Biodegradation **6:** 19–27, 1995.

Cometabolic transformation of *o*-xylene in a biofilm system under nitrate reducing conditions

Jean-Pierre Arcangeli & Erik Arvin
Department of Environmental Engineering/Groundwater Research Centre, Technical University of Denmark, Building 115, DK-2800 Lyngby, Denmark

Received 25 November 1993; accepted in revised form 9 February 1994

Key words: o-xylene, toluene, biofilm, denitrification, cometabolism, competitive inhibition, kinetics

Abstract

The purpose of this work was to investigate the anaerobic transformation of *o*-xylene in a laboratory biofilm system with nitrate as an electron acceptor. *o*-Xylene was degraded cometabolically with toluene as primary carbon source. A mass balance showed that *o*-xylene was not mineralized but transformed. *o*-Methyl-benzalcohol and *o*-methyl-benzaldehyde were identified as intermediates of *o*-xylene transformation which resulted in the formation of *o*-methyl-benzoic acid as an end product. A cross inhibition phenomenon was observed between toluene and *o*-xylene. The presence of toluene was necessary for stimulation of *o*-xylene transformation, but above a toluene concentration of 1–3 mg/L the *o*-xylene removal rate dramatically decreased. In return *o*-xylene inhibited the toluene degradation at concentrations above 2–3 mg/L.

Introduction

Monoaromatic compounds belong to a class of chemicals which are widely used in the industry and households, etc., for degreasing, as solvents and as primary constituent in gasoline. Therefore it is not surprising that they are often encountered in soil, groundwater and wastewater. These compounds are mostly benzene, toluene, ethylbenzene, and xylenes (BTEX).

It is well-known that these products are easily biodegradable in aerobic environments. However, aerobic biorestoration is limited by the difficulties to provide sufficient oxygen to the contaminated sites due to the low solubility of oxygen. Therefore, alternative electron acceptors are of interest when heavily contaminated sites are encountered. Denitrifying conditions have received much attention since the addition of nitrate to a contaminated site would be a feasible *in situ* technique due to the low cost and the high solubility of this electron acceptor. Several reports on anaerobic biodegradation of BTEX have been published. They show that this family of compounds can be degraded under denitrifying conditions (Hutchins & Wilson 1991; Evans et al. 1991). However, *o*-xylene degradation has been shown to be toluene-dependant, (Jensen et al. 1988; Evans et al. 1991).

Jørgensen (1992) investigated the pathway of the cometabolic transformation of *o*-xylene with toluene as primary substrate under denitrifying conditions. He showed that the initial steps of *o*-xylene degradation is an oxidation of a methyl group to form *o*-methyl-benzylalcohol, *o*-methyl-benzylaldehyde and *o*-methyl-benzoate (Fig. 1). Since the microbial consortium was able to degrade these metabolites without toluene present, it was concluded that the initial oxidation of the methyl group of *o*-xylene to *o*-methyl-benzylalcohol is catalysed by a toluene-induced enzyme. Evans et al. (1992) also found that the degradation of *o*-xylene was induced by toluene, but by a different mechanism. They reported the formation of dead-end products (2-methyl-benzylsuccinic-acid and 2-methyl-benzylfumaric-acid). Based on these observations, they suggested a totally different pathway involving an attack of the methyl group of *o*-xylene by succinyl-coenzyme-A.

The aim of the work described in this paper was to investigate the kinetics of *o*-xylene removal and formation of by-products, and finally to study the interactions

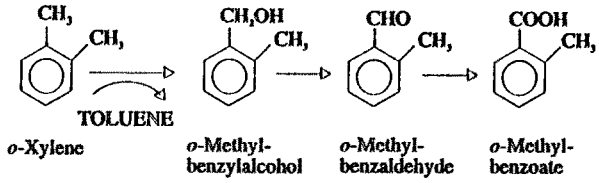

Fig. 1. Pathway of the degradation of *o*-xylene in a mixed nitrate reducing culture growing on toluene (Jørgensen 1992).

between toluene and *o*-xylene (cometabolism, competitive inhibition). These investigations were performed under circumstances where the biomass was grown with toluene as sole carbon source, in a continuously feed fixed film bioreactor.

Materials and methods

Biofilm reactor and culture conditions

The experimental system is depicted schematically in Fig. 2. The biofilm reactor is a so-called biodrum system (Kristensen & Jansen 1980). It consists of a cylinder rotating inside another with the biomass growing on the surface of the rotator and the stator. The rotation (200 RPM), as well as the recycling system ensures total mixing in the bulk liquid, and therefore provides a relatively uniform biofilm growth in the reactor.

The reactor was protected from the light in order to prevent growth of photosynthetic organisms. The characteristics of the biofilm reactor and the operating conditions for the experiments are summarized in Table 1.

The reactor was inoculated with an enrichment culture originating from denitrifying sewage sludge (Jørgensen 1992). The inoculation was done by injecting 100 ml of this culture into the reactor and by letting the biomass be attached to the walls of the rotator and stator. After two days, feeding of the reactor was initiated. All experiments were done under non-sterile condition at a temperature of 25 °C and at a pH of 7 ± 0.1.

Substrate

An artificial substrate was fed into the reactor typically with a flow rate of 4.5 L h^{-1}. The feed stream with a mineral medium was mixed with four stock solutions.

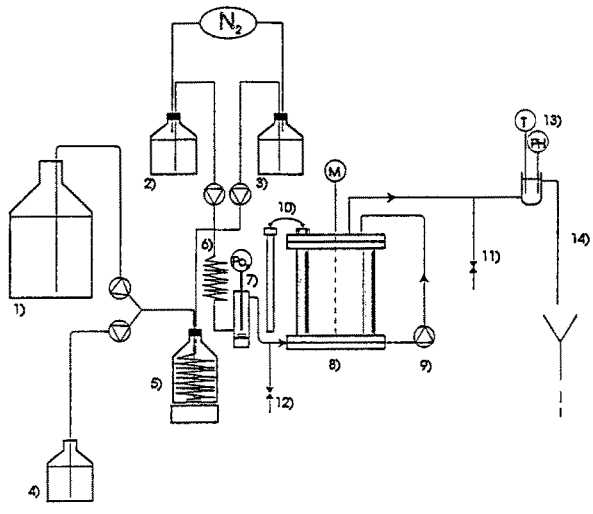

Fig. 2. Experimental setup: (1) mineral medium; (2) toluene stock solution; (3) *o*-xylene stock solution; (4) nitrate stock solution; (5) oxygen removal chamber; (6) mixing coil; (7) oxygen electrode; (8) biofilm reactor; (9) recirculation pump; (10) removable slides; (11) inlet sampling port; (12) outlet sampling port; (13) temperature and pH control; (14) outlet.

Table 1. Operational data for the biofilm reactor. During kinetics experiments the nitrate concentration was raised in order to keep it in excess.[a]

	Unit	Value
Surface area	m^2	0.16
Reactor volume	m^3	0.96×10^{-3}
Average flow of water	L/h	4.5
Toluene infl. conc.	mg/L	5
Nitrate infl.	mg N/L	50-200[a]
Phosphate infl.	mg P/L	46.5
Ammonium infl.	mg N/L	10.4
Temperature	°C	25 ± 1
Alkalinity	meq/L	20.8
pH in the reactor	6.9-7.1	

The major reasons for having several stock solutions were: (a) Toluene and *o*-xylene concentrations were varied independently, and (b) bacterial growth in the stock solution must be avoided.

The mineral medium contained the following salts dissolved in distilled water (mg/L): NH_4Cl, 198; $CaCl_2$, H_2O, 147; $NaHCO_3$, 1273; KCl, 500; Na_2SO_4, $10H_2O$, 161; $MgCl_2$, $6H_2O$, 407; KH_2PO_4, 204 and NaCl, 993.

The trace minerals were prepared in 1 litre of distilled water: $FeCl_2$, $4H_2O$, 1500 mg; $MnCl_2$, $4H_2O$, 100 mg; $CoCl_2$, $6H_2O$, 190 mg; $CuCl_2$, $2H_2O$, 2 mg; Na_2MoO_4, $2H_2O$, 36 mg; NiCl, $6H_2O$, 24 mg; H_3BO_4, 6 mg; $ZnCl_2$, 70 mg. The solution was acidified with 10 ml of a 7.7 N HCl. This microion stock solution was diluted 1,000-fold (1 ml solution in 1 L. mineral medium).

Nitrate was supplied in excess in order to restrict the reduction of nitrate to nitrite, and thus avoid the formation of nitrogen which might damage the biofilm.

The dissolved oxygen in the medium was continuously removed using a membrane chamber. The chamber consists of a five litre reservoir containing a solution of Na_2SO_3 (100 g/L) and $CoCl_2$ (200 mg/L) in which was immersed 20 m of silicone tubing through which the medium flowed (wall thickness 1 mm; inner diameter, 4 mm). Silicone is highly permeable to gases (Kjeldsen 1992; Zeyer et al. 1986); the oxygen diffuses through the silicone membrane and is absorbed in the sulphite solution. The presence of $CoCl_2$ catalyses the absorption process.

Using this setup the oxygen concentration in the substrate to the biofilm reactor could be reduced below the detection limit (0.05 mg/L). The influent oxygen concentration was constantly monitored with a Clark-type oxygen electrode (WTH oxy 196) and on a less frequent basis with the azide modification of the Winkler method (APHA 1989).

Analytical techniques

Analysis of toluene and *o*-xylene was performed with a Dani 8520 gas chromatograph (Merck) equipped with a 30 m J.& W. Scientific DB5 capillary column (i.d. 0.53 mm, film thickness 1.5 μm, cat n° 125–1032) and a FID detector kept at 275 °C. The injection port was 36 °C in 1 min. The injection port was purged with N_2 at a flow 10 times higher than the carrier gas flow until the rise of the injector temperature was increased by 30 °C per min to 250 °C, and kept there for 0.5 min. Nitrogen was used as the carrier gas at 10 ml/min. Extraction of the aromatic compounds was carried out by acidifying 10 ml of sample in a volumetric flask by four drops of a 3 M phosphoric acid, and adding 1 ml of diethyl ether containing heptane as an internal standard (4.1 mg/L). The sample was then shaken vigorously by hand for 3 min. As much as possible of the organic phase was transferred to a 0.9 ml vial and 1 μl of the organic phase was injected. At low concentration (< 500 μg/L) the organic phase was evaporated to about 25 μl under a stream of nitrogen gas to achieve a better detection limit. Samples were analyzed immediately because *o*-xylene by-products decayed completely in the organic phase within 24 hours. The peak areas were determined by integration on a MAXIMA 820 chromatographic workstation (Millipore Corporation, Massashusetts) and the concentrations of the aromatic compounds were calculated on the basis of standard curves.

Nitrate and nitrite were measured by spectrophotometry at 545 nm after reaction with sulphanilamide and N-(1-naphthyl)-ethylendiamine dihydrochloride at Ph 2. NO_3^- was measured as NO_2^- after reduction with hydrazinsulphate (Kamphake et al. 1967).

Experimental design

The biofilm was grown anaerobically with toluene as sole carbon source at an inflow concentration of 5 mg/L. During this time period various series of experiments were carried out (series X1–4). (1) Verification of the cometabolism between toluene and *o*-xylene, X1. (2) Identification of degradation products, and stoichiometry. (3) Influence of the primary substrate concentration (toluene) on the *o*-xylene removal rate, X2 and X4. (4) Inhibition effect of *o*-xylene on the toluene removal rate, X3.

Results

Cometabolic o-xylene removal

The evidence of cometabolism of *o*-xylene with toluene as primary substrate is supported by Fig. 3A,B which demonstrates that the *o*-xylene transformation and the associated by-products formation only occurred concomitant to toluene degradation. Furthermore, it is clear that the transformation of *o*-xylene leads to the formation of *o*-methyl-benzaldehyde (OMBA) and *o*-methyl-benzoic acid (OMBAC). The scattered data observed on OMBAC is due to its quick decay in the ether phase after extraction (see analytical techniques); the reason for that has not been identified.

Molar balance

The cumulative molar balances for four experiments (Fig. 4) suggest that *o*-xylene was not mineralized but transformed. The *o*-methyl-benzoic acid (OMBAC)

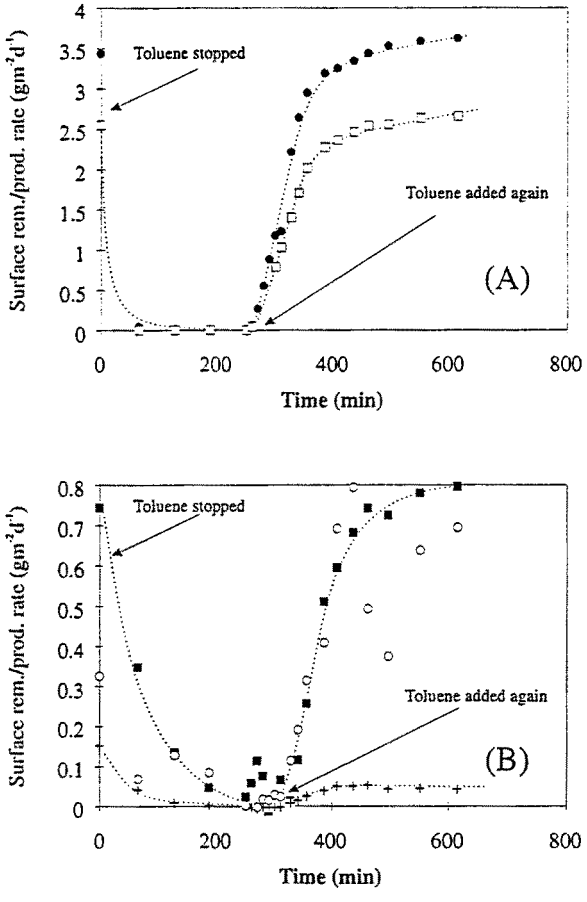

Fig. 3. Influence of toluene on the transformation rate of *o*-xylene. Toluene (□); nitrite (●); *o*-xylene (■); *o*-methyl-benzaldehyde (+); *o*-methyl-benzoic acid (○).

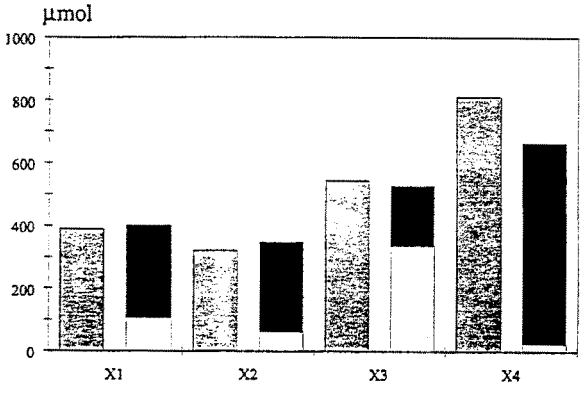

Fig. 4. Cumulative molar balance for kinetics test (X1–4). For each series two histograms are indicated: the first gives the accumulated *o*-xylene degraded; the second shows the accumulated product formed. *o*-xylene (▩); *o*-methyl-benzaldehyde (□); *o*-methyl-benzoic acid (■).

seems to be an end product in these experiments. The analytical procedure used did not allow a suitable quantification of *o*-methyl benzylalcohol. However, a molar balance suggests it was rapidly oxidized into the aldehyde. In the fourth test (X4) a discrepancy between *o*-xylene degraded and degradation products formed can be noticed. This could be the result of a partial mineralization of *o*-xylene or/and a formation of other by-products, not detected with the extraction/detection procedure employed.

Interaction between the degradation of o-xylene and toluene

Two tests (X2 and X4) were performed, where the *o*-xylene transformation was measured for different toluene concentrations in the reactor (Figs. 5 and 6). The *o*-xylene removal rate increased proportionally with the toluene bulk concentration. However, above a toluene concentration of approximately 2 mg/L the *o*-xylene removal rate dramatically decreased.

In test X4, the *o*-xylene and toluene removal rate was higher than in run X2, probably due to the larger amount of attached biomass. However, both tests showed that the maximum *o*-xylene removal rate occurred for a toluene bulk concentration in the order of 2 mg/L (Fig. 6A).

In these experiments, the *o*-xylene average concentration was 2.5 mg/L. This small concentration was sufficient to decrease the maximum substrate utilization rate, k_X, for toluene from 1.5 to 0.75 d^{-1}, whereas the apparent first- and half-order rate constant, $k_{1,a}$ and $k_{1/2,a}$, were not significantly affected (Table 3). The *o*-xylene inhibition of toluene degradation was demonstrated in test X3 (Fig. 7A). Both the toluene and *o*-xylene removal rate were measured for *o*-xylene bulk concentrations from 0 to 35 mg/L, and at a constant toluene inlet concentration of 12 mg/L. The toluene removal rate decreased linearly from 5 to 2 g m^{-2} d^{-1}. Besides, an accumulation of *o*-methyl-benzaldehyde, OMBA, was observed in the reactor outlet, indicating that oxidation to *o*-methyl-benzoic acid, OMBAC, was lowered (see molar balance test X3). This inhibition occurred when the *o*-xylene bulk concentration was above 3–5 mg/L.

Concerning the associated *o*-xylene removal rate (Fig. 7B), the degradation was first- order for an *o*-xylene bulk concentrations below 2 mg/L. Above a

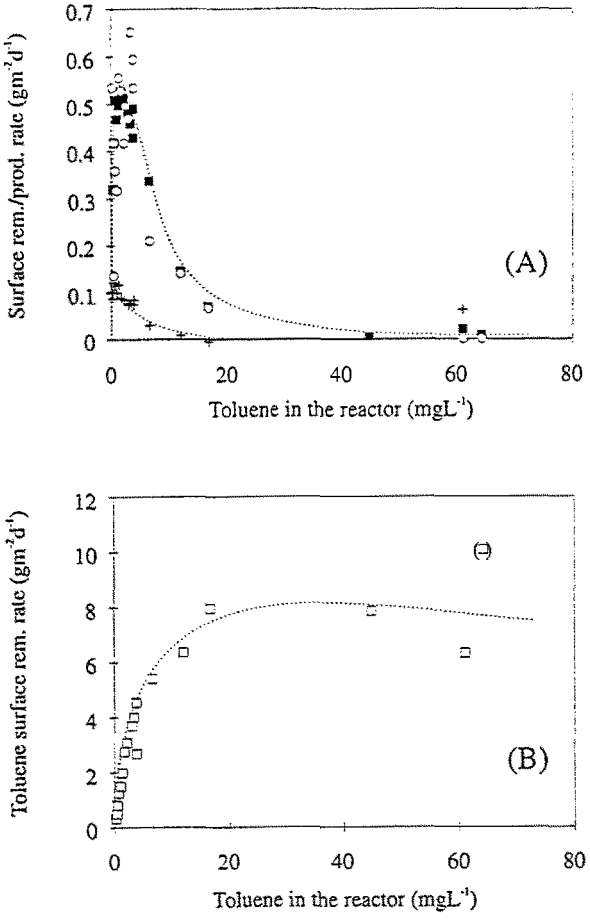

Fig. 5. Kinetic test X2. Toluene (□); *o*-xylene (■); *o*-methyl-benzaldehyde (+); *o*-methyl-benzoic acid (o). (A) Influence of toluene concentration on *o*-xylene transformation rate. (B) Associated toluene removal.

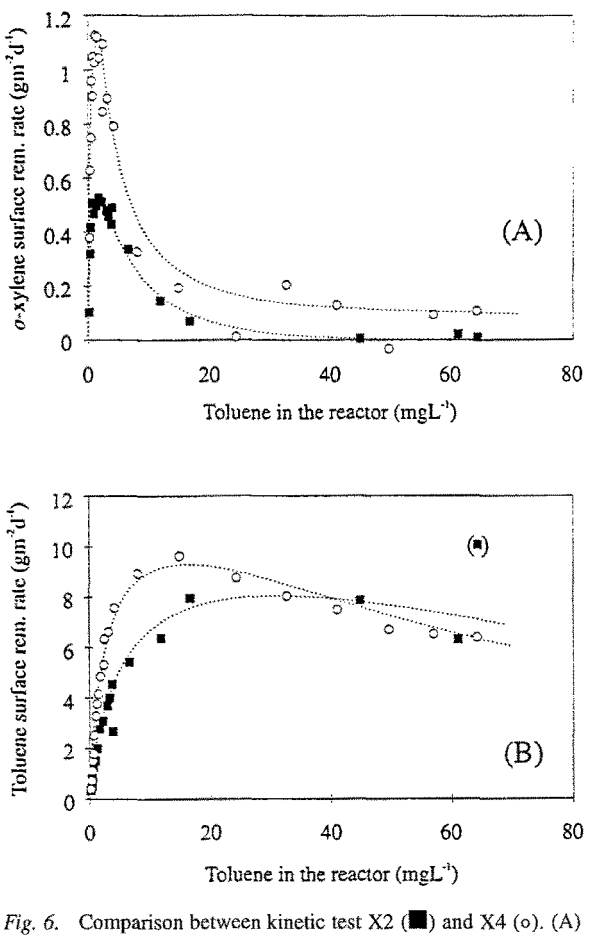

Fig. 6. Comparison between kinetic test X2 (■) and X4 (o). (A) Influence of toluene concentration on *o*-xylene transformation. (B) Associated toluene removal.

concentration of 5 mg/L, a zero-order reaction controlled the *o*-xylene degradation rate.

Kinetics of toluene biodegradation

The kinetics of toluene removal in the biofilm was interpreted according to the theory put forward by Harremoës (1978). Nomenclature and calculations are given in Appendix 1.

Kinetic data are summarized in Table 2. A first-order reaction controls the toluene degradation for a bulk concentration below 1–1.3 mg/L, leading to a half-saturation constant, K_S, of 1.71 and 0.77 mg/L for run X2 and X4, respectively. An average maximum substrate utilisation rate, k_X, of 0.75 d^{-1} was found for both tests.

Discussion

These results demonstrate that the degradation of *o*-xylene under nitrate reducing condition by a mixed culture is dependent on toluene as a primary substrate. It appears from the mass balance of the reaction that *o*-methyl-benzoic acid, OMBAC, is an end-product. In a batch system, the same culture was able to degrade *o*-methyl-benzoic acid subsequent to *o*-xylene transformation (Jørgensen 1992). However, *o*-methyl-benzoic acid degradation occurred within a time period of 10–15 days, while the toluene was depleted in only 1 day. Furthermore, the fate of *o*-methyl-benzoic acid could not be characterized. In our study, the residence time in the reactor was less than 13 min, which explains the lack of further transformation of *o*-methyl-benzoic acid. The same metabolite has been found to be an end-product for the methanogenic degradation of *m*-

Table 2. Kinetic parameters of toluene degradation. Nomenclature and calculation are shown in Appendix 1 (see Fig. 6B).

Run	L (μm)	$k_{1,a}$ (m/d)	$k_{0,a}$ ($gm^{-2}d^{-1}$)	$k_{1/2,a}$ ($g^{1/2}m^{-1/2}d^{-1}$)	D_f (10^4 m^2d^{-1})	ε	α	$k_{1,f}$ (d^{-1})	$k_{0,f}$ ($gm^{-3}d^{-1}$)	k_x (d^{-1})	K_s (g/m^3)	αNO_2^-
X2	311	1.5	7	3.00	1.75	0.37	2.69	13106	22508	0.71	1.71	1.2
X4	378	3.6	9.3	4.8	4.68	0.34	2.92	28011	24603	0.77	0.88	1.47

NB! Biomass dry weight density, X_f, was 31851 gm^{-3}.

Table 3. Kinetic parameters related to toluene biodegradation. Effect of *o*-xylene on the kinetic of toluene biodegradation.

	k_X (d-1)	$k_{1,a}$ (m/d)	$k_{1/2,a}$ ($g^{1/2}m^{-1/2}d^{-1}$)	K_S (mg/L)
Toluene sole carbon source	1.35-1.8	1.2-1.6	1.2-3.8	0.6-1.7
Toluene and *o*-xylene[a]	0.7-0.8	1.5-3.6	3.0-4.8	0.9-1.7

From Arcangeli and Arvin(1993b).
[a] average *o*-xylene inlet concentrations: 2.5 mg/L
The nomenclature is specified in the Appendix.

cresol (Robert et al. 1990). It could be degraded providing a lag time of 12 to 36 weeks (Londry & Fedorak 1992). The microbial degradation of *o*-methyl-benzoic acid has been investigated by Higson & Focht (1992). They reported the isolation of *Pseudomonas cepacia* MB2, which is believed to be the first microorganism able to utilize *o*-methyl benzoic acid as a sole carbon source.

Experiments, X2 and X4, reveal that the kinetics of *o*-xylene removal is strongly dependent upon the toluene concentration (Fig. 6A). It was found that lack of the primary carbon source, toluene, ceases the *o*-xylene transformation. This confirms results obtained by Jørgensen (1992) that the *o*-xylene transformation is enhanced by a toluene-induced enzyme. On the other hand, if provided in excess, the toluene inhibits *o*-xylene transformation. These interactions may be explained by competitive inhibition between both substrates if it is assumed that *o*-xylene and toluene are degraded by the same enzyme and that *o*-xylene has a lower affinity for this enzyme than toluene.

This competitive inhibition is of practical interest for groundwater remediation, since the degradation of some pollutants can be maximized using the optimum concentration of primary substrate. For example, Barbaro et al. (1992) showed that the anoxic degradation of ethylbenzene and xylenes in an aquifer was dependent upon the toluene-degrading bacteria, but could be inhibited competitively if the toluene was provided at too high concentrations.

The result from experiment X3 reveals a decreasing toluene consumption for increasing concentration of *o*-xylene (Fig.7A), which can be explained by a competition between toluene and the secondary substrate for the same enzyme. This inhibitory effect appears for a *o*-xylene concentration above 1–1.5 mg/L. However, kinetic data summarized in Table 3 show a significant decrease of the maximum substrate utilisation rate, k_X, even though the *o*-xylene concentration was low. An explanation could be a toxic effect on top of a competitive inhibition. The presence of *o*-xylene, even at very low concentrations, could decrease the biomass activity, leading to a lower toluene degradation rate. This is supported by the fact that although the toluene bulk concentration was increased in the reactor, this toxic effect still existed (Fig. 6B): the concentration threshold above which the toluene removal rate decreases was 15–20 mg/L, contrary to 40–50 mg/L when toluene was the sole substrate (data not shown). However, it seems that at low toluene concentration this toxic effect from *o*-xylene is unnoticeable: the toluene first- and half-order rate constants, $k_{1,a}$ and $k_{1/2,a}$, were not affected by the presence of *o*-xylene at low concentration (Table 3). The reason for that could not be identified.

These parameters are of practical interest for groundwater pollution since they describe the toluene degradation at low concentrations, typically in the

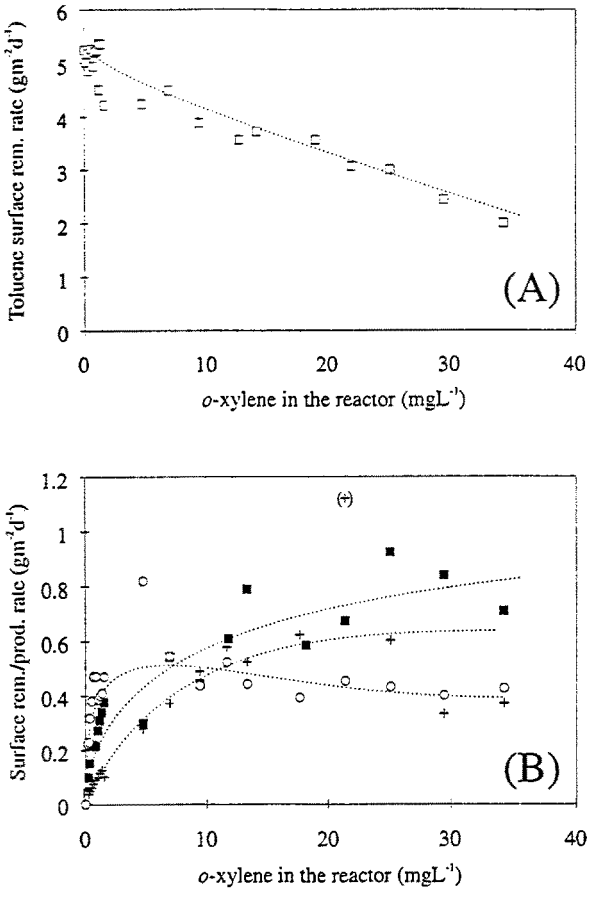

Fig. 7. Kinetic test X3. Toluene (□); *o*-xylene (■); *o*-methyl-benzaldehyde (+); *o*-methyl-benzoic acid (o). (A) Influence of *o*-xylene concentration on toluene removal. (B) associated *o*-xylene transformation and product formation.

range from 0 to 10 mg/L of toluene. Consequently, their small variability make those parameters suitable for the design of treatment processes for groundwater remediation.

The competition between toluene and *o*-xylene for the same enzyme reveals interactions where the presence of toluene affects the kinetics of *o*-xylene transformation and vice versa. Similar interactions have been observed for the aerobic biodegradation of benzene toluene and *p*-xylene (Jensen 1992; Chang et al. 1993□□□), or the biotransformation of TCE by methanotrophic bacteria (Broholm et al. 1992; Semprini et al. 1991□□□). Practically, it is very rare to find sites contaminated with a single pollutant. Consequently, the knowledge of mechanisms which control substrate interactions, and thus, the kinetics, is of practical interest for the design of treatment processes for groundwater remediation. A practical approach is to define a feasible region where the removal of the co-substrate is optimized. Fig. 6A suggests that the degradation of 2.5 mg/L of *o*-xylene was maximum when toluene was in the concentration range from 0.5 to 3 mg/L. A lower *o*-xylene concentration did not influence significantly this result (data not shown), which is relevant since low concentrations, typically below 3mg/L, are generally encountered in polluted groundwater. However, it is likely that higher *o*-xylene concentrations would require a higher amount of primary substrate, toluene, in order to induce enzymes for the *o*-xylene transformation. Furthermore, above 3 mg/L the *o*-xylene inhibits the toluene degradation (Fig. 7A). Consequently, for heavily polluted environments, the recourse to computer simulation would be necessary in order to predict the optimal amount of primary substrate to use. For that purpose, interactions between toluene and *o*-xylene have been used in order to calibrate a model. This model uses kinetics expressions, incorporating competitive inhibition and coupled cometabolic degradation. Results and details of this modelling are reported elsewhere (Arcangeli & Arvin 1993).

Conclusions

The experiments with a mixed culture of denitrifying bacteria showed that *o*-xylene could be transformed cometabolically with toluene as primary carbon source.

The first step in this transformation was a methyl group oxidation resulting in a transient accumulation of *o*-methyl benzaldehyde and *o*-methyl benzoic acid. The last was found to be an end product.

The kinetics of degradation reveals that the microbial transformation of *o*-xylene under nitrate reducing condition is dependant on a toluene-induced enzyme. However, if the concentration of toluene, the primary substrate, is too high, a competitive inhibition of *o*-xylene transformation will occur. In return, it was found that the toluene degradation was competitively inhibited by *o*-xylene. The reason for this cross-competitive inhibition is assumed to be a competition between toluene and *o*-xylene for the same enzyme.

Acknowledgements

The authors would like to thank Dr. C. Jørgensen for valuable discussions. This study was support by the Groundwater Centre at the Technical University of Denmark.

Appendix

Nomenclature:

S	:	substrate concentration (mg/L)
$r_{1,a}$, $r_{1/2,a}$ and $r_{o,a}$	:	1^{st}, 1/2 and 0′ order surface removal rates ($gm^{-2}d^{-1}$)
$k_{1,a}$, $k_{1/2,a}$ and $k_{o,a}$	:	1^{st}, 1/2 and 0′ order rate constants (md^{-1}, $g^{1/2}m^{-1/2}d^{-1}$ and $gm^{-2}d^{-1}$, respectively)
$k_{1,f}$ and $k_{o,f}$	:	1^{st} and 0′ order intrinsic reaction rate constants in the biofilm (d^{-1} and $gm^{-3}d^{-1}$, respectively)
k_x	:	maximum substrate utilisation rate (d^{-1})
K_s	:	Monod constant (half-saturation constant) (mg/L)
X_f	:	biofilm dry weight density (gm^{-3})
D	:	diffusion coefficient of the substrate in the biofilm (m^2/d)
L	:	biofilm thickness (m)
α	:	biofilm constant (–)
ε	:	1^{st} order efficiency (–)

Equations:

First-order removal rate : $r_{1,a} = k_{1,a} S$ (1)

Half-order removal rate : $r_{1/2,a} = k_{1/2,a} S^{1/2}$ (2)

Zero-order removal rate : $r_{o,a} = k_{o,a}$ (3)

With:

$$k_{1,a} = k_{1,f} L\varepsilon = \frac{k_x}{K_s} X_f L\varepsilon \quad (4)$$

$$k_{1/2,a} = \sqrt{2Dk_{0,f}} \quad (5)$$

$$k_{o,a} = k_x X_f L = k_{o,f} L \quad (6)$$

$$\varepsilon = \frac{\mathrm{Tanh}\,\alpha}{\alpha} \quad (7)$$

where

$$\alpha = \sqrt{\frac{k_{1,a} L}{\varepsilon D}} \quad (8)$$

Reaction rate constant are measured graphically in Fig. 6B. X_f and L were determined experimentally. The diffusion coefficient of toluene in the biofilm is found from Equation (5). After having determined ε through iteration from Equations (7) and (8), the Monod constant, K_S, can be estimated from Equation (4).

References

APHA (1989) Standard Methods for the Examination of Water and Wastewater, 17th Edition. American Public Health Association, Washington, DC

Arcangeli JP & Arvin E (1993a) Kinetics of toluene degradation in a biofilm reactor under denitrifying conditions. Accepted for presentation at the 2^{nd} International Specialized Conference on Biofilm Reactors, IAWQ, Paris, France, September 29 – October 1, 1993

Arcangeli JP & Arvin E (1993b) Modelling of *o*-xylene cometabolic transformation in a biofilm reactor under denitrifying conditions. Biodeg. 6: 27–36 (this issue)

Barbaro JR, Baker JF, Lemon LA & Mayfield CI (1992) Biotransformation of BTEX under anaerobic, denitrifying condition: field and laboratory observations. J. Contam. Hydrol. 11: 245–272

Broholm K, Christensen TH & Jensen BK (1992) Modelling TCE degradation by a mixed culture of methane-oxidizing bacteria. Wat. Res. 26: 1177–1185

Chang MK, Voice CV & Criddle CS (1993) Kinetics of competitive inhibition and cometabolism in the biodegradation of benzene, toluene, and *p*-xylene by two *Pseudomonas* isolates. Biotech. Bioengineer. 41: 1057–1065

Evans PJ, Mang DT, Kim KS & Young LY (1991) Anaerobic degradation of toluene by a denitrifying bacterium. Appl. Environ. Microbiol. 57: 1139–1145

Evans PJ, Ling W, Goldschmidt B, Ritter ER & Young LY (1992) Metabolites formed during anaerobic transformation of toluene and *o*-xylene and their proposed relationship to the initial steps of toluene mineralization. Appl. Environ. Microbiol. 58: 496–501

Harremoës P (1978) Biofilm kinetics. In: Mitchell R (Ed) Water Pollution Microbiology, Vol 2 (pp 82–109). John Wiley, New York

Higson FK & Focht DD (1992) Degradation of 2-methylbenzoic acid by *Pseudomonas cepacia* MB2. Appl. Environ. Microbiol. 58: 194–200

Hutchins SR & Wilson JT (1991) Laboratory and field studies on BTEX biodegradation in a fuel-contaminated aquifer under denitrifying condition. In: Hinchee RE & Olfenbuttel RF (Eds) *In Situ* Bioreclamation. Applications and Investigations for Hydrocarbon and Contaminated Site (pp 157–172). Butterworth-Heineman, Stoneham, MA

Jensen HM (1992) Kinetic of toluene and benzene biodegradation. Statistical analysis. Master Thesis Report (In Danish). The Institute of Mathematical Statistics and Operations Research, The Technical University of Denmark, DK 2800 Lyngby, Denmark

Jensen BK, Arvin E & Gundersen AT (1988) Biodegradation of phenolic and monoaromatic hydrocarbons by a mixed wastewater culture under denitrifying conditions. Presented at Cost 691/681 Workshop on Organic Contaminants in Wastewater, Sludge & Sediments: Occurrence, Fate and Disposal, Brussels, Oct. 26–27, 1988

Jørgensen C (1992) Anaerobic microbial degradation of aromatic hydrocarbons. Ph.D. Thesis. Department of Environmental Engineering, The Technical University of Denmark, DK 2800 Lyngby, Denmark

Kamphake LJ, Hannah SA & Cohen JM (1967) Automated analysis for nitrate by hydrazine reduction. Wat. Res. 1:205–216

Kjeldsen P (1992) Evaluation of gas diffusion through plastic materials used in experimental and sampling equipment. Wat. Res. 27: 121–131

Kristensen GH & Jansen JLC (1980) Fixed Film Kinetics. Description of Laboratory Equipment. Department of Environmental Engineering, Technical University of Denmark, DK 2800 Lyngby, Denmark

Londry KL & Fedorak PM (1992) Anaerobic degradation of 2-methylbenzoic acid by a methanogenic consortium. Submitted to Can. J. Microbiol.

Roberts DJ, Fedorak PM & Hrudey SE (1990) CO_2 Incorporation and 4-Hydroxy-2-Methylbenzoic Acid Formation during Anaerobic Metabolism of *m*-Cresol by a Methanogenic Consortium. Appl. Environ. Microbiol. 56: 472–478

Semprini L, Hopkins GD, Roberts PV, Grbic-Galic D & McCarty PL (1991) A field evaluation of *in-situ* biodegradation of chlorinated ethenes: Part 3, Studies of competitive inhibition. Ground Water 29: 239–250

Zeyer J, Kuhn EP & Schwarzenbach RP (1986) Rapid microbial mineralization of toluene and 1,3-dimethylbenzene in the absence of molecular oxygen. Appl. Environ. Microbiol. 52: 944–947