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Degradation of toxaphene in aged and freshly contaminated soil

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Abstract

Degradation of toxaphene in soil from both newly contaminated (from Sweden) and aged spills (from Nicaragua) were studied. The newly contaminated soil contained approximately 11 mg kg^{-1} toxaphene while the aged Nicaraguan soil contained approximately 100 mg kg^{-1}. Degradation was studied in anaerobic bioreactors, some of which were supplied with lactic acid and others with Triton® X-114. In this study we found that the lower isomers Parlar 11, 12 were degraded while the concentration of isomer Parlar 15 increased. This supported an earlier evaluation which indicated that less chlorinated isomers are formed from more heavily isomers. Lactic acid when added to the soil, interfere with the degradation of toxaphene. Lactic acid was added; several isomers appeared to degrade rather slowly in newly contaminated Swedish soil. The Swedish soil, without any external carbon source, showed the slowest degradation rate of all the compounds studied. When Triton® X-114 at 0.4 mM was added, the degradation rate of the compounds increased. This study illustrates that biodegradation of toxaphene is a complex process and several parameters have to be taken into consideration. Degradation of persistent pollutants in the environment using biotechnology is dependent on bioavailability, carbon sources and formation of metabolites.

Keywords: Aged contaminated soil; Biodegradation; Lactic acid; POPs

1. Introduction

Toxaphene is the trade name for an organochlorine pesticide comprised of a mixture of >200 polychlorinated C10-terpenes (WHO, 1984). It has been one of the most widely used organochlorine insecticides in many parts of the world (Voldner and Li, 1993, 1995).

The global use of toxaphene from 1950 to 1993 was estimated to be about 1 330 000 tonnes (Voldner and Li, 1993). The United States, the former Soviet Union,

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Central America and Brazil were using the largest amounts of the pesticide. The use of toxaphene was restricted and eventually banned in most western countries during the 1980s. However, many developing countries kept using large quantities of toxaphene and DDT and it is still being used in some parts of the world (Voldner and Li, 1993).

Toxaphene was widely used to replace DDT as an agricultural insecticide in 1970s (Saleh, 1991). More than 79000 metric tons have been produced in Nicaragua from 1974 to 1988 (Appel, 1991; Beck, 1997). Toxaphene in Nicaragua was often applied in formulations together with DDT, parathion or methyl-parathion. It was not until 1992 that toxaphene was banned in Nicaragua (Beck, 1997).

Between 1995 and 1997 a screening for persistent chlorinated hydrocarbons was carried out in the main coastal lagoons on the pacific coast of Nicaragua. Results for a wide range of organochlorine pesticides in lagoon sediments showed high concentrations of toxaphene and total DDT's attaining values of 1420 ng g^{-1} and 270 ng g^{-1} dry weight, respectively. The very high concentrations of toxaphene and DDT in these lagoons are the result from the intensive use of the pesticides in the district of Chinandega (Carvalho et al., 1999).

A number of studies have shown that toxaphene is biodegradable, especially by anaerobic processes (Parr and Smith, 1976; Smith and Willis, 1978; Mirsatari et al., 1987). The US EPA Environmental Response Team Centre (ERTC) and Response Engineering and Analytical Contract (REAC) have been conducting studies on the removal of toxaphene using anaerobic bioremediation technology since 1991.

Degradation of toxaphene can be done by aerobic or anaerobic processes in the environment. Aerobic degradation of toxaphene in soils is very slow (Nash and Woolson, 1967; Menzie, 1972). However, Fingerling et al. (1996) reported anaerobic degradation of six toxaphene components as well as technical toxaphene in soil. They found that toxaphene was degraded by reductive dechlorination in loamy silt under anaerobic conditions.

The aim of the present study was: (1) to degrade toxaphene by anaerobic treatment in aged contaminated soil, (2) to investigate the effect on bioavailability by the addition of Triton® X-114 on the biodegradation rate to determine what concentration would be the most efficient and (3) to test the effect of the addition of an extra carbon and energy source.

Surfactant molecules consist of a hydrophobic and a hydrophilic component and can interact with polar as well as apolar surfaces (Rosen, 1989). Above the critical micelle concentration (CMC), surfactant molecules aggregate and form micelles with a hydrophobic centre into which partitioning of chemicals is possible. The partitioning results in increased pseudo-solubility in water of hydrophobic compounds such as toxaphene, thereby increasing the concentration gradient and mass-transfer rates. CMC is a measure of surfactant efficiency. A lower CMC indicates that less surfactant is needed to saturate interfaces and form micelles. Typical CMC values are lower than 1% by weight. Triton® X-114, polyethylene glycol tert-octylphenyl ether (1,1,3,3-tetramethylbutyl) phenyl-polyethylene glycol is a non-ionic detergent, 100% active ingredient. It is often used in biochemical applications to solubilize proteins. Triton® X-114 has a CMC of approximately 0.2 mM or 0.009% (w/w in water) (Helenius and Simons, 1975). In this study Triton® X-114 was studied in two different concentrations, 0.2 and 0.4 mM.

Addition of lactic acid is regarded as one of the most effective ways to provide hydrogen and electrons to microbial populations as they undergo microbial breakdown. The hydrogen is then exploited by the microbial consortium for the availability of its electrons in chlorinated contaminants.

2. Materials and methods

2.1. Experimental systems

Six glass reactors with an internal diameter of 5 cm and a length of 26 cm, were used. The caps were made of PTFE material. Soil (150 g) was placed in each reactor. The first one, containing only sterile soil was used as control. The second reactor was loaded with aged contaminated soil from Nicaragua. The third, fourth, fifth and sixth reactors were filled with newly contaminated Swedish soil. Sterile water was added to the control reactor. All other reactors were supplemented with 100 ml of a bacterial mixture from Ellinge (anaerobic digestor) and 125 ml of medium. Lactic acid was added to the medium in reactors 2 and 3. For reactor 4, the medium was prepared without lactic acid. The medium used in reactors 5 and 6 was prepared with Triton® X-114 instead of lactic acid. The concentrations of Triton® X-114 in the fifth and sixth reactor were 0.4 mM and 0.2 mM, respectively (Table 1). All the reactors, except for the control, contained a mixed population of bacteria including sulphate-reducing bacteria.

2.2. Soil characteristics

The soil was sieved through a stainless steel net of 0.355 mm. The clean Swedish soil free of toxaphene and the aged contaminated Nicaraguan soil had an organic matter content of 0.7% and 2.0%, respectively. Swedish soil without any addition of toxaphene was divided into two parts. One part was autoclaved at 121 °C and the operation was repeated after 1 day to prevent the growth of bacteria. This soil was used as a sterile control. The second part (1 kg) was polluted with a solu-

Table 1
Setup of experiments

Reactor no.	Soil	Inoculation	External carbon source	Surfactant
1	Sterile conditions control	None	None	None
2	Nicaragua, aged toxaphene residues	Bacteria mixture	Lactic acid	None
3	Sweden, toxaphene added in study	Bacteria mixture	Lactic acid	None
4	Sweden, toxaphene added in study	Bacteria mixture	None	None
5	Sweden, toxaphene added in study	Bacteria mixture	None	Triton X-114 0.4 mM
6	Sweden, toxaphene added in study	Bacteria mixture	None	Triton X-114 0.2 mM

tion of Toxaphene (Promochem, 99.5%), which was dissolved in 500-ml acetone (Merck p.a.). The solution was evenly spread in 2–4 ml aliquots over the soil and mixed during a total time of 3 h. When the solvent was evaporated and dried, the soil fraction was stored in amber glass bottles. The final concentration of toxaphene in the soil was 11 mg kg^{-1} dry weight.

2.3. Culture

The reactors 2, 3, 4, 5 and 6 were inoculated with 100 ml of bacterial mixture from Ellinge anaerobic digestor (Sweden). Once a month the reactors were inoculated with 30 ml of the bacterial mixture from the same place.

2.4. Reactors

The reactors were operated with a constant flow rate of synthetic medium through the experiment at 5.0 ± 0.5 ml/h during 80 days. Peristaltic pumps were used for recirculation and the introduction of the influent. The reactors were operated with a hydraulic retention time of 3 days. The experimental setup is shown in Fig. 1.

2.5. Culture medium

The experiment was carried out with a synthetic medium containing the following (in grams per liter): $(NH_4)_2SO_4$, 1.17; K_2HPO_4, 0.5; $CaCl_2.2H_2O$, 0.10; Ethylenediamine-*N*,*N*,*N*′,*N*′-tetra-acetic acid ($C_{10}H_{16}N_2O_8$), 0.10; $Fe_2(SO_4)_3$, 0.10; $MgSO_4 \cdot 7H_2O$, 0.10; to 1 distilled water. The medium was not autoclaved. Lactic acid or Triton® X-114, was added to selected reactors according to Table 1.

2.6. Analytical methods

Soil samples from reactors were taken from day 0 when the experiment started, after 55 days, and when the experiment finished (79 days). All the samples were extracted and measured by GC–MS. The samples from the reactors were collected in anaerobic conditions with a large spoon. To avoid air contact with the reactor content, the sampling was done under a slow flow of

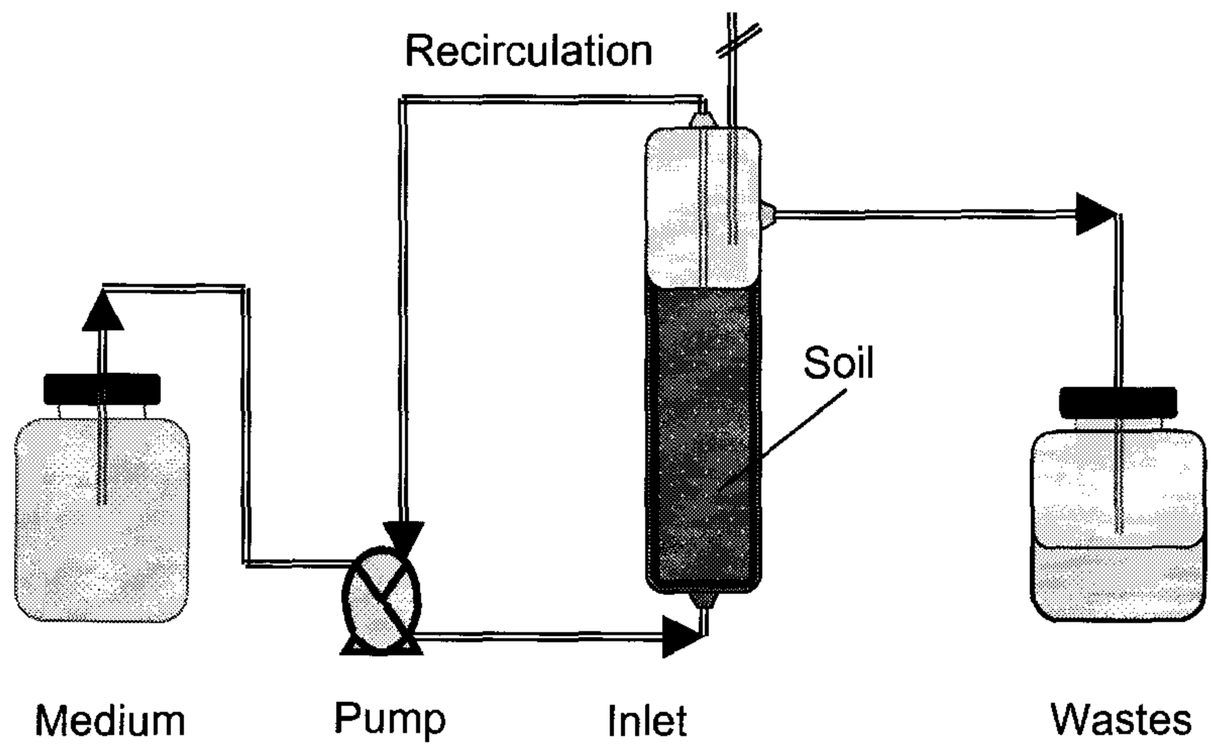

Fig. 1. Schematic representation of the sequential bioreactor setup. All the reactors except for the control contained a mixed population of bacteria including sulphate reducing bacteria. They were operated with a constant flow rate of synthetic medium through the reactor at 5.0 ± 0.5 ml/h during 80 days. The reactors were operated with a hydraulic retention time of 3 days.

nitrogen. All the samples were placed in a tray covered with aluminium foil. The soil samples were left in a fume hood until they were dried. About 10 g of sample was weighted accurately and placed in an extraction thimble. A volume of 500 μl of a solution of 80 pg μl^{-1} of octachloronaphtalene (OCN) was added as an internal standard and the sample was extracted for 8 h in a Soxhlet apparatus with hexane (250 ml). The hexane was replaced for methylene chloride (250 ml) and the extraction was continued for another 8 h. After extraction, the extracts were concentrated in a rotary evaporator to about 15 ml. The extract volume was adjusted to 1 ml by evaporation of the excess solvent under a gentle stream of clean, dry nitrogen. Elementary sulphur and sulphur compounds were removed with tetra butyl ammonium hydrogen sulphite (Jensen et al., 1977). The extracts were cleaned up using a modified method by Smith et al. (1984). The samples were evaporated to approximately 0.5 ml and then transferred to a 1 ml volumetric flask that was filled up with hexane. The toxaphene isomers studied are listed in Table 2.

2.7. GC–MS conditions

After sample extraction and clean up, 2 μl of the final volume of hexane was injected on a 30 m. Non-polar DB-5MS column (0.25 mm ID, 0.25 μm film thickness) connected with an Agilent gas cromatography mass selective detector GC/MSD 6890/5973. The following temperature programme was used: 100 °C (2 min), increased by 20 °C/min to 140 °C, then with 5 °C/min to 300 °C (Haglund et al., 1998). Detection was achieved by negative chemical ionisation (NCI) using methane as the reagent gas at 40% of a flow of 5 ml/min, resulting in a pressure of 2.2×10^{-4} Torr in the ion source. Both full scan and selected ion monitoring (SIM) were used. Identification and quantification of the different toxaphene isomers was done by comparison with a technical mixture of toxaphene (40 pg μl^{-1}), using octachloronaphtalene (OCN) as an internal standard. A reference mixture (Ehrenstorfer, Augsburg, GFR,) containing hepta- through decachlorobornanes and hexa and heptachlorocamphenes was used for peak assignments for GC/MS (Hainzl et al., 1995).

Table 2
Isomers of toxaphene used in this study

Compound designation	Chemical name (IUPAC)
P11	2,2,3-*exo*,8,9,10-Hexachlorocamphene
P12	2-*exo*,3-*endo*,8,8,9,10-Hexachlorocamphene
P15	2-*exo*,3-*endo*,7,8,9,10-Hexachlorocamphene
P26	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,10,10-Octachlorobornane
P31	2,2,3-*exo*,8,8,10,10-Octachlorocamphene
P32	2,2,5-*endo*,6-*exo*,8,9,10-Heptachlorobornane
P39	2,2,3-*exo*,5-*endo*,6-*exo*,8,9,10-Octachlorobornane
P40	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,9,10,10-Octachlorobornane
P41	2-*exo*,3-*endo*,5-*exo*,6-*exo*,8,9,9,10,10-Octachlorobornane
P42	2,2,5-*endo*,6-*exo*,8,8,9,10-Octachlorobornane and 2,2,5-*endo*,6-*exo*,8,9,9,10-Octachlorobornane
P44	2,-*exo*,5,5,8,9,9,10-Octachlorobornane
P50	2-*endo*,3-*exo*,5-*endo*,6-*exo*,8,8,9,10,10-Nonachlorobornane
P56	2,2,5-*endo*,6-*exo*,8,8,9,10,10-Nonachlorobornane
P58	2,2,3-*exo*,5,5,8,9,10,10-Nonachlorobornane
P59	2,2,5-*endo*,6-*exo*,8,9,9,10,10-Nonachlorobornane
P63	2-*exo*,3-*endo*,5-*endo*,6-*exo*,8,9,9,10,10-Nonachlorobornane
P69	2,2,5,5,,6-*exo*,8,9,9,10,10-Decachlorobornane

The "nominal" nomenclature system has been used according to Parlar (Burhenne et al., 1993).

2.8. Quality assurance–quality control

Quality assurance control precautions were taken during the present experiment. Recovery assays for the overall procedure were carried out with spiked soils. Recoveries of all the samples were between 70% and 100% and the RSD was below 15% ($n = 2$). Procedure blanks were processed in every set of samples. Blank samples and spiked controls without bacterial cultures were carried out for every series and over the same time range as the experiments.

3. Results and discussion

3.1. Control reactor

A mixture of sterilized soil and water was placed in a reactor in order to act as a control reactor. The toxaphene levels decreased in this reactor, to which neither an external carbon source nor bacteria were added. The toxaphene levels were reduced between 8% and 10% over the studied periods of 55 and 79 days, respectively.

3.2. Isomers of toxaphene studied in soil

The toxaphene congeners studied are described in Table 2. During the experiment the toxaphene congeners investigated in all the reactors were degraded more than 80% except for those in reactor 4. The soil in reactor 4 had been contaminated with toxaphene and had not been supplemented with any external carbon source. The compounds Parlar 11 and 12 were reduced between 78% and 99% at day 79 in all reactors (Table 3). Parlar 11 and 12 exhibited almost a total degradation between 88% and 99% in all reactors except for reactor 4 during

Table 3
Results of toxaphene congeners in aged contaminated Nicaraguan soil and in newly contaminated Swedish soil with an extra carbon and energy sources studied during the experiment

Days	P-11	P-12	P-15	P-26	P-31	P-32	P-39	P-40	P-41/42	P-44	P-50	P-56	P-58	P-59	P-63	P-69
Remaining toxaphene congeners studied in all bio-reactors, expressed in (%)																
Day 0[a]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Day 55[a]	2.8	1.1	31.7	25.0	26.9	9.43	1.08	10.0	20.0	25.1	21.4	23.8	9.9	4.9	9.5	19.4
Day 79[a]	11.1	10.1	15.6	1.9	1.97	1.08	0.36	0.94	1.03	6.6	2.1	1.1	3.4	0.9	4.7	2.7
Day 0[b]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Day 55[b]	12.50	10.61	30.02	15.06	24.73	10.58	14.29	14.10	22.58	26.20	24.40	21.45	13.67	23.16	22.73	15.44
Day 79[b]	21.43	22.17	15.01	7.83	9.62	4.46	9.82	9.25	10.39	10.25	9.90	10.03	10.03	14.55	9.09	8.82
Day 0[c]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Day 55[c]	34.3	45.5	49.6	42.8	49.2	50.7	73.2	58.1	59.7	44.6	53.6	69.9	56.1	70.1	63.6	61.0
Day 79[c]	3.5	1.6	12.5	7.2	3.3	4.4	6.2	10.5	3.9	4.5	29.4	12.0	25.0	25.6	36.3	47.0
Day 0[d]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Day 55[d]	5.3	1.4	25.7	15.0	24.5	14.4	2.6	10.1	20.0	25.0	24.8	19.7	10.0	20.0	18.1	19.1
Day 79[d]	12.5	5.2	14.5	2.4	1.2	2.5	1.3	1.3	1.4	4.9	5.0	7.8	2.4	4.9	4.5	1.4
Day 0[e]	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Day 55[e]	3.6	1.2	23.3	24.7	28.7	22.8	22.8	29.1	26.3	25.3	28.7	21.5	23.4	25.8	22.7	33.1
Day 79[e]	8.9	9.9	15.2	16.9	9.6	15.0	9.8	10.1	9.1	18.4	15.2	10.0	14.7	17.4	13.6	11.8

[a] Reactor 2 with Nicaraguan soil and lactic acid.
[b] Reactor 3 with Swedish soil and lactic acid.
[c] Reactor 4 with Swedish soil with toxaphene only.
[d] Reactor 5 with Swedish soil and Triton X-100 0.4 mM.
[e] Reactor 6 with Swedish soil and Triton X-100 0.2 mM.

the first period (day 55). This can be ascribed to the transformation of compounds with several chlorine atoms into compounds with a lesser amount of chlorine atoms. At day 75, the degradation decreased in a range between 79% and 98% in all reactors. These results correspond well with previous results (Buser et al., 2000; Lacayo et al., 2004). In reactor 4, the compounds P-11 and P-12 showed a performance completely different at day 55, being reduced 66% and 55% respectively. This behaviour can be due to the fact that congeners in reactor 4, containing only toxaphene and no other carbon source, required a longer time to undergo degradation. Parlar 15 showed a degradation rate lower than P-11 and 12 during the period studied. At day 79, the degradation of P-15 was between 84% and 87% and at day 55, the degradation rate was between 50% and 77% in all reactors. The higher chlorinated toxaphene congeners (octachlorocamphene, octachloro, nona and decachlorobornanes) showed degradation between 67% and 99% in all reactors studied except in reactor 4. These higher chlorinated toxaphene congeners were degraded at 30% for Parlar 59, 31% for Parlar 56, 36% for Parlar 63 and 39% for Parlar 69.

3.3. Influence of lactic acid

Table 3 show results of the aged contaminated soil from Nicaragua (reactor 2) and newly contaminated Swedish soil (reactor 3), both containing lactic acid. The remaining percentage of all toxaphene congeners studied in reactor 3 showed a degradation rate significantly lower than in reactor 2. Factors such as organic matter content, bioavailability, and native bacteria present in the Nicaraguan soil might be the cause for the increase in biodegradation.

The Swedish soil with toxaphene added more recently but without any external carbon source (reactor 4) showed a slow degradation rate with all the congeners of toxaphene studied during the whole experiment. They were reduced between 53% and 96% (see Table 3). This can be explained by the fact that microorganisms prefer to use lactic acid as a carbon source instead of use toxaphene. However, in absence of an external carbon source, they use the toxaphene.

Toxaphene consists of a large mixture of compounds, and for this reason it is extremely difficult to study the metabolism and degradation in detail with only limited data available. Only 14 congeners of a commercial toxaphene mixture were specifically analyzed. No metabolites were analyzed but it was clearly seen that other compounds, which are also regarded as pollutants, increased in concentration. Furthermore, the use of additional carbon sources may have different effects on the degradation pattern under different conditions. Bioavailability is a key issue in order to improve the remediation efficiency. This was clearly confirmed in this study.

3.4. Influence of Triton® X-114

Table 3 illustrate the results of reactors with Triton® X-114. The results of reactors 5 and 6 (Swedish soil with Triton® X-114 at 0.4 mM and 0.2 mM, respectively) showed a degradation between 67% and 91% during the whole period. The compounds studied in reactor 5 performed a degradation rate significantly higher than the compounds studied in reactor 6 during the whole period. The reduction of toxaphene in this reactor was due to the action of the surfactant molecules, which can contribute to make toxaphene more bioavailable (Rosen, 1989).

4. Concluding remarks

These experiments illustrate some interesting features that are important for soil remediation. An aged soil with relatively high content of toxaphene was efficiently remediated after addition of lactate and an anaerobic mixed bacterial culture. The aged soil remediated better than the freshly contaminated soil. This was unexpected. However, the soil characteristics are complex and may be difficult to draw any firm conclusions. Addition of carbon source as well as of a surfactant to the freshly contaminated soil improved degradation. The problem of aging and bioavailability when designing soil remediation is important.

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