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The influence of creosote compounds on the aerobic degradation of toluene

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Abstract

The inhibiting effect of 14 typical creosote compounds on the aerobic degradation of toluene was studied in batch experiments. Four NSO-compounds (pyrrole, 1-methylpyrrole, thiophene, and benzofuran) strongly inhibited the degradation of toluene. When the NSO-compounds were present together with toluene, little or no degradation of toluene was observed during 16 days of incubation, compared with a total removal of toluene within 4 days when the four compounds were absent. Indole (an N-compound) and three phenolic compounds (phenol, *o*-cresol, and 2,4-dimethylphenol) also inhibited the degradation of toluene, though the effect was much weaker that of the four NSO-compounds. *O*-xylene, *p*-xylene, naphthalene and 1-methylnaphthalene seemed to stimulate the degradation even though the influence was very weak. No effects of benzothiophene (an S-compound) and quinoline (an N-compound) were observed. Benzofuran (an O-compound) was identified as the compound that most inhibited the degradation of toluene. An effect could be detected even at low concentrations (40 μg/l).

Abbreviations: bf–benzofuran, bt–benzothiophene, dmp–2,4-dimethylphenol, GC–gas chromatograph, ind–indole, mnap–1-methylnaphthalene, MAH–monoaromatic hydrocarbons, mpyr–1-methylpyrrole, nap–naphthalene, *o*-cre–*o*-cresol, *o*-xyl–*o*-xylene, phe–phenol, pyr–pyrrole, *p*-xyl–*p*-xylene, tol–toluene, thi–thiophene, qui–quinoline

Introduction

At many coal-tar and related creosote waste sites, groundwater may be contaminated with a complex mixture of pollutants. Creosote/coal-tar consists of hundreds of compounds including the polyaromatic hydrocarbons (PAH), the monoaromatic hydrocarbons (MAH), the phenolic compounds and the heterocyclic aromatic hydrocarbons containing nitrogen, sulphur or oxygen (NSO-compounds). These compounds can all be dissolved in the groundwater but the concentrations vary widely and are mainly controlled by the solubilities of the compounds and the composition of the creosote. Observations from field sites show great variation in the composition of the contaminants in the groundwater (Ehrlich et al. 1982; Stuermer et al. 1982; Pereira et al. 1983; Goerlitz et al. 1985; Pereira & Rostad 1986; Turney & Goerlitz 1990; Godsy et al. 1992; Lotimer et al. 1992). Lotimer et al. (1992) observed high concentrations of MAH (up to 65000 μg/l) and PAH (2500 μg/l) but low concentrations of the phenolic compounds in a limestone aquifer, whereas high concentrations of phenol (10400 μg/l) and an N-compound (quinoline, 15600 μg/l) were observed in the groundwater contaminated by a wood preserving plant (Goerlitz et al. 1985). Other NSO-compounds were also detected, but in lower concentrations (<1,000 μg/l) (Pereira & Rostad 1986; Godsy et al. 1992). These observations show that the pollution from creosote-contaminated sites can be very complex because of the variety and concentrations of the organic compounds involved.

Different mechanisms for removing creosote compounds occur in the groundwater. Losses can happen through evaporation and sorption to sediment (abiotic processes), but the main mechanism is thought to

be biodegradation. Many studies have been carried out to investigate the degradation of creosote compounds under different redox conditions. In most cases, studies concentrated on the degradation of single compounds or of simple mixtures of compounds (Alvarez et al. 1991; Thomas & Lester 1993; Corseuil & Weber 1994). These studies may give an incorrect estimate of the biodegradation potential in the groundwater, since contamination in most cases consists of many different compounds. Studies about the degradation potential with complex mixtures have been reported (Mueller et al. 1991; Godsy et al. 1992; Nielsen et al. 1994) but only a few have investigated the interactions between different compounds and the resulting toxicity (Arvin et al. 1989; Alvarez & Vogel 1991).

The purpose of this work was:

- to study the inhibiting effect of 14 typical creosote compounds in relation to the aerobic degradation of toluene;
- to identify the compound that was most inhibitory to the degradation of toluene;
- to simulate the degradation potential in groundwater aquifers by conducting degradation experiments with complex mixtures of creosote compounds.

Materials and methods

Experimental systems

The degradation experiments (Experiments A, B and C, below) were all conducted as batch experiments in 117 ml serum bottles with Mininert valves. The serum bottles were rotated (2 rpm) in a dark box at room temperature (20±3 °C) throughout the experiments.Two or three bottles in each experiment acidified with 8 N sulphuric acid (pH≈1) were used for control of abiotic processes.

Experimental setup, experiment A

The purpose of Experiment A was to investigate the influence of fourteen typical creosote compounds on the aerobic degradation of toluene. The inoculum used for the experiment was an aerobic enrichment culture that originated from the groundwater at a creosote-contaminated aquifer in Fredensborg, Denmark. The site is polluted from a public gasworks operating between 1906 and 1968. The inoculum had been spiked twice with different mixtures of creosote compounds for one year before the start of Experiment A. The inoculum was transferred to a 10 l glass container containing tap water. NH_4Cl (4 mg N/l) was added as the nitrogen source, and Na_2HPO_4 (1 mg P/l) was added as the phosphate source. pH was adjusted to 7.5 with NaOH. 100 ml from the glass container was transferred to 67 serum bottles. Before a serum bottle was sealed, chemicals from stock solutions were added according to the statistical design. The initial concentrations of the compounds were (all concentrations in mg/l): phenol (phe) 6, *o*-cresol (*o*-cre) 5, thiophene (thi) 4, 1-methylpyrrole (mpyr) 4, pyrrole (pyr) 4, 2,4-dimethylphenol (dmp) 4, toluene (tol) 3, indole (ind) 3, quinoline (qui) 3, benzofuran (bf) 1.8, benzothiophene (bt) 1.4, *o*-xylene (*o*-xyl) 0.9, *p*-xylene (*p*-xyl) 0.9, naphthalene (nap) 0.4, and 1-methylnaphthalene (mnap) 0.2. The chemicals used were purchased from Merck, Germany and of analytical grade. Samples were taken from each bottle at time zero and after 4, 8, and 16 days.

Experimental setup, experiment B and C

In Experiment A it was shown that the combination of thi, pyr, mpyr, and bf had a significant effect on the degradation of toluene. The purpose of Experiment B was to identify the most inhibiting compound among thi, pyr, and bf, when other experiments had shown that mpyr did not affect the degradation of toluene. The purpose of Experiment C was to investigate the effect of the most inhibiting compound, bf. For both experiments the inoculum was prepared as follows: the remaining liquid from all serum bottles from the previous experiment was pooled together and centrifuged (2000 rpm for 20 min, IEC centra-7 centrifuge). Biomass was then transferred to a 5 l glass container containing tap water. NH_4Cl (4 mg N/l) and Na_2HPO_4 (1 mg P/l) were added to the container. pH was adjusted to 7.5 with NaOH. The suspended solids concentration was approximately 190 mg/l and the biomass concentration (volatile suspended solids, VSS) was approximately 30 mg/l. 100 ml from the glass container was transferred to each serum bottle. Before a serum bottle was sealed, chemicals from stock solutions were added according to the statistical design. The initial concentrations in Experiment B were (mg/l): pyr 4, thi 3, tol 2, and bf 1.5. In Experiment C the initial concentrations were: thi 3 mg/l, tol 3 mg/l, bf 0, 40, 90, 270, 450, 630, 900 and 1800 µg/l. Samples were taken at time zero and after 1, 4, 6, and 21 days in Experiment B, while in Experiment

C, samples were taken at time zero and after 1, 3, 5, 8, and 54 days.

The statistical design, experiment A

Experiment A was designed as a reduced $1/2 \cdot 2^6$ factorial experiment. The percent degradation of tol was the dependent variable while the six independent factors (A–F) were: A: thi, pyr, mpyr, and bf; B: bt; C: ind; D: qui; E: *p*-xyl, *o*-xyl, nap, and *m*-nap; F: phe, *o*-cre, dmp, and nap. By mistake, nap was included in both Factor E and F. The factorial experiment was designed so Factor F was confounded with the five-factor interaction A*B*C*D*E (alias relation was F=ABCDE). All other main factors were also confounded with a five-factor interaction. The two-factor interactions were confounded with the four-factor interactions, while the three-factor interactions were confounded with each other. It was assumed that interactions higher than third order were insignificant. This design required 32 bottles, but because duplicates were run for each bottle, 64 bottles were used. The statistical analysis was done with a statistical program (SAS 1985) using the procedure ANOVA. The analysis was based on the assumption of equal variances. An analysis was run separately on data from day 4, day 8, and day 16. The model included all main factors, all interactions between two factors and the interaction between Factors A, E, and F (or B, C, and D).

The statistical design, experiment B

Experiment B was designed as an incomplete 2^3 factorial experiment. Again the percent degradation of tol was the dependent variable while the 3 factors (A–C) were A: thi; B: pyr; C: bf. The only combination missing from a complete 2^3 factorial experiment was the interaction between Factor A and B, because a previous experiment showed that this combination had no significant effect on the toluene degradation (data not shown). Duplicates were run for each combination. This design required 14 batches.

The experimental design, experiment C

Experiment C was run as a dose-response experiment with the percent degradation of tol as the dependent variable, while bf was the independent variable. Eight different levels of bf concentrations were used. To prevent bf from being degraded, thi was added to all batches. Replicates were run for all batches.

Chemical analysis

Ten ml of sample from a serum bottle was withdrawn with a glass syringe and replaced by 10 ml of pure oxygen. It was calculated that the addition of pure oxygen would give sufficient oxygen to allow for complete mineralization of the organic compounds in a serum bottle. In similar experiments the oxygen concentration at the end of the experiment has been measured to above 8 mg/l. The sample was transferred to a 10 ml volumetric flask. 1 ml of diethyl-ether and 100 μl of pentane containing heptane and undecane as internal standards were added to the sample. The flask was then shaken vigorously by hand for 1 min. 250 μl of the organic phase was transferred to a 250 μl vial, which was sealed with a teflon lined cap. 1 μl of the organic phase was injected by a DANI ALS 3940 sampling unit into a DANI 8520 GC. The GC was equipped with a flame ionization detector (FID) and a 30 m J&W DB5 capillary column, i.d. 0.32 mm and film-thickness 0.25 μm. The detector-temperature and the temperature in the injector port was 275 °C. Two different temperature programs for the oven were used. For Experiment A and B the operational oven temperature was 35 °C for 3 min., followed by an increase to 125 °C at a rate of 30 °C/min. This temperature was held for 4 min before an increase of 30 °C/min to 185 °C, which was held for 1 min. For Experiment C the initial temperature was 35 °C for 1 min, followed by an increase to 125 °C at a rate of 30 °C/min. This temperature was held for 1 min. Nitrogen was used as carrier and make-up gas. Responses from the FID were collected, integrated and calculated on a Maxima 820 chromatographic workstation (Millipore Corporation, Massachusetts). The concentrations of the aromatic compounds in a sample were calculated using standards with known concentrations.

Results

Experiment A

The abiotic losses in the control batches were 3–19% during the 16 days of incubation, 3% for the less volatile compound, 1-methylnaphthalene, and 19% for the most volatile compound, toluene. An exception was quinoline, which could not be detected in the control bottles. The reason for this was probably the low pH in the samples from the control bottles. Even though the samples were supposed to be neutralized with a 10 N

Table 1. A. Percent degradation of different compounds in Experiment A. Data are from Day 4 and are average values of two replicates. () the factor where the compound was included. * indicates in which batch ind was initially present.

tol	bf (A)	bt (B)	qui (D)	*p*-xyl (E)	*o*-xyl (E)	mnap (E)	nap (E+F)	phe (F)	*o*-cre (F)	dmp (F)
34*	–	63	–	8	7	45	36	100	99	96
100*	–	–	–	100	71	99	98	–	–	–
100	–	100	–	–	–	–	99	100	100	97
80*	–	–	–	–	–	–	64	100	100	100
−1*	14	20	–	–	–	–	8	100	6	91
100	–	–	–	–	–	–	–	–	–	–
100*	–	72	–	–	–	–	–	–	–	–
100	–	100	13	100	26	74	100	100	100	90
100	–	98	–	100	34	92	97	–	–	–
−1	11	13	–	−2	−3	28	23	100	1	84
−4*	10	–	14	–	–	–	19	100	7	86
100	–	82	8	–	–	–	–	–	–	–
99*	–	28	−14	100	7	26	16	–	–	–
9	9	–	−4	–	–	–	–	–	–	–
1	4	6	3	–	–	–	11	70	−4	35
100	–	–	37	–	–	–	100	100	100	97
29*	–	–	−9	12	9	42	36	99	60	69
4*	8	–	–	–	–	–	–	–	–	–
3*	9	6	−13	4	7	9	4	91	−13	39
3	21	–	−4	1	−2	67	23	80	−9	35
2	12	–	–	–	–	–	30	83	−2	46
100	–	–	–	100	31	54	99	100	95	73
100	–	–	−2	100	68	100	100	–	–	–
100*	–	–	10	–	–	–	–	–	–	–
33	12	−1	−7	100	−3	8	7	–	–	–
41	23	–	–	100	17	93	75	–	–	–
−2*	6	5	10	–	–	–	–	–	–	–
30*	14	19	–	98	−2	15	10	–	–	–
−2*	10	–	–	−4	−5	26	20	41	−4	18
5	1	−4	–	–	–	–	–	–	–	–
13*	–	21	−16	–	–	–	2	63	6	18
23*	7	–	4	93	2	23	17	–	–	–

KOH, an analysis showed that the pH in the samples from the control bottles was around 3. This had a significant effect on the extraction of quinoline, because quinoline has a pK_a value of 4.87 (Katritzky 1963). The low pH had very little or no effect on the other compounds. The percent degradation of the different compounds in Experiment A is shown in Table 1A (incubation of 4 days), Table 1B (8 days) and Table 1C (16 days). The observations have been corrected for the observed abiotic losses, except for quinoline where the data presented is the observed removal divided with the initial concentration. The percent degradation shown is an average of two replicates. The replicates were within ±10% of the average, in most cases within ±2%. The standard error on the concentrations caused by analytical errors was 10%. The percent degradation of ind (Factor C) is not shown in Table 1A–1C, because ind was completely degraded in all batches within the first 4 days of incubation. Batches where ind was initially present are marked with a * at the percent degradation of toluene in Table 1A. Thi, pyr, and mpyr (part of Factor A) were not included in the tables either, since they were not degraded in any of the batches.

A short summary of the degradation of all compounds is shown in Table 2, except for toluene which will be presented in details in the following.

Table 1. B. Percent degradation of different compounds in Experiment A. Data are from Day 8 and are average values of two replicates. () the factor where the compound was included.

tol	bf (A)	bt (B)	qui (D)	*p*-xyl (E)	*o*-xyl (E)	mnap (E)	nap (E+F)	phe (F)	*o*-cre (F)	dmp (F)
100	–	100	–	100	100	100	100	100	100	100
100	–	–	–	100	100	100	100	–	–	–
100	–	100	–	–	–	–	100	100	100	100
100	–	–	–	–	–	–	100	100	100	100
7	15	53	–	–	–	–	68	100	19	94
100	–	–	–	–	–	–	–	–	–	–
100	–	74	–	–	–	–	–	–	–	–
100	–	100	28	100	96	100	100	100	100	100
100	–	100	–	100	100	100	100	–	–	–
10	23	74	–	9	7	90	93	100	21	97
5	17	–	5	–	–	–	73	100	17	94
100	–	88	100	–	–	–	–	–	–	–
100	–	100	100	100	100	100	100	–	–	–
32	6	–	83	–	–	–	–	–	–	–
7	10	36	8	–	–	–	56	100	17	94
100	–	–	80	–	–	–	100	100	100	100
100	–	–	45	100	100	100	100	100	100	100
14	5	–	–	–	–	–	–	–	–	–
6	17	42	–4	7	9	46	62	100	11	93
9	32	–	4	19	20	93	100	100	12	94
11	21	–	–	–	–	–	100	100	21	95
100	–	–	–	100	100	100	100	100	100	100
100	–	–	100	100	100	100	100	–	–	–
100	–	–	100	–	–	–	–	–	–	–
46	11	25	100	100	10	63	46	–	–	–
59	45	–	–	100	65	100	100	–	–	–
13	2	6	67	–	–	–	–	–	–	–
51	13	44	–	100	17	73	52	–	–	–
12	36	–	–	29	35	100	100	100	21	95
26	5	1	–	–	–	–	–	–	–	–
100	–	100	86	–	–	–	100	100	100	100
55	32	–	100	100	43	100	100	–	–	–

Degradation of toluene

Tol was degraded completely within the first 4 days in batches where no other compounds were present (Table 1A). A strong inhibitory effect on the tol degradation from Factor A was observed. In batches where Factor A was present, very little or no degradation of tol was observed (Table 1C). The phenolic compounds, too, seemed to have an inhibitory effect, but only in the beginning of the experiment.

The statistical analysis of the degradation of toluene

A statistical analysis was conducted with the statistical program package, SAS, using the procedure ANOVA. The percent degradation of tol was the dependent variable, while the six factors were the independent variables. A model with 22 independent variables was run separately on data for Day 4, Day 8, and Day 16. A small error might occur using the statistical factorial analysis, because 100% degradation of toluene was observed in the batches where Factor A, E, and F were absent (Table 1A–1C). This means that the assumption

Table 1. C. Percent degradation of different compounds in Experiment A. Data are from Day 16 and are average values of two replicates. () the factor where the compound was included.

tol	bf (A)	bt (B)	qui (D)	*p*-xyl (E)	*o*-xyl (E)	mnap (E)	nap (E+F)	phe (F)	*o*-cre (F)	dmp (F)
100	–	100	–	100	100	100	100	100	100	100
100	–	–	–	100	100	100	100	–	–	–
100	–	100	–	–	–	–	100	100	100	100
100	–	–	–	–	–	–	100	100	100	100
–2	15	100	–	–	–	–	100	100	25	100
100	–	–	–	–	–	–	–	–	–	–
100	–	76	–	–	–	–	–	–	–	–
100	–	100	100	100	100	100	100	100	100	100
100	–	100	–	100	100	100	100	–	–	–
4	25	80	–	12	14	100	96	100	23	96
–2	11	–	100	–	–	–	81	100	24	95
100	–	89	100	–	–	–	–	–	–	–
100	–	100	100	100	100	100	100	–	–	–
28	1	–	100	–	–	–	–	–	–	–
–1	7	44	70	–	–	–	65	100	24	95
100	–	–	100	–	–	–	100	100	100	100
100	–	–	100	100	100	100	100	100	100	100
16	2	–	–	–	–	–	–	–	–	–
0	18	63	37	4	6	97	86	100	25	95
14	59	–	100	48	55	100	99	100	25	98
8	19	–	–	–	–	–	90	100	30	95
100	–	–	–	100	100	100	100	100	100	100
100	–	–	100	100	100	100	100	–	–	–
100	–	–	100	–	–	–	–	–	–	–
42	9	34	100	100	7	91	63	–	–	–
60	48	–	–	100	67	100	100	–	–	–
33	2	13	100	–	–	–	–	–	–	–
49	13	54	–	100	14	100	71	–	–	–
19	66	–	–	58	72	100	100	100	39	98
26	0	6	–	–	–	–	–	–	–	–
100	–	100	100	–	–	–	100	100	100	100
56	35	–	100	100	51	100	100	–	–	–

of equal variance fails. However, a sensitivity analysis showed that this had no influence on the main results presented here. For Day 4, the significant factors (significance level, α=0.01) were: A, C, A*C, A*E, F and E*F, r^2 = 0.94. The asterisk (*) denotes interactions between factors. Factor A alone accounted for 73% of the total variance. For Day 8, the significant factors were: A, E, A*E, F, E*F and A*E*F, r^2 = 0.99. Again most of the variance could be attributed to Factor A (r^2 = 0.98). For Day 16, the significant factors were: A, E, A*E, F, E*F and A*E*F, r^2 = 0.98. Factor A contributed 87% of the total variance (r^2 = 0.87). It can therefore be concluded that Factor A had a very strong inhibitory effect on tol degradation on all three days.

Data from Day 4 showed that ind (Factor C) weakly inhibited the degradation of tol (Table 1A). If ind was present in a batch, less tol degradation was observed than in batches where no ind was present. This indicates that ind probably competitively inhibited the tol degradation. When both factors (+A +C) were present in a batch, a strong inhibitory effect on the tol degradation was observed (Fig. 1). The data shown are from Day 4 and were calculated as the average of the percent degradation of tol in the different batches. Factor C was not significant on Day 8 and Day 16, which is

Table 2. The degradation of fourteen creosote compounds in Experiment A.

Compound	Degradable	Comments
Thiophene	–	no degradation observed
Benzothiophene	(–)	degradation dependent on the degradation of other compounds
Benzofuran	(–)	degradation dependent on the degradation of other compounds
Pyrrole	–	no degradation observed
1-Methylpyrrole	–	no degradation observed
Quinoline	+	no signficant interactions
Indole	+	no interactions
Phenol	+	no interactions
o-Cresol	+	inhibited by Factor A
2,4-Dimethylphenol	+	no interactions
p-Xylene	+	inhibited by Factor A
o-Xylene	+	inhibited by Factor A
Naphthalene	+	weakly inhibited by Factor A
1-Methylnaphthalene	+	weakly inhibited by Factor A

+: easily degradable, –: difficult degradable

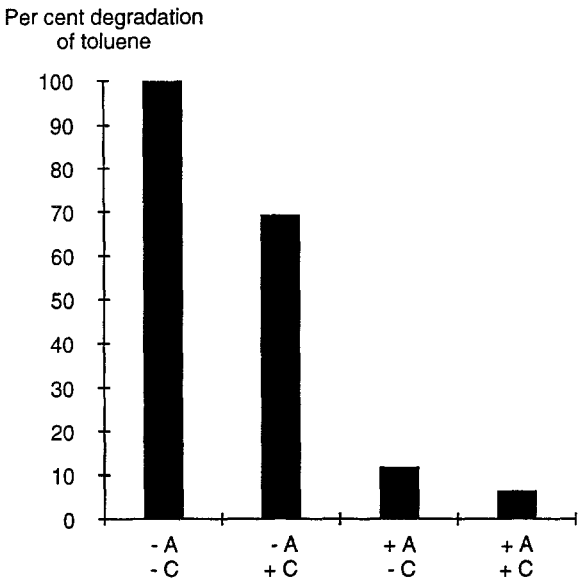

Fig. 1. Per cent degradation of toluene in Experiment A. Data are from Day 4 and are average values from batches where Factor A and C were absent (-A -C), Factor A absent and Factor C initially present (-A + A), Factor A initially present and Factor C absent (+ A -C), and Factor A and C initially present (+ A + C).

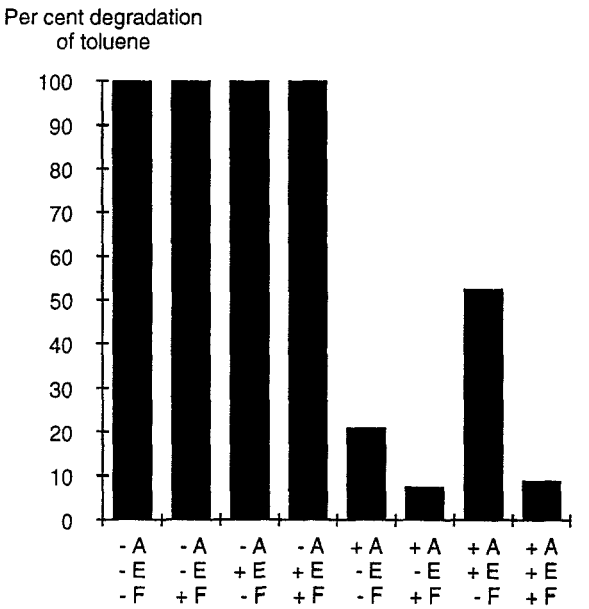

Fig. 2. Per cent degradation of toluene in Experiment A. Data are from Day 8. +/– means presence/absence of a factor.

consistent with the fact that ind was degraded before Day 4. The insignificance of bt (Factor B) and qui (Factor D) on Day 4, and of Factor B, C, and D on Day 8 and 16 shows that bt and qui did not have any influence on the tol degradation. It also indicates that no metabolites from the degradation of ind, bt and qui were produced in concentrations that were inhibiting for tol degraders.

Factor E seemed to stimulate the tol degradation even though the influence was weak, while Factor F had a negative effect, probably due to competitive inhibition. The same result was found for the interactions between all three factors (A, E and F), (Fig. 2, data

Table 3. Degradation time [days] for the four compounds used in Experiment B. () the percent degraded after 21 days if the compound was not completely degraded.

Factors	Tol	Thi (A)	Pyr (B)	Bf (C)
−A −B −C	4–6	–	–	–
+A −B −C	< 6	> 21 (26%)	–	–
−A +B −C	< 6	–	< 4	–
−A −B +C	< 21	–	–	< 21
+A −B +C	> 21 (99%)	> 21 (28%)	–	> 21 (75%)
−A +B +C	< 21	–	< 4	< 21
+A +B +C	> 21 (99%)	> 21 (61%)	< 6	> 21 (84%)

+/− means presence/absence of a factor
+ : Factor present, −: Factor not present.

from Day 8). The same result was observed on Day 16. Factor A inhibited tol degradation, Factor E stimulated the degradation, while Factor F had an inhibitory effect.

This experiment shows that a factorial experiment is a very powerful analytical tool to observe interactions between different compounds. It can also reveal if any toxic intermediates are formed.

Experiment B

A reduced factorial design was conducted to find out which compound (if any) accounted for the inhibitory effect in Factor A. While mpyr had no influence on the degradation of toluene (data not shown), three compounds were selected for Experiment B: thi, pyr and bf. The degradation time, defined as the time needed for complete degradation, and the percent degradation of a compound after 21 days is shown in Table 3. The left-hand column describes the experimental setup, eg. −A, −B, −C means that Factor A (thi), B (pyr) and C (bf) were not present in the batch. The results shown are the average of two replicates. The difference in the percent degradation between two replicates was less than 5%. Similar results for the degradation time were obtained for the replicates. The abiotic losses in the control batches showed similar losses as in Experiment A. The observations were corrected for abiotic losses. When tol was the single compound in a batch (−A, −B, −C), tol was quickly degraded after a lag-phase of 1 day. In one batch, a complete removal of tol was observed after 4 days of incubation, while 5% of tol remained in the other batch on Day 4. A simple average degradation rate was calculated to be 400–500 μg/l/day. When bf was present together with tol (−A, −B, +C),

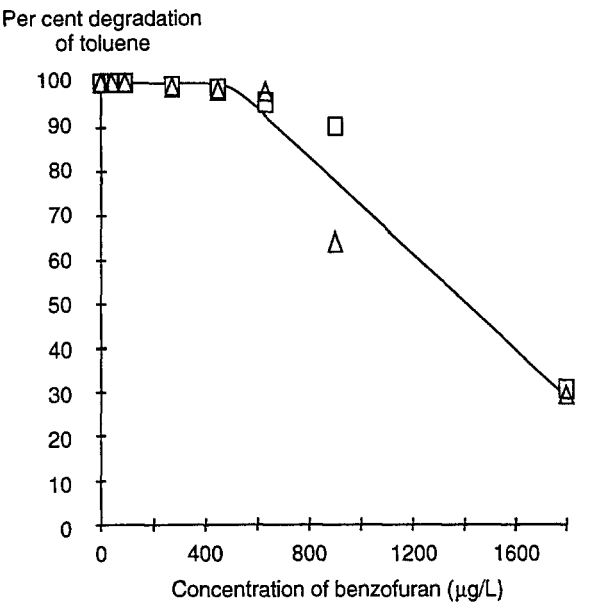

Fig. 3. Per cent degradation of toluene as a function of the benzofuran concentration in Experiment C. Data from Day 8. (□ Results from batch one. △ Results from batch two).

an inhibitory effect was observed, since the lag-time increased to four days. A decrease in the degradation rate was also observed. Concomitant with the tol degradation, a complete removal of bf was observed during the 21 days of incubation. Thi (+A, −B, −C) and pyr (−A, +B, −C) also had a small inhibitory effect, but it was significantly weaker than for bf. Because pyr in Experiment B was degraded faster than tol, a possible inhibitory effect was removed, and therefore no conclusion can be made about the inhibitory effect of pyr on the tol degraders. Interactions between three factors were clearly observed. If bf was present together with thi (+A, −B, +C), the degradation rate decreased to 80 μg/l/day, and tol could be detected even on Day 21.

Experiment C

A third experiment was carried out to study in detail the inhibitory effect of bf on the tol degraders. When thi had very little or no effect on the tol degraders, but a significant effect on the degradation of bf (Table 3), the experiment was run as a dose-response experiment where tol and thi were present together in all batches. The addition of thi should prevent the degradation of bf. Bf was the independent variable. Eight different concentrations of bf were used. The abiotic losses in the control batches at the end of the experiment were 26%

for tol and bf, and 11% for thi (average for the two control batches). The data presented have been corrected for the abiotic losses. The tol degradation started after a five-day lag-phase. Bf inhibited degradation of tol even at concentrations of 40 μg/l, where traces of tol (10–20 μg/l) could be detected on Day 54. The rate of tol degradation decreased significantly in the presence of high concentrations of bf (Fig. 3, data from Day 8). A difference in the percent degradation of tol was observed in duplicates where bf was present in an initial concentration of 900 μg/l (Fig. 3). In one batch, bf was degraded 60% after 8 days. In this batch, the rate of tol disappearance was higher than in the other batch, where bf was degraded only 35%. This, together with the high inhibiting effect on the tol degraders at a bf concentration of 1800 μg/l, indicates that bf probably had a toxic effect on the tol degraders, but also that the microorganisms were capable of overcoming this effect by degrading the inhibiting compound. Samples taken on Day 54 showed traces of tol (10–20 μg/l) in all batches where bf had been present.

Discussion

All 15 compounds investigated were degraded to some extent under aerobic conditions. Many different types of interactions between the different compounds were observed. The degradation of some compounds (thi, bf, and bt) seemed to be dependent on the concomitant degradation of another substrate, indicating that thi, bf, and bt might be degraded by cometabolism. This hypothesis is supported by Kropp et al. (1994) who observed that three *Pseudomonas* strains could not grow on bt, but bt could be biotransformed with 1-methylnaphthalene or glucose as substrate. Also Kanagawa and Kelly (1987) concluded that two strains of *Rhodococcus* could not grow on thi and bt. Mueller et al. (1991) observed some removal of benzothiophene in shake flask experiments using a relatively high concentration of surface soil micro organisms, though bt was not completely degraded within the 14 days of incubation. The removal of bt in their experiment was probably caused by cometabolism with other compounds as the primary substrate. No references on the aerobic degradation of bf and thi have been found. Degradation of pyr, mpyr (data not shown), qui, ind, phenol and dmp seemed to be independent of the other compounds. This is consistent with literature data showing that different cultures and strains are able to degrade these compounds as sole source of carbon and energy (Shukla 1987; Jensen et al. 1988; Mueller et al. 1991; Miethling et al. 1993; Nakhla and Al-Harazin 1993; Powlowski & Shingler 1994). The degradation of the xylenes was inhibited by the presence of thi, pyr, mpyr, and bf, but not as strongly as with tol. *P*-xyl was more rapidly degraded than *o*-xyl, which is consistent with the fact that *p/m*-xylene can be degraded by more microorganisms than *o*-xylene (Barbieri et al. 1993), probably because of the degradation pathway (Duetz et al. 1994). Nap and mnap degradation were also affected by Factor A, but an almost complete degradation was observed after 16 days. No other factors seemed to have any influence on the degradation of nap. Similar results were found by Jensen et al. (1988). They observed no inhibitory effect of pyr, *o*-cre or qui on the degradation of nap for a naphthalene-adapted culture. Mueller et al. (1991) observed a very rapid removal of the naphthalenes apparently with no influence of the other compounds.

The degradation of tol will be described more in detail. Factor A greatly inhibited the degradation of both tol and *o*-cre, but also ind had an influence on the aerobic degradation of tol, probably due to competitive inhibition, since it has been reported that both ind and tol could be degraded by the same enzyme system in *Pseudomonas putida* F39/D and *Escherichia coli* JM109(pDTG601) (Gibson et al. 1990). It was expected that pyr would be the most inhibiting compound of the four compounds in Factor A (thi, pyr, mpyr, and bf) because Arvin et al. (1989) found that pyr strongly inhibited the aerobic degradation of benzene at concentrations of 100–200 μg/l. Though Jensen et al. (1988) observed no inhibitory effect of pyr on the aerobic degradation of naphthalene. Surprisingly bf was identified as the compound most inhibitory to the toluene degraders. Even at low concentrations (40 μg/l) of bf, the tol degradation rate was reduced. Two different phenomena for the inhibiting effect were noticed. First, the lag-phase was extended in batches where bf was initially present. An explanation for this could be that the microorganisms had to adapt to the new conditions (the presence of bf). However, the problem with this explanation is that twice within a year before the start of Experiment A the microorganisms had been exposed to the NSO-compounds and therefore they may already have been acclimatized. The second phenomenon observed was the decrease in the toluene degradation rate. This is a typical phenomenon observed when two compounds are competing for the same enzyme system (i.e. competitive inhibition). This explanation, however, is not

very likely, because 1) no degradation of the inhibiting compounds was observed in Experiment A, and 2) bf was only degraded cometabolically with other compounds as primary substrate. Another explanation could be that there were at least two types of consortia of toluene degraders, i.e. fast-growing toluene degraders which were very sensitive to stress-factors, and slow-growing toluene degraders which were more tolerant. The hypothesis is that the NSO-compounds were toxic to the fast-growing degraders, while they were less toxic to the slow-growing tol degraders. It should be mentioned that the toluene-concentration used in the experiments reported here was below the toxicity limit for toluene degraders (Alvarez & Vogel 1991; Duetz et al. 1994). Button and Robertson (1986) studied toluene-degrading bacteria isolated from an oligotrophic marine environment. They concluded that only when the toluene concentration was > 20 mg/l, did inhibition begin to influence toluene oxidation.

The results presented in this paper suggest that interactions between different compounds can play a significant role during the aerobic degradation of the pollutants in creosote contaminated groundwater. Toluene might be found downstream a creosote contaminated site because of the inhibiting effect of the other compounds on the degradation although toluene is supposed to be very easily degradable under aerobic conditions. The indication of cometabolic degradation of the S- and O-heterocyclic compounds found in these experiments is important knowledge in relation to creosote contaminated groundwater. If the S- and O-heterocyclic compounds are found in the groundwater and the easily degradable compounds like toluene, benzene, naphthalene etc. are degraded already, the heterocyclic compounds can persist even if aerobic conditions exist.

Conclusions

A strong inhibiting effect on the aerobic degradation of toluene was observed when four NSO-compounds (thiophene, 1-methylpyrrole, pyrrole and benzofuran) were present. Indole, another N-compound, also had an inhibiting effect on the toluene removal, probably due to competitive inhibition, although the effect was much weaker. The effect disappeared when indole was degraded. A statistical analysis revealed that no toxic intermediates were formed during the degradation of indole. Two other NS-compounds (benzothiophene and quinoline) had no significant effect on the degradation of toluene. Four aromatic compounds (*o*-xylene, *p*-xylene, naphthalene and 1-methylnaphthalene) had a weak stimulating effect, while three phenolic compounds (phenol, *o*-cresol and 2,4-dimethylphenol) had a competitive inhibiting effect.

Benzofuran was identified as the most inhibiting compound among the four NSO-compounds. An effect on the toluene degraders could be observed even at very low concentrations of benzofuran (40 μg/l).

The interactions between compounds in a complex mixture can result in a very different degradation pattern than in simple mixtures. Therefore, the degradation potential in a contaminated aquifer can be incorrectly estimated, if experiments with only simple mixtures are carried out.

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