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Effects of creosote compounds on the aerobic bio-degradation of benzene

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Abstract

The inhibitory effect of creosote compounds on the aerobic degradation of benzene was studied in microcosm experiments. A total removal of benzene was observed after twelve days of incubation in microcosms where no inhibition was observed. Thiophene and benzothiophene, two heterocyclic aromatic compounds containing sulfur (S-compounds), had a significant inhibitory effect on the degradation of benzene, but also an inhibitory effect of benzofuran (an O-compound) and 1-methylpyrrole (a N-compound) could be observed, although the effect was weaker. The NSO-compounds also had an inhibitory effect on the degradation of *p*-xylene, *o*-xylene, and naphthalene, while they only had a weak influence on the degradation of 1-methylnaphthalene, *o*-cresol and 2,4-dimethylphenol. The phenolic compounds seemed to have a weak stimulating effect on the degradation of benzene whereas the monoaromatic hydrocarbons and the naphthalenes had no significant influence on the benzene degradation. The inhibitory effect of the NSO-compounds on the aerobic degradation of benzene could be identified as three different phenomena. The lag phase increased, the degradation rate decreased, and a residual concentration of benzene was observed in microcosms when NSO-compounds were present. The results show that NSO-compounds can have a potential inhibitory effect on the degradation of many creosote compounds, and that inhibitory effects in mixtures can be important for the degradation of different compounds.

Abbreviations: ben – benzene, bf – benzofuran, bt – benzothiophene, dmp – 2,4-dimethylphenol, GC – gas chromatograph, ind – indole, mnap – 1-methylnaphthalene, MAHs – monoaromatic hydrocarbons, mp – 1-methylpyrrole, nap – naphthalene, NSO-compounds – heterocyclic aromatic compounds containing nitrogen, sulphur or oxygen, *o*-cre – *o*-cresol, *o*-xyl – *o*-xylene, PAHs – polyaromatic hydrocarbons, phe – phenol, *p*-xyl – *p*-xylene, pyr – pyrrole, thi – thiophene, qui – quinoline

Introduction

Coal-tar creosote consisting of hundreds of organic compounds including monoaromatic hydrocarbons (MAHs), polyaromatic hydrocarbons (PAHs), phenolic compounds, and heterocyclic aromatic compounds containing nitrogen, sulphur or oxygen (NSO-compounds) can be found as a contaminant at many sites (Goerlitz et al. 1985; Sundström et al. 1986; Mueller et al. 1989a; Korsgaard et al. 1989; Raven & Beck 1992; Pyka 1993). The contamination of the groundwater from a creosote contaminated site is not only dependent on the composition of the creosote but also on the aqueous solubility of the compounds. The PAHs with three or more aromatic rings have a solubility less than one mg/l whereas the solubilities of the MAHs, phenols and NSO-compounds are orders of magnitude higher (Verschueren, 1983). Investigations carried out on field sites reflect the complexity of the creosote and a high variation in the composition and the concentration levels of the compounds have been observed (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). Considering the wide use of creosote, the degradability of the different compounds is of obvious interest. Many studies have been carried out to investigate the degradation of creosote compounds under different redox conditions. Often, studies are concentrated on the degradation of a single compound or simple mixtures of compounds (Alvarez et al. 1991; Flyvbjerg et al. 1993; Thomas & Lester 1993; Corseuil & Weber 1994), and usually they have focused on the PAHs, MAHs or the phenolic compounds. These studies may give an incorrect estimate of the biodegradation potential in the groundwater, since a contamination often consists of many different compounds. Studies on the degradation potential of complex mixtures have been reported (Mueller et al. 1989b; Mueller et al. 1991; Godsy et al. 1992) but only a few have investigated the interactions between different compounds and the resulting effects (Arvin et al. 1989; Alvarez & Vogel 1991; Millettte et al. 1995; Dyreborg et al. 1996).

In a previous paper (Dyreborg et al. 1996) we reported the effects of some typical creosote compounds on the aerobic biodegradation of toluene determined by batch experiments. Surprisingly, while phenolic compounds, MAHs, and PAHs showed little influence on the degradation of toluene, a highly significant inhibitory effect was observed from NSO-compounds. Benzofuran was identified as the compound that was most inhibitory to the toluene degradation, and an effect could be detected even at low concentrations (40 µg/l) of benzofuran. Also, Arvin et al. (1989) reported that NSO-compounds can have a potential inhibitory effect on the degradation of creosote compounds.

In an effort to generalize these results, experiments were conducted to investigate the influence of fourteen typical creosote compounds on the aerobic degradation of another MAH. Benzene was chosen as model compound because of its structural relationship with toluene. Also, the degradation pattern for the two compounds is supposed to be similar. The toluene dioxygenase enzyme has the ability to oxidize a wide range of organic compounds, including benzene, as discussed by Gibson et al. (1990) and Williams & Sayers (1994), and many toluene degraders are able to degrade benzene (Karlson & Frankenberger 1989; Alvarez & Vogel 1991; Chang et al. 1993). Therefore, one would expect that the inhibitory effect of creosote compounds on the degradation of benzene and toluene should be very similar.

Materials and methods

Experimental systems

The degradation experiments (Experiment 1 and 2) were conducted as microcosms experiments in 117-ml serum bottles with Teflon-coated Mininert valves. The serum bottles were covered with aluminium foil and dry-sterilized in an oven for four hours at 240°C before the start of an experiment. Mininert valves and a 5-l glass container containing tap water, NH_4Cl (4 mg N/l) as the nitrogen source, and Na_2HPO_4 (1 mg P/l) as the phosphate source were autoclaved for one hour at 125°C. After autoclaving, pH was adjusted to 7.5 with H_2SO_4 before the inoculum (see *Experimental setup*) was added to the container. 100 ml from the glass container was transferred to 35 serum bottles. Before a serum bottle was stoppered with a Mininert valve, benzene was added, and the other compounds were added from aqueous stock solutions according to the statistical designs in the two experiments. The chemicals used (Merck, Germany) were of analytical grade. Throughout the experiments the serum bottles were rotated (2 rpm) in a dark box at room temperature (20±3°C). Three bottles in each experiment were acidified (pH≈1) with 8 N sulphuric acid. They were used as control for abiotic processes. The data presented in this paper have been corrected for the observed abiotic losses in the acidified microcosms.

Experimental setup, Experiment 1

The purpose of Experiment 1 was to investigate the influence of fourteen creosote compounds in mixtures on the aerobic degradation of benzene. It was conducted as a reduced $\frac{1}{2} \cdot 2^6$ factorial experiment with benzene as the dependent variable. The six independent factors (A-F) contained combinations of creosote compounds. The factors are described in Table 1. 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). For further information regarding the statistical design, see Dyreborg et al. (1996). The statistical analysis of the degradation of benzene was conducted with a statistical program package (SAS 1985) using the procedure ANOVA for factorial analysis. The percent degradation of benzene was the dependent variable, while the six factors (Table 1) and all two-factor interactions were the twenty-one independent variables in the model.

Table 1. Factor description. The initial concentration of the different compounds in Experiment 1 if the factor is present in a microcosm is listed.

Factor	Compounds	Initial concentration [mg/l]
-	benzene (MAH)	3.0
A	indole (N-compound)	3.0
	quinoline (N-compound)	3.0
	thiophene (S-compound)	4.0
	benzothiophene (S-compound)	1.7
B	benzofuran (O-compound)	1.8
C	1-methylpyrrole (N-compound)	4.0
D	pyrrole (N-compound)	4.0
E	*p*-xylene (MAH)	0.9
	o-xylene (MAH)	0.9
	naphthalene (PAH)	0.3
	1-methylnaphthalene (PAH)	0.3
F	phenol (phenolic compound)	9.0
	o-cresol (phenolic compound)	9.0
	2,4-dimethylphenol (phenolic compound)	8.0

The inoculum for the experiment has been used previously in other experiments. It was an aerobic enrichment culture that originated from the groundwater at a creosote-contaminated aquifer in Fredensborg, Denmark. The initial concentrations of the different creosote compounds in the serum bottles are listed in Table 1. Samples were taken from each bottle at time zero and after 5, 12, 21, and 49 days.

Experiment 2, Experimental setup

The purpose of Experiment 2 was to identify the compound among thiophene, benzothiophene, benzofuran and 1-methylpyrrole that had the strongest inhibitory effect on the benzene degradation. It was designed as a complete 2^4 factorial experiment where benzene was the dependent variable while the four factors (1–4) were 1: thiophene; 2: benzothiophene; 3: benzofuran; 4: 1-methylpyrrole. Duplicates were run for each combination. The statistical analysis was conducted in the statistical program package, SAS. A model with the four factors and their interactions was used (15 variables).

The inoculum for this experiment was prepared as follows: the remaining liquid from all serum bottles from Experiment 1 was pooled together and centrifuged (2,000 rpm for 20-min, IEC centra-7 centrifuge). The biomass was then transferred to a 5-l glass container as described in *Experimental systems*. The initial concentrations in Experiment 2 were (mg/l): benzene 2.3, 1-methylpyrrole 6, thiophene 4, benzothiophene 2, and benzofuran 1.2. Samples were taken at time zero and after 6, 9, 16, and 23 days.

Chemical analysis

A ten ml water-sample from a serum bottle was withdrawn with a glass syringe and replaced by ten ml pure gaseous oxygen to maintain aerobic conditions throughout the experiment. The water-sample was transferred to a volumetric flask, extracted with one ml of diethyl-ether and 100 µl of pentane containing heptane and undecane as internal standards before it was analyzed on a DANI 8520 GC with a flame ionization detector (FID). Two different temperature programs for the oven were used. For Experiment 1 the operational oven temperature was 35°C for three min, followed by an increase to 125°C at a rate of 30°C/min. This temperature was held for four min before an increase of 30°C/min to 185°C, which was held for one min. For Experiment 2 the initial temperature was 35°C for two min, followed by an increase to 185°C at a rate of 30°C/min. This temperature was held for one min. The detection limit for the organic compounds was below 4 µg/l. For further information regarding the chemical analysis, see Dyreborg et al. (1996).

Results

Experiment 1

The percent degradation of the different compounds after twelve days of incubation in Experiment 1 is shown in Table 2. The abiotic losses were in general 0–10%, probably due to evaporation to the headspace in the serum bottles.

Indole, quinoline, and phenol were quickly degraded to below the detection limit in the microcosms. Also 1-methylpyrrole was completely removed within the incubation period of 49 days although the degradation was slower compared with indole, quinoline, and phenol (Table 2). No effects were observed on the degradation of the four compounds. Pyrrole, 1-methylnaphthalene, and *o*-cresol were completely removed after 49 days. Though, the degradation of pyrrole (indicated by the results from Day 5, data not shown), 1-methylnaphthalene, and *o*-cresol was inhibited by the presence of Factor A (Table 2). The

Table 2. Percent degradation of different compounds in Experiment 1. Data are from Day 12. No data for indole, quinoline, pyrrole, and phenol are shown since a 100 percent degradation of these compounds was observed in all microcosms after twelve days. The residual concentration of benzene after 49 days is listed in the last column.

ben	thi	bt	bf	mp	*p*-xyl	*o*-xyl	nap	mnap	*o*-cre	dmp	res. ben [µg/l]
100	-	-	-	-	-	-	-	-	-	-	0
100	-	-	-	-	99	82	98	73	100	93	0
100	-	-	-	-	-	-	-	-	100	67	0
100	-	-	-	-	100	100	100	100	-	-	0
74	-	-	-	68	-	-	-	-	100	49	0
10	-	-	-	10	100	97	100	100	-	-	0
80	-	-	-	53	-	-	-	-	-	-	0
93	-	-	-	72	74	42	95	48	100	68	0
57	-	-	12	-	-	-	-	-	100	21	0
40	-	-	73	-	100	77	100	98	-	-	0
59	-	-	0	-	-	-	-	-	-	-	0
80	-	-	15	-	2	0	84	45	100	27	0
24	-	-	0	6	-	-	-	-	-	-	34
5	-	-	23	7	12	8	100	55	100	7	0
46	-	-	16	25	-	-	-	-	100	8	0
49	-	-	65	25	100	73	100	100	-	-	12
62	54	94	-	-	-	-	-	-	100	39	28
89	66	70	-	-	98	0	3	18	-	-	0
14	37	51	-	-	-	-	-	-	-	-	0
23	48	65	-	-	17	9	5	8	99	19	17
7	1	44	-	8	-	-	-	-	-	-	353
41	34	90	-	29	28	11	0	0	99	14	782
39	58	86	-	22	-	-	-	-	100	29	0
13	38	60	-	20	99	2	58	35	-	-	90
17	7	37	2	-	-	-	-	-	-	-	60
4	5	25	2	-	3	3	6	16	98	21	0
12	35	32	17	-	-	-	-	-	99	24	29
32	32	32	22	-	23	19	14	26	-	-	29
2	0	24	5	3	-	-	-	-	99	16	170
2	0	13	0	11	94	0	0	13	-	-	789
7	25	20	1	13	-	-	-	-	-	-	281
0	12	16	6	7	2	0	0	7	90	7	1262

-: Compound not present
ben:benzene; thi:thiophene; bt:benzothiophene; bf:benzofuran; mp:1-methylpyrrole; *p*-xyl:*p*-xylene; *o*-xyl:*o*-xylene; nap:naphthalene; mnap:1-methylnaphthalene; *o*-cre:*o*-cresol; dmp:2,4-dimethylphenol; res.ben.:residual concentration of benzene after 49 days of incubation.

degradation of *p*-xylene, *o*-xylene, naphthalene, and 2,4-dimethylphenol was also inhibited by the presence of Factor A (Table 2), and no complete removal of these compounds was observed at the conclusion of the experiment (data not shown). The degradation of thiophene, benzothiophene, and benzofuran was also incomplete resulting in residual concentrations, and the degradation was apparently dependent on the concomitant degradation of other compounds (data not shown).

Degradation of benzene

A strong inhibitory effect of the creosote compounds on the degradation of benzene could be observed after twelve days of incubation (Table 2). Benzene was completely removed within the twelve days in micro-

cosms where no other compounds were present. In the microcosm in which benzene was present with pyrrole and the phenolic compounds, benzene was completely removed within the first five days (data not shown). Benzene was degraded to below the detection limit after 49 days of incubation in fourteen out of sixteen microcosms where Factor A was absent (Table 2). A residual concentration of benzene of 12–34 µg/l was observed in the last two microcosms. It should be noticed that benzofuran and 1-methylpyrrole were present in these two microcosms (Table 2). When Factor A was present, only a partial removal of benzene was observed and a residual concentration of benzene of 17–1262 µg/l was observed after 49 days of incubation. This suggested that Factor A had a strong inhibitory effect on the degradation of benzene. Also benzofuran and 1-methylpyrrole had an inhibitory effect although the effect was weaker. The phenolic compounds and pyrrole apparently had a stimulatory effect on the degradation of benzene.

Statistical analysis of the degradation of benzene

A statistical analysis was done on data from Day 12 because hardly any degradation of benzene was observed after five days of incubation. Since a 100% removal of benzene was observed in four microcosms at Day 12 (Table 2), a small error in the statistical analysis might occur as described in Dyreborg et al. (1996), but it has no influence on the main results presented here. The significant factors for the model were (significance level, α=0.01): A, B, and C (r^2 = 0.91). Factor A, Factor B, and Factor C accounted for 34%, 21%, and 13% of the total variance, respectively, and all three factors had a significant inhibitory effect. No interactions between two factors were significant. Therefore, it can be concluded that Factor A had the most inhibitory effect on the degradation of benzene. Because two of the four compounds in Factor A (quinoline and indole) were degraded before Day 12, it can be assumed that quinoline and indole did not affect the degradation of benzene, although they were part of Factor A. However, it cannot be excluded that formation of metabolites during the degradation of quinoline and indole inhibited the benzene degradation, but no metabolites were detected with the analytical method we were using. A statistical analysis of data from Day 5 (data not shown) suggested that the phenolic compounds had a stimulatory effect on the degradation of benzene probably due to an enhanced biomass-production, whereas no effect was observed for pyrrole, the xylenes and the naphthalenes.

The inhibitory effect of thiophene, benzothiophene, benzofuran, and 1-methylpyrrole was further investigated in Experiment 2.

Experiment 2

A complete 2^4 factorial experiment was conducted to investigate the effect of the four compounds on the degradation of benzene. The recovery percent from the acidified control microcosms were 90–95% for benzofuran and benzothiophene, 80–90% for benzene and thiophene, and 50% for 1-methylpyrrole. The low recovery for 1-methylpyrrole was probably due to analytical problems (neutralization of the samples from the control microcosms), whereas the other losses most likely are caused by evaporation to headspace in the serum bottles.

Degradation of thiophene, benzothiophene, benzofuran, and 1-methylpyrrole

A small degradation of benzothiophene (0–44% removal) and benzofuran (0–26% removal) was observed in some microcosms during the experiment, whereas thiophene was partly removed in all microcosms (17–81% removal) (Table 3). The removal of thiophene seemed to happen concomitant with the degradation of benzene (Figure 1), but it is difficult to make a strong conclusion because of the sparse amount of data. When the degradation of benzene started, a removal of thiophene was observed, and when benzene was completely degraded or its degradation stopped, the removal of thiophene stopped too. 1-Methylpyrrole was completely degraded in microcosms where it was present alone with benzene, while it was only partly degraded (63–95%) when other compounds were present. 1-Methylpyrrole degradation was not dependent on the concomitant degradation of benzene (data not shown). This suggests that 1-methylpyrrole could be degraded as a sole source of carbon and energy, but also that the three compounds (thiophene, benzothiophene, and benzofuran) tested in this experiment had an effect on the degradation of 1-methylpyrrole.

Degradation of benzene

Figure 2 shows some typical examples of the normalized concentration (C/C_0) of benzene as a function of incubation time. Benzene was rapidly

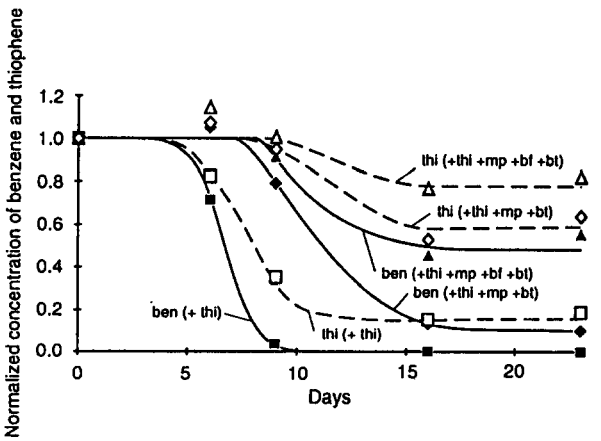

Figure 1. The normalized concentration of benzene (filled, full line) and thiophene (empty, dotted line) as a function of incubation time in Experiment 2. □,(+thi): benzene and thiophene as the sole sources of carbon and energy; ◇,(+thi+mp+bt): benzene, thiophene, 1-methylpyrrole, and benzothiophene; △,(+thi+mp+bt+bf): benzene, thiophene, 1-methylpyrrole, benzothiophene, and benzofuran. Data are an average of two replicates. Lines are smooth curves.

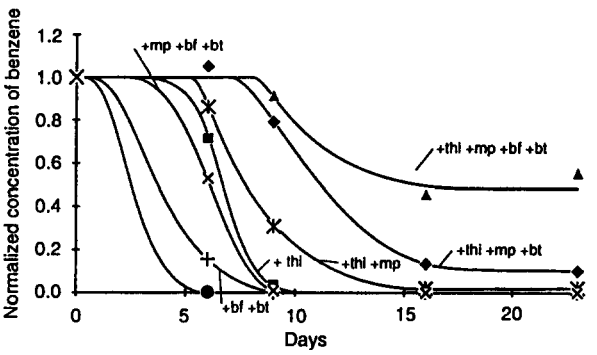

Figure 2. The normalized concentration of benzene as a function of incubation time in Experiment 2. ●: benzene; ■,+thi: benzene and thiophene; ∗,+thi+mp: benzene, thiophene, and 1-methylpyrrole; ♦,+thi+mp+bt: benzene, thiophene, 1-methylpyrrole, and benzothiophene; ▲,+thi+mp+bt+bf: benzene, thiophene, 1-methylpyrrole, benzothiophene, and benzofuran; +,+bt+bf: benzene, benzothiophene, and benzofuran; x,+mp+bt+bf: benzene, 1-methylpyrrole, benzothiophene, and benzofuran. Data are an average of two replicates. Lines are smooth curves.

Table 3. Percent degradation of the different compounds in Experiment 2. The residual concentration of benzene is listed in the last column. Data are from Day 23.

ben	thi	bt	bf	mp	res. ben [µg/l]
100	-	-	-	-	0
100	-	-	-	100	0
100	-	-	23	-	0
100	-	-	0	63	0
100	-	44	-	-	0
99	-	17	-	95	12
100	-	0	26	-	0
100	-	5	0	76	0
100	81	-	-	-	0
97	57	-	-	87	52
97	74	-	15	-	65
86	34	-	0	75	277
98	77	1	-	-	40
90	36	13	-	80	210
97	77	0	2	-	65
45	17	0	0	74	1162

-: compound not present.

degraded within the first six days of incubation when no other compounds were present (●, Figure 2). When thiophene was present as the sole compound with benzene (■), an indication of an extended lag phase was observed, although no strong conclusions can be made due to the sparse amount of data. When thiophene and benzene were present with 1-methylpyrrole (∗), 1-methylpyrrole and benzothiophene (♦), and 1-methylpyrrole, benzofuran, and benzothiophene (▲) two other phenomena were observed. A decrease in the degradation rate of benzene and a residual concentration of benzene. The influence on the degradation of benzene from benzothiophene, benzofuran, and 1-methylpyrrole (+ and x) was much weaker than the influence of thiophene.

Statistical analysis of the degradation of benzene.

A statistical analysis was conducted on the degradation of benzene after six days of incubation. The model could explain most of the variation in the data ($r^2 = 0.98$). Only four variables were significant (α=0.01) and they all inhibited the biodegradation of benzene: thiophene, benzothiophene, benzofuran, and 1-methylpyrrole. No interactions between the factors were significant. Especially thiophene accounted for the variance in the data (85%), while benzothiophene, 1-methylpyrrole, and benzofuran only accounted for 5, 4, and 1%, respectively. This suggested that thiophene was the compound that was most inhibitory to the degradation of benzene.

Table 4. Comparison between results reported in this paper and by Dyreborg et al. (1996). See text for explanation. References where a compound is reported to be degraded as a sole source of carbon and energy are listed.

Compound	Degradation of compound inhibited by[A]	Degradation of compound inhibited by[B]	Can be degraded as sole source of carbon and energy
indole	**none**	**none**	yes[2]
quinoline	**none**	**none**	yes[3,4]
thiophene	**d.o.d.**	n.d.	no
benzothiophene	**d.o.d.**	**d.o.d.**	no
benzofuran	**d.o.d.**	**d.o.d.**	yes[5]
1-methylpyrrole	**none**	n.d.	yes[1]
pyrrole	weakly (thi, bt, ind, qui)	n.d.	yes[1,2]
p-xylene	(thi, bt, ind, qui), bf, (phe, *o*-cre, dmp)	(thi, bt, bf, pyr)	yes[6,7,8]
o-xylene	(thi, bt, ind, qui), bf	(thi, bt, bf, pyr)	yes[6,7,8]
naphthalene	(thi, bt, ind, qui)	weakly (thi, bt, bf, pyr)	yes[8,9,10]
1-methylnaphthalene	weakly (thi, bt, ind, qui), (phe, *o*-cre, dmp)	weakly (thi, bt, bf, pyr)	yes[9,10,11]
phenol	**none**	**none**	yes[12,13]
o-cresol	weakly (thi, bt, ind, qui), bf	(thi, bt, bf, pyr)	yes[12,13]
2,4-dimethylphenol	weakly (thi, bt, ind, qui)	**none**	no

[A]: in this experiment.
[B]: results presented in Dyreborg et al. (1996).
none: no inhibitory effect on the degradation of the compound was observed; **d.o.d.**k: degradation dependent on the degradation of other compounds; **n.d.**: not degraded.
[1]: Dyreborg et al. (1996); [2]: Jensen et al. (1988); [3]: Shukla (1987); [4]: Miethling et al. (1993); [5]: Dyreborg et al. (unpublished); [6]: Gibson et al. (1990); [7]: Barbieri et al (1993); [8]: Fredrickson et al. (1991); [9]: Volkering et al. (1993); [10]: Stringfellow & Aitken (1995); [11]: Mahajan et al. (1994) [12]: Powlowski & Shingler (1994); [13]: Hinteregger et al. (1992); no: no references found.

Discussion

In this paper we have presented data that show the complexity in the degradation pattern when running degradation experiments with mixtures of compounds. The phenolic compounds, the MAHs, and the PAHs did not seem to affect the degradation of benzene very strongly, while the presence of NSO-compounds had a great influence not only on the degradation of benzene but also on the degradation of the other MAHs and the PAHs. This is consistent with the results presented by Arvin et al. (1989) and Dyreborg et al. (1996). Arvin et al. (1989) reported that pyrrole even at low concentrations (100–200 µg/l) strongly inhibited the aerobic degradation of benzene. Only minor effects of other compounds (toluene, *o*-xylene, naphthalene, 1,4-dimethylnaphthalene, and phenanthrene) were observed in their experiments. Dyreborg et al. (1996) observed a strong inhibitory effect of NSO-compounds on the aerobic degradation of toluene. They identified benzofuran as the compound that had the strongest inhibitory effect on the degradation, and the effect could be detected at very low concentrations (40 µg/l). Those results, and the results presented here, show that the NSO-compounds can have an inhibitory effect on the aerobic degradation of many aromatic hydrocarbons.

Similarities in the data presented by Dyreborg et al. (1996) and the results presented here can clearly be observed. Compounds that inhibit the degradation of the different compounds are summarized in Table 4. The results presented in this paper are listed in the second column, while the third column in Table 4 gives the results presented in Dyreborg et al. (1996). Effects from compounds surrounded by ( ) cannot be distinguished from each other since they were included in a factor and therefore present together. One major difference in the results is the degradation of pyrrole and 1-methylpyrrole. In the previous experiment no degradation of pyrrole and 1-methylpyrrole was observed, whereas the microorganisms could degrade both compounds in the experiment reported here. In other experiments reported in the work of Dyreborg et al. (1996) degradation of pyrrole and 1-methylpyrrole was observed. It has not been identified whether the microorganisms adapted to pyrrole and 1-methylpyrrole during the pre-exposure in the first experiment or whether the two other compounds

in Factor A (thiophene and benzofuran, (Dyreborg et al. 1996)) in that experiment had a high inhibitory effect on the degradation of the two compounds. Arvin et al. (1988) observed no degradation of pyrrole during 45 days of incubation using free-living bacteria from a groundwater aquifer at a field site contaminated with heavy fuel, whereas Jensen et al. (1988) observed a slow degradation of pyrrole after a lagphase of 20 days in a single substrate experiment. In a mixed substrate experiment they observed no degradation of pyrrole during 55 days of incubation. This suggests that microorganisms have to adapt to pyrrole before they can degrade the compound and that the lag phase can be very long.

The insignificant effect of pyrrole on the aerobic degradation of benzene as presented in this paper is in contrast to the findings of Arvin et al. (1989). There are at least two possible explanations. First, different inocula were used in the two experiments. This can clearly be observed in the differences in the degradation pattern. In the experiment reported in this paper, pyrrole was degraded completely within the first 12 days of incubation, whereas Arvin et al. (1989) observed only 17–51% degradation of pyrrole during 11 days of incubation. Secondly, different initial concentrations were used. We used an order of magnitude higher concentrations of benzene and pyrrole.

It is interesting to notice that the NSO-compounds have an inhibitory effect not only on the aerobic degradation of benzene and toluene but also on the degradation of other aromatic hydrocarbons and phenolic compounds. Whereas the other compounds have been observed to be degradable as the sole source of carbon and energy, no observations have been found for thiophene and benzothiophene and only one report on the degradation of benzofuran (Table 4). Not only have the S- and O-compounds potential inhibitory effects on the degradation of many creosote compounds, but they are also difficult to degrade themselves. Thiophene, benzothiophene and benzofuran were only degraded if a concomitant degradation of other compounds occurred. This is consistent with the literature. Bohonos et al. (1977) showed for four different cultures that benzothiophene only could be degraded when naphthalene was added to the cultures. Fedorak and Grbić-Galić (1991) observed that a *Pseudomonas* strain could degrade benzothiophene cometabolically with 1-methylnaphthalene, glucose or peptone as primary substrates. Also isopropylbenzene can be used as primary substrate for cometabolic degradation of benzothiophene (Eaton & Nitterauer 1994). Mueller et al. (1991) observed partial removal of benzothiophene in shake-flask experiments with pentachlorophenol and coal-tar creosote contaminated groundwater using surface soil microorganisms. The removal of benzothiophene in their experiment was probably caused by cometabolism with other compounds as primary substrate. No data have been found on the aerobic degradation of thiophene, but several attempts have been made to demonstrate the degradation of thiophene as reported by Fedorak (1990), but none have succeeded yet. Therefore, it is likely that thiophene and benzothiophene cannot be used for growth. To our knowledge no references have been reported on the degradation of benzofuran, though we have seen degradation of benzofuran as a sole source of carbon and energy in some experiments (Dyreborg et al. unpublished). In others experiment reported in Dyreborg et al. (1996), benzofuran could not be degraded as a sole source of carbon and energy. It should be mentioned that both *di*benzothiophene and *di*benzofuran can be used as a sole source of carbon and energy (Bohonos et al. 1977; Fredrickson et al. 1991; Monna et al. 1993). Yet, some experiments show that dibenzothiophene and dibenzofuran can only be degraded cometabolically (Constanti et al. 1994).

Thiophene was identified as the compound that had the most inhibitory effect on the aerobic degradation of benzene, but also benzothiophene, benzofuran, and 1-methylpyrrole had a small but significant inhibitory effect. The effect was observed as an increase in the lagphase, a decrease in the degradation rate and, interestingly, a residual concentration of benzene. The benzene concentration at the end of the experiment was highly dependent on the presence and concentration of other compounds. Benzene was completely removed when no other compounds were present. In Experiment 1, a remaining concentration of benzene of 12-1262 µg/l was observed in some microcosms after 49 days of incubation, whereas the remaining benzene concentration in Experiment 2 varied between 12–1162 µg/l. Also Karlson & Frankenberg (1989) reported remaining benzene in degradation studies using inoculum from a gasoline-contaminated aquifer. They observed a rapid degradation of benzene from an initial concentration of 500–600 µg/l to 22–35 µg/l during 20 hours of incubation. In contrast to our findings, they suggested that 22–35 µg/l of benzene did not provide enough carbon to sustain an active microbial population. The reason for the residual concentration of benzene in our experiments has not yet been identified.

Elsewhere we reported that benzofuran was the most inhibitory compound to the aerobic degradation of toluene (Dyreborg et al. 1996). In contrast, thiophene had the strongest inhibitory effect on the degradation of benzene. This suggests that two different inhibition phenomena for the degradation of toluene and benzene exist. The reason for this may be identified in different degradation pathways for benzene and toluene. Toluene is degraded through three different pathways; the TOL pathway, where the methyl-group is oxidized by a monooxygenase (Worsey & Williams 1975; Duetz et al. 1994), another monooxygenase, where the aromatic nucleus is hydroxylated forming cresols (Kukor & Olsen 1990; Shields et al. 1991; Whited & Gibson 1991; Duetz et al. 1994), and the dioxygenase (the TOD pathway) resulting in catechols as intermediates (Zylstra & Gibson 1989; Lee et al. 1994). The toluene *ortho*-monooxygenase in *Pseudomonas cepacia* G4 was able to degrade benzene (Shields et al. 1991), but benzene can also be utilized by the TOD pathway (Gibson et al. 1990; Lee et al. 1995). As discussed in Dyreborg et al. (1996), one explanation for the inhibitory effects of benzofuran on the degradation of toluene was that benzofuran might be toxic to some fast-growing toluene degraders, whereas it might be less toxic to some slow-growing degraders. A hypothesis why benzofuran was the strongest inhibiting compound to toluene degradation and not to benzene degradation could be that the fast-growing toluene degraders were using the TOL-pathway, whereas the slow-growing toluene degraders, which also could degrade benzene, were using the other degradation pathways. This is supported by the fact that benzofuran also had an inhibitory effect on the degradation of benzene, even though the effect was much weaker than for thiophene and benzothiophene. An explanation why thiophene was the strongest inhibiting compound on the benzene degradation and not on toluene degradation could be that thiophene acted as a specific enzyme inhibitor in the benzene degradation. In contrast to the shared enzyme system for mono- and dioxygenase in the toluene degradation, a benzene dioxygenase from *Moraxella* was specific for benzene as discussed in Gibson and Subramanian (1984).

Conclusion

It is well known that inhibitory and stimulating effects are likely to occur when degradation of mixtures of organics is investigated. The results presented in this paper show that the effects can have a significant role on the aerobic degradation of aromatic hydrocarbons, and especially the NSO-compounds have a strong inhibitory effect. Thiophene was identified as the compound that was most inhibitory to the aerobic degradation of benzene, but also a significant inhibition from benzothiophene, benzofuran, and 1-methylpyrrole was observed. Three different phenomena for the inhibitory effect were identified. The lag phase was extended when one or more of the NSO-compounds were present. A decrease in the degradation rate of benzene and a residual concentration of benzene were observed. The remaining benzene concentration varied between 12–1262 µg/l, and it was highly dependent on the presence and concentrations of NSO-compounds.

All fifteen creosote compounds investigated were degraded to some extent during 49 days of incubation, but a large variation in the degradation pattern was observed. The NSO-compounds inhibited not only the degradation of benzene but also the degradation of *o*-xylene, *p*-xylene, and naphthalene. A weak effect of the NSO-compounds was observed on the degradation of 1-methylnaphthalene, *o*-cresol and 2,4-dimethylphenol. The degradation of quinoline, indole, phenol, and 1-methylpyrrole was unaffected by the presence of the NSO-compounds. Three of the inhibiting compounds (thiophene, benzothiophene, and benzofuran) could not be degraded as a sole source of carbon and energy, but the degradation was dependent on the concomitant degradation of other compounds.

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