Bull. Environ. Contam. Toxicol. (1997) 58:227–233

Bulletin of Environmental Contamination and Toxicology

Kinetics of the Sequential Dechlorination of Chloroorganics in an Anaerobic Sediment

S. Susarla, S. Masunaga, Y. Yonezawa

Ecological Chemistry and Microbiology Division, National Institute for Resources and Environment, 16-3 Onogawa, Tsukuba, Ibaraki, 305, Japan

Received: 8 April 1996/Accepted: 1 September 1996

Chloroorganic compounds (e.g., Chlorophenols, chlorobenzenes and chloroanilines) are widely used as solvents, fungicides and as major intermediates for various manufacturing processes (Pearson, 1982). These compounds are very toxic and their toxicity tends to increase with their degree of chlorination (Liu *et al.*, 1982). Many of these compounds tend to persist in the environment and pose potential health risk.

Anaerobic dechlorination of chlorobenzenes, chlorophenols and chloroanilines have been well documented. For example, Mikesell and Boyd (1986), Nicholson *et al.* (1992) have examined the dechlorination of 3,4,5-TCP and identified its metabolites and pathway of degradation. Similarly, Wang and Jones (1994) and Ramanand *et al.* (1993) and others have reported on the dechlorination of chlorobenzenes, while Kuhn and Suflita (1989) have reported on the transformation of chloroanilines in sediments. However, in all these studies, information on the kinetics of transformations are very limited and very few reports have been published on the modeling of dechlorination reactions.

Recently, Armenante *et al.* (1995) have developed a model to predict the sequential dechlorination of 2,4,6-TCP using Michaelis-Menten kinetics. Similarly, Suflita et al. (1983) reported the dechlorination kinetics for chlorobenzoates using Michaelis-Menten kinetics. However, the authors have not included all possible dechlorination products in their analysis. The objective of the present work was to model the sequential dechlorination of 1,2,3-TCB, 3,4,5-TCP and 3,4,5-TCA using a first-order reaction kinetics. The model parameters for all possible intermediate metabolites were obtained from independent experiments and the results were compared with the model predictions.

Correspondence to: S. Susarla

From the identified intermediate metabolites (Masunaga *et al.*, 1996a), the transformation of 3,4,5-TCP occurred in the following steps.

$$\text{3,4,5-TCP} \rightarrow \text{3,4-DCP} + \text{3,5-DCP} \tag{1}$$

$$\text{3,4-DCP} \rightarrow \text{3-CP} + \text{4-CP} \tag{2}$$

$$\text{3,5-DCP} \rightarrow \text{3-CP} \tag{3}$$

$$\text{3-CP} \rightarrow \text{phenol} \tag{4}$$

$$\text{4-CP} \rightarrow \text{phenol} \tag{5}$$

Similar transformation mechanisms were established for 1,2,3-TCB and 3,4,5-TCA based on the formation of intermediate metabolites. The metabolites for 1,2,3-TCB were 1,2-DCB, 1,3-DCB and MCB, while in case of 3,4,5-TCA, 3-CA and 3,5-DCA were identified as the major metabolites. From the transformation pathways, the mass balance equations for the 3,4,5-TCP sequential dechlorination are expressed as:

$$\frac{dC[3,4,5\text{-}TCP]}{dt} = -k(3,4,5\text{-}TCP)\,C[3,4,5\text{-}TCP] \tag{6}$$

$$\frac{dC[3,4\text{-}DCP]}{dt} = k(3,4,5\text{-}TCP)\,C[3,4,5\text{-}TCP] - k(3,4\text{-}DCP)\,C[3,4\text{-}DCP] \tag{7}$$

$$\frac{dC[3,5\text{-}DCP]}{dt} = k(3,4,5\text{-}TCP)\,C[3,4,5\text{-}TCP] - k(3,5\text{-}DCP)\,C[3,5\text{-}DCP] \tag{8}$$

$$\frac{dC[3\text{-}CP]}{dt} = k(3,4\text{-}DCP)\,C[3,4\text{-}DCP] + k(3,5\text{-}DCP)\,C[3,5\text{-}DCP] - k(3\text{-}CP)\,C[3\text{-}CP] \tag{9}$$

$$\frac{dC[4\text{-}CP]}{dt} = k(3,4\text{-}DCP)\ C[3,4\text{-}DCP] - k(4\text{-}CP)\ C[4\text{-}CP] \tag{10}$$

$$\frac{dC[phenol]}{dt} = k(3\text{-}CP)\ C[3\text{-}CP] \tag{11}$$

$$\frac{dC[phenol]}{dt} = k(4\text{-}CP)\ C[4\text{-}CP] \tag{12}$$

where, *k* is the rate constant of the compound, which is identified by the respective compound and *C* is the concentration in moles/L. The main assumptions in the above model equations were: i) there were no interactions between different intermediate metabolizes, and ii) all the metabolites formed follow a first-order reaction mechanism.

However, the latter assumption is not always applicable in heterogeneous systems (Suflita *et al.,* 1983). The above model equations were solved simultaneously using a fourth–order Runge-Kutta method. Similar model equations were written for 1,2,3–TCB and 3,4,5-TCA and solved using the above procedure to predict the sequential dechlorination.

MATERIALS AND METHODS

The sediment was collected from the mouth of Tsurumi river which flows into Tokyo Bay. River water above the sampling point was also collected. The characteristics of the sediment are shown in Table 1.

All chemicals were obtained from Tokyo Kasei Kogyo Co. Ltd., Japan. The chemicals were of high purity and used directly as obtained.

Table 1. Characteristics of the sediment and details of the experimental runs

Interstitial water		Sediment slurry	
NaCl (%)	1.7	pH	5.6
Sulfate (mmole/L)	20.6	Total solids (g/kg)	272.0
Nitrate (mg/L)	ND		
River water		C_0 (µmole/L)	4.0
Temperature (°C)	24.0	Recovery (%)	80-100
NaCl (%)	1.5		
Sulfate (mmole/L)	19.2		
Nitrate (mg/L)	4.0		
Oxygen (mg/L)	7.0		

The sediment and river water were mixed to obtain a sediment-slurry. For each test tube (total 25 for each compound), 5 mL of sediment slurry was added, which was then spiked with 10µL of the test compound (Table 2). All the test tubes were kept at 25 °C during the kinetic experiments. Sampling was done at frequent intervals till the end of the experiment. After the completion of the experiment, the loss of parent compound and formation of possible intermediate metabolites were analyzed (Masunaga *et al.,* 1996a,b; Susarla *et al.,* 1996). All samples were analyzed at the end of the experiment using GC/MS (HP 5971 MSD) by a selected-ion-monitoring method. Details of the analytical procedures used were discussed in detail elsewhere (Masunaga *et al.,* 1996a,b) .

RESULTS AND DISCUSSION

From the transformation experiment for each compound studied (listed in Table 2), the amount of compound remaining (C_t) at a given time *t* relative to the amount initially present (C_0) was plotted against time on a semi-log graph, the slope of the line gave the first-order rate constant, *k*. Linear regression analysis was carried out to determine the value of the rate constant, the number of data points was kept at a minimum of 8 for all calculations. Table 2 lists the calculated *k* values for various compounds. The *k* value for each compound shown in table was obtained from an independent experiment. The results of typical sequential dechlorination run for 1,2,3-TCB is shown in Figure 1. 1,2,3-TCB dechlorinated to 1,2-DCB, 1,3-DCB and MCB during the course of the experiment. Further degradation of MCB was not observed in the incubation experiment (Masunaga *et al.*, 1996b).

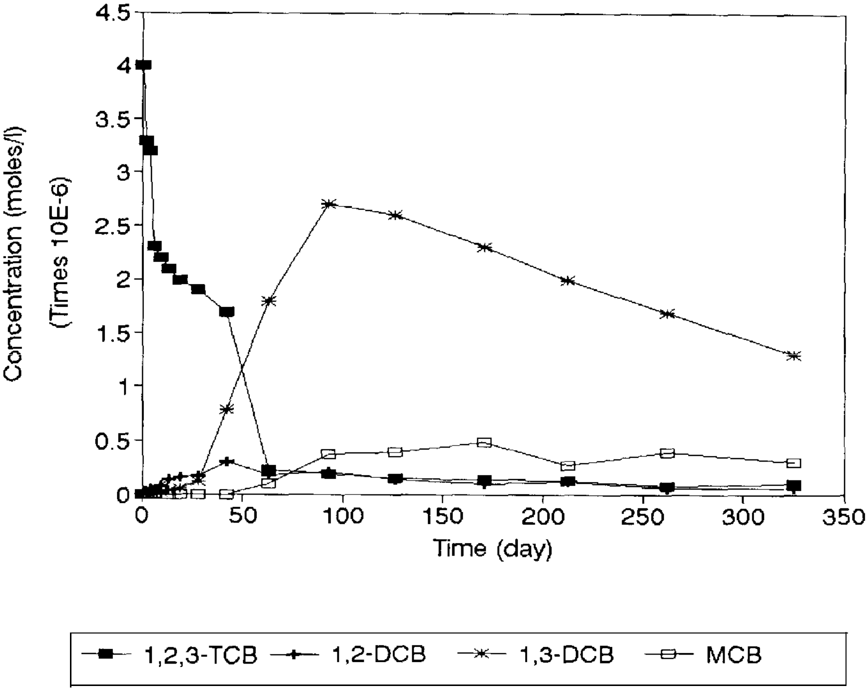

Figure 1. Reductive dechlorination of 1,2,3-TCB in anaerobic estuarine sediment.

Table 2. First-order rate constants for chloroorganics in anaerobic sediment

CP	*k* (day^{-1})	CB	*k* (day^{-1})	CA	*k* (day^{-1})
3-CP	0.020	MCB	0.0150	3-CA	0.001
4-CP	0.060	1,2-DCB	0.0016	3,5-DCA	0.005
3,4-DCP	0.001	1,3-DCB	0.0190	3,4,5-TCA	0.005
3,5-DCP	0.040	1,2,3-TCB	0.0300		
3,4,5-TCP	0.030				

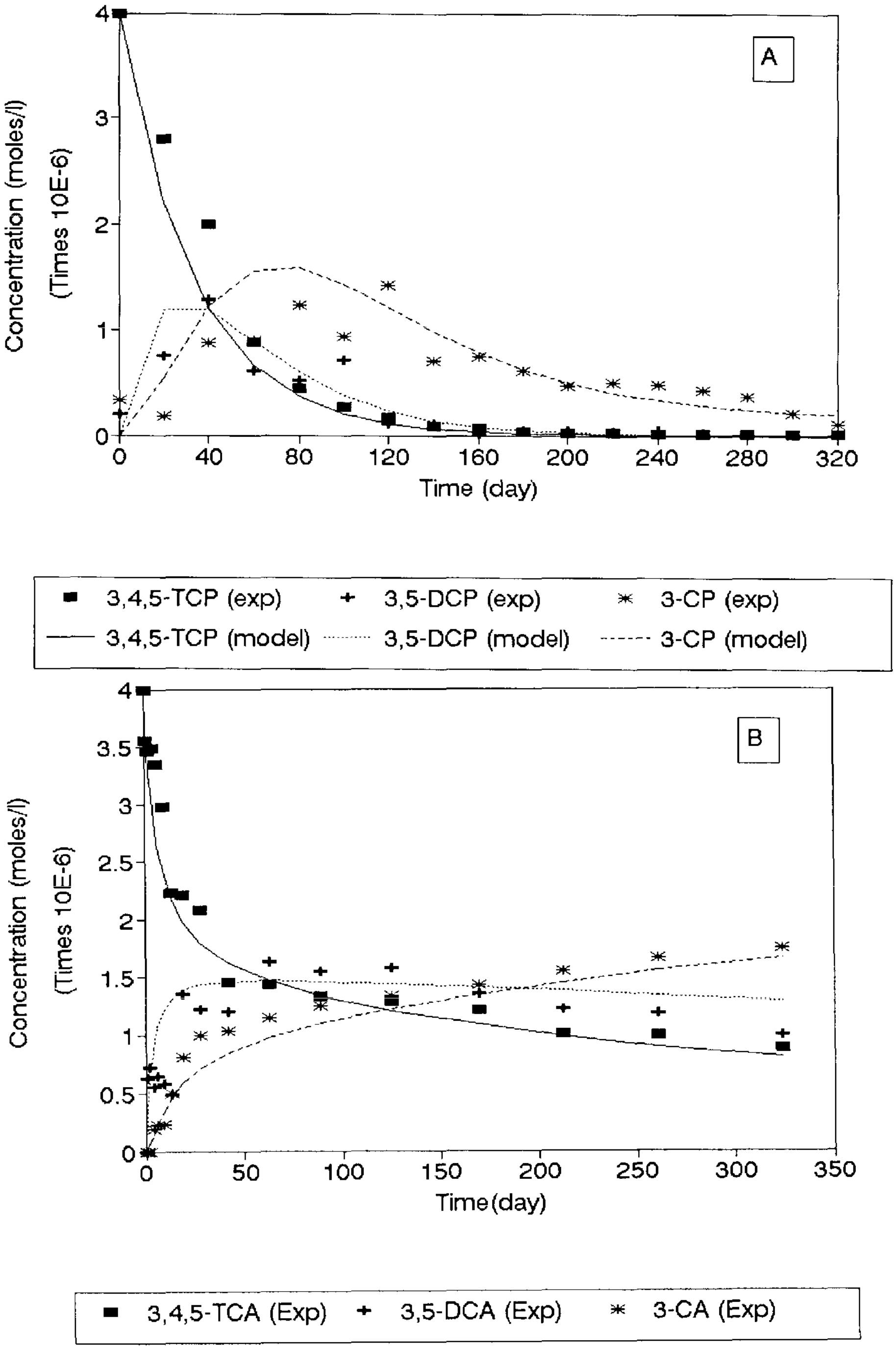

Figure 2. Comparison between experimental data and model predictions using the kinetic constants listed in Table 2. a) 3,4,5-TCP and b) 3,4,5-TCA.

To model the sequential dechlorination, for example, in case of 3,4,5-TCP, the *k* values determined for parent compound and for each metabolite were used in Equations (6-12) to obtain the model solution. As shown in Figure 2a, the experimental run of sequential dechlorination was compared with model predictions, in general, a good agreement between observed data and predicted profiles was obtained. A similar analysis was carried out for 1,2,3-TCB (figure not shown) and for 3,4,5-TCA as shown in Figure 2b. From, the analysis, it is clear that the first-order kinetic model satisfactorily describes the sequential dechlorination of various chloroorganics in the sediment.

The reductive dechlorination of priority pollutants by anaerobic bacteria represents an important step in the detoxification mechanism. The sequential dechlorination reaction mechanism was described using a mathematical model in this work. The model described the experimental data satisfactorily using the parameters obtained from the kinetic experiments. The model is very simple and easy to use and the only parameters required are the first-order rate constants.

Acknowledgments. We thank Mr Takao Yamagishi for his help with anion analysis, Ms Mayumi Tamura for her support in chlorophenol analysis and data calculation, and Ms Ruri Shinozuka and Ms Keiko Sugawara for their assistance in the experiments.

REFERENCES

Armenante PM, Shu HY, Huang CR, Kung CM, Kafkewitz D (1995) Kinetics of the sequential dechlorination of 2,4, 6-trichlorophenol by an anaerobic microbial population. Biotech Lett 17: 663-668.

Kuhn EP, Suflita JM (1989) Sequential reductive dehalogenation of chloroanilines by microorganisms from a methanogenic aquifer. Environ Sci Technol 23:848-852.

Liu D, Thompson K, Kaiser KLE (1982) Quantitative structure-toxicity relationship of halogenated phenols in bacteria. Bull Environ Contam Toxicol 29:130-136.

Masunaga S, Susarla S, Gundersen JL and Yonezawa Y (1996a) Pathway and rate of chlorophenol transformation in anaerobic estuarine sediment. Environ Sci Technol Vol 30: 1253-1260

Masunaga S, Susarla S, Yonezawa Y (1996b) Dechlorination of chlorobenzenes in anaerobic estuarine sediment. Wat Sci Technol Vol. 33:173-180

Mikesell MD and Boyd SA (1988) Enhancement of pentachlorophenol degradation in soil through induced anaerobiosis and bioaugmentation with anaerobic sewage sludge. Environ Sci Technol 22:1411-1414

Nicholson DK, Woods SL, Istok JD, Peek DC (1992)

Reductive dechlorination of chlorophenols by a pentachlorophenol-acclimated methanogenic consortium, Appl Environ Microbiol 58:2280-2286

Pearson CR (1982) Halogenated aromatics, In The handbook of environmental chemistry, Vol 3, Part B: Anthropogenic compounds, O. Hutzinger (ed), Springer-Verlag, New York

Ramanand K, Balba MT, Duffy J (1993) Reductive dehalogenation of chlorinated benzenes and toluenes under methanogenic conditions. Appl Environ Microbiol 59:3266-3272

Suflita JM, Robinson JA, Tiedje JM (1983) Kinetics of microbial dehalogenation of haloaromatic substrates in methanogenic environments. Appl Environ Microbiol 45:1466-1473

Susarla S, Masunaga S, Yonezawa Y (1996) Reductive dehalogenation of chloroanilines in anaerobic estuarine sediment. Environ Technol (In press)

Wang MJ, Jones KC (1994) Behaviour of chlorobenzenes (CBs) introduced into soil-plant systems by sewage sludge application: A review. Chemosphere 28:11325-1360