Influence of Ethanol and Methyl Groups on the Degradation of Xylenes in Soil

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Abstract: The main objective of the present study was to assess the influence of ethanol on the evaporation profile of xylenes (ortho + meta and para isomers) in active (with microorganisms) and inert (without microorganisms) soils. The vapors from four sealed flasks containing inert soil + neat xylenes, active soil + neat xylenes, inert soil + xylenes + ethanol and active soil + xylenes + ethanol were monitored during 20 days by Gas Chromatography using a Flame Ionization Detector (GC/FID). No statistical differences were observed comparing the concentrations of the meta and para isomers in all samples. The differences among the average concentrations of meta, para and ortho isomers were relevant only in the active soil samples without ethanol (95% confidence interval). Ethanol may enhance the ortho isomer volatilization and delay the degradations of meta and para isomers.

Keywords: Xylenes, Gas Chromatography, Volatilization, Biodegradation, Soil

Introduction

After the first oil crisis in the 1970s, with the increasing pollution levels and the need for clean energy production, several nations around the world developed programs to support the use of alternative fuels, including ethanol and gasohol (a gasoline-ethanol blend), as automobile fuels. The main advantages of the ethanol-blend fuels are the reduction of greenhouse gas emissions and the higher octane number of gasoline (Fedrizzi et al., 2013).

Despite the benefits provided by the mixture, the presence of ethanol in gasoline may affect the BTEX (benzene, toluene, ethylbenzene and xylenes) volatilization and these compounds may evaporate from spills of underground storage tanks or by accidental releases (Fedrizzi et al., 2013). The gas transport through the vadose zone can be influenced by moisture content due to variations in gaseous permeability, phase partitioning and aerobic biodegradation (Nerantzis and Dyer, 2010).

The main target organ for acute and chronic exposures to xylenes are the central nervous system (Manuela et al., 2012), the respiratory and cardiovascular systems and kidneys (Sarigiannis and Gotti, 2008).

In the case of soil contamination, remediation procedures are required, such as stabilization, containment or decontamination (Balseiro-Romero and Monteroso, 2012). The most common methods for decontamination are: bioremediation employing special cultures (Cozzarelli et al., 2010; Jahn et al., 2005; Meckenstock et al., 2012; Morasch et al., 2004), phytoremediation (Boonsaner et al., 2011) and bioaugmentation (Fantroussi and Agathos, 2005). However, despite the recent study published by Chemlal et al. (2012), there is a lack of studies evaluating the degradation/volatilization of xylenes isomers in natural soil (without introduction of exogenous cultures) and in presence of ethanol.

The purpose of the present study is to determine the influence of ethanol on the degradation/volatilization of the xylene isomers in crude soil.

Materials and Methods

Soil Samples

The method of soil collection was modified from Cardoso et al. (2011). The sample was composed by 100g of soil collected near the Center of Exact Sciences and technology of the University of Caxias do Sul, from a depth of 10 cm and placed in a plastic bag. The soil was dried at room temperature and passed through a 2 mm sieve.

The physical-chemical characteristics of the soil were determined based on Cachada et al. (2012). The measured parameters were: Moisture (by gravimetry);
Total Organic Carbon (TOC, by Walkley method); Total Nitrogen (TN, by Kjeldahl method); Total Phosphorus (TP, by acidic double extractor, Mehlich-1) and Total Volatile Solids (TVS, by gravimetric method).

**Microcosm’s Preparation**

The microcosms were prepared in transparent glass serum bottles (50 mL capacity, base diameter = 3.3 cm and high = 4.8 cm) as follows:

- **Inert soil**: Ten grams of soil and a serum bottle were treated in an autoclave (121 °C for 15 min). One milliliter of the xylenes standard solution (750 mg L\(^{-1}\) in \(n\)-hexane) was added to the sample that was homogenized with a glass stick, closed with rubber caps and aluminium rings and left to rest overnight.
- **Active soil**: The same procedures (item a) were applied, except by the autoclave step.
- **Inert soil + ethanol**: The same procedures (item a) were applied, plus the sample contamination with the mixture of xylenes standard solution (750 mg L\(^{-1}\) in \(n\)-hexane) and ethanol (25% (v/v)).
- **Active soil + ethanol**: The same procedures (item a) were applied, except by the autoclave step, plus the mixture of xylenes standard solution (750 mg L\(^{-1}\) in \(n\)-hexane) and ethanol (25% (v/v)). After the solvent evaporation the theoretical concentration of xylenes was 0.75 mg g\(^{-1}\).

Figure 1 illustrates the microcosm’s preparation.

**Vapors Collection**

The vapours in the microcosms were sampled using a gas-tight syringe (3 mL). Two mL of the vapours were transferred to autoinjector vials (1.5 mL of capacity with a screw top). The autoinjector vials were previously evacuated by flushing 3 mL of the inner air. The vapours were injected (2.0 µL in triplicate) in the injection port of a gas chromatograph. After each sampling, 20 mL of the headspace vapours were flushed with a gas-tight syringe to force the system to reach a new equilibrium state by mass transfer of xylenes to the headspace. In this way, the environment inside the microcosms was gradually oxygen depleted. The interval between each sampling was 24 h. Even though phase equilibrium should be within in few hours for NAPL (Non-Aqueous Phase Liquids) such as xylenes, the interval of 24 h was necessary due to the GC availability for the instrumental analysis. The experimental period was of 20 days.

**Instrumental Analysis**

Instrumental analysis was performed with a gas chromatograph (Agilent 7820) equipped with autoinjector (7693A) and flame ionization detector.

![Microcosm’s preparation](image)

A 30 m column (HP-5, film thickness 0.32 mm, internal diameter 0.25 mm) was temperature programmed from 40°C (held for 10 min) to 220°C (held for 0 min) at 5°C min\(^{-1}\). Helium was the carrier gas with a flow rate of 1.0 mL min\(^{-1}\). The Limit of Quantitation (LOQ) was evaluated by injection of the headspace vapours of standard solutions at 0.3, 0.5, 1.0, 2.5, 5.0, 10.0 and 20.0 mg L\(^{-1}\) obtained by successive dilutions of a xylenes standard solution in \(n\)-hexane (50 mg L\(^{-1}\)).

In the present work the LOD is defined as the lowest amount of an analyte in a sample that can be quantified with acceptable precision and accuracy (Shrivastava and Gupta, 2011). The LOQ was calculated from the signal-to-noise ratio of 10 (Shrivastava and Gupta, 2011).

Kanai et al. (2005) used the intermolecular forces theory to explain the chromatographic separation of meta and para isomers of xylenes. However, in the present study we refer to these compounds as \(m+p\)-xylenes since their retention times were very close in the gas chromatographic analysis. This approach seems to be usual, according to the publications of (Eshaghi et al., 2011; Simantiraki et al., 2013).

For the LOQ calculations, the relation between the cylinder volume in the headspace (\(πR^2h\)) and the chromatographic peak area were used. The results were expressed in mg/cm\(^3\).

**Results**

For the \(m+p\)-xylenes, the LOQ was 0.8115 mg/cm\(^3\) and the detector response was linear between 0.8115 and 3.246 mg/cm\(^3\) (\(R^2 = 0.9972\); equation: \(y = 4419.1x – 1546.5\)). For the \(o\)-xylene, the LOQ was 0.4058 mg/cm\(^3\) and the detector response was linear between 0.4058 and 6.492 mg/cm\(^3\) (\(R^2 = 0.9103\); equation: \(y = 1637.5x – 2741.8\)). For the soil samples without ethanol no differences between the concentrations of the \(m+p\)-xylenes isomers in the headspace of the inert and active soil were observed (this is the null hypothesis and the \(p\)-value was > 0.05). On the other hand, considering the final and initial collection days, the concentration of \(o\)-xylene in the active soil’s headspace decreases by 31.95% (Fig. 2) (null hypothesis: No decrease of the concentration of \(o\)-xylene during the experimental time, \(p\)-value < 0.05). This difference was relevant in a 95% confidence interval.
Discussion

The results suggest that polarity may exert a key role on the xylenes degradation. The Table 1 shows that the m+p-xylene isomers are by far less polar than the ortho-xylene. This may explain the lower concentration of ortho-xylene in the active soil’s headspace.

According to Sun and Cupples (2012), the initial step of the biodegradation depends on electron transfer reactions. In this way, the most polar isomer may suffer higher degradation.

This hypothesis is supported by several studies focused on the degradation of xylenes. Early reports of anaerobic xylenes degradation under nitrate-reducing conditions indicated that both para and meta (but not ortho) xylene were degraded. In some cases the presence of meta-xylene inhibited concomitant ortho and para xylene degradation (Jahn et al., 2005; Meckenstock et al., 2012; Morasch et al., 2004).

Cozzarelli et al. (2010) have used an in situ microcosm in a crude-oil contaminated aquifer to measure the biodegradation of benzene, toluene, ethylbenzene, ortho, meta and para-xylenes and four pairs of C8- and C9-benzenes over a 3-year period. According to the authors the apparent order of persistence is ethylbenzene > benzene > meta, para-xylenes > ortho-xylene ≥ toluene.

However, none of the studies cited above were performed with crude soil (without chemical modification or introduction special cultures). Also there is a lack of publications evaluating the specific influence of ethanol on the biodegradation of the xylenes isomers. Furthermore, no explanations were found about the differences of the degradation kinetics of those compounds. In this way, the results obtained in the present study cannot be directly compared.

Table 1. Dipole moments of ethanol and xylenes (Fedrizzi et al., 2013)

| Compound     | Dipole moment (D) |
|--------------|-------------------|
| Ethanol      | 1.69              |
| o-xylene     | 0.59              |
| m-xylene     | 0.30              |
| p-xylene     | 0.02              |

Another reason for the higher degradation of ortho-xylene may be the spatial hindering caused by the methyl groups (Fig. 3). According to Balseiro-Romero and Monteroso (2012), the presence of a methyl group at the meta or para positions of the benzene ring would impart a greater sorptive affinity in soil than a methyl group at the ortho position in the ring. A possible steric repulsion between the two adjacent methyl groups in ortho-xylene could lower its sorption compared with meta and para-xylene. In this way, the methyl groups in the ortho position may be more available for biodegradation than the meta and para methyl groups. Kube et al. (2004) proposed that the initial step of the toluene biodegradation, at anaerobic conditions, is the oxidation of the methyl group.

Figure 4 shows the effect of ethanol on the volatilization of xylenes in the active soil with ethanol.

The concentrations of the m+p-xylenes in the active soil samples were not different in an 95% confidence interval (this is the null hypothesis and the p-value was > 0.05). On the other hand, the highest increase of the ortho isomer concentration in the ethanol sample was relevant (null hypothesis: No increase of the ortho isomer concentration, p-value < 0.05).

Due to its higher polarity (Table 1) the ortho isomer may be preferentially soluble in ethanol, reducing the mixture boiling point. This effect is called cosolvency and may increase the o-xylene volatilization.

Many works has been published regarding the cosolvency promoted by gasoline-ethanol mixtures. According to Yu et al. (2009), the proportions of BTX (benzene, toluene and xylenes) in ethanol-amended gasoline are changed by the cosolvent behavior of ethanol. Cataluña and Silva (2006) described the development of a device to determine the vapor pressure and the vaporization enthalpy of formulations containing volumes of 5, 15 and 25% of ethanol in four base gasolines. Their results showed that the addition of ethanol to the gasoline hydrocarbons generates a mixture with a boiling point smaller than the original.

Ethanol and n-hexane showed very close retention times at the instrumental analysis conditions adopted in the present study. This means that complete chromatographic separation was not achieved. The variation of ethanol concentration was obtained by differences between the peak solvent areas of the samples with and without ethanol, at each sampling. Because of this, the following discussion will be developed in relation to the percent chromatographic peak areas that were calculated by comparison of the initial (100%) and final absolute peak areas.
Fig. 3. Chemical structures of the xylene isomers

As can be seen in Fig. 5, ethanol presents the lowest percent area increase in the active soil + ethanol samples. These results may be explained by the preferential degradation of ethanol. According to Österreicher-Cunha et al. (2009), ethanol enhances BTEX retention in soil, boosts microbial activity but delays BTEX biodegradation.

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**Author’s Contributions**

Irajá Nascimento: Conceived this study and wrote the paper.

Ricardo Baldasso:Performed GC investigation, interpreted the results and critically reviewed the manuscript.

Kira Manfredini: Performed GC investigation, interpreted the results and critically reviewed the manuscript.

All authors read and approved the final manuscript.

**Ethics**

No ethical issues may arise after the publication of this manuscript.

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