Biodegradability and biodegradation kinetics of creosote compounds in a biofilm in a fractured clay flow model

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Abstract If contaminated sites are situated in moraine clay areas, there may be a risk that the leachate is penetrating fractures to the underlying groundwater and thereby compromising the water quality. This study addresses the biodegradability and biodegradation kinetics of creosote compounds in a model fracture system. Eleven compounds typical of the water-soluble fraction of creosote were added to a mineral medium, each in a concentration of 0.5–1 mg/L: phenol, o-cresol, 2,4- and 3,5-dimethylphenol, naphthalene, 1-methylnaphthalene, 2,3-dimethylnaphthalene, indole, quinoline, benzothiophene, and dibenzofuran. The total concentration was around 7 mg/L. All the compounds were removed biologically by a biofilm in the model fracture under aerobic conditions. The compounds could be divided into readily biodegradable, moderately biodegradable and partly biodegradable compounds. The first order surface removal rates, $k_{1,a}$, were in the range 0.02–0.1 m/d.

Keywords Biodegradation; biofilm; coal tar; creosote; fractured clay; kinetics

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Introduction

The moraine clay, which is deposited in major parts of the Northern Hemisphere, was previously considered as an adequate protective layer against contamination of the underlying aquifers due to its low permeability. However, recent research has shown that the contamination reaches the groundwater, even that of deep aquifers. This is due to the presence of fractures in the clayey till (Jørgensen and Fredericia, 1992; Klint and Fredericia, 1995). In Denmark, excavations have revealed visible fractures in clayey tills to depths of 4–6 m (Jørgensen and Fredericia, 1992; Klint and Fredericia, 1995). A tritium profile measured in Denmark indicated that the transport into the clayey till was not only controlled by diffusion and convective transport, but also by the presence of fractures to depths of 6–10 m (Jørgensen and Fredericia, 1992). At another site in Denmark the free phase of creosote was observed to a depth of 9 m in the fractures in clayey till (Jakobsen and Klint, 1999). The results of a pump test indicated hydraulically active fractures to a depth of 18 m in Canada (Keller *et al.*, 1986). Results from other pump tests indicate that extensive clay layers have been penetrated by hydraulically conductive fractures (D'Astous *et al.*, 1989; Thomson, 1990; Rudolph *et al.*, 1991; Ruland *et al.*, 1991). A tracer test at a site in Denmark also indicated the presence of hydraulically active fractures in 15 m of clayey till within 2 weeks after the initiation of the test, since the tracer was found in the underlying groundwater (Nilsson *et al.*, 2001). The presence of fractures in clayey till may increase the bulk hydraulic conductivity by several orders of magnitude (Fredericia, 1990).

Broholm *et al.* (1995; 1999a,b) studied the fate of dissolved creosote compounds in an intact fractured clay column. Their column experiments revealed that transport through

fractures in clay is an important process. Jørgensen and Foged (1994), Hinsby *et al.* (1996), Jørgensen *et al.* (1998a), and Jørgensen *et al.* (1998b) obtained the same result when they studied various pesticides, viruses, creosote or DNAPL (trichloroethylene) transport in intact blocks of clayey till. Field experiments in fractured clayey tills (McKay *et al.*, 1993; Broholm *et al.*, 2000; Nilsson *et al.*, 2001) have also revealed that dissolved contaminants, although retarded by matrix diffusion, can still migrate at significant rates in some fractured clay deposits.

However, if the contaminants are biodegradable, it may be that they can be destroyed within the time of transport. This is a realistic scenario according to studies with a clay till column and a pilot field study where creosote compounds were infiltrated into the groundwater (Broholm *et al.*, 1999a, b and Broholm *et al.*, 2000). However, such field studies cannot provide quantitative information on the kinetics of biodegradation.

The purpose of this study was to investigate the biodegradability and biodegradation kinetics of creosote compounds in a fractured clay flow model in order to create a basis for risk assessment in relation to creosote waste sites.

Experimental system

The studies were conducted in a fractured clay flow model system consisting of two Vermiculite clay blocks put together and sealed within a Plexiglas box (see Figure 1). Thus, the flow system was constructed with a single fracture. The aperture of the fracture was approximately 500 μm. A pump controlled the flow through the system. The average flow rate was 2.16 L d^{-1} during the experimental period resulting in a residence time of approximately 15 min in the flow system based on flow through the fracture. A stainless steel pipe connected the influent to a 10 L glass bottle, and a stainless steel pipe connected the effluent to a pump. A riser pipe was placed at both the influent and effluent of the system where the difference in hydraulic head through the flow model was monitored. An outline of the experimental set-up is shown in Figure 2.

Eleven compounds typical of the water-soluble fraction of creosote were added to a mineral medium consisting of (mg/L): Fe^{++}, 1, K^{+}, 5, Mg^{++}, 5, NH_4^{+}-N, 1, Ca^{++}, 10, Na^{+}, 1,400, PO_4^{3-}, 15, SO_4^{--}, 31, Cl^{-}, 50, HCO_3^{-}, 3,600, Mn^{++}, 0.027, Co^{++}, 0.03, B^{3+}, 0.006, Zn^{++}, 0.032, Mo^{6+}, 0.048, Ni^{++}, 0.015, I^{-}, 0.013, EDTA, 0.001. Each of the creosote compounds had concentrations of 0.5–1 mg/L: phenol, o-cresol, 2,4- and 3,5-dimethylphenol, naphthalene, 1-methylnaphthalene, 2,3-dimethylnaphthalene, indole, quinoline, benzothiophene, and dibenzofuran. The compounds represent phenols,

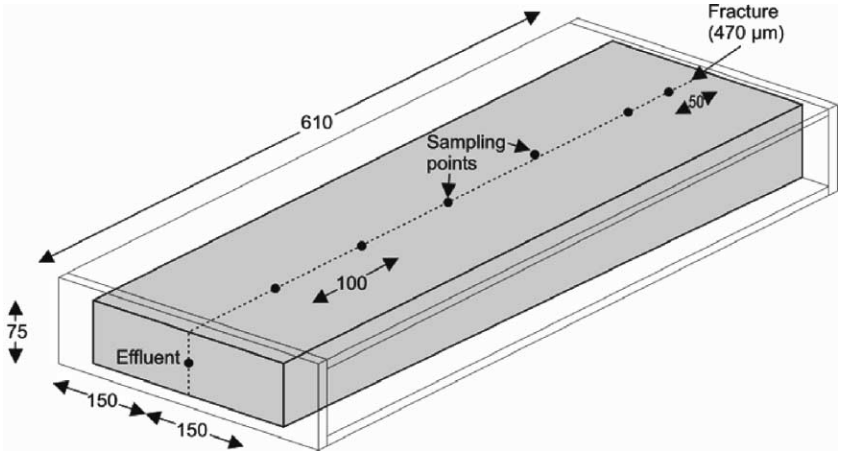

Figure 1 The flow model. All the numbers are in mm except where noted in the figure

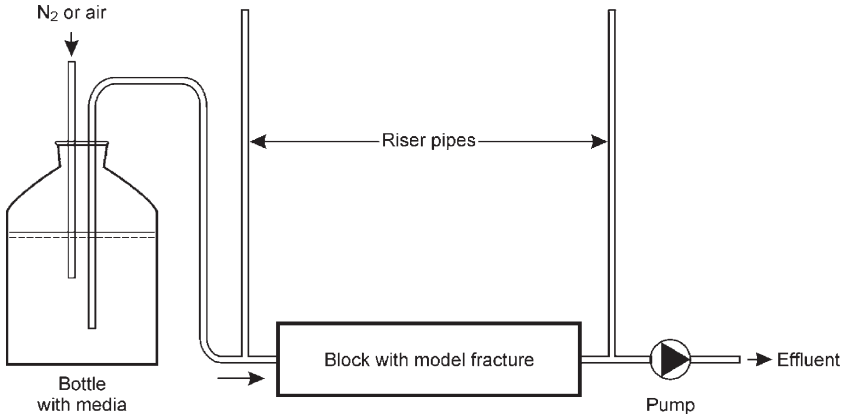

Figure 2 The experimental set-up

aromatic hydrocarbons, and heterocyclic compounds with nitrogen, sulphur and oxygen in the aromatic ring (NSO-compounds). The total concentration was around 7 mg/L. The compounds were analysed by GC after extraction by a mixture of pentane (15%) and ether (85%). In addition, oxygen, chloride, nitrate, nitrite, pH and the pressure drop across the fracture were measured. The total bacterial concentration was measured by the AODC (Acridine Orange Direct Count) method. The influent oxygen concentration was 9–9.5 and the oxygen concentration in the effluent never went below 1.5 mg/L. The pH in the influent was 6.5 and the pH was 7–8 in the effluent. The rise in pH was due to the alkaline properties of the Vermiculite. The bacteria present in the distilled water supply (PVC pipes) inoculated the system. The number of bacteria in the influent water was 1.2×10^6 cells/mL. The experiment started with an aerobic phase (15 days), followed by an anoxic phase (13 days) and then followed by an aerobic phase of 64 days.

Results and discussion

Biodegradability

The biodegradation of some of the eleven compounds is shown in Figures 3 and 4. This represents the aerobic period from day 28 to day 92. The biodegradability pattern fell into three groups: 1, the readily biodegradable compounds: phenol, o-cresol, indole and

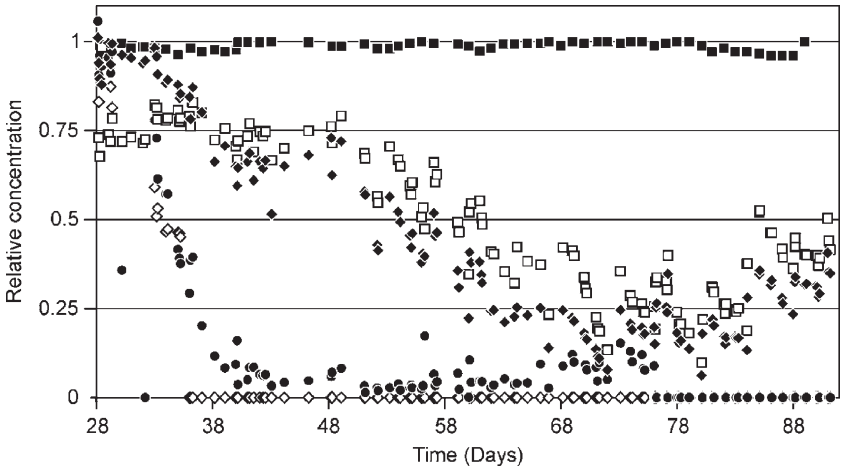

Figure 3 Relative concentrations of phenol (◇), o-cresol (•), 2,4-dimethylphenol (□), 3,5-dimethylphenol (◆), and chloride (■) as a function of time from day 28 to 92

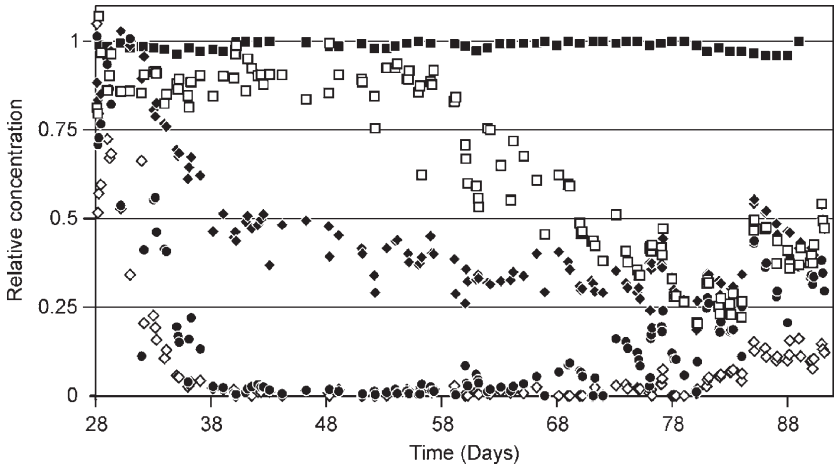

Figure 4 Relative concentrations of indole (•), quinoline (◇), benzothiophene (◆), dibenzofuran (□), and chloride (■) as a function of time from day 28 to 92

quinoline that were removed within 8–12 days; 2, the moderately biodegradable compounds: 2,4- and 3,5-dimethylphenol, naphthalene, 1-methylnaphthalene, 2,3-dimethylnaphthalene, and dibenzofuran; and 3, the partly biodegradable compound benzothiophene, which was most likely removed by co-metabolism.

O-cresol was degraded to 5% of the influent concentration. This might be because o-cresol is biodegraded co-metabolically but it could also be because one or more of the other compounds inhibit the biodegradation of o-cresol. The biodegradation of benzothiophene represents a typical co-metabolic picture. Biodegradation started after only 4 days and 45% was biodegraded after 10–12 days, after which the biodegradation almost stopped. The stagnation occurred when the biodegradation of the readily biodegradable compounds was completed (phenol, o-cresol, indole and quinoline). Phenol and o-cresol were removed from the creosote mixture in order to examine whether these two compounds were used as primary substrates in the co-metabolic biodegradation of benzothiophene (days 76–92). The results showed that the biodegradation of the nine remaining compounds decreased when the two compounds were removed. This indicates that phenol and o-cresol have an effect on the biodegradation of not only benzothiophene but also the other creosote compounds. The primary substrate could as well have been indole and quinoline or a combination of the four easily degradable compounds.

Considering that the residence time in the model fracture was only 15 minutes, the biodegradation potential of the biofilm in the fracture was surprisingly high. This fits well with the experimental results obtained from clay till column experiments and from field experiments with the same type of aromatic compounds (Broholm *et al.*, 1999a, b, 2000).

Biodegradation kinetics

If we assume that the biodegradation takes place only in the fracture, the reaction volume without biofilm is 22.9 cm^3, the surface area is 916 cm^2 (both sides of the fracture), and the specific surface area is 4,000 m^{-1}. Furthermore, if we assume a first order reaction (rate per unit area: $r_a = k_{1,a} \cdot C$), then the first order rate coefficient k_{1a} (area based) was 0.05–0.1 m/d for o-cresol, indole and quinoline. It was higher for phenol since the effluent phenol concentration fell below the detection limit. For the rest of the compounds, 2,4- and 3,5-dimethylphenol, naphthalene, 1-methylnaphthalene, 2,3-dimethylnaphthalene, dibenzofuran and benzothiophene the $k_{1,a}$-value was 0.02–0.035 m/d.

The $k_{1,a}$-values derived from this study were more than ten times lower than the ones determined in previous biofilm studies (Arcangeli and Arvin, 1995). This is most likely because of the much thinner biofilm in the fracture than in the studies referred to. The $k_{1,a}$-value is proportional to the biofilm thickness. According to the pressure drop measurements (increase in hydraulic gradient from 4 to 7.4 per mille), the fracture aperture fell from 470 μm to 380 μm during the experiment, i.e. the average biofilm thickness increased to a maximum of 45 μm if we assume that the biofilm was equally distributed on both sides of the fracture.

Detachment of bacteria from biofilm

To study how much biofilm was detached from the fracture surface the difference in number of bacteria in the influent and effluent of the system was measured using the AODC method. The average concentration of bacteria in the influent was 1.22×10^6 cells mL^{-1} and in the effluent 2.84×10^6 cells mL^{-1}, i.e. about twice as many bacteria were found in the effluent as in the influent. The shape of the bacteria was also different. In the effluent the bacteria were thicker and longer compared with the bacteria in the influent. These results indicate that bacteria detached from the biofilm on the surface of the fracture.

Conclusions

This study has shown that phenols, aromatic hydrocarbons and heterocyclic compounds being leached from creosote waste sites are biodegradable under aerobic conditions in a model fracture system. Considering that the residence time in the model fracture was only 15 minutes, the biodegradation potential of the biofilm in the fracture was surprisingly high. The first order surface removal rates, $k_{1,a}$, were in the range 0.02–0.1 m/d.

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