CHEMICAL ENGINEERING TRANSACTIONS
VOL. 54, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Selena Sironi, Laura Capelli

ISBN 978-88-95608-45-7; **ISSN** 2283-9216
DOI: 10.3303/CET1654020

Interactions between BTEX Compounds during their Anoxic Degradation

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Benzene, toluene, ethyl-benzene and o-xylene degradation was evaluated under anoxic denitrifying conditions. These aromatic hydrocarbons were supplemented as single carbon sources or in dual or quaternary mixtures in order to identify key interactions during the degradation process. The results showed that toluene, together with ethyl-benzene, were the most readily biodegradable compounds under anoxic conditions among the aromatic compounds and their combinations tested. However, ethyl-benzene degradation time was significantly increased when combined with toluene as carbon source. Both benzene and o-xylene demonstrated to be highly recalcitrant compounds for anoxic denitrification, their degradation being rapidly inhibited likely as a result of the accumulation of toxic metabolites excreted during the degradation process. In this sense, mineral medium renewal was required to achieve complete degradation of these pollutants. Any combination of the aromatic compounds revealed an inhibitory interaction during the biodegradation process, an increase in the biodegradation time and therefore a decrease in pollutant degradation rates being observed upon addition of more than one compound.

1. Introduction

Benzene (B), toluene (T), ethylbenzene (E) and xylene (X) are volatile aromatic hydrocarbons frequently used as solvents in many industrial sectors such as petrochemistry, pulp and paper or paint and dye (Lu et al., 2002).These BTEX compounds account for up to 59 % (w/w) of gasoline pollutants and represent about 80 % of total volatile organic compounds (VOCs) emissions in petrochemical plants (Barona et al, 2007, El Naas et al., 2014). They pose a global threat to human health and the environment due to their genotoxic properties and their widely extended use. The increasing public concern on environmental and health issues and the more stringent legislations on VOC emissions worldwide result in the need for effective minimization and abatement of these BTEX emissions.

Up to date, conventional physical–chemical processes such as absorption, adsorption or combustion have been used for BTEX removal, although recently developed biological treatment alternatives provide similar removal efficiencies with lower operating and maintenance costs, based on an environmentally-friendly process and avoiding the production of toxic by-products (Trigueros et al., 2010, Lebrero et al., 2010). In this sense, several authors have demonstrated the possibility of aerobically degrading these BTEX-loaded emissions in different bioreactor configurations such as biofilters, bioscrubbers and biotrickling filters (Robledo-Ortiz et al., 2011; Singh et al., 2010). However, these aerobic biological processes are strongly limited in O_2-free petrochemical industry emissions due to potential explosion risks, and cannot be implemented as end-of-pipe technologies (Muñoz et al., 2013)

In order to overcome this problem it is necessary to develop alternative biotechnologies able to use an electron acceptor other than O_2 within the biodegradation process. In this context, anoxic BTEX mineralization via denitrification offers a potential solution for the removal of these pollutants from O_2 deprived emissions. However, the number of studies devoted to anoxic BTEX removal is still scarce and little is known regarding the interactions among these pollutants during the degradation process.

Please cite this article as: Akmirza I., Carvajal A., Munoz R., Lebrero R., 2016, Interactions between btex compounds during their anoxic degradation, Chemical Engineering Transactions, 54, 115-120 DOI: 10.3303/CET1654020

2. Materials and Methods

2.1 Inoculum Conditions

Activated sludge from the anoxic denitrification tank of the wastewater treatment plant of Valladolid (Spain) was used as inoculum in order to promote the adaptation of the microbial community to anoxic conditions.

2.2 Chemicals and Mineral Salt Medium

All chemicals for mineral salt medium (MSM) preparation were purchased from PANREAC (Barcelona, Spain) with a purity of at least 99 %. Benzene, toluene, ethylbenzene and o-xylene (99.0 % purity) were obtained from Sigma–Aldrich (Madrid, Spain). The MSM was composed of (g L^{-1}): $Na_2HPO_4 \cdot 12H_2O$, 6.15; KH_2PO4, 1.52; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2$, 0.038; and 10 mL L^{-1} of a trace element solution containing (g L^{-1}): EDTA, 0.5; $FeSO_4 \cdot 7H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 0.01; $MnCl_2 \cdot 4H_2O$, 0.003; H_3BO_3, 0.03; $CoCl_2 \cdot 6H_2O$, 0.02; $CuCl_2 \cdot 2H_2O$, 0.001; $NiCl_2 \cdot 6H_2O$, 0.002; $NaMoO_4 \cdot 2H_2O$, 0.003 (Muñoz et al., 2013). The final MSM pH was adjusted to 7. NO_3^- (supplemented as $NaNO_3$) was used as electron acceptor for BTEX oxidation and as nitrogen source for microbial growth. In order to ensure similar conditions in each bottle, from 1.6 ml to 8.1 mL of a 10 g $NaNO_3$ L^{-1} stock solution were added to the bottles according to the carbon load.

2.3 Experimental Setup and Cultivation Procedure

Twenty-two 1 L air-sealed bottles with 100 mL of MSM were inoculated with anoxic activated sludge, reaching a final concentration of ~ 200 mg volatile suspended solids (VSS) L^{-1}. Two additional bottles containing MSM without inoculum were used as controls to rule out any possible BTEX volatilization or abiotic loss. Bottles were closed with rubber stoppers and sealed with aluminium caps. The gas headspace of the bottles was washed with N_2 (Linde Spain, purity > 99.999 %) for at least 10 minutes in order to remove any oxygen (the required time to reach anoxic conditions in the headspace was verified by O_2 analysis). Single, dual and quaternary combinations of BTEX compounds were injected into the bottles at the following initial concentrations: (1) B: 465 ± 33 mg m^{-3} (2) T: 564 ± 77 mg m^{-3} (3) E: 533 ± 40 mg m^{-3} (4) X: 564 ± 100 mg m^{-3} (5) B-T: 560 ± 50.2 mg m^{-3}, 557 ± 16.7 mg m^{-3}, respectively (6) B-E: 495 ± 57 mg m^{-3}, 584 ± 79 mg m^{-3}, respectively (7) B-X: 479 ± 128 mg m^{-3}, 511 ± 88 mg m^{-3}, respectively (8) T-E: 499 ± 33 mg m^{-3}, 505 ± 38 mg m^{-3}, respectively (9) T-X: 439 ± 36.7 mg m^{-3}, 440 ± 28 mg m^{-3}, respectively (10) E-X: 560 ± 56 mg m^{-3}, 552 ± 40 mg m^{-3}, respectively (11) B-T-E-X: 522 ± 50 mg m^{-3}, 541 ± 57 mg m^{-3}, 600 ± 115 mg m^{-3}, 553 ± 34 mg m^{-3}, respectively. The bottles were continuously agitated at 320 rpm by magnetic stirring and kept at constant room temperature of 25 ºC. Three times per week samples were taken from the headspace using a gas-tight syringe (Hamilton, USA) and the BTEX concentration was analysed by GC-FID.

Weekly, 30% of liquid media was exchanged with new MSM to provide sufficient nutrients for microbial growth, and every two weeks the corresponding amount of the 10 g $NaNO_3$ L^{-1} stock solution was added to the bottles to prevent NO_3^- limitation. Liquid samples were taken before $NaNO_3$ addition for nitrate analysis by HPLC. Five complete biodegradation cycles were performed for the acclimation of the microorganisms to BTEX as single carbon source.

2.4 Kinetic Experiment

After the 5^{th} biodegradation cycle and before the kinetic experiment, 30% of the media was exchanged with new MSM and $NaNO_3$ was added to guarantee sufficient nutrients and nitrate during the test. The corresponding contaminant or BTEX mixture was added to the headspace, and gaseous samples were periodically taken and analysed by GC-FID until the BTEX compounds were totally degraded or until the biodegradation stopped. Before and after the kinetic test, liquid samples were taken in order to determine the NO_3^- consumption during the test. Finally, biomass samples were taken at the end of the experiment for total and volatile suspended solids (TSS and VSS) analysis.

2.5 Analytical Procedure

BTEX concentration in the gas phase was analysed by a Bruker 3900 gas chromatograph (Palo Alto, USA) equipped with a flame ionization detector and a Supelco Wax (15 m × 0.25 mm × 0.25 µm) capillary column. Oven temperature was initially maintained at 50 ºC for 1 minute, increased at 50 ºC min^{-1} up to 70 ºC and then at 65 ºC min^{-1} to a final temperature of 140 ºC. VSS and TSS were measured according to Standard Methods (American Water Works Association, 2012). Nitrite and nitrate concentrations in the liquid phase were analysed via HPLC-IC using a Waters 515 HPLC pump coupled with a conductivity detector (Waters 432) and equipped with an IC-PAK Anion HC column (4.6 × 150 mm) and an IC-Pak Anion Guard-Pak (Waters). Samples were eluted isocratically at 2 mL min^{-1} (at room temperature) with a solution of distilled water/acetonitrile/n-butanol/buffer at 84/12/2/2% v/v (Muñoz et al, 2013).

3. Results and Discussion

3.1 Acclimation

Before the kinetic experiments, 5 cycles of biodegradation were completed in each bottle in order to adapt microorganisms to BTEX compounds. During the acclimation period 30 % of MSM was periodically exchanged with a fresh medium in order to prevent microbial inhibition by the toxic metabolites excreted during the biodegradation process. Whereas toluene and ethyl-benzene were completely removed before each MSM exchange, degradation of benzene and o-xylene was rapidly inhibited probably as a result of the accumulation of potentially toxic by-products (Figure 1). The degradation capacity of the microbial community was restored after MSM renewal, which decreased metabolites concentration in the cultivation broth.

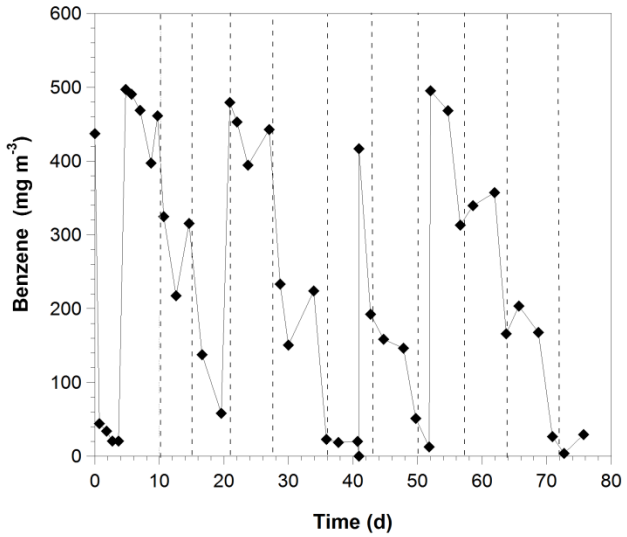

Figure 1. Time course of benzene concentration during the adaptation period. Vertical lines represent MSM exchange.

3.2 Kinetic experiments

After the start-up of the kinetic experiment, T and E were completely degraded under anoxic conditions within 5 and 6 hours, respectively, when present as single carbon sources in the batch test bottles (Figure 2B and 2C). Toluene degradation was negatively affected by B or X, the time for complete degradation increasing up to 25 ± 2 h (B-T test), 54 ± 1 h (X-T test) and 51 ± 5 h (BTEX test). However, only 2 additional hours were necessary for complete T degradation in the presence of E. Ethyl-benzene biodegradation was observed when combined with T as carbon sources in 12 ± 1 h (Figure 3D). Ethyl-benzene biodegradation was also hampered when either B (52 ± 2 h) or X (48 ± 1) were added to the bottles headspace (Figure 3B). Moreover, 47 ± 1 h were necessary for complete degradation of E when all BTEX were present (Figure 4).

While T and E were readily degraded under anoxic conditions, both B and X required several MSM exchanges for complete removal. In this sense, the concentration of B decreased by 34 ± 9 % within the first 11 h when fed as the sole carbon source, its concentration stabilizing afterwards (Figure 2A). This inhibition of B degradation was attributed to the accumulation of toxic metabolites in the liquid cultivation media, similar to the pattern observed during the acclimation cycles. When T or E were supplemented together with B, substrate interactions started to occur, and only 19 ± 4 % and 27 ± 2 % of the initial B concentration was degraded, respectively, within the first 25 and 27 h of the kinetic assay (Figures 3A and 3B). Likewise, the combination of B with X underwent inhibition of B degradation after 22 hours of biodegradation (corresponding to a removal of B of 16 ± 9 %) (Figure 3C*)*. Only a slight B degradation of 12 ± 7 % was observed upon addition of all the BTEX (Figure 4). O-xylene showed a similar behaviour than B when supplemented as single carbon source, reaching 49 ± 4 % removal within the first 24 hours (Figure 2D). Inhibitory competition was observed when X was fed together with either T or E, o-xylene biodegradation stopping in the presence of T after a removal of 42% ± 10 and of 37% ± 7 in the presence of E (Figures 3E and 2F). O-xylene degradation was inhibited after 22 h of assay when combined with B, which supported a removal of X of 12 ± 10 % (Figure 2C). Finally, 29 ± 7 % of X was degraded upon addition of all BTEX (Figure 4).

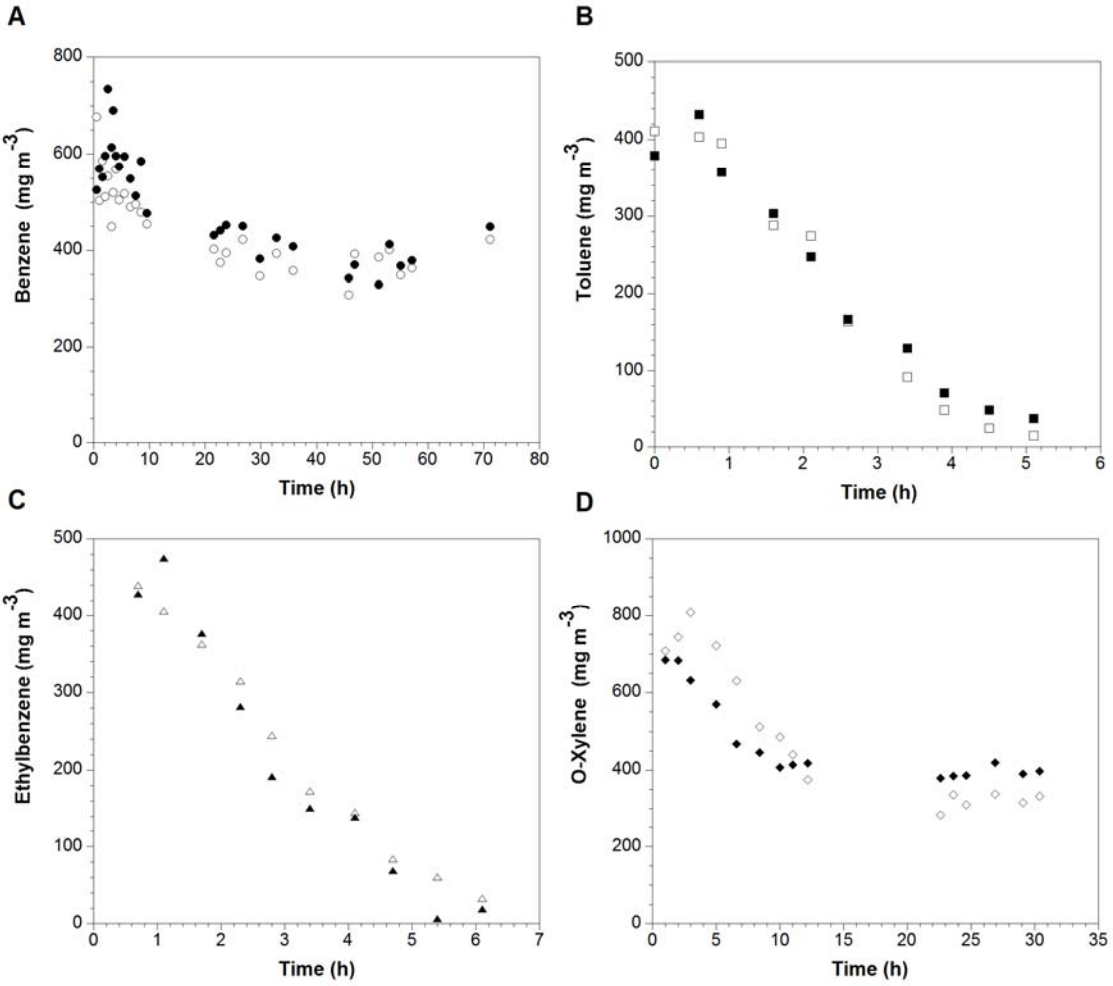

Figure 2. Time course of benzene (A, circle), toluene (B, square), ethyl-benzene (C, triangle) and o-xylene (D, diamond) concentration during the kinetic tests. White and black symbols represent bottle duplicates.

These kinetic results confirmed the results previously observed during the acclimation period cycles and the fact that T was the most readily biodegradable compound under anoxic conditions among the aromatic compounds and their combinations tested. Also E showed similar behaviour to T under anoxic conditions during the kinetic tests but in the presence of T, E degradation time increased by a factor of 2. Toluene and E have been also shown to be readily biodegradable under aerobic conditions when present as the sole carbon and energy source (Littlejohns et al., 2008). A rapid T and E degradation was observed under aerobic conditions in previous studies, and the addition of other carbon sources also increased the degradation time due to substrate interactions similar to the results here obtained under anoxic conditions (El Naas et al., 2014). The fact that B and X were partially degraded under anoxic conditions confirmed the high recalcitrance of X under aerobic conditions reported by Littlejohns et al. (2008). At both aerobic and anoxic conditions the dual combination of the aromatic compounds evaluated resulted in a competition for carbon source, which increased the biodegradation time and lowered the pollutant biodegradation rates. A supplementary analysis for the identification of the trace level toxic metabolites accumulated in the cultivation broth under the different BTEX mixtures should be conducted in order to further understand the inhibition of BTEX biodegradation.

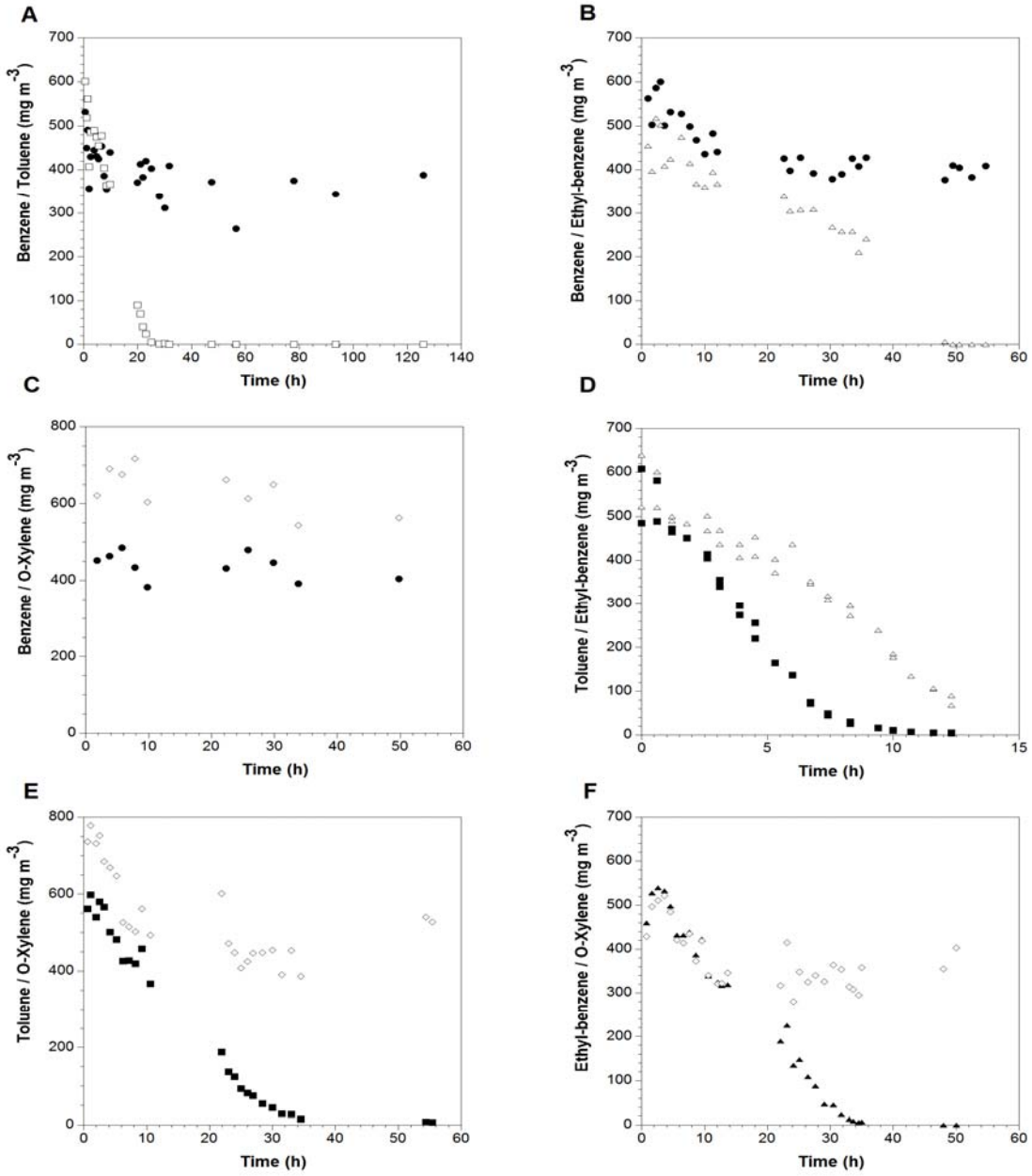

Figure 3. Time course of benzene - toluene (A, circle - square), benzene - ethyl benzene (B, circle - triangle), benzene-o-xylene (C, circle - diamond), toluene-ethyl benzene (D, square - triangle), toluene - o-xylene (E, square - diamond), ethyl benzene - o-xylene (F, triangle - diamond) concentration during the kinetic tests.

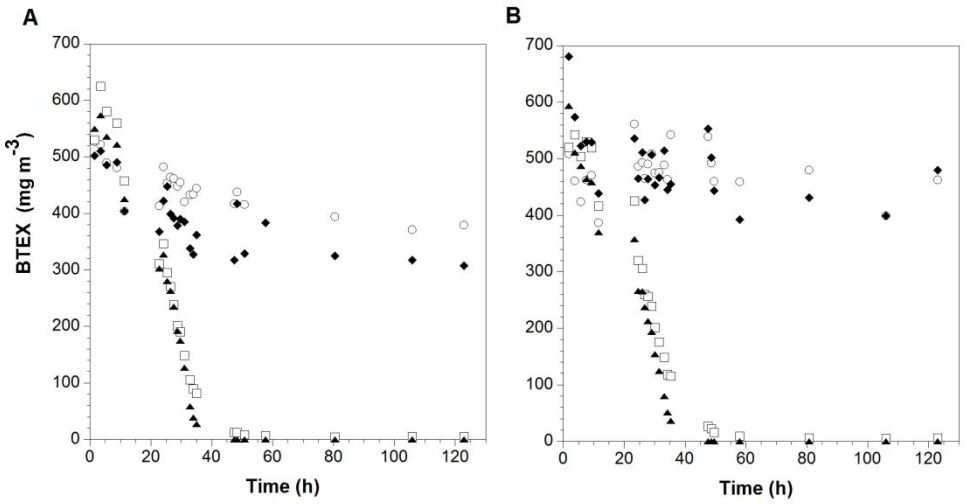

Figure 4. Time course of benzene (circle), toluene (square), ethyl-benzene (triangle) and o-xylene (diamond) concentration during the quaternary mixture kinetic test.

4. Conclusions

The kinetic studies of BTEX biodegradation under anoxic conditions confirmed that E and T are readily biodegradable when present as the sole carbon and energy source. The addition of other carbon sources increased the time needed for biodegradation as a result of competitive inhibition. The degradation of B and X took place at lower rates as a result of the likely toxic effects of their biodegradation products. These results showed that interactions such as substrate competition and metabolite inhibition can influence the degradation kinetic compared to the biodegradation of the single compounds.

References

Barona A., Elias A., Arias R., Acha E., Cano I., 2007, Desorption and biolfiltration for the treatment of residual organic gases evolved in soil decontamination processes, Chemical Engineering Technology, 30 (11), 1499–1505

El-Naas M.H., Acio A.J., El Telib A.E., 2014, Aerobic biodegradation of BTEX: Progresses and Prospects, Journal of Environmental Chemistry, Eng. 2, 1114-1122

Lebrero R., Rodriguez E., Martin M., Garcia-Encina P.A., Munoz R., 2010, H_2S and VOCs abatement robustness in biofilters and air diffusion bioreactor: a comparative study, Water Resource 44, 3905–3914

Littlejohns J.V., Daugulis A.J., 2008, Kinetics and interactions of BTEX compounds during degradation by a bacterial Consortium, Process Biochemistry, 43, 1068–1076

Lu C., Lin M., Chu C., 2002, Effects of pH, moisture, and flow pattern on trickle-bed air biofilter performance for BTEX removal, Advances in Environmental Research, 6, 99-106

Muñoz R., Souza T.S.O., Glittmann L., Pérez R., Guillermo G., 2013, Biological Anoxic Treatment of O2-Free VOC Emissions From The Petrochemical Industry: A Proof Of Concept Study, Journal of Hazardous Material, 260, 442-450

Rahul, Mathur A.K., Balomajumder C., 2013, Biological treatment and modelling aspect of BTEX abatement process in a biofilter, Bioresource Technology, 142, 9-17

Robledo-Ortiz J.R., Ramirez-Arreola D.E., Perez-Fonseca A.A., Gomez C., Gonzalez-Reynoso O., Ramos-Quirarte J., Gonzalez-Nunez R., 2011, Benzene, toluene, and o-xylene degradation by free and immobilized P. putida F1 of postconsumer agave-fiber/polymer foamed composites, International Biodeterioration & Biodegradation, 65, 539– 546.

Singh K., Singh R.S., Rai B.N., Upadhyay S.N., 2010, Biofiltration of toluene using wood charcoal as the biofilter media. Bioresource Technology, 101, 3947–3951.

Trigueros D.E.G., Módenes N.A., Kroumov D.A., Espinoza-Quiñones F.R., 2010, Modeling of biodegradation process of BTEX compounds: Kinetic parameters estimation by using Particle Swarm Global Optimizer, Process Biochemisty, 45, 1355-1361