC from the vapor phase and from the liquid reservoir (liquid volume 2.375 L). The reactor was separated from the headspace of the liquid reservoir (initial head space volume approximately 45 mL) by a stainless steel mesh (d = 4.1 cm, pore size = 1 mm) that was covered with a double layer of PTFE beads (n = 66, d = 6 mm) to avoid the formation of a liquid barrier at the base of the reactors. All reactors were open to the atmosphere on their top to allow vertical diffusion of the VOC emanating from the liquid reservoir. The upper 5 cm of the reactors were filled with dry glass beads to prevent microbial contamination from the atmosphere.

2.2.2 Reagents and analytical procedures

Toluene (h-toluene), methyl tertiary butyl ether MTBE (99 %) and $HgCl_2$ (99.5 %) were obtained from Merck KGaA. Per-deuterated toluene (99.6 atom % D, d-toluene) was purchased from Sigma-Aldrich Chemie Germany. Liquid and gasphase VOCs samples were analysed with a Hewlett-Packard Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID). The automated injection using the headspace auto-sampler (Hewlett-Packard 7694) with 1 mL injection volume and oven temperature of 95°C for liquid samples and 70 °C for gas samples. To separate the liquid phase d- and h-toluene, fused silica capillary column (Optima d-3, length 60 m, I.D. 0.32 mm, film thickness 0.35 μm; Macherey-Nagel, Duren Germany) was used. Temperature sequence was as follows; 35°C for 2 min, heated to 100°C at a rate of 6 °C min-1 for liquid samples; and 80 °C at a rate of 9 °C min-1 for gas samples , cooled down to 35°C. The

separation of D/H was achieved at 105.7 kPa with a N2 flowrate of 15 mL min-1 and with a split of 5:1. The FID was operated at 280 °C, and N2 was used as carrier gas.

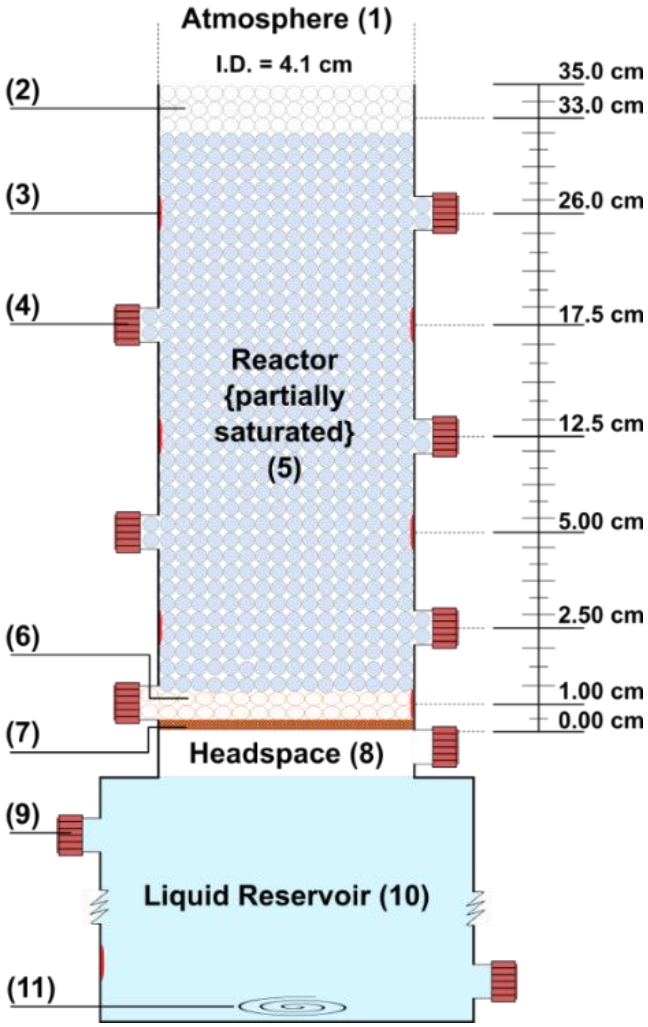

Figure 1: Schematic view of the experimental setup to mimic a shallow aquifer. It consists of a stirred (11) liquid reservoir (10) containing the dissolved VOC and a vertical reactor column (5) filled with partially saturated glass beads. The reactor is filled with dry glass beads (2) at the top, is open to the atmosphere at its top (1) and separated from the headspace (8) of the liquid reservoir by a steel mesh (7) covered by a thin layer of PTFE beads (6). Dissolved and gasphase VOC can be sampled by gas sampling point (4) and liquid sampling points (9), and oxygen by sensing spots (3). The scale represents the distance in cm from the steel mesh above the headspace of the reservoir.

2.2.3 Cultivation of bacteria and preparation of the inocula

Pseudomonas putida KT2442 DsRed pWW0 gfp, a rod-shaped, toluene-degrading bacterium as previously described by Nancharaiah et al. (2003), was pre-cultured in 250 mL gastight bottles up to the exponential phase (≈ 23 h; 30 oC; rotary shaker at 150 rpm) in 50 mL of minimal medium, sodium succinate, supplemented with toluene (50 mgL-1), was then cultivated in 2 L gastight bottles up to the late exponential phase (≈ 40 h; 30 oC; rotary shaker at 125 rpm) in 800 mL of minimal medium supplemented with toluene (200 mgL-1) (Kampara et al., 2008). The cultures were centrifuged at 7000 rpm, at 20 oC for 10 min. The pellet was washed with 100 mM phosphate buffer saline and then re-suspended in PBS at pH = 7. This suspension was then re-suspended in sterile minimal medium to make a total volume of 33 mL containing 0.3 % agar. This bacteria-agar suspension was then mixed with 700 g of glass beads (d =2.9 − 3.5 mm; Th. Geyer GmbH) under gentle shaking resulting in an approximate loading of 5 x 107 cfu per g of glass beads. Bacteria were quantified in triplicate by colony forming units on LB agar plates.

2.2.4 Reactor loading and running conditions

Presented reactor experiments include an abiotic "Control" experiment as well as a set of two bioreactive experiments ("Bioreactor 1" and "Bioreactor 2", operated as replicates). Additional reactor experiments with higher initial reservoir concentrations ("HC Control" and "Bioreactor 3") are presented in the Supporting Information. In all experiments, the agar covered glass beads were carefully transferred to autoclaved reactors and slight under-pressure (- 500 mbar) was applied to remove excess water from the system which resulted in a water saturation of approximately 14 % of the pore volume. Abiotic control reactors were identically treated except for the presence of bacteria. In addition, the control reactor was spiked with HgCl2 to exclude any microbial activity during the experiment. The column reactors were finally tightly connected to the reservoirs.

Reactors inoculated with strain KT2442 and their abiotic controls (cf. SI) were operated in duplicate for 7 days at standard pressure (1 atm) and T = 24°C. To provide quasi steady-state conditions, an observation period between day 2 and day 5 was selected for the evaluation of the vapor-phase results. Reactors were sterilized prior to use and operated under sterile conditions to avoid cross contamination. The liquid reservoirs contained 2.375 L of deionized water, a 1:1 mixture of h-toluene and d-toluene as well as MTBE as a non-reactive tracer (at the given experimental conditions) with expected concentrations of 18.5 mg L-1, 18.5 mg L-1 and 20 mg L-1 respectively, HgCl2 (2 μg L-1) was added to avoid biodegradation in the liquid reservoirs. All VOCs were spiked for 12 hours prior to the experiments to allow equilibration.

2.2.5 Sampling

500 μL of each, vapor-phase and liquid samples were taken approximately every 24 hours with gas tight syringes (1 mL for gas and 500 μL for liquid sampling; Hamilton, Switzerland). Samples were transferred to 10 mL-gas chromatograph (GC) sterilized vials, closed with Teflon coated crimp caps and analyzed within 24 h. Small gasphase and liquid sampling volume (i.e., per sampling event 2% of 140 mL of gas phase and 0.5‰ of 2375 mL of liquid) was considered to have limited effect on observed concentrations. The microbial biomass was quantified by sacrificial sampling from an extra reactor at the start and from experimental reactors at the end of the experiments i.e. at the end of the experimental runs sub-samples were taken from three different locations within the reactors and pooled into a single sample.

Oxygen concentrations in the reactors were measured at the same time of gas sampling, by a Fibrox 3 optical oxygen meter (PreSens Precision Sensing GmbH, Germany) using fixed sensing spots located at the same distance as sampling ports from the liquid reservoirs.

Flux and biodegradation rate calculations

For each sampling event, fluxes of volatile compounds between two neighboring sampling locations along the reactors were determined using Fick's law (eq.1).

$$J = -D \cdot ((C_A - C_B))/((X_A - X_B)) \cdot \tau \cdot \Phi_a \cdot A \quad \text{(eq. 1)}$$

where D is the molecular diffusion coefficient of the compound in the vapor phase. Considered values for h-toluene (D = 8.24·10-2 cm2s-1) and MTBE (D = 8.18·10-2 cm2s-1) were taken from (USEPA, 2015). For d-toluene a value of D = 8.16·10-2 cm2s-1 was derived from the associate value for h-toluene following (Bouchard et al., 2008) or (Mahieu et al., 2008). CA, B are the vapor-phase concentrations of the compound at sampling locations A and B, XA, B are the distances of these locations from the inlet of the reactor, τ is the tortuosity and ϕa the air filled porosity of the glass bead packing, and A is the cross sectional area of the reactor. Fluxes from all individual sampling events during the observation period (day 2 to day 5) of the experiment were used to estimate the average fluxes for the entire observation period.

Mass losses from the reservoirs during the observation period were calculated from the concentrations measured at the beginning and the end of the period. To estimate the average degradation rate of d-toluene and h-toluene, average fluxes along the reactors and mass losses from the reservoirs (plotted as flux at 0 distances from the reactor inlet) were fitted by an (arbitrarily chosen) exponential fit. The resulting slope of the fitting function at the inlet was used to estimate the maximum biodegradation rate for a compound in the reactor, assuming that any changes of the fluxes along the reservoirs were caused by biodegradation.

2.3 Results and Discussion

2.3.1 Measured concentrations

Measured concentrations showed a gradual depletion of all VOCs from the reservoirs (**Figure 2**) associated with pronounced and highly reproducible concentration profiles along the column reactors (Figure 3). Concentration profiles during the observation period (i.e. after 2 and 5 days) showed minor variations only. This indicates that during the observation period (quasi-) steady-state vapor-phase conditions were achieved determined by the respective concentration in the reservoir, the later showing only a gradual depletion. In abiotic controls, vapor-phase MTBE and toluene concentrations (Figure 3 and SI, Figure S1) exhibited a linear profile throughout the entire observation period. This indicates that no VOC sinks existed and that molecular diffusion was the dominating transport process in the vapor phase with no physical heterogeneities affecting the vapor-phase transport.

The concentration profiles of the bioreactors (**Figure 3**) showed strong depletion of h-toluene, which decreased to concentrations below the detection limit values already at 5 cm above the reactor inlet. Although isotope labelling has only minor effects on the gasphase diffusion coefficients (Kampara et al., 2008; Morasch et al., 2001), decrease of d-toluene concentrations was less pronounced with significant concentrations observed up to the top outlet of the column reactors. Such differences of the concentration profiles changes

of the toluene species is in accordance with the known better degradability of h-toluene under oxic conditions (Kampara et al., 2008). The supplemental experiment with approximately 70% increased toluene concentration in the reservoir water phase and consequently higher concentration in the reactors' inlets led to similar observations increasing only the penetration length of the compounds into the reactors (SI, Figure S1).

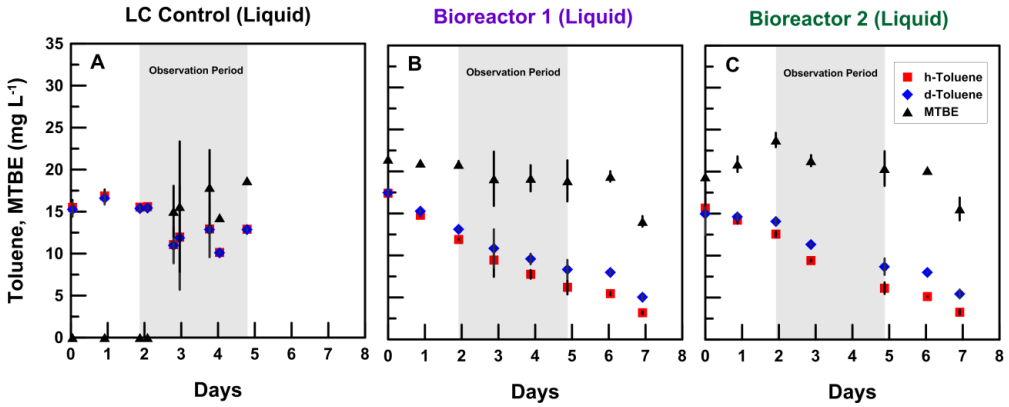

Figure 2: Evolution of h-toluene, d-toluene and non-reactive MTBE in the liquid reservoir of the control reactor (panel A) as wells as bioreactor 1 (panel B) and bioreactor 2 (panel C). Standard deviations reflect the analytical error.

Oxygen was present in the reactor throughout the experiments (75-100% air saturation) and allowed for aerobic growth of strain KT2442 from 4.7 x 107 ± 1.9 107 CFU per gram of glass beads at the beginning of the experiments to 2.5 x 109 and 1.5 x 109 CFU per gram of glass beads for reactors 1 and 2 at the end of the experiments, respectively. Typical water contents were 14% of the pore volume at the beginning and 7 - 9% of the pore volume after 7 days.

2.3.2 Flux and degradation rate analysis

Comparing the liquid concentrations in the reservoir with vapor-phase concentrations showed good agreement with Henry's law for h-toluene in the abiotic control experiments (**Figure 4**). In the abiotic control, concentration ratios for d-toluene were slightly smaller than those for h-toluene, which is in agreement with the (to our knowledge) only available literature value for d-toluene (dimensionless Henry volatility, K = 0.20) being in the lower range of the values reported for h-toluene (Sander, 2015). The similarity of the concentration ratios of the two toluene species supports the assumption that biodegradability is the only major difference between both species. The same observations were made for the high concentration control experiment (SI, Figure S3). Concentration ratios for MTBE are consistent with Henry's law yet located in the upper part or even slightly exceeding the data range from the literature (Sander, 2015). In presence of active biomass vapor-phase to water-phase toluene concentration ratios were consistently lower than expected from Henry's law (an effect observed also in earlier studies, e.g., Kurt and Spain (2013) with discrepancies being less explicit for the less reactive d-toluene (Figure 4). This implies that the steeper concentration gradients resulting from toluene degradation led to increased diffusive fluxes, which in the reservoirs could not be fully compensated by the mass transfer from the liquid to the vapor

phase (headspace). This simultaneous assessment of gasphase and liquid-phase concentration thus provided an additional perspective on the dynamics of the processes controlling the fate of toluene in the reactors.

In contrast to quasi constant vapor-phase MTBE fluxes throughout the reactor, h-toluene and d-toluene fluxes decreased rapidly in the reactors as a result of biodegradation (Figure 3). Fluxes for the sampling interval closest to the inlet (0-1 cm) were in approximate agreement with mass losses from the reservoirs. In spite of the simplified assumptions, estimations derived from vapor-phase concentrations thus provided a useful estimate for the VOC fluxes. A comparison of the toluene fluxes in the bioreactors with those of the control confirmed that biodegradation led to clearly enhanced h-toluene and d-toluene fluxes out of the water reservoirs relative to the abiotic control (**Figure 3**). For the high concentration experiments an analogous observation was made (SI, Figure S1). Based on the fluxes the toluene biodegradation rates were estimated considering again an average water saturation of 12% of the pore space. In the vicinity of the inlet of the reactors biodegradation rates were 338 and 246 mg h-1 Lwater-1for h-toluene, and 39 and 34 mg h-1 Lwater-1 for d-toluene in bioreactor 1 and 2, respectively. Results for the supplemental high-concentration (bioreactor 3) experiment revealed similar biodegradation rates of 305 mg h-1 and 26 mg h-1 Lwater-1 for h- and d-toluene, respectively. Such difference of approximately one order of magnitude in the degradation rate of the two toluene compounds is in agreement with previous observations (Kampara et al., 2008; Morasch et al., 2001). For h-toluene the estimated rates were similar to Picone et al. (2013) who assessed toluene biodegradation rates in unsaturated column reactors of similar water saturation (10%) and concentrations yet in presence of advective vapor-phase transport. This shows that independent of the specific vapor-phase transport process, biodegradation rates in the unsaturated zone can be much higher than rates reported for water-saturated systems. H-toluene degradation rates were also close to values proposed by De Biase et al. (2013) for the aerobic degradation of benzene in unsaturated soil filters at the pilot scale or by Sihota and Mayer (2012) for aerobic methane oxidation in the unsaturated zone of a petroleum contaminated size, and microbial abundances encountered in the column reactors have been found in field studies too e.g., (Bundt et al., 2001). This indicates that the column reactors are suitable for studying reactive transport processes taking place in the unsaturated zone, and that the results presented here might also be relevant for the field.

Compared to earlier vapor phase experiments with porous media reported in the literature (e.g., Hohener et al., 2003; Jin et al., 1994), where diffusive travel paths of several decimeters were needed to remove toluene from soil air, the results of this study indicate that in presence of sufficient microbial activity lower distances of several centimeters might be sufficient. Furthermore, by using glass beads as a solid matrix and two toluene isotopologues of (nearly) identical physical properties but highly differing degradability was possible to attribute the observed high removal rates exclusively to biodegradation and to show that no abiotic processes are needed to achieve such removal. The high spatial resolution of this setup was a prerequisite for resolving the fast reactive turnover of the degraded toluene.

2.3.3 Environmental implications

The obtained results confirm that even in the presence of vapor-phase diffusion, biodegradation is able to significantly alter concentration gradients of volatile species at the centimeter scale (Hanzel et al., 2011). Compared to other VOCs toluene has a relatively high volatility (Sander, 2015) and a relatively high diffusion coefficient, and the used aqueous concentrations are typical, yet in the upper range, for BTEX concentrations at contaminated groundwater sites (e.g., Martienssen et al., 2006; Schafer, 2001). Thus, biodegradation in an

unsaturated zone of 5 - 10 cm thickness can lead to a full removal of a volatile contaminant (i.e. prevents any emissions to the atmosphere) if site specific conditions support similar degradation rates as observed in our experiments.

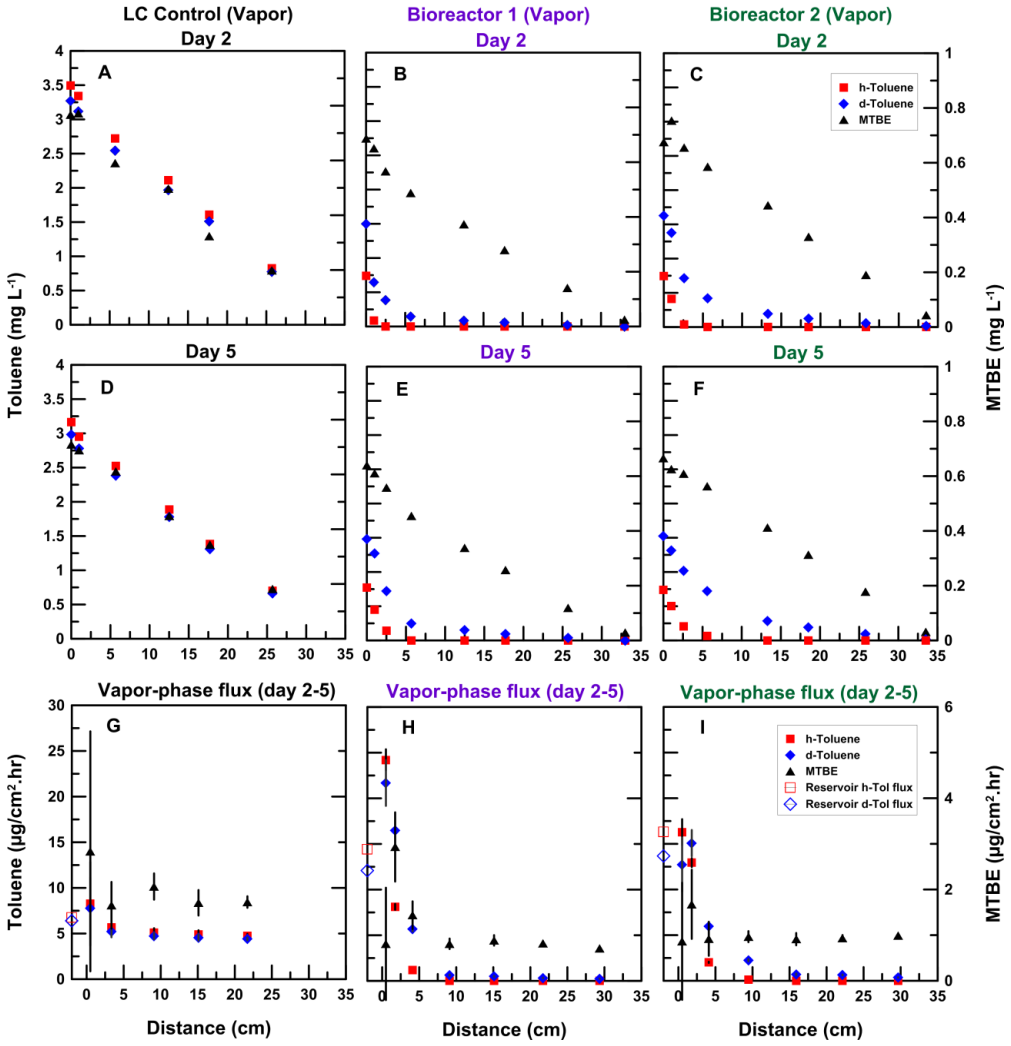

Figure 3: Top and middle row: Concentration profiles of gas phase h-toluene, d-toluene and non-reactive MTBE along the abiotic control column (left) and column bioreactors 1 (middle) and 2 (right) after 2 days (panels A to C) and after 5 days (panels D to F). Values as x = 0 cm represent head space concentrations. Bottom row: Cumulative gas phase fluxes of h-toluene, d-toluene and non-reactive MTBE along the column reactors during days 2 to 5 (panels G & I considering an average water saturation of 12% of the pore space). Empty symbols refer to the loss of h-toluene and d-toluene from the reservoirs between days 2 and 5. Standard deviations reflect uncertainty due to analytical errors.

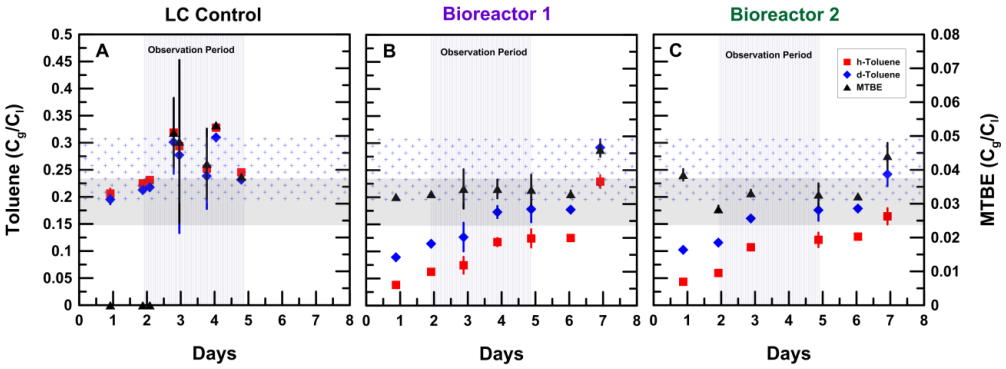

Figure 4: Development of the measured gas to liquid concentration ratios of h-toluene, d-toluene and MTBE in the control reactor (panel A) as well as bioreactor 1 (panels B) and reactor 2 (panel C). Marked horizonal zone represent ranges of literature values of Henry volatilities (taken from Sander (2015) ignoring 2-3 highest and lowest values reported there), blue dots for toluene and grey for MTBE.

Our results further show that biodegradation in the vadose zone can increase the flux of volatile biodegradable contaminants out of underlying groundwater, which can decrease the groundwater contaminant concentrations (Cussler, 2009; Kurt & Spain, 2013; Pasteris et al., 2002; Rivett et al., 2011), and that the air present in the top layer of the vadose zone contains enough oxygen to support sufficient microbial activity without any additional aeration needed. For technical systems such as biofilters a rather small unsaturated cover layer would be sufficient to avoid volatile emissions of compounds injected into the filter underneath such cover layer, which confirms findings from pilot scale vertical-flow soil filter systems (De Biase et al., 2013; De Biase et al., 2011; van Afferden et al., 2011). The water saturation used in the present study was relatively low not imposing a major limitation of diffusive transport in the gas phase. Increased water saturation would have reduced diffusive transport rates which would have facilitated the removal of VOC from the gas phase. Similarly, the presence of organic material in the soils or other natural unsaturated porous media would lead to a retardation of the diffusive transport which would again support VOC removal across short distances (Bushnaf et al., 2011). In any case, sufficiently high degradation rates require the right combination of field conditions and compound properties and future research is needed to determine where and when such combinations are met.

2.4 Acknowledgments

This research was supported by the funding from Helmholtz Centre for Environmental Research – UFZ in the frame of the SAFIRA II and contributes to the research topic Chemicals in the Environment (CITE) within the research programme Terrestrial Environment of the Helmholtz Association. The authors thank colleagues from the UFZ Departments Environmental Microbiology and Soil Physics for their support of the experimental work, and Dr. Cecilia De Biase and Dr. Sajid Ali for their critical comments on this manuscript and help throughout the study.

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SUPPORTING INFORMATION

Results of high concentration reactors

Concentrations of h- and d-toluene and the non-degradable tracer MTBE exhibited throughout the entire observation period a linear profile along the gas phase of the high concentration abiotic control column reactor (**Figure S1**). This indicates that no physical heterogeneities were affecting the gas phase transport. Concentration profiles showed only minor variations during the observation period, which are attributed to the gradual depletion of the compounds from the reservoirs (**Figure S2**). The concentration profiles in the high concentration bioreactor 3 showed strong depletion of h-toluene, reaching negligible values (**Figure S1**) at 10-12 cm above the reactor inlet. D-toluene concentrations showed less depletion, compared to h-toluene. Concentrations in the liquid reservoirs showed for both toluene species a gradual decrease during the duration of the experiment (**Figure S2**). Compared to h-toluene, d-toluene exhibited a slightly stronger decrease. Compared to this the decrease in the abiotic control was much smaller showing nearly identical trends for both toluene species (**Figure S2**).

Concentration ratios between headspace and liquid reservoir concentrations showed for both isotopologues good agreement with literature data. Measured values for d-toluene were slightly smaller than for h-toluene. For both high concentration reactors measured values for MTBE were in the upper range or slightly exceeding the data range from the literature. In the bioreactors 3 measured concentration ratios for h-toluene were consistently lower than expected from Henry's law, while values for d-toluene were higher and remained close to the predictions from Henry's law.

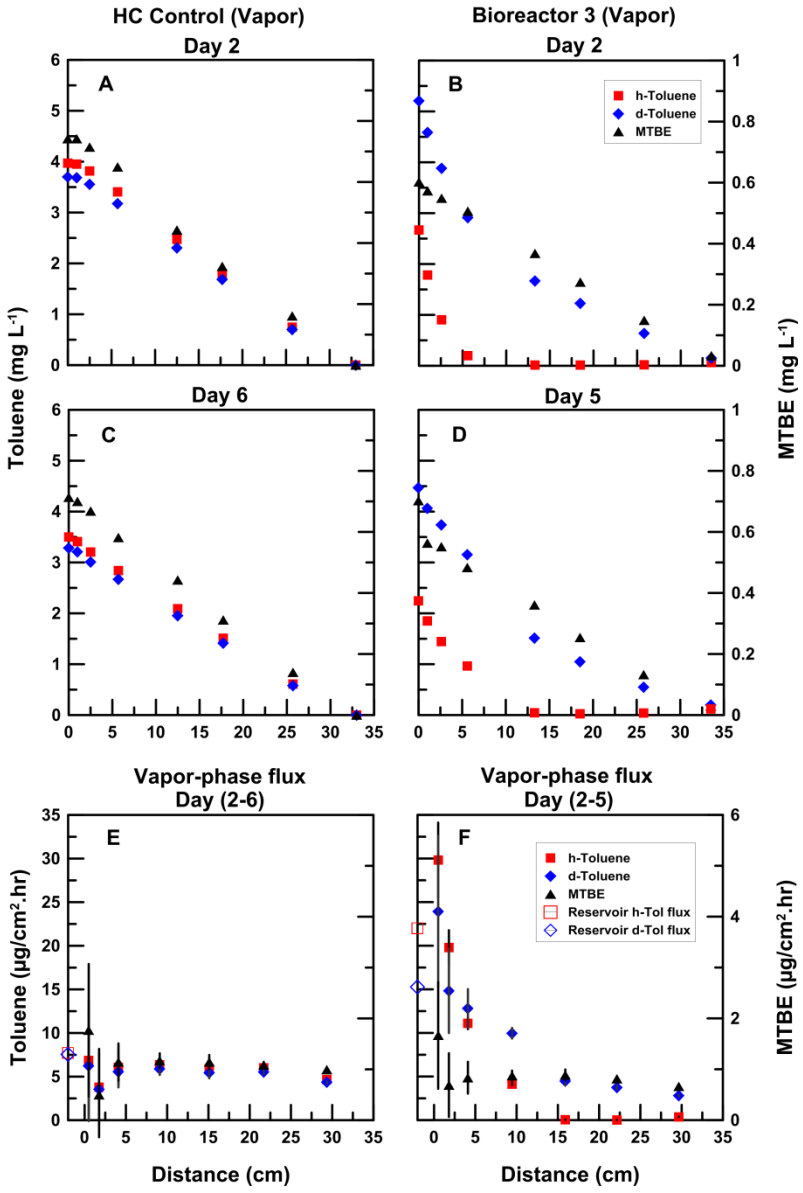

Figure S1: Top and middle row: Concentration profiles of gas phase h-toluene, d-toluene and non-reactive MTBE along the high concentration abiotic control column reactor (left) and high concentration column bioreactor 3 (right) after 2 days (panels A & B) and after 5/6 days (panels C & D). Observation period of the control column reactor lasted until day 6 due to sampling difficulties on day 5. Values as x = 0 cm represent headspace concentrations. Bottom row: Average gas phase fluxes of h-toluene, d-toluene and non-reactive MTBE along the column reactors during days 2 to 5 (panels E & F; considering an average water saturation of 12% of the pore space). Empty symbols refer to the loss of h-toluene and d-toluene from the reservoirs between days 2 and 5. Standard deviations reflect uncertainty due to analytical errors.

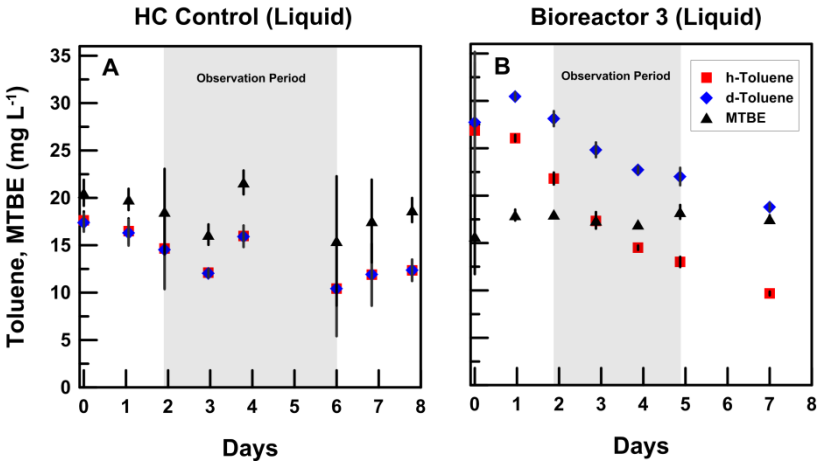

Figure S2: Evolution of h-toluene, d-toluene and non-reactive MTBE in the liquid reservoir of the high concentration abiotic control reactor (panel A) and the high concentration bioreactor 3 (panel B). Standard deviations reflect the analytical error.

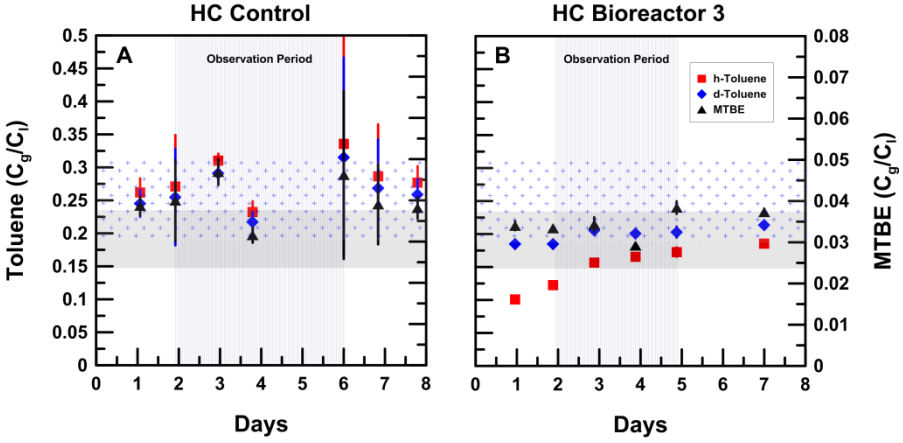

Figure S3: Development of the measured gas to liquid concentration ratios of h-toluene, d-toluene and MTBE in the high concentration control reactor (panel A) and the high concentration bioreactor 3 (panel B). Marked horizontal zones represent ranges of literature values of Henry volatilities (taken from Sander (2015) ignoring 2-3 highest and lowest values reported there), blue dots for toluene and grey shade for MTBE.

Chapter 3

"The right question is usually more important than the right answer."

Plato (428 BC – 348 BC)

Applying the Rayleigh approach for stable isotope-based analysis of VOC biodegradation in diffusion-dominated systems

Abstract

Compound-specific stable isotope analysis (CSIA) has become an established tool for assessing biodegradation in the subsurface. Diffusion-dominated vapor phase transport thereby is often excluded from quantitative assessments due to the problem of diffusive mixing of concentrations with different isotopic signatures for CSIA interpretation. In soils and other unsaturated porous media volatile organic compounds (VOCs) however, are mainly transported via gas-phase diffusion and may thus prohibit a CSIA-based quantitative assessment of the fate of VOCs. The present study presents and verifies a concept for the assessment of biodegradation-induced stable isotope fractionation along a diffusive transport path of VOCs in unsaturated porous media. For this purpose data from batch and column toluene biodegradation experiments in unsaturated porous media were combined with numerical reactive transport simulations; both addressing changes of concentration and stable isotope fractionation of toluene. The numerical simulations are in good agreement with the experiment data, and our results show that the presented analytically derived assessment concept allows using the slope of the Rayleigh plot to obtain reasonable estimates of effective in situ fractionation factors in spite of diffusion-dominated transport. This enlarges the application range of CSIA and provides a mean for a better understanding of VOC fate in the unsaturated subsurface.

Keywords: Volatile organic compounds (VOC), Subsurface processes, Reactive transport modeling, Compound-specific stable isotope analysis (CSIA), Biodegradation, Bioremediation, Unsaturated zone, Outgasing.

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3.1 Introduction

Biodegradation of volatile organic compounds (VOCs) in the unsaturated subsurface has been observed for different laboratory and field conditions,[1-4] indicating that natural attenuation may be a feasible remediation option for VOCs in the unsaturated subsurface. However, the fate of subsurface vapor-phase VOCs depends on a multitude of hydrological, geochemical, and microbiological processes. These processes are not only highly interlinked and dependent on temperature, water saturation, pH and many other environmental factors, but also act in parallel, making the *in-situ* identification and quantification of the key processes controlling the system dynamics difficult. In order to distinguish biodegradation from other processes, Compound-Specific Stable Isotope Analysis (CSIA) is widely accepted as a monitoring strategy and as a powerful tool in studying the fate and behavior of contaminants in groundwater systems.[5-8] The application of CSIA makes use of the fact that the stable isotope fractionation of the biodegradation reaction dominates the change of the stable isotope signature of the contaminants. Especially for a quantitative analysis of biodegradation using CSIA, it is required that contributions from mixing,[8-10] sorption,[11-13] small-scale mass transfer,[14-15] dispersion[8, 16-18] or the regeneration of a degraded compound[19] can be either neglected or their influence be adequately considered. If these assumptions are met, the analytical Rayleigh model[20-21] is frequently used to deduce the extent of biodegradation from the degree of isotopic enrichment[22-23] in groundwater systems with advection-dominated transport.

In the gas phase, molecular diffusion coefficients are up to four orders of magnitude larger than in the aqueous phase. Thus - in contrast to groundwater systems - transport in the gas phase of the unsaturated subsurface is more easily dominated by diffusion in the absence of relevant pressure gradients. As diffusion coefficients in the gas phase[24-25] as well as the aqueous phase[26-28] can differ between isotopologues (i.e. between chemically identical species with different isotopic composition), diffusion-dominated transport systems may exhibit significant stable isotope fractionation even in the absence of biodegradation.[27, 29-31] Furthermore, even if diffusive transport is not leading to any fractionation effects, diffusive mixing along concentration gradients mitigates changes in stable isotope signatures caused by biodegradation. As a consequence it has been considered that the standard Rayleigh-equation based analysis approach of stable isotope fractionation is not applicable for diffusion-dominated transport systems.[29] This would mean that for diffusion-dominated transport systems CSIA could at best be used as qualitative biodegradation indicator only. However, for the related case of soil organic matter decomposition quantitative assessment approaches could be obtained describing the fractionation of CO_2 as volatile reaction product in spite of the diffusion dominated transport regime.[25, 32]

The aim of this study is to show that even for diffusion-dominated systems CSIA data might still be used to obtain a quantitative understanding of VOC biodegradation. For this we use experimental results published in Khan, et al.[4] showing efficient biodegradation in column reactor systems mimicking the conditions in the unsaturated subsurface above the groundwater table. Data from the column reactors and additional batch experiments are analyzed regarding stable isotope fractionation and interpreted using a combination of analytical calculations and numerical modeling. To address the complex interplay of processes and their impact on the fate of bioreactive species in the subsurface, numerical reactive transport models are powerful means[33] and have shown their potential also for the analysis of VOC biodegradation in unsaturated systems.[34-35] In recent years, reactive transport modeling concepts have been expanded to consider isotope-specific processes and the resulting stable isotope fractionation.[1, 15, 36-44] This provides an approach to

disentangle the potential influence of different processes on stable isotope fractionation effects experimentally observed in subsurface compartments.

In this study a combination of simplified analytical calculations with numerical reactive transport simulations is used to determine to which extent the simplified calculations lead to acceptable estimates of the fractionation effects observed experimentally and to show that also for diffusion-dominated transport system a quantitative analysis of CSIA can be obtained via a modified interpretation of the analytical Rayleigh model.

3.2 Materials & Methods

3.2.1 Batch Reactors

Batch reactor systems were used to quantify stable hydrogen isotope fractionation factors during biodegradation of vapor-phase toluene. Gastight chromoflax glass bottles with total volume of 1150 mL were used as batch reactors (Supporting Information (**SI**)**, Figure S1**). Reactors were filled with 50 mL glass beads (d = 2.9 – 3.5 mm), coated with minimal media agar that contained toluene degrading bacteria (Pseudomonas putida KT2442 DsRed pWW0 gfp) at a density of 3.95 x 10^8 cfu per gram of glass beads as previously described by Khan, et al.[4] The minimal medium agar layer contained all nutrients relevant for bacterial activity and growth.[45] The headspace of the batch reactor (1100 mL) provided sufficient oxygen for bacterial activity during the entire experimental period. As sorption of nonionic, hydrophobic organic chemicals to mineral surfaces is expected to be negligible,[46] no controls assessing the adsorption of agar-born MTBE and toluene to glass were performed. Four different operation modes were applied each characterized by specific period of time (1 to 4 days) the reactors were first kept at room temperature under sterile conditions for 1 to 4 days before toluene was added. After this reactor-specific resting period, a known concentration of a 1:1 mixture of toluene and perdeuterated toluene was spiked to the internal glass wall close to the neck of the reactor. Methyl tert-butyl ether (MTBE) was additionally added as a non-reactive VOC control. This allowed us to get 20 mg L^{-1} total gas phase concentration of the two toluene isotopologues, and 5 mg L^{-1} gas phase concentration of MTBE.

After spiking of the VOCs, the batch reactors were let to equilibrate regarding volatilization for 20 minutes (allowing vapor-phase toluene concentrations to achieve calculated equilibrium values) before the start of sampling (marked as time t_0 = 0 hours). Subsequent samples were taken every hour until t = 8 hours. Gas-phase VOC samples were taken and analyzed as mentioned previously in Khan, et al. [4] (see also Supporting Information). The observation period was selected for the isotope analysis and the measured data (toluene concentration c and stable (hydrogen) isotope ratio R in the gas phase) were analyzed using Rayleigh plots (i.e., plotting the logarithmic form of the Rayleigh equation:[21, 47] $ln(R/R_0)$ against $ln((c/c_0)/((R+1)/(R_0+1))$ for the large values of R given here;[48] the subscript 0 refers to the initial conditions) to determine stable isotope fractionation factors.

3.2.2 Column Reactors

The column reactor experiments are described in detail in Khan, et al.[4] (see also SI) and only a brief overview is given here: The setup consisted of vertical chromoflax glass column reactors (l = 35 cm, *i.d.* = 4.1 cm) packed with agar-coated 700 g glass beads (d = 2.9–3.5 mm), separated with 45 mL headspace from the liquid reservoir of 2.375 L volume (**SI Figure S2**). Column reactors were open to the atmosphere on top to allow

sufficient oxygen for biodegradation.[4] Known concentrations of VOCs (toluene 37 mg L^{-1} and MTBE 20 mg L^{-1}) were spiked in the liquid reservoirs with magnetic stirrer bars and were kept on magnetic shakers for 12 hours prior to the start of experiments to equilibrate. $HgCl_2$ (2 µg L^{-1}) was added to avoid biodegradation in the liquid reservoirs. To avoid cross contamination the columns were sterilized and were attached to the liquid reservoirs under sterile conditions.

Data were taken from two abiotic experiments ("Control 1" and "Control 2", termed "Control" and "Control HC" in Khan, et al.[4]) as well as a set of three bioreactive experiments ("Column 1", "Column 2" and "Column 3", termed "Bioreactor 1" to "Bioreactor 3" in Khan, et al.[4]) where the glass bead packing was inoculated with *Pseudomonas putida* KT2442 DsRed pWW0 gfp. Reactors were operated for 7 days at standard pressure (1 atm) and T = 22 °C. Vapor-phase and liquid samples (500 µL) were taken every day. To provide quasi steady-state conditions, an observation period between day 2 and day 5 was selected for the evaluation of the vapor-phase results.

3.3 Theoretical Approaches

In this study, two different computational approaches are applied: an analytical approach relying on a simplified description of transport and degradation in the columns, and a numerical approach providing a more detailed description of the processes in the gas phase and in the aqueous phase of the combined reservoir-column system.

3.3.1 Analytical solutions for diffusive-reactive transport with first order degradation and stable isotope fractionation.

The fractionation of stable isotopes by (bio-)reactive transformations is described by the isotope fractionation factor $\alpha_b = \frac{{}^{h}r/{}^{h}c}{{}^{l}r/{}^{l}c}$, where ${}^{h}r$ and ${}^{l}r$ are the reaction rates, and ${}^{h}c$ and ${}^{l}c$ are the gas phase concentrations of reactants containing the light or the heavy isotope, the latter denoted by the superscripts *l* and *h*, respectively. If the degradation reaction is following first order kinetics (${}^{h}r = {}^{h}k \cdot {}^{h}c$ and ${}^{l}r = {}^{l}k \cdot {}^{l}c$, with ${}^{h}k$ and ${}^{l}k$ as first order degradation rate parameters) this simplifies to $\alpha_b = \frac{{}^{h}k}{{}^{l}k}$. Analogously the stable isotope fractionation due to diffusive transport can be described by a factor $\alpha_d = \frac{{}^{h}D}{{}^{l}D}$, with ${}^{h,l}D$ as effective molecular diffusion coefficients.

If in a one-dimensional system diffusion and such degradation are the only processes acting on the concentration distribution of the compound, concentration changes are given as

$$\frac{\partial {}^{h,l}c}{\partial t} = {}^{h,l}D \cdot \frac{\partial^2 {}^{h,l}c}{\partial x^2} - {}^{h,l}k \cdot {}^{h,l}c \qquad (1)$$

with *t* and *x* as temporal and spatial coordinate, respectively.

For steady-state conditions ($\partial {}^{h,l}c/\partial t = 0$) and boundary condition of ${}^{h,l}c(x=0) = {}^{h,l}c_0$ and ${}^{h,l}c(x=L) = 0$ the solution of Eq. 1 is given by Wilson[49] and Pasteris, et al.[50]

$$ {}^{h,l}c(x) = {}^{h,l}c_0 \cdot \frac{\sinh\left(\sqrt{{}^{h,l}Da}\cdot\left(1-\frac{x}{L}\right)\right)}{\sinh\left(\sqrt{{}^{h,l}Da}\right)} \quad (2) $$

with ${}^{h,l}Da = {}^{h,l}k \cdot L^2 / {}^{h,l}D$ as Damköhler number describing the ratio between the time scales of transport and of reaction.

In the case of $L \rightarrow \infty$ Eq. 2 simplifies to

$$ {}^{h,l}c(x) = {}^{h,l}c_0 \cdot \exp\left(-\sqrt{\frac{{}^{h,l}k}{{}^{h,l}D}}\cdot x\right) \quad (3) $$

Using Eq. 3 the isotope ratio $R = {}^{h}c/{}^{l}c$ is given as $R = \frac{{}^{h}c_0\cdot\exp\left(-\sqrt{\frac{{}^{h}k}{{}^{h}D}}\cdot x\right)}{{}^{l}c_0\cdot\exp\left(-\sqrt{\frac{{}^{l}k}{{}^{l}D}}\cdot x\right)} = \frac{{}^{h}c_0\cdot\exp\left(-\sqrt{\frac{\alpha_b\ {}^{l}k}{\alpha_d\ {}^{l}D}}\cdot x\right)}{{}^{l}c_0\cdot\exp\left(-\sqrt{\frac{{}^{l}k}{{}^{l}D}}\cdot x\right)}$ which can be transformed into

$$ \frac{R}{R_0} = \frac{\left({}^{l}c/{}_{{}^{l}c_0}\right)^{\sqrt{\alpha_b/\alpha_d}}}{\left({}^{l}c/{}_{{}^{l}c_0}\right)} = \left(\frac{{}^{l}c}{{}^{l}c_0}\right)^{\left(\sqrt{\alpha_b/\alpha_d}-1\right)} = \left(\frac{c/c_0}{(R+1)/(R_0+1)}\right)^{\left(\sqrt{\alpha_b/\alpha_d}-1\right)} \quad (4) $$

with $c = {}^{h}c + {}^{l}c$ and the subscript *0* denoting conditions at $x = 0$. Note that assuming $c \approx {}^{l}c$ (i.e. $R << 1$) simplifies Eq. 4 to $\frac{R}{R_0} = \left(c/c_0\right)^{\left(\sqrt{\alpha_b/\alpha_d}-1\right)}$. When plotting concentration and isotope data in a Rayleigh plot (i.e., plotting the logarithmic form of the Rayleigh equation: $ln(R/R_0)$ against $ln(c/c_0)$[21, 48]), the slope m of the Rayleigh plot would thus be given by

$$ m = \sqrt{\alpha_b/\alpha_d} - 1 \quad (5) $$

(and not by $m = \alpha_b - 1$ as predicted by the classical Rayleigh equation for advection dominated transport or for batch systems). For other conditions, in particular for finite size systems (finite L) and other than first order degradation kinetics, no closed form analogue for Eq. 4 exists to our knowledge and it is not clear to which extent Eq. 5 can be used as an approximate solution. Note that Eq. 5 is valid for systems with biodegradation. In the absence of biodegradation, no fractionation effects are present at steady state.

3.3.2 Numerical simulations

The simulations of the column reactors presented in Khan, et al.[4] consider processes in both parts of the reactors: the reservoir and the column. The reservoir is assumed to contain a well-mixed liquid phase and a well-mixed gaseous head space. The exchange of volatile compounds between these two phases is controlled by a linear exchange term (Eq. 6). The column is spatially discretized along its length and is also assumed to contain at each length a liquid and a gas phase using again a linear term for the exchange of volatile compounds between the phases (Eq. 8). At the bottom of the column, concentration in the gas phase are coupled to those in the head space of the reservoir using again such an exchange term (Eq. 7). Diffusive transport is assumed to take place in the gas phase along the length of the column, no transport is considered

along the aqueous phase of the column. Biodegradation of toluene (i.e., $C_7H_8 + 9O_2 \rightarrow 7CO_2 + 4H_2O$) is restricted to the liquid phase of the first 30 cm of the column (from 30 to 35 cm the glass bead packing had not been inoculated in the experiments). Growth of degrading microorganisms is not considered. To describe degradation and stable isotope fractionation of toluene in the column reactors, deuterated and non-deuterated toluene are simulated as individual species using Michaelis-Menten kinetics (isotope-specific version adapted from Thullner, et al.[37]) for the degradation reaction (Eq. 9 and 10). This results in the following set of expressions for the kinetics of the individual processes.

$$ {}^{h,l}r_1 = {}^{h,l}k_1 \cdot \left({}^{h,l}c_{r,g} - {}^{h,l}c_{r,a} \cdot {}^{h,l}H \right) \quad \text{(phase exchange reservoir)} \quad (6) $$

$$ {}^{h,l}r_2 = {}^{h,l}k_2 \cdot \left({}^{h,l}c_{x=0,g} - {}^{h,l}c_{r,g} \right) \quad \text{(exchange head space – column)} \quad (7) $$

$$ {}^{h,l}r_3 = {}^{h,l}k_3 \cdot \left({}^{h,l}c_{x,a} \cdot {}^{h,l}H - {}^{h,l}c_{x,g} \right) \quad \text{(phase exchange column)} \quad (8) $$

$$ {}^{l}r_4 = k_4 \cdot \frac{{}^{l}c_{x,a}}{K_s + {}^{l}c_{x,a} + {}^{h}c_{x,a} \cdot \alpha_b} \quad \text{(degradation non-deuterated toluene)} \quad (9) $$

$$ {}^{h}r_4 = k_4 \cdot \alpha_b \cdot \frac{{}^{h}c_{x,a}}{K_s + {}^{l}c_{x,a} + {}^{h}c_{x,a} \cdot \alpha_b} \quad \text{(degradation deuterated toluene)} \quad (10) $$

with subscripts *g* and *a* denoting gas phase and liquid (aqueous) phase, respectively. Subscript *r* refers to the reservoir while *x* refers a location in the column; $x = 0$: bottom of the column, $x = L$ top of the column. ${}^{h,l}H$ is the dimensionless Henry volatility, $k_{...}$ are rate parameters, K_s is the Michaelis-Menten constant and α_b is the stable isotope fractionation factor of the degradation reaction. Eq. 6 and 7 describe the mass flux (mass per time) between the different compartments, while Eq. 8 directly describes the concentration change (mass per volume per time) in the gas phase of the column. No further species are considered in the simulations. In particular, no oxygen limitation is considered for the degradation kinetics as preliminary simulations have shown that aerobic conditions are maintained for all parts of the systems throughout the experiments, which is in agreement to the experimental observations of Khan, et al.[4] . The kinetic expressions were implemented into the Biogeochemical Reaction Network Simulator[51-53] using a regular spatial discretization of the column of 0.5 cm. Effective gas phase diffusion coefficients are derived from molecular diffusion coefficients ${}^{h,l}D_m$ and the tortuosity τ of the glass bead packing (${}^{h,l}D = {}^{h,l}D_m \cdot \tau$) (note that partitioning effects between gas phase and aqueous phase are explicitly described in the simulations).

Parameter values used for the simulations (**Table 1**) were either derived directly from the experimental systems or were fitted to match the experimental observations. For this purpose first the control experiments were used to adjust the parameters of the non-reactive processes. Then parameters describing biodegradation were determined using the data from the systems with biodegradation. The target of the parameter estimation was to obtain simultaneously a good match of the total toluene concentrations in the reservoirs and in the columns, and of the slopes of the Rayleigh plots for the reservoirs and the columns. Parameters were varied without using any automated algorithm. All parameters describing transport and reactions are assumed to be constant in space and time. Exceptions are the water saturation of the columns which is assumed to decrease linearly from initially 14% to 7% after 7 days reflecting the experimental observations (note that this also affects the gas phase volume in the column and it is assumed that no concentration changes are directly induced by the volume changes due to the fast relaxation of the system compared to the time scale of the volume changes) and k_4 (maximum rate of the degradation reaction) which is considered to decrease according to $k_4(t) = k_4(t = 0) \cdot \exp(-\lambda \cdot t)$. Reasons for this decrease in reactivity are not apparent from

Table 1: Parameter values used for the numerical simulations.

Param-eter	**Description**	**Value**	**Origin**
L	column length	35 cm	measured
d	column inner diameter	4.1 cm	measured
V_r	reservoir: liquid volume	2375 cm^3	measured
V_h	reservoir: head space volume	45 cm^3	measured
Φ	porosity	0.39	measured
τ	tortuosity	0.5	measured
S	initial water saturation	14%	measured
${}^hD_m$, ${}^lD_m$	gas phase molecular diffusion coefficient	297 $cm^2\ h^{-1}$, 294 $cm^2\ h^{-1}$	USEPA [27] for toluene and modified according to Bouchard, et al. [12a] for deuterated toluene
${}^hH$, ${}^lH$	Henry volatility	0.30, 0.30	fitted, constrained by Sander [28]
K_s	Michaelis-Menten constant	0.5 mg L^{-1}	fixed to reasonable value
${}^hk_1$, ${}^lk_1$	time constant for phase exchange in reservoir	100 $cm^3\ h^{-1}$, 100 $cm^3\ h^{-1}$	fitted, constrained by assuming diffusion through liquid boundary layer
${}^hk_2$, ${}^lk_2$	time constant for exchange between head space and column	10^4 $cm^3\ h^{-1}$, 10^4 $cm^3\ h^{-1}$	fixed to high value
${}^hk_3$, ${}^lk_3$	time constant for phase exchange in column	100 h^{-1}, 100 h^{-1}	fitted, constrained by assuming diffusion through liquid boundary layer
k_4	initial maximum rate parameter of biodegradation reaction	552 mg $L^{-1}\ h^{-1}$ (Column 1) 276 mg $L^{-1}\ h^{-1}$ (Column 2) 138 mg $L^{-1}\ h^{-1}$ (Column 3)	fitted
λ	time constant for reactivity decrease	0.01 h^{-1}	fitted

α_b	isotope fractionation factor of biodegradation reaction	0.05	fitted, constrained by batch experiment from this study
${}^{h,l}c_i$	initial concentration in reservoir liquid phase	15.5 mg L^{-1} (Control 1) 17.4 mg L^{-1} (Control 2) 17.4 mg L^{-1} (Column 1) 17.4 mg L^{-1} (Column 2) 32.0 mg L^{-1} (Column 3)	adjusted to experimental observations

the experimental data, but the decrease might have been caused by the decreasing water content or a depletion of some trace nutrients. If not stated otherwise parameter values do not differ between the different column reactors, i.e. the presented parameter values describe simultaneously all column reactors. Initial concentrations were set to 0 in the entire systems except of in the liquid phase of the reservoir where concentration values were adjusted to match experimental observations.

3.4 Results and Discussion

3.4.1 Vapor-phase hydrogen stable isotope fractionation in batch reactors

Vapor-phase toluene biodegradation was studied in the batch systems containing deuterated and non-deuterated toluene to obtain the hydrogen stable isotope fractionation factor of toluene by *Pseudomonas putida* KT2442 DsRed pWW0 gfp. All batch reactors exhibited a similar behavior showing a strong hydrogen stable isotope fractionation due to biodegradation (**SI, Figure S3**) with slopes of the Rayleigh plots ranging between -0.86 and -0.97; i.e. stable isotope fractionation factors in the range of 0.03 to 0.14. An additional replicate for Day 1 yielded unreasonable results and was omitted from further analysis. No temporal shifts in fractionation of vapor-phase toluene was observed and the average stable isotope fractionation factor was $\alpha_b = 0.08 \pm 0.05$. This value obtained from vapor-phase toluene data is similar to values reported in Kampara, et al.[45] ($\alpha_b = 0.07 \pm 0.02$) and Morasch, et al.[54] ($\alpha_b = 0.09 \pm 0.07$) for liquid batch systems where fully deuterated toluene was degraded by a closely related bacterial strain having the same TOL plasmid as *P. putida* KT2442. In general, phase transitions may contribute to the stable isotope fractionation in a system.[55-56] The similarity between the results from the two phase system and those reported for the liquid systems suggests that the transition between gas phase and liquid/agar phase did not have any impact on the magnitude of the observable fractionation effects in this study or that any possible effects were in the order of the uncertainties of the measurements.

3.4.2 Hydrogen stable isotope fractionation in column reactors

Control experiments: Results of the two control column reactors showed continuous yet moderate depletion (approx. *10 mgL^{-1}* throughout the experimental period) of toluene in the liquid reservoirs attributed to the losses by diffusion through the column reactors (**SI, Figure S4**). Compared to the strong fractionation

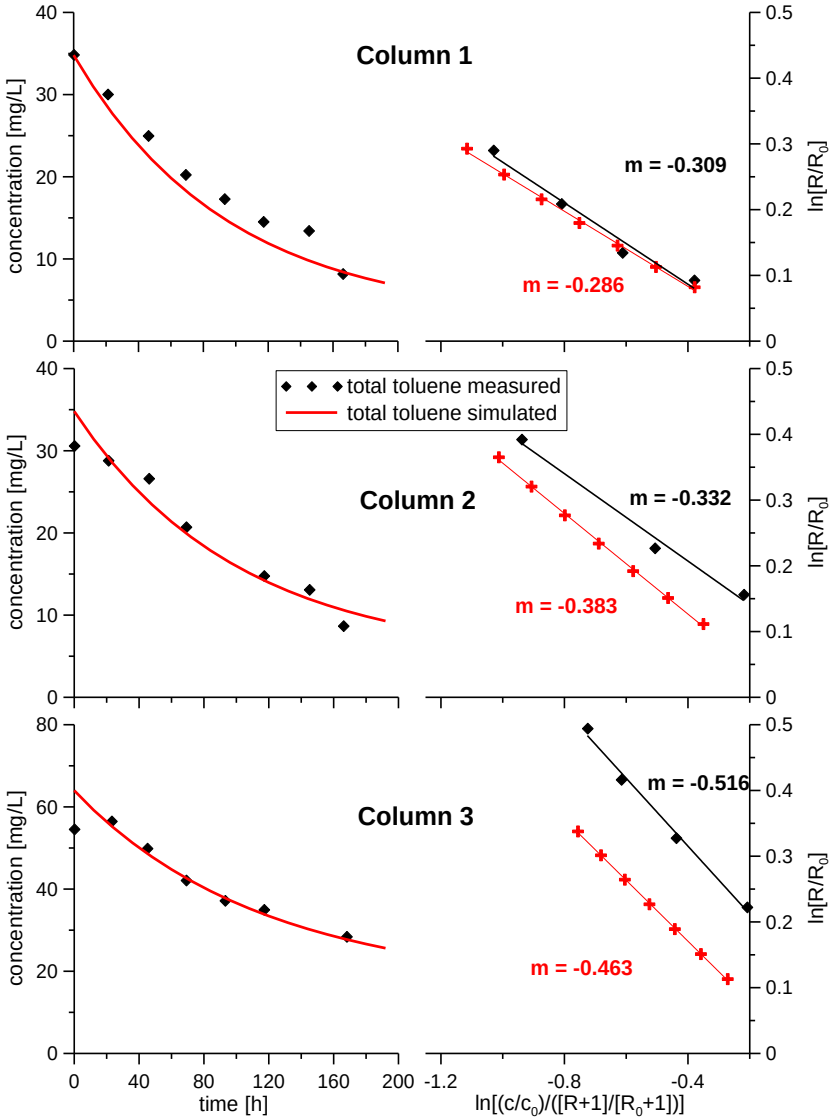

Figure 1: Left: Concentration changes in the liquid reservoirs of the bioreactive column reactors. Symbols mark experimental results, solid lines show simulations results. Right: Rayleigh plots of the column reactors. Black diamonds mark experimental results, red crosses mark simulation results at 12 h intervals. m is the slope of the linear regression fitted to the data.

observed in the batch reactors (see above), only minor fractionation effects (slopes of the Rayleigh plots of -0.010 to -0.006) were observed in the reservoir indicating that fractionation effects caused by the diffusive transport and or the phase exchange between liquid reservoir and head space are relatively small. In the absence of an isotopologue-specific Henry's law constant and any effects (masking of fractionation or causing additional fractionation) due to the mass transfer from liquid to water fractionation in the reservoir should

be given by a_b[29] which is in agreement with the measured data given the rather strong signal to noise ratios. The gas to liquid concentration ratios between liquid reservoir and its headspace were nearly constant during the experiment (**SI, Figure S5**). Along the columns of the control systems, linear concentration profiles were observed indicating quasi-steady state conditions of the diffusive transport (**SI, Figures S6 and S7**). This is in agreement with the approximate relaxation time (time approximately needed to establish steady state conditions) $t_r = L^2/D \approx 8$ h of the diffusion along the column, which is comparably small to the time scale of concentration changes in the reservoir. At steady state, differences in the diffusion coefficients between the two isotopologues would not lead to any fractionation along the column as the steady-state linear concentration profiles are not affected by the values of the diffusion coefficients.[29] This is in agreement with the negligible fractionation effects (trends in the Rayleigh plots rather reflecting the noise level of the measurements) observed along the control reactor columns considered to be at (quasi-)steady state.

The behavior of the control reactors was well captured by the numerical model (**SI, Figures S4-S7**) with the simulated results matching the measured concentrations as well as stable isotope signatures in the reservoir and in the columns. Parameters describing the diffusive transport (**Table 1**) are taken directly from the experimental setup or from the literature, indicating that the model represents a valid conceptualization of the experimental system and that the description of the abiotic processes provides a reliable basis for the simulation of the reactive processes.

Biodegradation experiments – experimental observations: Measured changes in concentrations in the reservoirs of the biodegradation reactors show a decrease in total toluene over an experimental period of seven days (**Figure 1**) which is stronger than observed for the control systems. Column 1 and Column 2 were operated as replicates and exhibit very similar results while Column 3 was operated with a higher initial concentration (approx. *35 mg L^{-1}* vs. *55 mg L^{-1}*) to test the behavior of the setup under different conditions. In contrast to the control systems (**SI, Figure S3**) all bioreactive systems showed pronounced hydrogen stable isotope fractionation with slopes of the Rayleigh plots in the range of -0.3 for Column 1 and Column 2 and -0.5 for Column 3 (**Figure 1**). the course of the experiment (from approx. 0.1 to 0.3; **Figure 2**) indicates a rate limiting effect of the phase exchange from liquid reservoir to its head space for the entire losses of toluene from the system. This is further confirmed by comparing measured slopes of the Rayleigh plots with predictions of the 'source fractionation factor' by Bouchard et al.[44] When neglecting finite-size effects of the column and isotopologue-specific Henry's law constants the source fractionation factor should be equal to $\sqrt{\alpha_b \cdot \alpha_d}$ with α_d derived from **Table 1** and α_b as determined from the batch experiments, the slopes of the Rayleigh plots for the reservoirs should be -0.719 ± 0.088. The observed differences between predicted and measured values indicate a masking of the fractionation in the reservoir due to the rate-limiting phase exchange. Concentration profiles along the columns of the bioreactive systems observed at (quasi-)steady-state conditions at two different observation days clearly deviate from the linear profiles observed for the control systems, which confirms biodegradation to have taken place. This was associated with strong hydrogen stable isotope fractionation along the columns (**Figures 3 and 4**). For Column 1 the slopes of the Rayleigh plots were in the range of -0.55 to -0.6 and for Column 2 and Column 3 slopes were in the range of -0.7 and below. This indicates biodegradation leading to higher losses of toluene to the unsaturated part of the system Khan, et al.[4] and that the fractionation caused by the biodegradation leading to enrichment of the heavy isotopes in the liquid reservoir representing the source zone of the VOC as previously reported by Bouchard, et al.[29] As already discussed in Khan, et al.[4] the increase of the gas to liquid concentration ratios in the reservoir during While these slopes indicated a strong fractionation due to biodegradation, their values

are higher (less negative) than the slopes observed for the batch reactor systems. This is in agreement with the analytical calculations predicting slopes to be controlled by $\sqrt{\alpha_b}$ rather than by α_b as in the batch experiments, see Eq. 5. Using Eq. 5, with α_d again derived from **Table 1** and α_b as determined from the batch experiments, the slopes of the Rayleigh plots for the columns should be -0.716 ± 0.089 which covers the observed values for Column 2 and Column 3. Slopes for Column 1 were slightly below this range which indicates for this system a possible masking of the stable isotope fractionation, e.g. due to mass transfer limitations.[37, 45, 57]

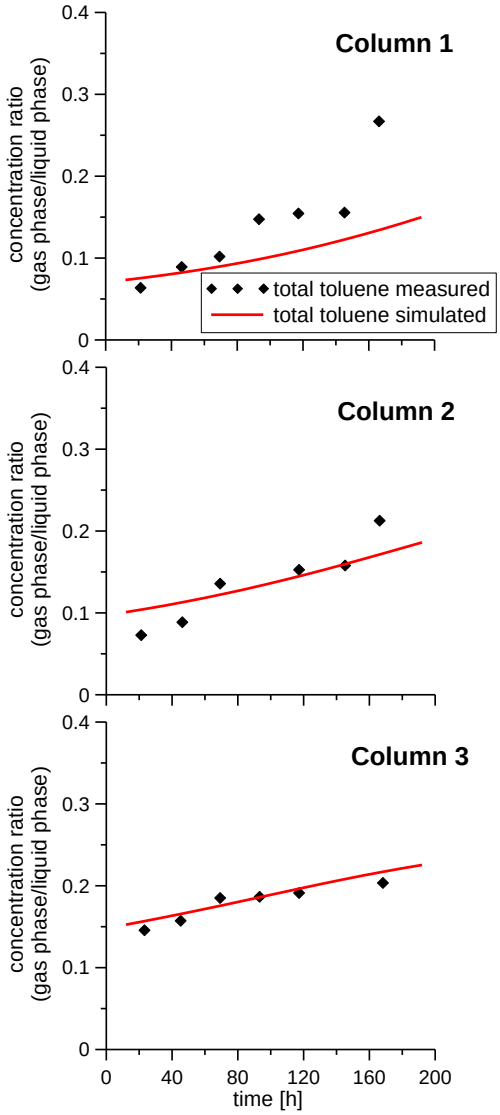

Figure 2: Gas to liquid concentration ratios in the reservoirs of the bioreactive column reactors. Symbols mark experimental results, solid lines show simulation results.

Biodegradation experiments - numerical simulations: Results of the simulations allowed for a good fit between simulated and experimental data (**Figures 1-4**). Both, concentration changes and stable isotope fractionation were well described with the used modeling concept. Values of the fitting parameters (**Table 1**) were adjusted in a non-automated procedure and are in good agreement with literature values (for the Henry volatilities) or predictions from boundary layer theories (time constants for phase exchange). In particular, for the fractionation factor of the biodegradation reaction the value of $\alpha_b = 0.05$ obtained by the model fitting coincided well with the observed range of 0.08 ± 0.05 obtained in the batch experiments.

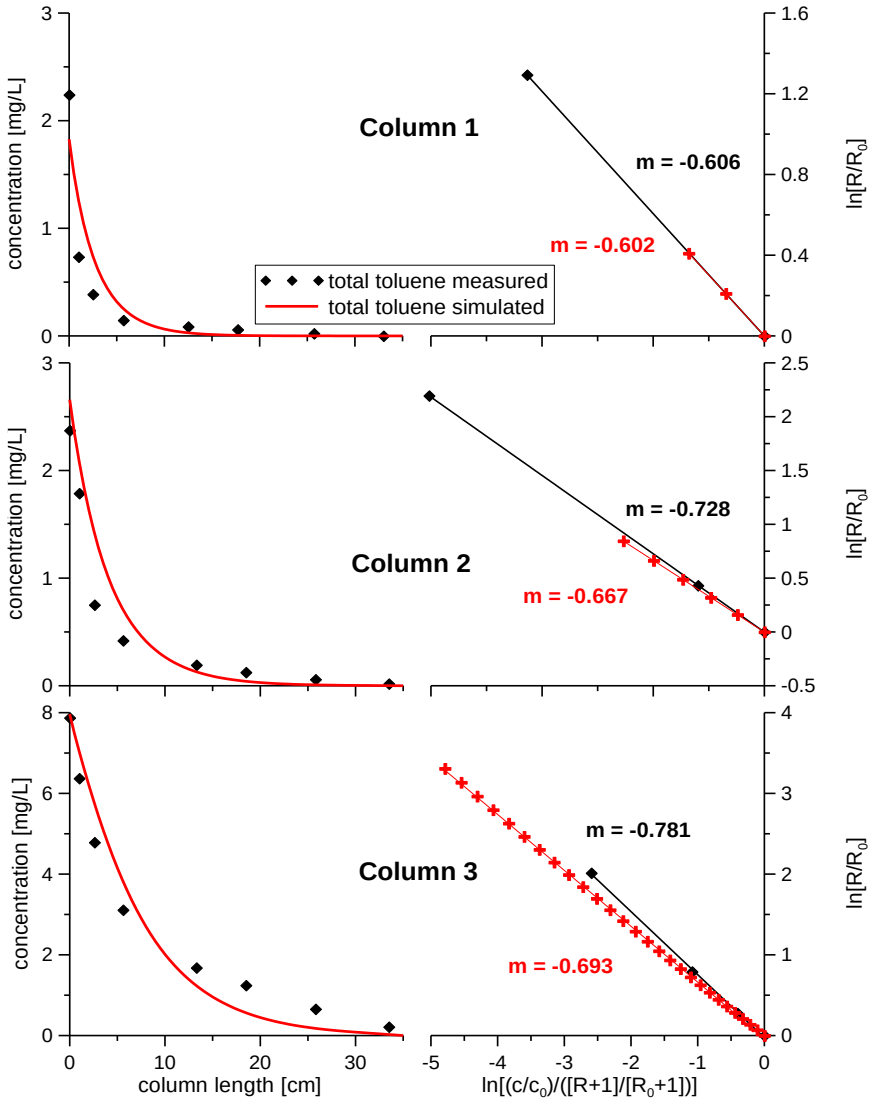

Figure 3: Left: Concentration profiles along the gas phase of the bioreactive columns after 2 days. Symbols mark experimental results, solid lines show simulation results. Right: Rayleigh plots of the bioreactive columns after 2 days. Black diamonds mark experimental results, red crosses mark simulation results. m is the slope of the linear regression fitted to the data.

Furthermore, this suggests that the model was able to provide a valid description of the reactive transformations in the column reactors. The simulation results also showed that the microbial reactivity of the columns decreased over time as is likely to be explained by a gradual exhaustion of nutrients during the course of the experiments.

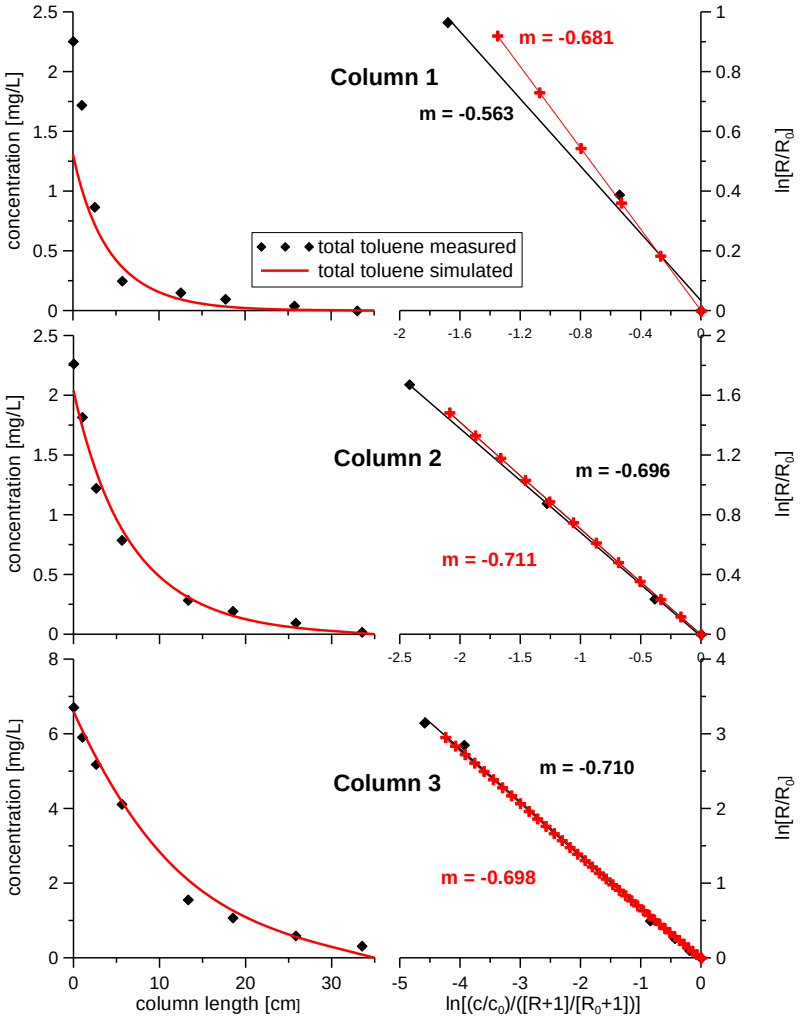

Figure 4: Left: Concentration profiles along the gas phase of the bioreactive columns after 5 days. Symbols mark experimental results, solid lines show simulation results. Right: Rayleigh plots of the bioreactive columns after 5 days. Black diamonds mark experimental results, red crosses mark simulation results. m is the slope of the linear regression fitted to the data.

Simulation results also show that although the three biodegradation columns performed similarly their initial reactivity varied by a factor of up to 4 (**Table 1**). As the columns were all inoculated similarly, these variations might be caused by random/natural variations of microbial abundance and activity in the inoculum. The

simulation results confirm that isotope fractionation in the reservoirs was masked by a rate-limiting phase exchange between the liquid reservoir and its headspace an observation made for several mass-transfer limited systems [14]. The same limitation is also the reason for the disequilibrium of gas to liquid concentration ratios in the reservoirs (**Figure 2**) confirming previous interpretations of the experimental results.

3.4.3 Factors affecting isotope fractionation of vapor-phase toluene during diffusive transport in column experiments

General considerations: The slopes of the Rayleigh plots obtained from the studied columns do not match the fractionation factors of the microbial degradation reaction observed in the batch reactors. This was expected giving the diffusion-dominated transport regime in the column reactors. Both, experimental observation and simulation results also reveal that the slopes show a deviation from the predictions of m = -0.775 provided by Eq. 5 (using the fitted value for a_b) with strongest deviations observed for the reactor Column 1. As will be discussed below, potential reasons for this behavior are two inherent assumptions in Eq. 5 that are not met in the column reactors: the column length was not infinitely long and the degradation was not following first order kinetics. If the columns are not well described by a semi-infitite system (see requirement for Eq. 3) finite size effects can lead to less negative slopes of the Rayleigh plot; especially when analyzing data up to the outlet (i.e., the zero concentration end of the column; **SI, Figure S8**). These effects are observed when reaction is slow compared to diffusive transport (i.e. for small Damköhler numbers; Da < 10^2-10^3) or in practical terms whenever concentrations are not fully depleted well before the zero concentration end. Less negative slopes than predicted by Eq. 5 may also arise if degradation processes follow Michaelis-Menten kinetics instead of first order kinetics (**SI, Figure S9**). Such effects are most pronounced close to the source of the concentration where higher concentrations lead to a stronger deviation from first-order kinetics. Consequently, the higher the source concentration (i.e., the higher the ratio between source concentration and Michelis-Menten constant) the stronger the deviation of the Rayleigh plot slopes from the theoretical prediction. Furthermore, mass transfer limitations inside the column reactor packing may have masked the microbially induced isotope fractionation. Mass transfer related limitations of substrate bioavailability are known to lead to less observable fractionation (i.e. less negative slopes).[14-15, 37, 45, 57] This effect is more pronounced for lower concentrations (i.e. low ratios between concentration and Michaelis-Menten constant) than for higher concentrations.[37] Consequently, each of these effects or any combination of them could be the reason for deviations between observed and predicted slopes of the Rayleigh plots. The dependency of these effects on concentration or distance to the column ends can also lead to changes of the slopes along the diffusive path and thus to a dependency of the obtained slopes on the analyzed data range (**SI, Figures S8 and S9**). Additional transient effects (i.e. deviations from steady state) are not considered due to the short relaxation time of the system compared to the slow gradual changes of reservoir concentrations and microbial reactivity.

Analysis of individual factors – sensitivity analysis: To determine the contribution of each of these processes to the observed fractionation effects and resulting slopes of the Rayleigh plots in the three-bioreactive column reactors a number of additional simulations were made to test the sensitivity of the results to variations of different parameters. Variations include an increase of the column length from 35 cm to 70 cm to test for finite size effects, an increase of the phase exchange time constant between vapor and liquid phase in the columns by different factors to test for bioavailability restrictions and the associated masking of the fractionation, and an increase of the Michaelis-Menten constant and the initial maximum biodegradation rate parameter (both by the same factor) to test for effects from using non-first order kinetics. These

variations also lead to (minor to major) changes of the concentration profiles along the column reactors, which challenges the comparison of slopes from different simulations. For the comparison between experimental and simulated results, model data were analyzed for the same column segments for which isotope ratios were measurable in the experiments (i.e. non-deuterated toluene above detection limit). Using these segments for all sensitivity tests lead to different concentration ranges analyzed each time. Thus simulated slopes were additionally analyzed for a range defined by an arbitrary limit of $\ln(R/R_0) = 7$ covering variation of R by approximately three orders of magnitude. An overview of these results is provided in the Supporting Information (**Table S1**).

The obtained results show that deviations between observed and predicted slopes could mainly be attributed to mass transfer induced limitations of substrate bioavailability. This effect is most pronounced for reactor Column 1 which had the highest reactivity and least negative slopes. In turn, for reactor Column 3 at day 5 which had the lowest reactivity and high reservoir concentration an increased bioavailability had the least effects on the observed fractionation effects. The lower reactivity and higher concentrations of the latter case also explain why only in this case an increase of the column length had a minor effect on the observed fractionation effect as non-negligible concentration values were found in the vicinity of the zero-concentration boundary (for the original column length). For the other two reactors an increase of the column length had no (or negligible) effects on the slopes of the Rayleigh plots. An analysis of the influence of the degradation kinetics on the slopes was not straightforward as these changes had also a major effect on the concentration profiles. Furthermore, according to Thullner, et al.[37] the substrate bioavailability depends on two quantities: the ratio between concentration and Michaelis-Menten constant and the ratio between the specific affinity and the time constant of the phase-exchange in the columns. While the specific affinity (i.e. the ratio between maximum degradation rate parameter and Michaelis-Menten constant) was kept constant, the ratio between concentrations and Michaelis-Menten constant was not and thus a variation of this parameter led to differing trends depending on the relevance of bioavailability restrictions. Using the $\ln(R/R_0) \leq 7$ criterion for comparison showed all in all a rather limited sensitivity of the slopes to the choice of reaction kinetics: Those data sets showing highest influence of bioavailability restrictions (Column 1 and Column 2, day 2) exhibited slightly less negative slopes if the reaction kinetics became closer to first-order kinetics, while the other data set exhibited slightly more negative slopes. The only exception was again found for reaction Column 3 (day 5) where initial concentrations were higher and thus degradation kinetics differing more from first order. To isolate effects from the used reaction kinetics in a better way simulations were also performed combining conditions with no bioavailability restrictions (i.e. high phase-exchange time constant) with an increased value of the Michaelis-Menten constant. High bioavailability and increased column length led to slopes deviating only up to 0.030 (using the $\ln(R/R_0) \leq 7$ criterion for comparison) from the theoretically expected value of -0.775. A shift of the degradation kinetics toward first-order kinetics decreased this deviation to 0.009 or less.

In summary, the performed sensitivity analysis showed that all three tested factors had some influence on the slopes of the Rayleigh plots along the column reactors. The most significant factor was the limitation of bioavailability while the other two factors had only minor to negligible effects on the slopes. All tested factors led to less negative slopes than theoretically predicted, which in turn means that using Eq. 5 for converting an experimentally determined slope of a Rayleigh plot into and apparent stable isotope fractionation factor would lead to an overestimation of the fractionation factor (i.e., estimated values of a_b are closer to 1).

However, estimation errors are in the same range as experimental uncertainties in measuring fractionation factors.

3.5 Implications for other studies

Our findings reflect that compound-specific stable isotope analysis can be a tool for qualitative as well as quantitative estimates of the major subsurface processes in diffusion-dominated systems. This enlarges the range of application of CSIA for the assessment of (contaminant) biodegradation in the subsurface. In spite of the contribution of diffusive mixing and diffusion induced fractionation,[28] our results show that the magnitude of isotope fractionation due to biodegradation can be quantitatively estimated if concentration gradients have approximately achieved a steady-state. The application of the presented concepts is not limited to the high stable isotope fractionation factor associated with the biodegradation but may also be used for conditions encountered in real world systems as neither the basic principles nor the computational procedures depend on the magnitude of the fractionation factors or the relative abundance of the different isotopologues. Biodegradation of VOC in the unsaturated subsurface can mitigate emissions of contaminants to the atmosphere[2-3, 35, 58] or may reduce the chance of vapor-phase intrusion into buildings.[59-61] An assessment of such degradation *in situ* is possible using concentration data[50] yet it is challenging given the problems associated obtaining a sufficient number of *in-situ* samples. The presented concepts allow using CSIA as an additional and highly beneficial source of information for an existing number of samples even if diffusion is the dominant transport process. Furthermore, our results confirm that in cases where the stable isotope fractionation factors of the biodegradation reaction are close to those of diffusion a lack of fractionation along a diffusive flow path (as has been observed for systems with proven biodegradation when approaching steady state[29, 44]) is not necessarily an indication for the absence of biodegradation.

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SUPPORTING INFORMATION

DETAILS OF THE EXPERIMENTAL SETUP

Batch Reactors

Gastight chromoflax glass bottles with total volume of 1150 mL, filled with 50 mL glass beads, coated with minimal media agar and bacteria leaving 1100 mL headspace. That is, equivalent to 300 mg of oxygen sufficient to degrade the spiked total amount of toluene.

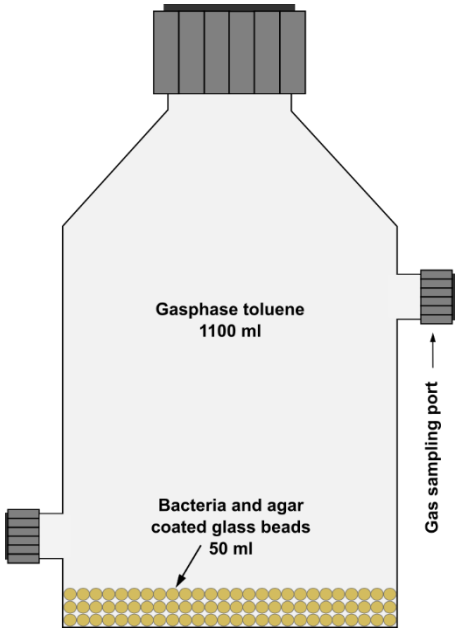

Figure S1: Glass beads batch reactor containing 50 mL of agar + bacteria coated glass beads at the base and 1100 mL of VOC vapors in the headspace. The reactor consists of a three neck bottle as illustrated, with a main opening at the top and sampling ports on the top right and bottom left side.

Column Reactors

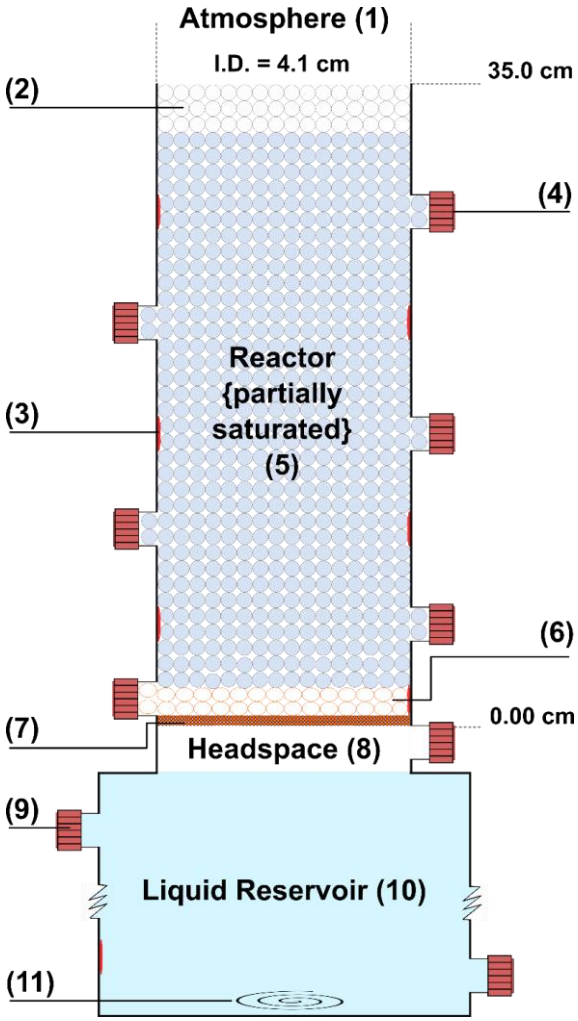

Figure S2: Column reactors consisting of vertical chromoflax glass columns. With a stirred (11) liquid reservoir of 2.375 L volume (10) containing the dissolved VOCs and a vertical reactor column with l = 35 cm, *i.d.* = 4.1 cm (5) filled with partially saturated 700 g glass beads (d = 2.9–3.5 mm). The reactor is filled with dry glass beads (2) at the top, it is open to the atmosphere (1) and separated from the headspace of 45 mL (8) of the liquid reservoir by a steel mesh (7) covered by a thin layer of PTFE beads (6). Dissolved and gas-phase VOCs can be sampled by gas sampling points (4) and liquid sampling points (9), and oxygen by sensing spots (3). The total columns length is 35.00 cm from the steel mesh above the headspace of the reservoir until the top of the column.

DETAILD DESCRIPTION OF THE COLUMN REACTOR EXPERIMENTS AS PROVIDED IN KHAN, ET AL. [1]:

Reactor design and operation

Reactor design

Vertical chromoflax glass columns (l = 35 cm, i.d. = 4.1 cm; cf. Figure 1) packed with agar-covered (approximate agar layer thickness of 60 μm) glass beads (d = 2.9 − 3.5 mm) were used to study the spatiotemporal concentration of the VOC in a water-unsaturated fixed-bed reactor (tortuosity, τ = 0.5, derived from measured drainage curves using standard soil physical procedures; porosity, ϕ = 0.39). Sampling ports allowed the sampling of VOC from the vapor phase and from the liquid reservoir (liquid volume 2.375 L). The reactor was separated from the headspace of the liquid reservoir (initial head space volume approximately 45 mL) by a stainless steel mesh (d = 4.1 cm, pore size = 1 mm) that was covered with a double layer of PTFE beads (n = 66, d = 6 mm) to avoid the formation of a liquid barrier at the base of the reactors. All reactors were open to the atmosphere on their top to allow vertical diffusion of the VOC emanating from the liquid reservoir. The upper 5 cm of the reactors were filled with dry glass beads to prevent microbial contamination from the atmosphere.

Reagents and analytical procedures

Toluene (h-toluene), methyl tertiary butyl ether MTBE (99 %) and HgCl2 (99.5 %) were obtained from Merck KGaA. Per-deuterated toluene (99.6 atom % D, d-toluene) was purchased from Sigma-Aldrich Chemie Germany. Liquid and gasphase VOCs samples were analysed with a Hewlett-Packard Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID). The automated injection using the headspace auto-sampler (Hewlett-Packard 7694) with 1 mL injection volume and oven temperature of 95°C for liquid samples and 70 °C for gas samples. To separate the liquid phase d- and h-toluene, fused silica capillary column (Optima d-3, length 60 m, I.D. 0.32 mm, film thickness 0.35 μm; Macherey-Nagel, Duren Germany) was used. Temperature sequence was as follows; 35°C for 2 min, heated to 100°C at a rate of 6 °C min-1 for liquid samples; and 80 °C at a rate of 9 °C min-1 for gas samples , cooled down to 35°C. The separation of D/H was achieved at 105.7 kPa with a N2 flowrate of 15 mL min-1 and with a split of 5:1. The FID was operated at 280 °C, and N2 was used as carrier gas.

Cultivation of bacteria and preparation of the inocula

Pseudomonas putida KT2442 DsRed pWW0 gfp, a rod-shaped, toluene-degrading bacterium as previously described by Nancharaiah, et al. 2, was pre-cultured in 250 mL gastight bottles up to the exponential phase (≈ 23 h; 30 oC; rotary shaker at 150 rpm) in 50 mL of minimal medium, sodium succinate, supplemented with toluene (50 mgL-1), was then cultivated in 2 L gastight bottles up to the late exponential phase (≈ 40 h; 30 oC; rotary shaker at 125 rpm) in 800 mL of minimal medium supplemented with toluene (200 mgL-1) 3. The cultures were centrifuged at 7000 rpm, at 20 oC for 10 min. The pellet was washed with 100 mM phosphate buffer saline and then re-suspended in PBS at pH = 7. This suspension was then re-suspended in sterile minimal medium to make a total volume of 33 mL containing 0.3 % agar. This bacteria-agar suspension was then mixed with 700 g of glass beads (d =2.9 − 3.5 mm; Th. Geyer GmbH) under gentle shaking resulting

in an approximate loading of 5 x 107 cfu per g of glass beads. Bacteria were quantified in triplicate by colony forming units on LB agar plates.

Reactor loading and running conditions

In all experiments, the agar covered glass beads were carefully transferred to autoclaved reactors and slight underpressure (- 500 mbar) was applied to remove excess water from the system which resulted in a water saturation of approximately 14 % of the pore volume. Abiotic control reactors were identically treated except for the presence of bacteria. In addition, the control reactor was spiked with HgCl2 to exclude any microbial activity during the experiment. The column reactors were finally tightly connected to the reservoirs.

Reactors inoculated with strain KT2442 and their abiotic controls (cf. SI) were operated in duplicate for 7 days at standard pressure (1 atm) and T = 24°C. To provide quasi steady-state conditions, an observation period between day 2 and day 5 was selected for the evaluation of the vapor-phase results. Reactors were sterilized prior to use and operated under sterile conditions to avoid cross contamination. The liquid reservoirs contained 2.375 L of deionised water, a 1:1 mixture of h-toluene and d-toluene as well as MTBE as a non-reactive tracer (at the given experimental conditions) with expected concentrations of 18.5 mg L-1, 18.5 mg L-1 and 20 mg L-1 respectively, HgCl2 (2 μg L-1) was added to avoid biodegradation in the liquid reservoirs. All VOC were spiked for 12 hours prior to the experiments to allow equilibration.

Sampling

500 μL of each, vapor-phase and liquid samples were taken approximately every 24 hours with gas tight syringes (1 mL for gas and 500 μL for liquid sampling; Hamilton, Switzerland). Samples were transferred to 10 mL-gas chromatograph (GC) sterilized vials, closed with Teflon coated crimp caps and analyzed within 24 h. Small gas-phase and liquid sampling volume (i.e., per sampling event 2% of 140 mL of gas phase and 0.5‰ of 2375 mL of liquid) was considered to have limited effect on observed concentrations. The microbial biomass was quantified by sacrificial sampling from an extra reactor at the start and from experimental reactors at the end of the experiments i.e. at the end of the experimental runs sub-samples were taken from three different locations within the reactors and pooled into a single sample.

Oxygen concentrations in the reactors were measured at the same time of gas sampling, by a Fibox 3 optical oxygen meter (PreSens Precision Sensing GmbH, Germany) using fixed sensing spots located at the same distance as sampling ports from the liquid reservoirs.

RESULTS OF THE BATCH EXPERIMENTS

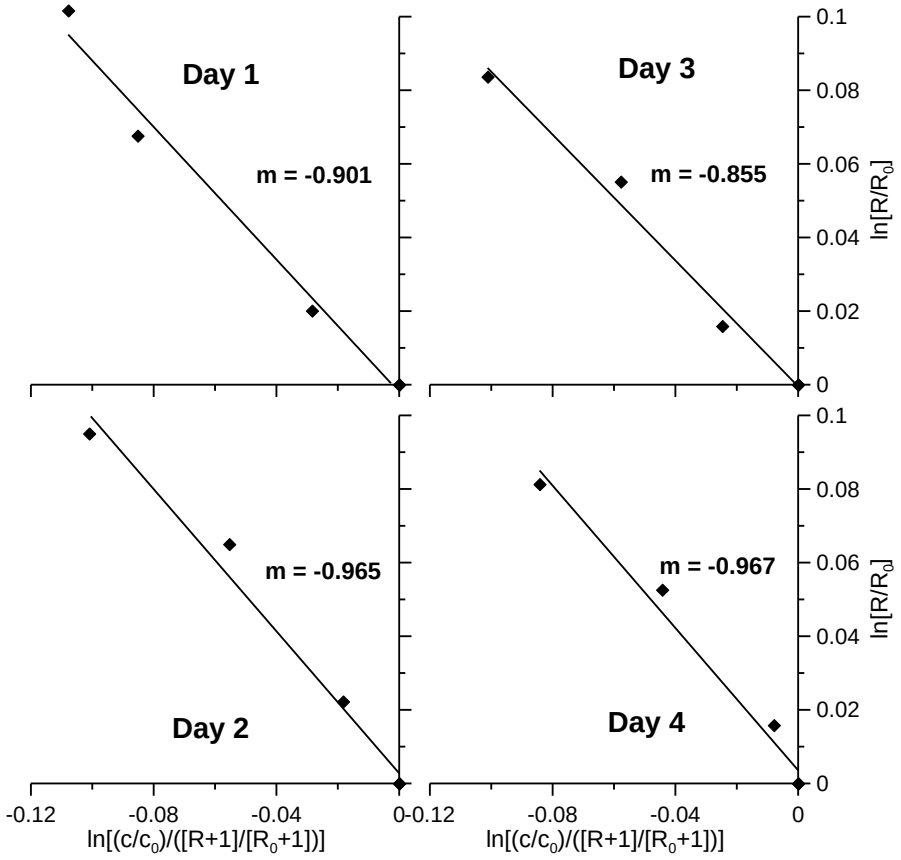

Figure S3: Rayleigh plots for the batch experiments (gas phase data). Black diamonds mark experimental results. m is the slope of the linear regression fitted to the data.

RESULTS OF THE CONTROL COLUMNS

Reservoirs

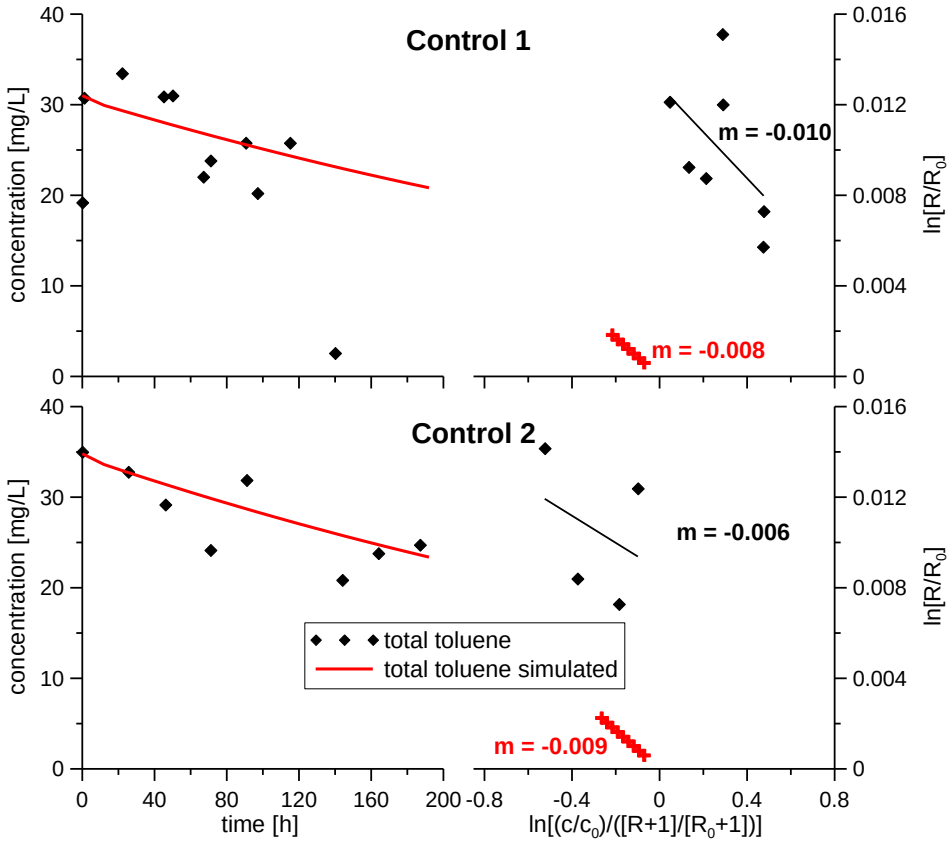

Figure S4: Left: Concentration changes in the reservoirs of the control column reactors. Symbols mark experimental results, solid lines show simulation results. Right: Rayleigh plots of the control column reactors. Black diamonds mark experimental results, red crosses mark simulation results at 12-hour intervals. m is the slope of the linear regression fitted to the data. For the Control 1 experiment, the low concentration value at 144 h was considered an artefact and excluded from the Rayleigh plot.

Phase exchange ratios

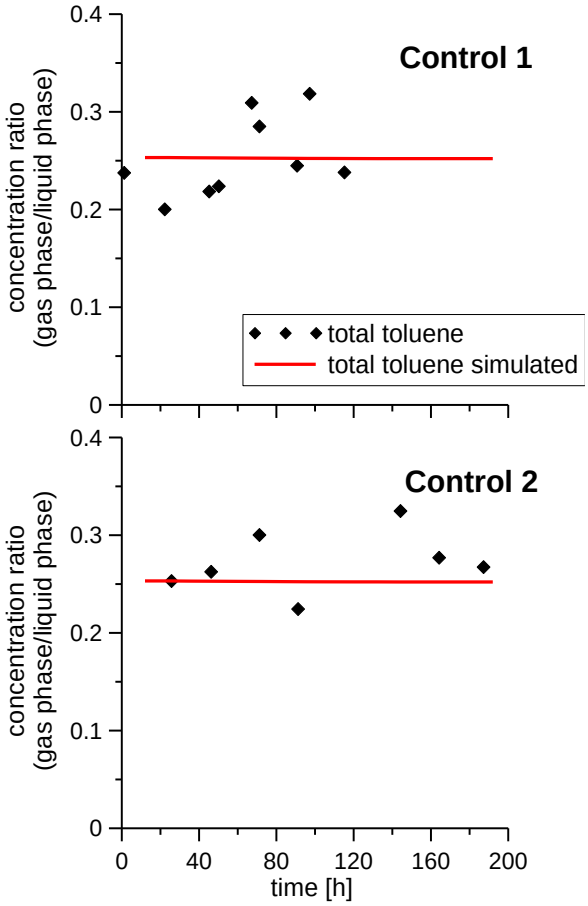

Figure S5: Gas to liquid concentration ratios in the in the reservoirs of the control column reactors. Symbols mark experimental results, solid lines simulation results.

Vapor-phase day 2

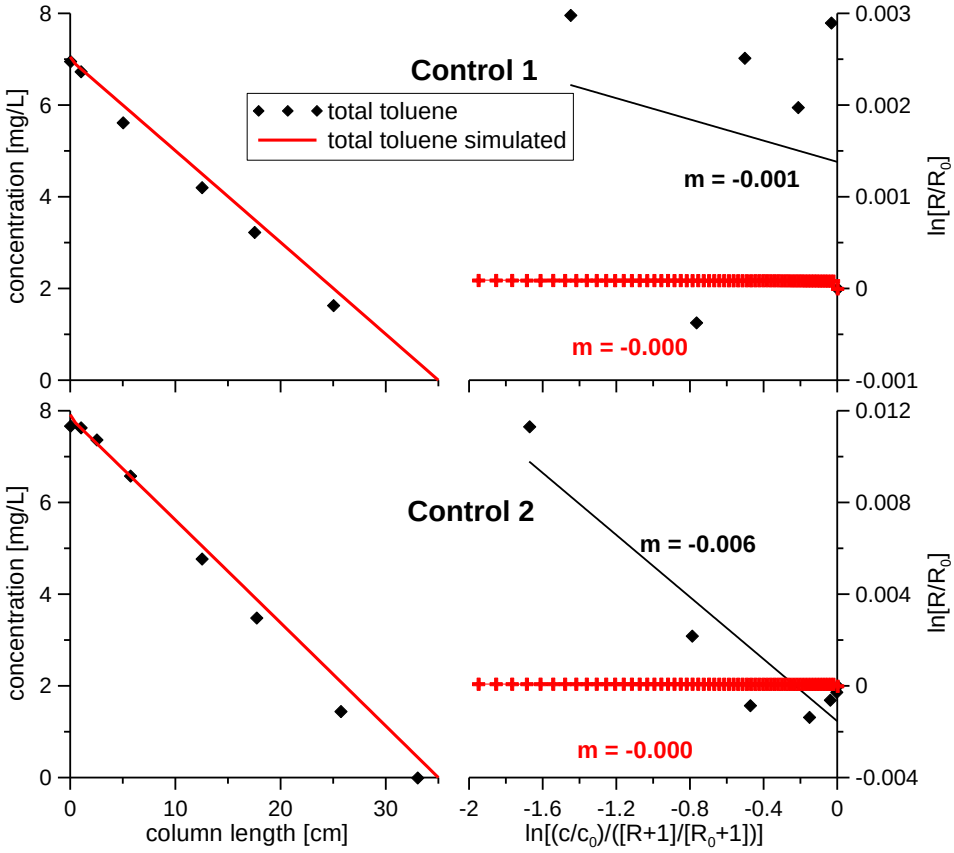

Figure S6: Left: Concentration profiles along the gas phase of the control columns after 2 days. Symbols mark experimental results, solid lines show simulation results. Right: Rayleigh plots of the control columns after 2 days. Black diamonds mark experimental results, red crosses mark simulation results. m is the slopes of the linear regression fitted to the data.

Vapor-phase day 5

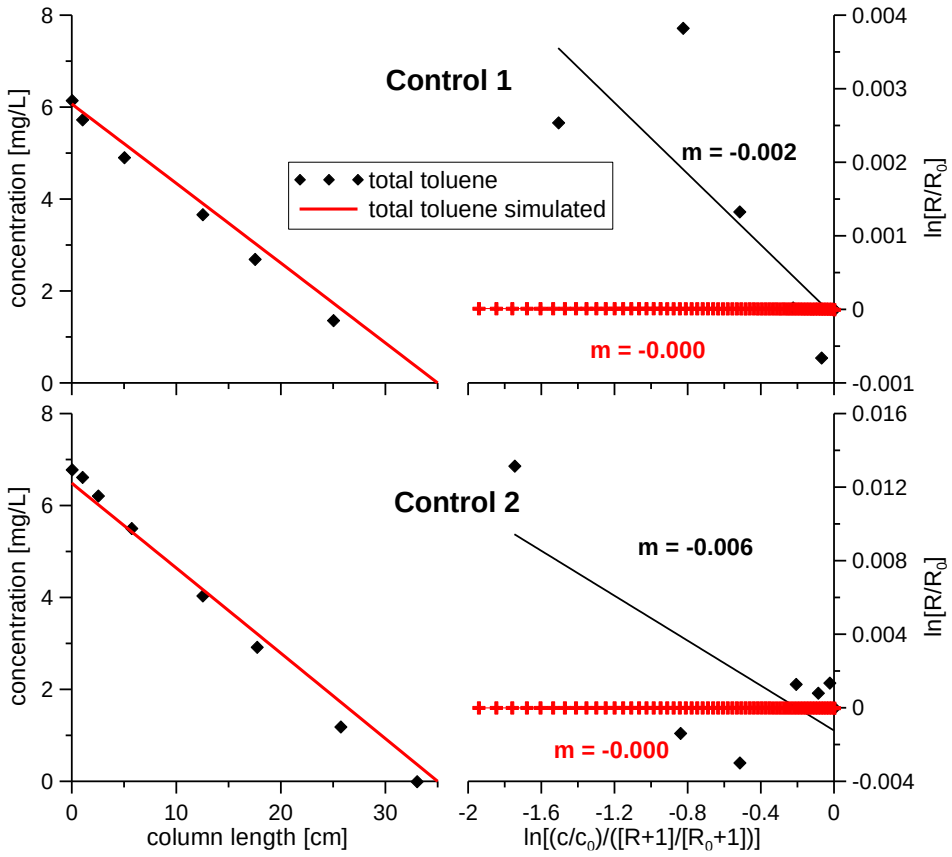

Figure S7: Left: Concentration profiles along the gas phase of the control columns after 5 days. Symbols mark experimental results, solid lines show simulation results. Right: Rayleigh plots of the control columns after 5 days. Black diamonds mark experimental results, red crosses mark simulation results. m is the slope of the linear regression fitted to the data.

DEPENDENCY OF RAYLEIGH-PLOT SLOPES ON DIFFERENT FACTORS

Finite column length

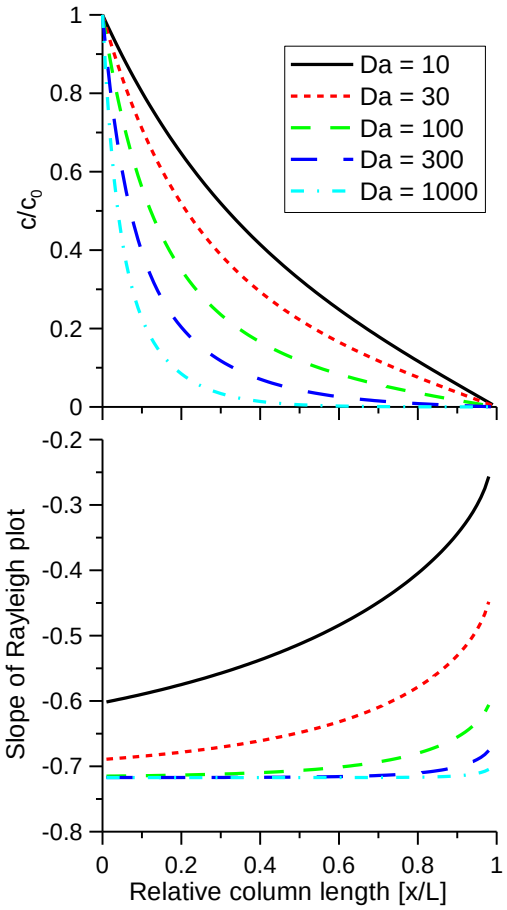

Figure S8: Concentration changes and changes of the slope of the Rayleigh plot along a finite column of length L for different Damköhler numbers. Shown results a derived from concentrations calculated using Eq. 2 and calculating the slope for data points up the respective relative column length. Degradation follows 1st order kinetics with $\alpha_b = 0.08$. α_d is set to 0.99 resulting in $\sqrt{\alpha_b/\alpha_d} - 1 = -0.716$.

Michaelis-Menten kinetics

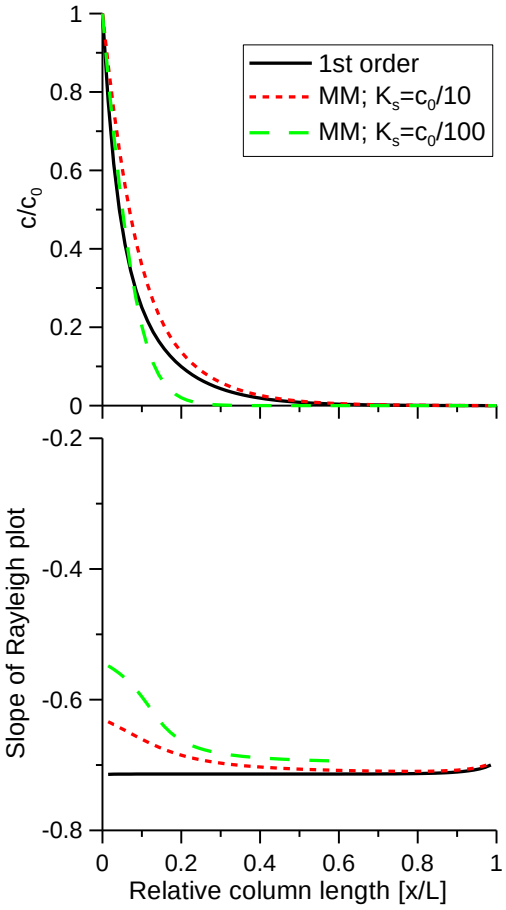

Figure S9: Concentration changes and changes of the slope of the Rayleigh plot along a finite column of length L=35 for different degradation kinetics. Results are obtained by numerical simulations using a simplified version (only gas-phase diffusion and degradation reaction; fixed concentration boundary at x=0) of the set-up described in the manuscript. Results show comparison between first-order kinetics (^1k = 100 h^{-1}, i.e. Da ≈ 800) and Michaelis-Menten kinetics with $K_s = c_0/10$ and $^1k_{max}/K_s$ = 100 and with $K_s = c_0/100$ and $^1k_{max}/K_s$ = 1000. In all cases fractionation factors were set to α_b = 0.08 and α_d = 0.99 resulting in $\sqrt{\alpha_b/\alpha_d} - 1 = -0.716$.

Sensitivity analysis

Reactor		Analyzed data range	L = 35 cm							L = 70 cm		
			Table 1	$k_{exch}=10\cdot k_{fit}$	$k_{exch}=100\cdot k_{fit}$	$k_{exch}=1000\cdot k_{fit}$	K_s, $k_{max}=3\cdot...fit$	K_s, $k_{max}=10\cdot..fit$	k_{exch} = 1000·k_{fit}; K_s, $k_{max}=10\cdot...fit$	Table 1	$k_{exch}=1000\cdot k_{fit}$	k_{exch} = 1000·k_{fit}; K_s, $k_{max}=10\cdot...fit$
Column 1	Day 2	Exp. data range	-602	-717	-731	-733	-575	-566	-767	-602	-733	-767
		ln(R/R₀) ≤ 7	**-563**	**-733**	**-758**	**-760**	**-559**	**-558**	**-766**	**-563**	**-760**	**-766**
	Day 5	Exp. data range	-681	-740	-747	-748	-676	-674	-771	-681	-748	-771
		ln(R/R₀) ≤ 7	**-675**	**-758**	**-765**	**-766**	**-673**	**-672**	**-771**	**-675**	**-766**	**-771**
Column 2	Day 2	Exp. data range	-667	-716	-721	-722	-661	-655	-768	-667	-722	-768
		ln(R/R₀) ≤ 7	**-658**	**-748**	**-759**	**-760**	**-653**	**-652**	**-770**	**-658**	**-760**	**-770**
	Day 5	Exp. data range	-711	-737	-740	-740	-721	-723	-771	-712	-740	-771
		ln(R/R₀) ≤ 7	**-720**	**-759**	**-763**	**-763**	**-722**	**-722**	**-772**	**-722**	**-764**	**-773**
Column 3	Day 2	Exp. data range	-693	-721	-724	-724	-705	-705	-770	-693	-724	-770
		ln(R/R₀) ≤ 7	**-702**	**-740**	**-744**	**-744**	**-705**	**-705**	**-770**	**-703**	**-745**	**-770**
	Day 5	Exp. data range	-698	-709	-710	-710	-735	-741	-767	-710	-722	-771
		ln(R/R₀) ≤ 7	**-**	**-**	**-**	**-**	**-**	**-733**	**-765**	**-**	**-**	**-772**
		All data till 30 cm	-687	-705	-707	-708	-714	-719	-748	-729	-746	-772

Table S1: Slopes of Rayleigh plots for different parameter variations. 'Table 1' refers to the best fit parameter set presented in Table 1. Other parameters than those explicitly mentioned are have not been changed. k_{exch} refers to the time constant for phase exchange in the column, K_s to the Michaelis-Menten constant and k_{max} to the initial maximum rate parameter of biodegradation reaction. k_{fit} denotes the respective best fit value. '-' denotes concentration profiles not reaching the selected criterion until 30 cm. In that case, the data range till 30 cm was used for analysis.

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Chapter 4

"It is the mark of an educated mind to be able to entertain a thought without accepting it."

Aristotle (384 BC – 322 BC)

Combined effects of phase exchange and biodegradation on stable isotope fractionation observed in the gas and the liquid phase

Abstract

Stable isotope fractionation of toluene under dynamic phase exchange was studied aimed at ascertaining the effects of gas-liquid partitioning and biodegradation on toluene stable isotope composition in liquid-air phase exchange reactors (LAPER). The liquid phase consisted of a mixture of water including minimal media and known amount of VOC and was inoculated with a toluene degrading bacterial strain. A mixture of deuterated and non-deuterated toluene was introduced into the system. During biodegradation experiments, the liquid and air-phase concentrations of both toluene isotopologues were monitored to determine the stable isotope fractionation observable in each phase. The results show a strong fractionation in both phases with apparent enrichment factors beyond -800‰. An offset was observed between enrichment factors in the liquid and the gas phase with gasphase values showing a strong fractionation in the gas phase than in the liquid phase. As biodegradation takes place in the liquid phase, only these results are not caused by any masking effects due to a rate limiting phase transition from gas to liquid phase and point towards further fractionation effects associated with the phase transition.

Keywords: VOC, dynamic phase exchange, stable isotope fractionation, bioavailability, biodegradation.

This chapter is in preparation as: *Khan, A. M., Wick, L. Y. & Thullner, M., (2018). Combined effects of phase exchange and biodegradation on stable isotope fractionation observed in the gas and the liquid phase*

4.1 Introduction

BTEX (i.e., benzene, toluene, ethylbenzene, and xylene) are frequently found contaminants in soils and aquatic environments and continue to be the major source of direct and indirect air pollution. These aromatic hydrocarbon pollutants are of particular concern due to their relatively high solubility, mobility, and toxicity (Rolle & Jin, 2017; Wiedemeier et al., 1999). Compound specific isotope analysis (CSIA) is a powerful tool to trace the fate of organic contaminants in the environment based on the shift in isotope composition of the target compounds due to chemical reactions or biodegradation (Elsner, 2010; Elsner et al., 2012; Hunkeler et al., 2009; Kopinke et al., 2017; Thullner et al., 2012). To understand and quantify contaminant transport and transformation mechanisms (bioremediation and natural attenuation), labelled and non-labelled organic compound mixtures have been often applied as a diagnostic tool (Horst et al., 2016). In two-phase (air-water) systems, many volatile organic compounds (VOC) show inverse isotope effects for elements such as hydrogen and carbon, which means that the liquid phase becomes more depleted in the heavier isotopes during volatilization, and approaches to explain these inverse effects were presented by (Baertschi & Kuhn, 1957; Bigeleisen, 1961; Wolfsberg, 1963) and (Horst et al., 2016). Usually, the obtained fractionation data are explained and quantitatively interpreted in terms of the established two-film theory (Schwarzenbach et al., 2003) resulting in diffusion-controlled and equilibrium-controlled fractionation coefficients (Kopinke et al., 2017).

Despite the large amount of literature, the vast majority of studies have focused only on vapor pressure isotope effects of pure organic compounds (Horst et al., 2016), and only some studies reported isotope effects for organics dissolved in water and under equilibrium conditions (Horst et al., 2016; Daniel Hunkeler & Aravena, 2000; Slater et al., 1999). However, in the environment, organic compounds are often dissolved in water and phase transfer may occur under non-equilibrium or kinetic conditions (Horst et al., 2016). The observed fractionation effects are quite different for the 'forward' and the 'reverse' partitioning, i.e. from the aqueous into an organic solvent phase and vice versa (Kopinke et al., 2017).

Motivated by this lack in database and basic understanding, we measured relative phase exchange between gas phase and aqueous phase of isotopologues (non-labelled and deuterated) of toluene by means of a two-phase partitioning approach under dynamic conditions driven by the biodegradation of toluene in the aqueous phase. To the best of our knowledge, relevant to contaminant science and field studies under natural conditions, no study has reported a comparison of stable isotope fractionation in the aqueous phase and the gas phase simultaneously.

The purpose of this short communication is to report the unexpected fractionation behavior of toluene in gas phase and liquid phase. We present isotope effects associated with non-equilibrium volatilization of toluene dissolved in water that we have observed in a series of experiments performed in liquid-air phase exchange reactors (LAPER).

4.2 Materials & Methods

4.2.1 Liquid-Air Phase Exchange Reactors (LAPER)

Gastight chromoflax glass bottles with a total volume of 1150 mL (series A) and 2375 mL (series B) were used as batch system reactors (SI: Figure S1). The reactors were filled with liquid minimal media, 200 mL in

series-A and 400 mL in series-B, one abiotic "control" and duplicate bioreactive LAPER in each series, and were run in parallel at 22-23 °C. The remaining volume 950 mL and 1975mL in series-A and series-B were categorized as headspace respectively. Series-A reactors have sufficient amount of oxygen for complete biodegradation of the known amounts of VOCs toluene-h (20 µL pure phase), toluene-d (20 µL pure phase). Additionally methyl tert-butyl ether (MTBE) (20 µL pure phase) was added as tracer considered to be non-reactive at the given conditions (no MTBE degrading strain present). The reactors were spiked to the liquid media with magnetic stirrer bars and were kept on magnetic shakers for 12 hours to equilibrate prior to the start of the sampling. Calculated equilibrium concentrations of total toluene were 12 mg/L non-deuterated and deuterated toluene (1:1) in the gas phase and 37.21 mg/L in the liquid phase; and the MTBE gas phase concentrations were 2.1 mg/L (series-A) and 1 mg/L (series-B) and 33.34 mg/L in the liquid phase.

The bacterial strain Pseudomonas putida KT2442 DsRed pWW0 gfp (OD578nm = 0.1 equivalent to 2 × 107 cfu/ml at start) was cultured following the protocol mentioned in (Kampara et al., 2008; Khan et al., 2016). The cells were added in the LAPER just before the start of the experiments. LAPER batches allowed gastight sampling of vapor-phase VOCs closer to the main opening on top of the reactor and the liquid phase towards the bottom of the reactor. First samples were taken just before and immediately after the addition of bacteria and this was marked as t0 (0 hours), subsequent samples were taken every hour until t8 (8 hours). Vapor-phase and liquid samples (500 µL) were taken, handled and analysed as mentioned previously in Khan et al. (2016). An observation period between t0 and t8 was selected for the isotope analysis. The measured data were then analyzed by plotting the logarithmic form of the Rayleigh equation (Mariotti et al., 1981), to determine stable isotope enrichment factors.

4.3 Results and Discussion

Series-A 200 mL LAPER - The results of the 200mL LAPER are presented in **Figure 1**. These closed systems are suitable to be analyzed by the Rayleigh model approach. Liquid phase concentrations of total toluene and MTBE are shown in panels A, D, G and J, which are in agreement (within an error margin) with the calculated concentration of 37 mg/L (total toluene) and 33 mg/L (MTBE). Similarly, the corresponding gasphase concentrations of the same VOCs (panels B, E, H and K) match the calculated initial values. While there are slight experimental variations in the liquid concentrations, the gasphase concentrations are much more precise and have negligible experimental error. Column in the right (panels C, F, I and L) shows the plotted isotope data using Rayleigh analysis.

Toluene concentrations from the abiotic control experiment and MTBE-tracer concentrations show no major changes during the experiment and indicate that – besides biodegradation – there are no underlying processes leading to a removal of the VOCs from these gastight systems. The isotope data of the control experiment (**Figure 1C**) confirms that there is no microbial activity as the values of the stable isotope enrichment factors of ε = -4 to -5‰ in the gas phase and ε = -1 to -2‰ in the liquid phase are too negligible compared to enrichment factors of up to -950‰ reported for the used bacterial strain (Khan et al., 2018). These negligible values can be attributed to the combined physical processes such as sorption, isotopic mass differences, but might also reflect the noise level of the measurements.

In contrast to the control experiments, the concentration trends LAPER-1, LAPER-2 and LAPER-3 were different, where the toluene concentrations were fast depleted over time due to the presence of bacteria,

within 8 hours 50% of toluene was biodegraded. The observed average hydrogen isotope enrichment factors were (ε = -888‰, Stdev = 19‰) for the gas phase and (ε = -797‰, Stdev = 52‰) for the liquid phase. The standard deviation values were significantly reduced when we analysed the data without outliers (i.e. ε = -879‰, Stdev = 6‰ for the gas phase and ε = -814‰, Stdev = 4‰ for the liquid phase). This confirms that the predominant removal process is biodegradation due to the presence of microbes, which prefer normal toluene over the per-deuterated counterpart (**Figure-2**).

Series-B 400 mL LAPER - A very similar trend was observed in the parallel experiments of 400 mL LAPER. Although there was large surface area in this series, the liquid-air ratio was the same as in the 200 mL series. Concentrations of both species of toluene and MTBE-tracer were observed very close to the calculated values. Similarly, the liquid concentrations showed bigger variations than the gasphase concentrations mainly due to the experimental error. The obtained hydrogen isotope enrichment factors were considered in the average values presented above.

Series-B 400 mL LAPER - A very similar trend was observed in the parallel experiments of 400 mL LAPER. Although there was large surface area in this series, the liquid-air ratio was the same as in the 200 mL series. Concentrations of both species of toluene and MTBE-tracer were observed very close to the calculated values. Similarly, the liquid concentrations showed bigger variations than the gasphase concentrations mainly due to the experimental error. The obtained hydrogen isotope enrichment factors were considered in the average values presented above.

4.3.1 Dynamic phase exchange implications

Our reactor setups showed that the phase exchange between liquid phase – gas phase has an influence on the observed stable isotope fractionation. Stronger fractionation (i.e. less negative hydrogen isotope enrichment factors) was observed in the gas phase with difference in the order of 50-100‰ not only for the average values but also observed for each single experiment. This trend is opposite to what would have been expected when the phase-exchange between gas phase and liquid phase would have been rate limiting for the biodegradation taking place in the liquid phase only, which would have caused as masking of the fractionation observed in the gas phase. Equilibrium fractionation effects can also be excluded as the control experiment did not show any indications for this. The observed effects could be explained by the phase transition itself causing a fractionation. While such effects have been observed before (Jeannottat & Hunkeler, 2012, 2013), they would have to be extremely strong given that fast (i.e. not strongly rate limiting) phase exchange and the observed differences between the enrichment factors observed for the two phases. Further research is thus needed to identify the processes responsible for the effects reported in this study.

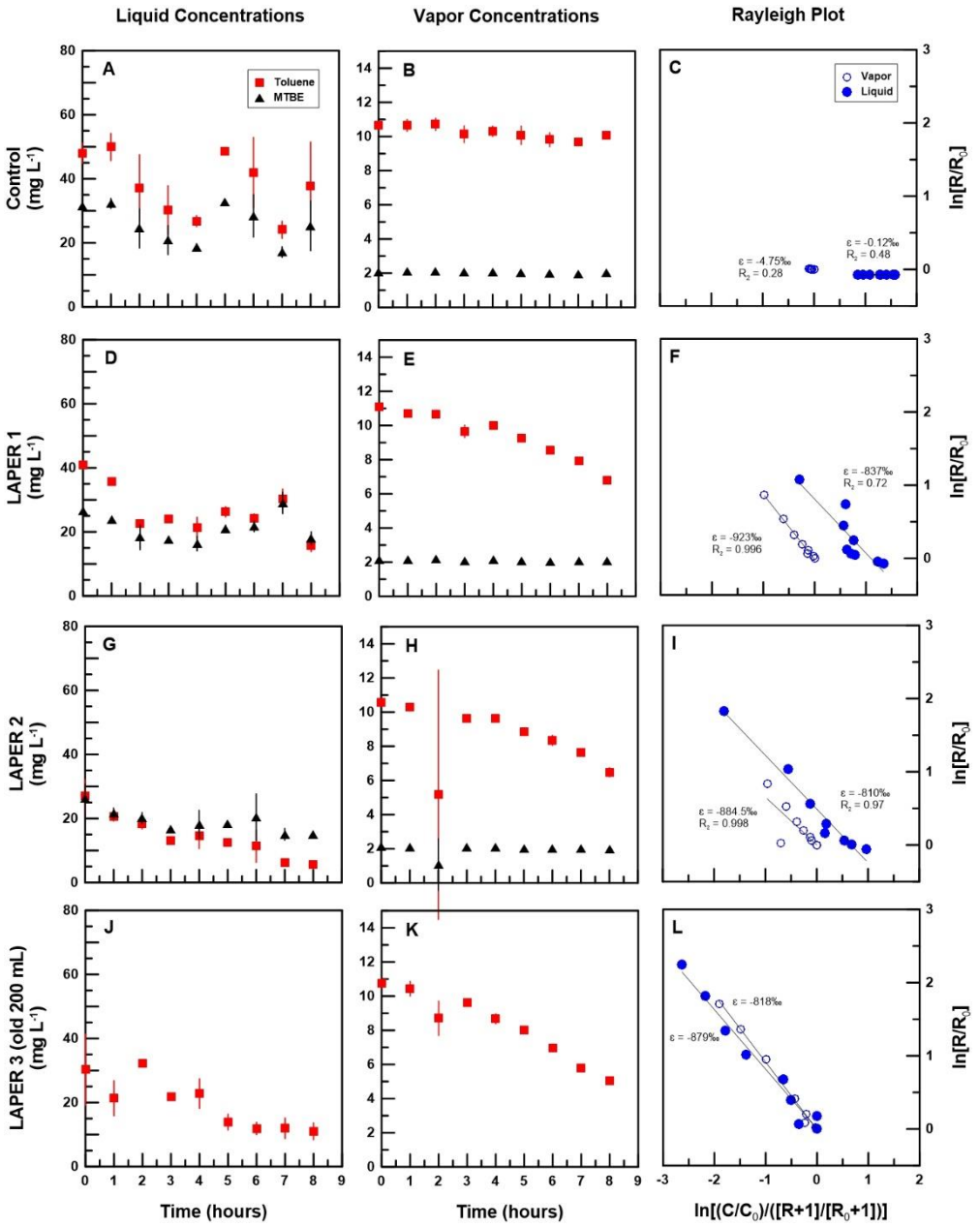

Figure 1: LAPER-200 mL. Left column (panels; A, D, G & J) show the liquid phase concentrations of toluene and MTBE. The middle column (panels; B, E, H & K) illustrate the corresponding gasphase concentrations of the same VOCs. The right column (panels; C, F, I & L) show the Rayleigh plots for the gas and the liquid phase data. The first row of the plots represents abiotic control, 2nd shows LAPER-1 and 3rd LAPER-2, the last row shows LAPER-3, which had no MTBE.

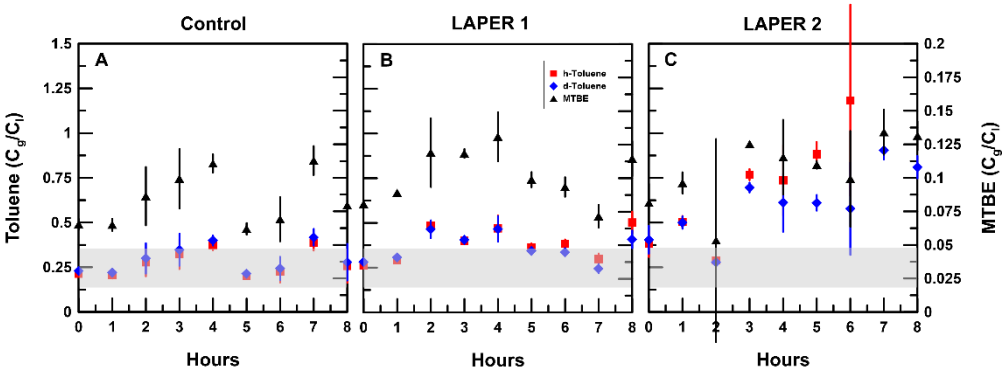

Figure-2: LAPER-200 mL. Development of concentrations of h-toluene, d-toluene and non-reactive MTBE in the gas to liquid phase ratios from the control reactor (panel A) as wells as LAPER-1 (panel B) and LAPER-2 (panel C). Standard deviations relate to the analytical error. Shaded horizontal zones represent ranges of literature values of Henry volatilities for toluene (taken from Sander (2015) ignoring 2-3 highest and lowest values reported there).

4.4 Acknowledgments

This research was supported by the funding from Helmholtz Centre for Environmental Research – UFZ in the scope of the SAFIRA II Research Programme: Revitalization of Contaminated Land and Groundwater at Megasites, project Compartment Transfer II, and via the integrated project Controlling Chemicals Fate (CCF) of the research topic Chemicals in the Environment (CITE) within the research programme Terrestrial Environment. The authors thank colleagues from UFZ Leipzig for their technical support in the laboratory and to Asif Ali, Dr. Mohsin Kamal, and Dr. Sajid Ali for their critical comments and moral support during the course of this study.

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Chapter 5

"If you can't explain it simply, you don't understand it well enough."
Albert Einstein (1879 - 1955)

Mass-transfer & toxicity limitations of isotope fractionation in diffusion driven gasphase biodegradation of VOCs

Abstract

Fate of biodegradable compounds in the subsurface is governed by several abiotic factors. This includes the mass transfer of chemicals to the microbial cells, which is limiting the biochemical activity of the microbes. Furthermore, the presence of chemicals may have toxic effect on the microorganisms and also limit their degradation activity. This study addresses the qualitative and quantitative effects variable concentrations have on the biodegradation and stable isotope fractionation of substrate serving as sole carbon and energy source of the degrading microbial strain. We investigate both, bioavailability and toxicity, by exposing a toluene degrading bacterial strain to two VOCs, toluene and benzene. Our results show that biodegradation of vapor-phase toluene was limited by the toluene mass-transfer at low concentration and by toxicity at the high concentrations. Gas-phase toluene was more toxic to the cells in higher concentrations than benzene. This finding was verified the observed variations of the biodegradation induced stable isotope fraction of toluene.

Keywords: Volatile organic compounds (VOC), intrinsic cellular activity, compound-specific stable isotope analysis (CSIA), biodegradation, toxicity, biochemical activity, mass transfer.

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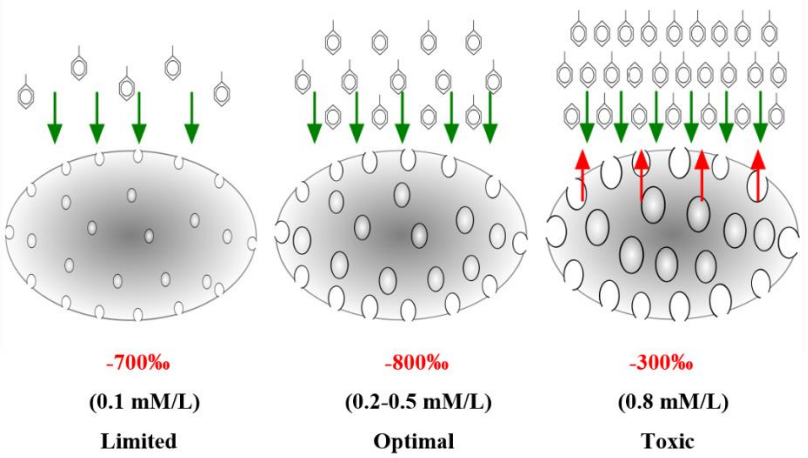
Graphical Abstract Art



5.1 Introduction

Toluene toxicity to the microbial cells have been previously described (Alagappan & Cowan, 2003). However, the concentration at which toluene exhibit toxic effects on the intrinsic biochemical activity of the microbes is larger than its concentration found in most of the contaminated sites (Bosma et al., 1997). Several petroleum hydrocarbons coexist in the contaminated sites and this may affect their physical properties and their interaction to the microbial community in these sites (Bosma et al., 1997). Combined effects of volatile organic compound (VOCs) such as benzene and toluene on the microbial activity have not been extensively studied. Toluene a potential respiratory disease toxin and benzene being carcinogen are of great attention. Biodegradation of these compounds is governed by (1) the rate of uptake by the microbial cells and (2) intrinsic biochemical transformation (Bosma et al., 1997). The microbes followed a pattern of degradation of first order (Michaelis-Menten) kinetics for toluene (Khan et al., 2016) and for benzene (no degradation was observed with current bacterial strain (Pseudomonas putida KT2442 DsRed pWW0 gfp). However, inclusion of benzene in the substrate showed unexpected effects on the bioactivity of the cells; apparent in the sudden change and stress/reaction to benzene. Toluene alone proved to be more lethal in high concentration to the cells than the combined mixture of benzene and toluene. The changes were obvious in the CSIA signatures; and have not been documented in relevant studies as far as we are aware. Gasphase concentrations of these chemicals are toxic to the cells (Hanzel et al., 2012; Khan et al., 2016) however, microbes also showed a great potential to degrade up to a certain concentration of these vapor phase VOCs (Khan et al., 2016).

This lead us to investigate the optimal concentration at which microbes don't respond to any environmental stress/toxicity and whether there is any evidence of effects of other combination VOCs in the vicinity; i.e.

usually the case of contaminated sites with petroleum hydrocarbons and will extend the knowledge on in-situ bioremediation of the contaminated sites.

5.2 Materials & Methods

5.2.1 Gasphase Toxicity Reactors (GTR)

Following the protocol of (Khan et al., 2016), gastight chromoflax glass bottles with total volume of 1150 mL were used as batch reactors. A brief description of the reactors is mentioned here: The reactors have with three openings, one main opening at the top and two small openings located on shoulder top and bottom of the reactors. The main opening remained closed while the small openings were used for gasphase sampling.

Warm liquid agar (45 mL) was poured at the base of the reactors and was cooled down for 10 minutes before adding in (5 mL) swimming agar at 20°C containing (OD578nm = 0.1 equivalent to 1 x 10^9 total cfu) of Pseudomonas putida KT2442 DsRed pWW0 gfp. Reactors were then kept overnight in sterile environment, as settling time and to starve the microbes before the experiments, similarly as described in (Khan et al., 2016). Reactors were closed gastight prior to the start of the experiments. A mixture of 20 µL toluene (1:1) deuterated and non-deuterated isotopologues was spiked close to the internal wall of the neck of the reactors to get a total concentration of 18 mg/l. The same procedure was followed for methyl tertiary butyl ether – MTBE, that was added as non-reactive tracer and the end concentration was 5 mg/l. Similarly, benzene was spiked as toxin in varying concentrations, between 0 mg/l and 250 mg/l. There was 1100 mL of headspace, stoichiometrically equivalent to 330 mg oxygen which was enough to biodegrade total amount of added toluene.

All VOCs were gasified within 20 minutes after spiking, which was verified by the calculated concentrations. This stage was marked as t0 (0 hours), subsequent samples were taken every hour until t12 (12 hours). Vapor-phase and liquid samples (500 µL) were taken, handled and analysed as mentioned previously in (Khan et al., 2016). To provide quasi steady-state conditions, an observation period was selected for the isotope analysis using Rayleigh plots (Mariotti et al., 1981), to determine hydrogen stable isotope enrichment factors.

5.3 Results and Discussion

Hydrogen stable isotope enrichment factors obtained from the batch-type systems (**Figure 1**) show that that under normal conditions (without external stresses), there was an optimal concentration range of 0.3-0.4 mmol/L for which stable isotope fractionation was strongest (ε = -880‰). At lower concentration less fractionation was observed, which indicate bioavailability limitation.

After this optimal range, any higher concentration of toluene seems to act as a toxin and the microbes react to stress by reducing the permeability of the membrane (Griepentrog, 2009) which also lead to less exhibited fractionation effects (**Figure 2**).

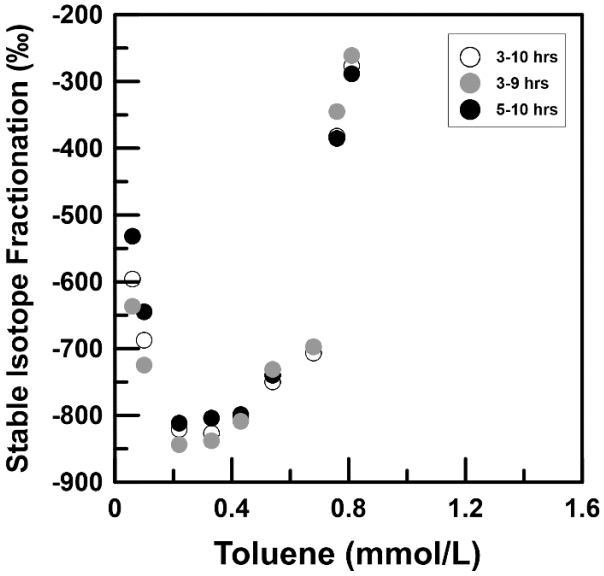


Figure 1: Illustrates the stable isotope fractionation corresponding to variable concentrations of toluene; within (5-10 hours) of the start of the experiments. The legend shows the range of different time windows of the data analysis.

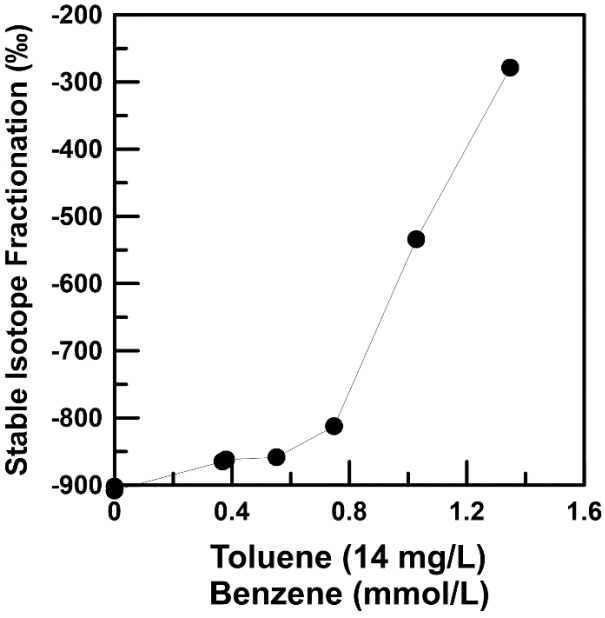


Figure 2: Illustrates the stable isotope fractionation corresponding to the batch reactor set with constant initial toluene concentrations of 14 mg/L and variable benzene concentrations.

Batch experiment with bacteria exposed to toluene and benzene simultaneously showed maximum fractionation effects similar to the toluene-only experiments (**Figure 2**). While low concentration of toluene combined with variable concentration of benzene extended the resilience of the microbial cells to toxicity; any further increase of toluene shows more toxic effects (**Figure 3**). However, an interesting phenomenon was observed in the range we categorized as mass-transfer limited zone. The cells fractionated maximal in the presence of benzene and high toluene. Opening of the cell membranes can be associated with this (Heipieper et al., 1992). The mass transfer limitation was (reduced) and the cells showed maximum values of ε for this strain (TOL plasmid pWW0), literature values of which are ε = -900‰ (Kampara et al., 2008) in a closely related strain with the same plasmid.

The presented results show that both, bioavailability limitations and toxicity effects can have an impact on the observed stable isotope fractionation. Therefore, the apparent stable isotope fractionation factors can vary with concentration and factors obtained from one systems cannot necessarily be applied to another system with different concentrations or environmental conditions.

The introduction of an additional toxin may further reduce the observable fractionation. One way to answer this is to attribute these effects to the microbes reducing the permeability of their membrane to limit their exposure to the toxin (Heipieper et al., 1994). Consequently, the substrate availability is also reduced leading to a masking of the fractionation. However, for some circumstances this additional toxin led to maximum fractionation. This is attributed to the toxin decomposing the cell membrane (Alagappan & Cowan, 2003), which increases the membrane permeability and thus increases the bioavailability of the substrate. Further research must show to which extent these effects are given attention in natural or man-made systems where microbes are exposed to toxic levels over long periods of time which may lead to adaptation effects or contrarily to lethal effects on the microbes.

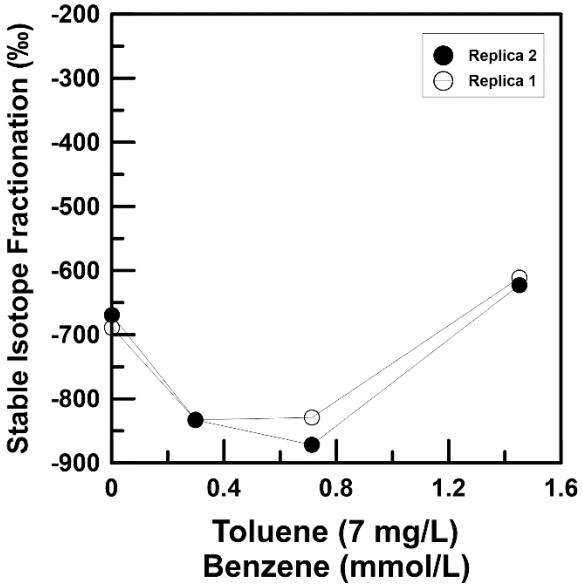


Figure 3: Shows the stable isotope fractionation from two identical replicates. Toluene initial concentrations was fixed at 7 mg/L and benzene concentration varied.

5.4 Acknowledgments

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"Education is vital to discern between knowledge and learning."

Bulleh Shah (1680 - 1757)

Summary

Reactive transport of vapor-phase VOCs in the subsurface is controlled by a multitude of interrelated factors, such as properties of the chemicals, the presence of microbial communities and the soil properties. The approach used in this thesis is to highlight and interpret the data obtained by multiple experiments and modeling scenarios. To fill the knowledge gap and to improve the understanding of dynamics of vapor-phase VOCs in the unsaturated subsurface environment, various sets of laboratory column experiments were performed. Furthermore, an approximation of Rayleigh-model was derived, which in turn allowed derivation of a stable isotope fractionation factor for diffusion and biodegradation from various depth profiles of vapor-phase VOC concentrations and the associated isotope ratios. The thesis in general consists of: *laboratory experiments* dealing with vapor transport, phase exchange, biodegradation, CSIA, and *numerical model simulations* of biodegradation using parameters derived from experiments.

Biodegradation is an important removal process of gas phase VOCs in unsaturated porous media. Toluene (non-deuterated and deuterated) has been studied as a model VOC in this work to assess biodegradation by combining data on the concentration gradients in the column and batch reactors and on the associated stable isotope fractionation with numerical modeling. A conservative volatile tracer (MTBE, non-reactive with the given bacterial strain) was used to quantify physical transport mechanisms independently of biological or chemical interactions. Our results show that the presence of bacteria along with sufficient amount of VOCs, moisture, and oxygen lead to total removal of the VOCs. Hence, the unsaturated zone can be an efficient biofilter for VOCs emanating from the groundwater and helps to avoid emissions to the atmosphere even at high gas-phase diffusion rates. However, our findings are based on the key assumptions that there is no governing process for mass transfer except diffusion and the whole reactor setups represents an unsaturated zone with only gas-phase chemicals. Therefore, the results would be misleading if extrapolated to the entire vadose zone. Our results further show that biodegradation can be limited by mass transfer of contaminants to the cells and by toxicity effects due to the combination of chemicals present in the system.

Experimental setups consisting of vertical column reactors were used to study biodegradation of gas-phase VOCs, their transport in the subsurface and the interrelation of relevant processes in **Chapter-2**. The obtained results show that biodegradation can limit the penetration of VOCs

into the unsaturated zone to only a few centimeters and confirm earlier hypotheses on the efficiency of the vadose zone as an efficient biofilter for volatile contaminants.

In-situ assessment of bioremediation is complex not only regarding the measurements, but also regarding the interpretation of the measured results. In this study, this limitation gap is filled partly by compound-specific stable isotope analysis and partly by numerical modeling. A combined approach of the experiments mentioned above, CSIA and numerical modeling was investigated in **Chapter-3**. Parameters obtained by a series of lab experiments were used in a 1-D diffusion-reaction model. The model accounted for gas-phase diffusive transport and biodegradation, resolved isotope ratios, and allowed for simulating the dynamic subsurface processes. This study elaborated a new quantification approach for stable isotope fractionation of biodegraded VOCs during their diffusion-controlled passage through the unsaturated zone.

The insight into the processes of phase exchange between vapor-phase and liquid phase and their impacts on the observed isotope fractionation effects are described in **Chapter-4**. Different sets of batch type reactors were used, which allowed simultaneous gas and liquid samples to study the influence of phase exchange on the biodegradation induced stable isotope fractionation of VOCs.

Mass transfer of chemicals to the cells is known to limit the biodegradation rate of bacterial cells. **Chapter-5** deals with the VOC toxicity and its limiting effects on the biochemical activity of the microbes. The qualitative and quantitative effects of variable VOC concentrations have shown varied isotope signatures and it was the subject of investigation in this chapter. Bioavailability and toxicity were taken into account in this chapter showing that both of them can have a limiting effect on the extent of stable isotope fractionation observed in the systems.

Samenvatting

Reactief transport van gasfase VOC's in de ondergrond wordt gecontroleerd door meerdere gerelateerde factoren, zoals de eigenschappen van de chemicaliën, de aanwezigheid van micro-bacteriële gemeenschappen en de bodemeigenschappen. De in deze thesis gebruikte aanpak is het uitlichten en interpreteren van data die door meerdere experimenten en modellen verzameld zijn. Om de kennisklоof te vullen en het verkrijgen van inzicht in de dynamiek van gasfase VOC's in de onverzadigde ondergrond zijn verschillende kolom experimenten in een laboratorium uitgevoerd. Daarnaast is een benadering van het Rayleigh-model afgeleid, wat toestond een stabiele isotopenfractioneringsfactor voor diffusie en biologische afbraak van verschillende diepteprofielen van gasfase VOC concentraties en de geassocieerde isotoopverhoudingen af te leiden. De thesis zelf bestaat uit; laboratorium experimenten met betrekking tot gas transport, fase uitwisseling, biologische afbraak, component specifieke isotopen analyse (CSIA) en meerdere model simulaties van biologische afbraak, waarbij gebruik is gemaakt van parameters afgeleid van experimenten.

Biologische afbraak is een belangrijk verwijderingsproces van gasfase VOC's in onverzadigde, poreuze media. Tolueen (niet-gedeutereerd en gedeutereerd) is bestudeerd als model VOC in dit werk om biologische afbraak te karakteriseren, door data van de concentratiegradiënten in de kolom en batch reactoren en de geassocieerde stabiele isotoop fractionering te combineren met numerieke modelering. Een conservatieve vluchtige tracer (MTBE, non-reactief met de gegeven bacteriestam) is gebruikt om fysieke transportmechanismen onafhankelijk van biologische of chemische interacties te kwantificeren. Onze resultaten laten zien dat de aanwezigheid van bacteriën, naast voldoende VOC's, vocht en zuurstof, geleid heeft tot de verwijdering van de VOC's. Hierom kan de onverzadigde zone een efficiënt biologisch filter voor uit grondwater afkomstige VOC's zijn en helpt dit emissies naar de atmosfeer zelfs bij hoge gasfase diffusiesnelheden te voorkomen. Hier moet bij gezegd worden dat onze vindingen gebaseerd zijn op belangrijke aannames dat transport alleen plaatsvindt door diffusie en dat de hele reactoropstellingen een onverzadigde zone met alleen gasfase chemicaliën vertegenwoordigt. Hierom zouden de resultaten misleidend kunnen zijn als deze geëxtrapoleerd zouden worden naar de gehele vadose zone. Onze resultaten laten verder zien dat biologische afbraak gelimiteerd kan worden door massaoverdracht van vervuilingen van de cellen en door toxische effecten van in het systeem aanwezige gecombineerde chemicaliën.

De experimentele opstellingen, bestaande uit verticale kolomreactoren, zijn gebruikt om biologische afbraak van de gasfase VOC's te bestuderen, hun transport in de ondergrond en de verwevenheid van relevante processen in **Hoofdstuk 2**. De verkregen resultaten laten zien dat biologische afbraak de penetratie van VOC's in de onverzadigde zone kan limiteren tot een paar centimeter en bevestigen de eerdere hypothese over de effectiviteit van de vadose zone als efficiënt filter voor vluchtige verontreinigingen.

In-situ beoordelingen van bioremediatie zijn complex, niet alleen vanwege de metingen maar ook vanwege de interpretatie van de meetresultaten. In deze studie is de kloof van limiteringen deels gevuld door verbindingsspecifieke stabiele isotoop analyse, en deels door numerieke modelering. Een gecombineerde aanpak van voorgenoemde experimenten, CSIA en numerieke modelering is onderzocht in **Hoofdstuk 3**. Parameters verkregen door een serie laboratoriumexperimenten zijn gebruikt in een 1-D diffusie-reactie model. Het model hield rekening met gasfase diffusief transport, biologische afbraak, stabiele isotoop verhoudingen en stond toe de dynamische processen in de ondergrond te simuleren. Deze studie werkt een nieuwe kwantitatieve aanpak voor stabiele isotoop fractionering van biologisch afgebroken VOC's tijdens hun diffusie-gecontroleerde doorgang door de onverzadigde zone uit.

Het inzicht in het proces van uitwisseling tussen gasfase en vloeibare fase en hun impact op de waargenomen isotoop fractionerende effecten zijn beschreven in **Hoofdstuk 4**. Verschillende sets batch-type reactoren zijn gebruikt, wat toestond gelijktijdig vluchtige en vloeibare monsters en de invloed van fase uitwisseling op de biologisch afbreekbare stabiele isotoop fractionering van VOC's te bestuderen.

Transport van chemicaliën naar de cellen kan de biologische afbraak limiteren. **Hoofdstuk 5** behandelt de giftigheid van VOC en de gevolgen voor van de biochemische activiteit van de microben. De kwalitatieve en kwantitatieve effecten van verschillende VOC concentraties hebben invloed op de isotopische ratios, de focus of dit hoofdstuk. Er is rekening gehouden met de bio-beschikbaarheid en giftigheid in dit hoofdstuk, waarbij aangetoond is dat beiden een beperkend effect hebben op de hoeveelheid stabiele isotoop fractionering die in de systemen waargenomen is.

Zusammenfassung

Reaktiver Stofftransport von Dampfphasen-VOCs im Untergrund wird durch eine Vielzahl zusammenhängender Faktoren gesteuert, wie zum Beispiel den Eigenschaften der Chemikalien, dem Vorhandensein von mikrobiellen Gemeinschaften und der Beschaffenheit des Bodens. Die in dieser Dissertation verwendete Vorgehensweise ist es, die aus einer Vielzahl an Experimenten gewonnenen Informationen darzustellen und zu interpretieren. Um die Wissenslücke zu schließen und das Verständnis über die Dynamik der Dampfphasen-VOCs im ungesättigten Untergrund zu verbessern, wurden verschiedene Laborsäulenversuche durchgeführt. Darüber hinaus wurde eine Näherungslösung das Rayleigh-Modells abgeleitet, was wiederum eine Abschätzung von Fraktionierungsfaktoren von stabilen Isotopen für Diffusion und biologischen Abbau aus Profilen der Konzentration und dem Isotopenverhältniss von Dampfphasen-VOCs erlaubt. Die Dissertationsschrift beinhaltet Laborversuche zum Wasserdampftransport, Phasenübergang, biologischen Abbau, der dadurch verursachten Fraktionierung stabiler Isotope und der numerischen Modellsimulationen dieser Prozesse basierend auf aus den Experimenten abgeleiteten Parametern.

Der biologische Abbau ist ein wichtiger Prozess zur Entfernung von Gasphasen-VOCs in ungesättigten porösen Medien. Toluol (deuteriert und nicht-deuteriert) wurde als ein Modell-VOC untersucht. Durch eine Kombination aus Daten von Konzentrationsgradienten in Säulenexperimenten und von Experimenten mit Batch-Reaktoren und der dabei beobachteten Fraktionierung von stabilen Isotopen mit numerischer Modellierung wurde der biologischen Abbau bewertet. Ein konservativer volatiler Tracer (MTBE, nicht-reaktiv mit dem gegebenen Bakterienstamm) wurde genutzt, um physikalische Transportmechanismen unabhängig von biologischen oder chemischen Wechselwirkungen zu messen. Die Ergebnisse zeigen, dass das Vorhandensein von Bakterien zusammen mit einer ausreichenden Menge an VOCs, Feuchtigkeit und Sauerstoff zum vollständigen Abbau der VOCs führt. Deshalb kann die ungesättigte Schicht ein effizienter Biofilter für aus dem Grundwasser stammenden VOCs sein und hilft somit, Emissionen in die Atmosphäre sogar bei hohen Gasphasendiffusionsraten zu vermeiden. Abgesehen davon basieren unsere Ergebnisse auf den wesentlichen Voraussetzungen, dass es außer der Diffusion keinen vorherrschenden Prozess für Stofftransport gibt und dass die Versuchsaufbauten eine ungesättigte Zone beschreiben, die nur Gasphasen-Chemikalien enthält. Aus diesem Grund können die Ergebnisse nicht direkt auf die komplette ungesättigte Bodenzone übergeleitet werden. Unsere Ergebnisse zeigen darüber hinaus, dass der biologische Abbau durch den Stofftransport von Schadstoffen zur Zelle sowie durch toxische Effekte verursacht durch die Kombination der vorhandenen Chemikalien im System begrenzt werden kann.

Um den biologischen Abbau von Gasphasen-VOCs, deren Transport im Untergrund und die Wechselwirkung der entsprechenden Prozessen zu untersuchen, wurden in **Kapitel 2** Versuchsaufbauten bestehend aus vertikalen Säulenreaktoren genutzt. Die beobachteten

Ergebnisse zeigen, dass der biologischer Abbau das Eindringen von VOCs in die ungesättigte Zone bis auf wenige Zentimeter begrenzen kann und bestätigen somit vorherige Hypothesen zur Effizienz der ungesättigten Bodenzone als ein effizienter Biofilter für leichtflüchtige Schadstoffe.

In situ Bewertungen von Bioremediation sind komplex, nicht nur in Bezug auf die Messungen sondern auch bezogen auf die Interpretation der Messergebnisse. In dieser Studie wird diese Wissenslücke einerseits durch stoffspezifische Analysen stabiler Isotope (CSIA) und andererseits durch numerische Modellierungen geschlossen. Ein kombinierter Ansatz der oben erwähnten Experimente, CSIA sowie numerischer Modellierungen wurde in **Kapitel 3** untersucht. Durch eine Reihe an Laborversuchen wurden Parameter bestimmt, die in einem 1-D-Diffusions-Reaktions-Modell angewandt wurden. Dieses Modell berücksichtigt den diffusiven Gasphasentransport, den biologischen Abbau sowie die Verhältnisse stabiler Isotope und kann für die Simulation der dynamischen Prozesse im Untergrund genutzt werden. Diese Studie erarbeitet einen neuen Quantifizierungsansatz für die Fraktionierung stabiler Isotope biologisch abbaubarer VOCs während ihres diffusionsgesteuerten Transports durch die ungesättigte Zone.

Einblicke in den Prozess des Phasenübergangs zwischen Gasphase und Flüssigphase sowie dessen Einfluss auf die beobachteten Isotopenfraktionierungseffekte sind in **Kapitel 4** beschrieben. Dazu wurden verschiedene Arten von Batch-Reaktoren verwendet, was die gleichzeitige Entnahme von gasförmigen und flüssigen Proben erlaubt, um so den Einfluss des Phasenwechsels auf den biologischen Abbau, der die Fraktionierung der stabile Isotopen von VOCs hervorruft, zu untersuchen.

Der Stofftransport von Chemikalien zu den Zellen ist bekannt dafür, die Geschwindigkeit der biologischen Abbauleistung der Bakterienzellen zu begrenzen. **Kapitel 5** beschäftigt sich mit der Toxizität von VOCs und deren hemmende Wirkung auf die biochemische Aktivität der Mikroorganismen. Die qualitativen und quantitativen Auswirkungen von variierenden VOC-Konzentrationen haben verschiedene Auswirkungen auf die beobachteten Änderungen der Isotopensignaturen, was der Untersuchungsgegenstand in diesem Kapitel ist. Bioverfügbarkeit und Toxizität wurden in diesem Kapitel untersucht und es wird aufgezeigt, dass beide eine hemmende Wirkung auf das Ausmaß der Fraktionierung stabiler Isotope haben können.

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Curriculum Vitae

Ali M. Khan was born in Pakistan. He finished his multiple Masters at University of Kiel, Germany in 2011. The same year he started his PhD in the faculty of geosciences at Utrecht University, the Netherlands. Helmholtz Centre for Environmental Research – UFZ hosted his research work under the supervision of Prof. Philippe van Cappellen, Dr. Martin Thullner and Dr. Lukas Wick.

کون کہتا ہے تم برے آخرؔ
اچھا اچھا! ہم بہت سنا کیجیے

چشمِ باطن سے تو دیکھ لیا
جو دل میں ہے کہا کیجیے

دو گھڑی کا ساتھ مانگا ہے
جلدی جلدی سے فیصلہ کیجیے

آ گیا محفل میں اجنبی کوئی
بھاگیے۔۔۔ دوڑیئے! پتہ کیجیے

آگے چل کر اسی کا ساتھ ملا
پھر اسی شخص سے وفا کیجیے

گریہ زاری ہے موت سے پہلے
شیخ کہتے ہیں کہ حوصلہ کیجیے

وقت کس کا ہے ہوا ساتھی
حسنِ آخرؔ سے اب چلا کیجیے

آخرؔ