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Effects of electron acceptors, reducing agents, and toxic metabolites on anaerobic degradation of heterocyclic compounds

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Abstract

Degradation of four heterocyclic compounds was examined under nitrate-reducing, sulphate-reducing and methanogenic conditions. Soil samples from a creosote-polluted site in Denmark were used as inoculum. Indole and quinoline were degraded under all redox conditions with the highest degradation rates obtained under sulphate-reducing conditions. Benzothiophene and benzofuran were not degraded during the observation period of 100 days under any of the redox conditions. Indole and quinoline degrading cultures could be repeatedly transferred under all redox conditions, except for degradation of quinoline under sulphate-reducing conditions which was inhibited by sulphide at concentrations above 0.8 mM. Degradation of quinoline under methanogenic conditions was also inhibited by 3.2 mM sulphide used as a reducing agent, but sulphide had no inhibitory effect on the degradation of indole in methanogenic and sulphate-reducing soil slurries.

Introduction

Creosote waste sites are one of the oldest types of chemical deposits, and during the last decade old abandoned gasworks have become the object of increasing concern. Contamination with creosote originates from its storage in basins of concrete, transportation in pipes and from the wood-preserving industry. Creosote is a complex mixture that consists of polynuclear aromatic hydrocarbons (PAH), phenolic compounds and heterocyclic compounds, i.e. nitrogen, sulphur, and oxygen-containing compounds (Mueller et al. 1989). This study focuses on the biological degradation of heterocyclic compounds, which constitute approximately 5% of creosote (Mueller et al. 1989). However, due to their relatively high water solubility (Kuhn & Suflita 1989) and thus weak sorption they can amount to 35–40% of the water soluble fraction of creosote. Heterocyclic compounds are therefore potential ground water contaminants.

Several investigations have described the degradation of heterocyclic compounds, but most studies have focused on one compound and its derivatives, whereas comparative studies of different heterocyclic compounds under several redox conditions are sparse.

The initial degradation step of indole is hydroxylation to oxindole, regardless of the redox conditions being denitrifying (Madsen & Bollag 1989; Madsen et al. 1988), sulphate-reducing (Shranker & Bollag 1990) or methanogenic (Berry et al. 1987; Gu & Berry 1991; Shranker and Bollag 1990; Madsen et al. 1988). Indole can be completely mineralized under methanogenic conditions to CH_4 and CO_2 (Berry et al. 1987; Wang et al. 1984). A sulphate-reducing marine bacterium, *Desulfobacterium indolicum*, grows with both indole and quinoline as sole carbon sources when sufficient vitamin B_{12} and NaCl are present (Bak & Widdel 1986).

Quinoline was completely mineralized to CH_4 and CO_2 in methanogenic aquifer material and ground water (Godsy et al. 1992). A methanogenic consortium degraded quinoline via hydroxylation to 2(1H)quinolinone, possibly using the oxygen-atom from water for the hydroxylation (Pereira et al. 1988).

At the Pensacola site in Florida, U.S.A., the presence of 2(1H)quinolinone, 1(2H)quinolinone and several isomers of their methylated and oxygenated derivatives were assumed to be products of anaerobic transformation of quinoline (Pereira et al. 1987).

Documentation of anaerobic biodegradation of benzothiophene is sparse. An undefined anaerobic culture degraded up to 75% of the 0.83 mM benzothiophene added (Maka et al. 1987). However, no growth was observed when a methanogenic culture of aquifer material was fed benzothiophene as sole carbon source. Benzothiophene was initially oxidized and the S-heterocyclic ring cleaved, and after a series of transformations, benzothiophene was mineralized to CH_4, CO_2, and H_2S (Godsy & Grbić-Galić 1988). A sulphate-reducing bacterium, *Desulfovibrio desulfuricans* M6, degraded benzothiophene and dibenzothiophene; biphenyl was the major product of dibenzothiophene degradation (Kim et al. 1990).

There are no reports of anaerobic degradation of benzofuran, whereas furan was degraded in a methanogenic aquifer sample (Kuhn & Suflita 1989).

The most extensive study of anaerobic degradation of heterocyclic compounds showed that 9 out of 12 chemicals tested were degraded by sulphate-reducing and/or methanogenic aquifer slurries (Kuhn & Suflita 1989). The authors found degradation of pyridine, nicotinic acid, furan, and 2-furoic acid under both sulphate-reducing and methanogenic conditions; degradation of 2- and 3-methylpyridine, and 2-methylfuran under sulphate-reducing conditions and degradation of 4-methylpyridine and 2-thiophene carboxylic acid under methanogenic conditions. They concluded that nitrogen and oxygen-containing heterocyclic compounds are more susceptible to anaerobic degradation than sulphur heterocyclic compounds.

We have found no reports on the effect of reducing agents or on toxicity of sulphide on degradability of heterocyclic compounds. These factors may be important in transferring laboratory results to field conditions.

On the basis of the state of knowledge presented, the purpose of this study was to investigate the degradation potential of heterocyclic compounds in two soil samples under denitrifying, sulphate-reducing, and methanogenic conditions. The inhibitory effect of heterocyclic compounds (the N-containing indole and quinoline, the S-containing benzothiophene and the O-containing benzofuran) to the soil samples was examined. We also investigated the effect of different reducing agents on the degradation of indole and quinoline under sulphate-reducing and methanogenic conditions, and the toxicity of sulphide on degradation of quinoline under sulphate-reducing conditions.

Materials and methods

Site description and inoculum

Soil samples were collected at a former gasworks on Zealand, Denmark. During operation of the gasworks from 1906 to 1963, creosote in particular, but also petrol and oil, were spilled at the site. The soil samples were a mixture of clay and sand. The two soil samples used in this study were collected at a depth of approximately 1.5 m. The soil sample named "the low contaminated soil" contained no measurable naphthalene, which is the most abundant compound of creosote, whereas the sample named "the highly contaminated soil" contained 4.1 mmol naphthalene per kg soil. In the laboratory, the soil samples were homogenized and mixed with anaerobic mineral medium, 25% soil w/vol (Madsen & Aamand, 1991), reduced with 0.35 mM titanium(III)-citrate (Zehnder & Wuhrmann 1976) and stored in the dark at 10 °C until used.

Culture methods and growth conditions

Experiments were done in 117 ml serum bottles sealed with butyl rubber stoppers. Anaerobic mineral medium was supplemented with vitamins (Madsen & Aamand 1991) and buffered with $NaHCO_3$ (3.8 g/L), with an atmosphere of N_2-CO_2 (4:1), so that the final pH was 7.0 to 7.1. To obtain denitrifying conditions, the medium was reduced with 0.80 mM sulphite (Na_2SO_3) and 2.0 mM nitrate was added as electron acceptor. To obtain sulphate-reducing and methanogenic conditions, the medium was reduced with 0.35 mM titanium(III)-citrate (Zehnder & Wuhrmann 1976), and 2.0 mM sulphate was added as electron acceptor to the sulphate-reducing soil slurries. The medium was inoculated with 1.0% soil (w/vol), to a final volume of 100 mL.

A set of soil slurries were autoclaved twice for 20 min at 121 °C with a 24 hour interval and used as sterile controls. Another set of soil slurries was used for quantification of the background redox processes, and thus not amended with heterocyclic compounds. Heterocyclic compounds were added separately to individual serum bottles from anaerobic, aqueous stock solutions.

The initial concentrations were 80 mM indole, 50 mM quinoline, 30 mM benzothiophene or 20 mM benzofuran. The bottles were incubated at 37 °C in the dark. All investigations were made in triplicate series.

Effect of additional electron donors on quinoline degradation in sulphate-reducing soil slurries from the low contaminated soil

To produce enrichment cultures, indole and quinoline were readded to the soil slurries after degradation. Under sulphate-reducing conditions degradation of quinoline could not be repeated and therefore the following tests were made: a) flushing with anaerobic gas for 5 min to remove any possible toxic volatile metabolites, b) 10 times dilution to reduce the effect of possible non-volatile toxic metabolites, c) 10 times dilution plus amendment with yeast extract (1.0 g L^{-1}) to test for the influence of primary substrates, minerals or vitamins and d) 10 times dilution plus one of the following amendments: 0.50 mM of acetate, pyruvate, lactate, propionate, butyrate, benzoate, toluene, or phenol, or addition of hydrogen (21 kPa) or 10 times concentrated vitamin solution to test for more specific needs. All cultures were respiked with quinoline and sulphate. These tests were made in medium reduced with 0.35 mM titanium(III)-citrate or 3.2 mM sulphide. To test for maintenance of the quinoline degrading ability, quinoline and sulphate were respiked after degradation.

Effect of reducing agents on the degradation of indole and quinoline in sulphate-reducing and methanogenic soil slurries from the low contaminated soil

To test for the effect of different reducing agents on the degradation potential of indole and quinoline in the sulphate-reducing and methanogenic soil slurries, media were reduced with the following reducing agents: 0.35 mM titanium(III)-citrate, 3.2 mM Na-sulphide, or 0.029 mM Na-dithionite. The effect of sulphide precipitates produced during sulphate-reduction was examined by addition of 1.0 mM Fe^{2+} as $FeCl_2$ to sulphate-reducing cultures reduced with titanium(III)-citrate and dithionite at the same concentrations as above.

To test for effects of sulphide on the degradation of quinoline in the sulphate-reducing enrichment culture, medium was reduced with 0.35 mM titanium(III)-citrate and Na-sulphide was added at various concentrations up to 6.4 mM.

Effects of additional electron donors on the degradation of benzothiophene and benzofuran in the nitrate-reducing soil slurries from the low contaminated soil

To enhance degradation of benzothiophene and benzofuran in the denitrifying soil slurries after reduction of added nitrate, the following treatments were tested: a) addition of 2.0 mM nitrate as electron acceptor to prolong the test period/adaptation period, b) addition of 2.0 mM nitrate plus yeast extract (1.0 g/L w/vol) to test for need of a primary substrate and c) amendment with 2.0 mM iron(III)-citrate to test for iron as an alternative electron acceptor and citrate as electron donor. This procedure was done only with the nitrate-reducing soil slurries because benzothiophene and benzofuran were expected to be most susceptible to degradation under nitrate-reducing conditions due to the higher redox potential compared to sulphate-reducing and methanogenic conditions.

Analysis of heterocyclic compounds by GC

For quantification of the heterocyclic compounds, 1.0 mL sub-samples were made alkaline to pH 12 with 25 μL 4.0 M KOH, and extracted with 500 μL diethylether plus 50 μL pentane with 0.38 mM undecane added as an internal standard. The organic phase was analyzed on a Carlo Erba Mega gas chromatograph with a flame ionization detector and a CP-Sil 8 CB column, 25 m, ID 0.32 mm, film 0.25 mm. The column temperature started at 60 °C, raised by 30 °C min^{-1} to 150 °C and raised by 20 °C min^{-1} to 200 °C in one min. Detector temperature was 300 °C and injector temperature was 285 °C.

Quantification of methane

Methane formation was determined by analysis of headspace samples on a Shimadzu GC-9A gas chromatograph with a flame ionization detector. The injection temperature was 200 °C, detector temperature 275 °C, and the column temperature was 40 °C. N_2 was carrier gas.

Quantification of sulphate

Sulphate was measured spectrophotometrically at 420 nm after precipitation of sulphate as barium sulphate (Madsen & Aamand, 1991). Sulphide production was

measured by the addition of iron(II)-chloride to precipitate sulphide as iron sulphide, which is black.

Quantification of nitrate, nitrite, and N_2O

Nitrite were quantified using a Technicon Autoanalyzer II at 545 nm after reaction with sulphanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride at pH 2. Nitrate was measured as nitrite after reduction with hydrazinsulphate. Denitrification was assayed by addition of acetylene (10% in headspace) and analysis of accumulated N_2O in headspace on a Carlo Erba Gas Chromatograph, Vega series 2, with an electron capture detector.

Results

Degradability of heterocyclic compounds in slurries of the low contaminated soil

In soil slurries amended with sulphate, indole and quinoline were degraded within 9 days. In the methanogenic slurries, indole was degraded within 25 days, while 74 days were needed for full degradation of quinoline. In slurries with nitrate added, two serum bottles in a triplicate series degraded indole within 42 days, while 16 μM still remained after 100 days in the last serum bottle (Table 1). Quinoline was degraded within 56 days, but again only in two out of three cultures (Table 1). In the third bottle quinoline was not degraded.

Benzothiophene and benzofuran were not degraded under any of the redox conditions tested within the test period of 100 days. Further, no degradation after amendment with yeast extract as primary substrate or with iron(III)-citrate was observed, indicating that iron(III) as electron acceptor together with citrate as electron donor did not have any effect on benzothiophene or benzofuran degradation (data not shown).

Effect of addition of heterocyclic compounds on anaerobic respiration in slurries of the low contaminated soil

In the low contaminated soil without addition of heterocyclic compounds, 2.0 mM nitrate was reduced to nitrite and then denitrified within approximately 30 days. Within the same time span, 2.0 mM sulphate was reduced and sulphide was produced in the sulphate-reducing culture (Table 2). After 30 days, production of methane was 3 mmol methane per litre methanogenic culture, approximately 70% of the total methane-production after 60 days of incubation.

Addition of heterocyclic compounds to the slurries generally decreased the rates of the anaerobic respiratory processes slightly. On average, cultures with quinoline were the least inhibited, while cultures with indole and benzothiophene showed the greatest inhibition. The various respiratory processes showed approximately equally sensitivity towards the heterocyclic compounds (Table 2).

Anaerobic activity in slurries of the highly contaminated soil

In an experiment with slurries of the highly contaminated soil sample, no degradation of either indole, quinoline, benzothiophene or benzofuran was observed within a test period of 80 days. Production of methane was used as an indicator of anaerobic activity, and there was a lag phase of 30 days before methane production started in slurries of the highly contaminated soil; there was no lag phase in the slurries of the low contaminated soil. After the lag phase, methane was produced at the same rate in the highly contaminated soil as in the less contaminated soil sample (data not shown). A total concentration of 41 μM naphthalene was measured in slurries inoculated with 1.0% of the highly contaminated soil, indicating that this soil sample already contained high concentrations of creosote contaminants.

Effect of electron donors and reducing agents on the degradation of quinoline in sulphate-reducing slurries of the low contaminated soil

Initially, quinoline was degraded under all redox conditions, with the highest rate under sulphate-reducing conditions. However, when quinoline and sulphate were again added to sulphate-reducing slurries, no further degradation of quinoline was observed. To examine this phenomenon, the slurries were, as detailed in the Materials and methods section, a) flushed, b) diluted, c) diluted and yeast extract was added, or d) diluted and one of nine different electron donors was added. No degradation of quinoline was observed in flushed undiluted slurries in medium reduced with titanium(III)-citrate. Degradation of quinoline was stimulated in diluted soil slurries amended with yeast extract, lactate or hydrogen, as well as in diluted slurries without addition of an electron donor. However, when

Table 1. Maximum rates of anaerobic degradation of indole and quinoline under denitrifying, sulphate-reducing and methanogenic conditions in slurries of the low contaminated soil.

Redox-condition	Indole (μmol L^{-1} d^{-1})[a]	Quinoline (μmol $L^{-1}d^{-1}$)[a]
Denitrifying	1.9 ± 0.5	0.5 ± 0.6
Sulphate-reducing	16.0 ± 1.8	13.1 ± 1.4
Methanogenic	6.6 ± 0.6	1.8 ± 0.6

[a] Degradation rates determined from the linear part of a concentration versus time plot by linear regression of at least 15 measurements ± standard error.

Table 2. Range of maximum rates of anaerobic respiration in the low contaminated soil in triplicates with no heterocyclic compounds and with addition of individual heterocyclic compounds.

Heterocyclic compound added	Nitrate-reduction (μmol nitrate reduced L^{-1} d^{-1})	Sulphate-reduction (μmol sulphate reduced L^{-1} d^{-1})	Methanogenesis (μmol methane produced $^{-1}d^{-1}$)
None	79 ± 11	101 ± 10	90 ± 10
70 μM indole	45 ± 8	88 ± 14	70 ± 9
60 μM quinoline	91 ± 9	108 ± 9	51 ± 9
30 μM benzothiophene	54 ± 12	69 ± 8	73 ± 9
20 μM benzofuran	68 ± 8	ND	70 ± 10

Respiration rates determined from the linear part of a concentration versus time plot by linear regression of at least 15 measurements ± standard error. ND: not determined.

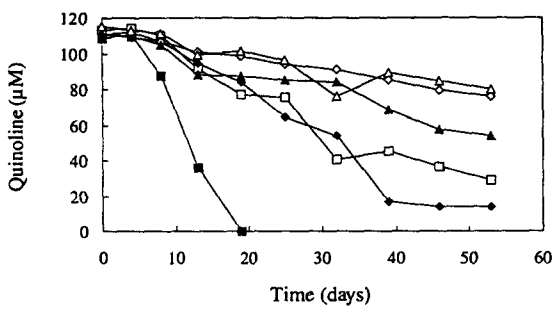

Fig. 1. Effect of sulphide on quinoline degradation in sulphate-reducing slurries from the low contaminated soil in medium reduced with titanium(III)-citrate. No sulphide added (■), Sulphide added in concentrations of 0.8 mM (□), 1.6 mM (♦), 2.4 mM (◇), 3.2 mM (▲), and 6.4 mM (△).

quinoline was readded to the original soil slurries, it was only partially degraded or not degraded, while dilution of the cultures re-established the quinoline-degradation. Soil slurries reduced with 3.2 mM sulphide only showed partial degradation of quinoline, with the most pronounced inhibitory effect in slurries amended with hydrogen, lactate, propionate and citrate (data not shown).

Addition of sulphide to sulphate-reducing slurries reduced with titanium(III)-citrate showed that sulphide inhibited degradation of quinoline even at a concentration as low as 0.80 mM sulphide. Quinoline was degraded within 19 days in slurries with no sulphide added, while 10% of the quinoline remained after 50 days in the slurries with 0.80 mM sulphide added. No quinoline degradation occurred at 2.4 mM sulphide or above (Fig. 1).

Effect of reducing agents on degradability of indole and quinoline in methanogenic and sulphate-reducing slurries of the low contaminated soil

No reducing agent was optimal under all conditions tested (Fig. 2). In general, degradation of indole and quinoline was slow but constant in slurries reduced with dithionite. In methanogenic slurries reduced with sulphide a lag phase of about 25 days occurred before degradation of indole and quinoline, whereas indole and quinoline degradation were almost completely inhibited in sulphate-reducing slurries reduced with sulphide. Precipitation of sulphide in sulphate-reducing cultures had no effect on the degradation of indole, whereas addition of Fe^{2+} had a marked stim-

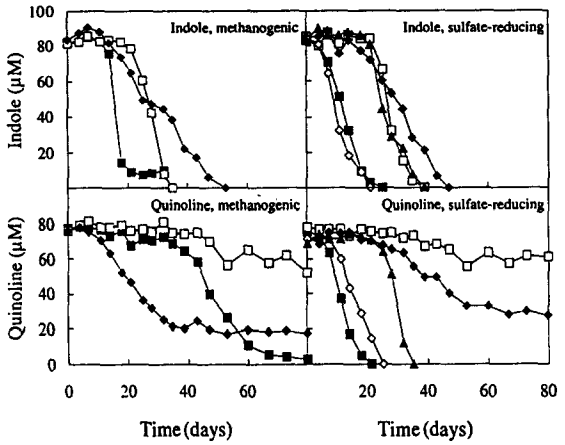

Fig. 2. Effect of reducing agents on degradation of indole and quinoline in methanogenic and sulphate-reducing slurries of the low contaminated soil. The tested reducing agents are titanium(III)-citrate (■), Na-dithionite ♦ and sulphide (□) Sulphide-precipitation was tested by addition of $FeCl_2$ to medium reduced with titanium(III)-citrate (◇), and Na-dithionite (▲).

ulating effect on degradation of quinoline in slurries reduced with dithionite (Fig. 2). When titanium(III)-citrate was used as a reducing agent under sulphate-reducing and methanogenic conditions the degradation rate of indole and quinoline was generally high except for degradation of quinoline under methanogenic conditions where a lag phase of 40 days preceded degradation.

Discussion

Soil slurries from the low contaminated soil degraded both indole and quinoline under denitrifying, sulphate-reducing and methanogenic conditions. Generally, indole was degraded more easily than quinoline, and the degradation rates of indole and quinoline were highest under sulphate-reducing conditions. The background rates of anaerobic respiration in the soil slurries were all of the same order of magnitude. Thus, the activities of anaerobic respiration does not explain the high degradation rates of indole and quinoline under sulphate-reducing conditions, and the slow and variable degradation rates under nitrate-reducing conditions. It should be noted that an extra carbon source, citrate, was added to the sulphate-reducing and methanogenic slurries by reducing the medium with titanium(III)-citrate, whereas the denitrifying medium was reduced with the inorganic sulphite. However, as rates of nitrate-reduction, sulphate-reduction and methanogenesis were approximately the same, the degradation of the heterocyclic compounds should be comparable. Similar observations were made by Liu et al. (1994), who found that indole was degraded under denitrifying, sulphate-reducing and methanogenic conditions, within 18, 27 and 17 days, respectively. Quinoline was completely transformed under sulphate-reducing and methanogenic conditions within 45 and 23 days, whereas only 23% of quinoline was transformed under denitrifying conditions after 83 days. This could indicate that the native populations of sulphate-reducing and methanogenic quinoline-degrading bacteria are larger and than the denitrifying populations of quinoline-degrading bacteria in the examined soil samples.

The resistance of benzothiophene to degradation was unexpected, since several studies have demonstrated anaerobic degradation of benzothiophene (Maka et al. 1987; Godsy & Grbić-Galić 1988). It was also expected that benzofuran would be degraded since oxygen-containing heterocyclic compounds have been reported to be more susceptible to anaerobic degradation than the sulphur-containing heterocyclic compounds (Kuhn & Suflita 1989). The oxygen-containing heterocyclic compounds examined by Kuhn & Suflita (1989) were furan and 2-furoic acid, which were, respectively, 90 and 99% degraded within one month, and 2-methyl furan which was 70% degraded within three months.

The anaerobic respiration rates measured as nitrate-reduction, sulphate-reduction and methanogenesis indicated that none of the heterocyclic compounds were added at inhibitory concentrations. Toxic effects of the added heterocyclic compounds can thus not explain the lack of degradation of benzothiophene and benzofuran. Our results indicate that no organisms able to degrade benzothiophene or benzofuran were present in our soil samples. A great abiotic loss of benzothiophene excluded extension of the incubation time beyond 100 days. The same phenomenon has been observed for thiophene, 2-methylthiophene and 3-methylthiophene by Kuhn & Suflita (1989). The general nature of our results was further supported by investigations made with a number of soil and sludge samples from different creosote or oil-contaminated spots. These investigations all showed the same tendency for resistance of benzothiophene and benzofuran and degradability of indole and quinoline under

nitrate-reducing, sulphate-reducing and methanogenic conditions (manuscript in preparation).

Slurries of the highly contaminated soil showed no degradation potential for any of the heterocyclic compounds tested. Production of methane showed a lag phase of 30 days compared with the low contaminated soil. After this lag phase, the rate of methane-production corresponded to the rate in slurries of the low contaminated soil. The lag phase could be due either to a low number of organisms in the soil sample or inhibition of the microbial activity by creosote compounds in the soil. As the inhibitory effect was partially relieved by the 100-fold-dilution of the soil sample, the most likely explanation is inhibition by creosote in the soil.

Degradation of quinoline under sulphate-reducing conditions always stopped before all substrate was converted. The reasons for the loss of quinoline-degrading activity in the sulphate-reducing culture, and re-establishment of the degrading activity of the culture by dilution with new medium could be explained by 1) further need for minerals or vitamins deficient in the original culture, 2) depletion of citrate from titanium(III)-citrate used as a primary substrate, or 3) by the presence of an inhibitory metabolic product. Dilution of the soil slurries after degradation of quinoline was the only treatment by which quinoline degradation could be maintained. Supplementation of yeast extract, other carbon sources, vitamins, or hydrogen did not re-establish the quinoline-degrading activity. This led to exclusion of theory one. Citrate from titanium(III)-citrate could possibly have been used as a primary substrate, but since Na-citrate added as electron donor did not stimulate the degradation of quinoline in media reduced with sulphide, and since degradation of quinoline was observed in cultures reduced with dithionite, theory 2 was also excluded. The most likely theory (no. 3) is that sulphide acts as an inhibitory metabolite on the quinoline-degrading bacteria, which again was confirmed by experiments with sulphide added to slurries reduced with titanium(III)-citrate. It is, therefore, likely that the cultures are slowly toxified by sulphide produced by sulphate-reduction. Normal concentrations of sulphide used as a reducing agent in anaerobic media average from 2.9 to 7.4 mM (Jain et al. 1991), which is even higher than the concentrations found to inhibit the degradation of quinoline in our study, of 0.8 to 2.4 mM. This indicates that the population of quinoline-degrading organisms were sensitive to low concentrations of sulphide. Sulphide has a direct and reversible toxicity on some sulphate-reducing bacteria, with complete inhibition of culture growth at 16.1 mM (Reis et al. 1992). Complete mineralization of quinoline has been shown with the sulphate-reducing bacterium *Desulfobacterium indolicum* in medium reduced with 5.1 mM sulphide (Bak & Widdel, 1986).

Comparison of effects on degradation in slurries reduced with different reducing agents were expected to show that titanium(III)-citrate would give the best degradation of indole and quinoline under methanogenic and sulphate-reducing conditions. The argument for this hypothesis was that titanium(III)-citrate is an organic reducing agent which supplies an extra carbon source to the system as well as the fact that titanium(III)-citrate is believed to give a lower redox potential than sulphide (Zehnder & Wuhrmann, 1976). However, titanium(III)-citrate was not the best reducing agent for degradation of quinoline under methanogenic conditions which could be due to substrate competition between quinoline and citrate. Sulphide inhibition during sulphate-reducing conditions only showed a significant effect on the degradation of indole and quinoline in sulphate-reducing slurries reduced with dithionite. This could be explained by the fact that about 2 mM of sulphide had to be produced by mineralization of organic matter from the soil by sulphate-reduction in order to get a pronounced inhibition. However, as about 30 days proceeded before this concentration was reached, the concentration of sulphide was too low for the first month of incubation to have any effect on degradation of indole and quinoline in sulphate-reducing slurries reduced with titanium(III)-citrate.

Conclusions

Indole and quinoline were easily degraded under nitrate-reducing, sulphate-reducing and methanogenic conditions in slurries inoculated with samples of a slightly contaminated soil. However, benzothiophene and benzofuran were not degraded under any of the redox conditions tested. Degradation of indole and quinoline could be repeated by readdition of indole and quinoline, except for quinoline under sulphate-reducing conditions, where degradation could only be maintained by simultaneous dilution of the culture. Results showed that this was due to inhibition by sulphide at a concentration above approximately 1 mM. Titanium(III)-citrate was an effective reducing agent that supported degradation of indole and quinoline

under methanogenic and sulphate-reducing conditions. However, during incubations longer than about one month, sulphide production had an inhibitory effect on degradation of quinoline. This sulphide inhibition was more pronounced when not only organic matter from the soil was present, but also for example citrate from titanium(III)-citrate. The best way to enrich for sulphate-reducing bacteria degrading quinoline was in media reduced with dithionite and with sulphide precipitated by Fe^{2+}. By this method quinoline was the only carbon source present and there was no inhibitory effect of sulphide.

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