



STUDY OF SORPTION, BIODEGRADATION AND ISOMERIZATION OF HCH IN STIMULATED SEDIMENT/WATER SYSTEM

W.Z. Wu^{1,3}, Y. Xu¹, K.-W. Schramm², A. Kettrup^{2,3}

¹ State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Wuhan 430072, China

² GSF-Forschungszentrum für Umwelt und Gesundheit, Institut für ökologische Chemie, Ingolstädter Landstr. 1, D-85764 Oberschleißheim, Germany

³ Lehrstuhl für Ökologische Chemie und Umweltanalytik, Techn. Universität München, Germany

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Abstract

This paper reported the sorption, biodegradation and isomerization of hexachlorocyclohexane (HCH) in laboratory sediment/water system under aerobic and anaerobic conditions, respectively. The effect of organic nutrient addition to the sorption of HCH was also investigated. It indicates that HCH is highly adsorbed on sediments under both conditions. During the tests, the biodegradation and isomerization of HCH were dramatically speeded up after organic nutrient additions, especially in the case of the observation under aerobic condition. It was found, β -HCH was the most persistent in the environment, that is due to the isomerization of α -HCH in a big amount to β -HCH, besides its chemical stability. © 1997 Elsevier Science Ltd

Key words sorption biodegradation isomerization HCH

Introduction

Due to the low price, hexachlorocyclohexane (HCH) was produced in considerable amount and widely used as a broad spectrum insecticide in many countries of the world. In China, the annual production of HCH once reached 200,000 tons, in spite of the ban of the use of HCH since 1983. In China, the farmers took the powder formulation of HCH, the mixture of α , β , γ and δ four isomers to spray it into the agriculture fields. Meanwhile,

lakes were seriously polluted by HCH because the waste water from chemical plant was discharged directly into the lake. Through a long term movement and transfer, the most of them was finally deposited into sediment.

In order to understand the releasing behavior of HCH from field sediment and its long-term effect to aquatic environment, as well as verifying the obtained results from environment, the sorption, biodegradation and isomerization of α and γ -HCH isomers under aerobic and anaerobic conditions were reported in this paper.

Materials and Methods

1. Solvent

Hexane, acetone, ethyl acetate and benzene used were of analytical grade and redistilled. Sodium sulfate and sea sand were activated in a Muffle furnace at 600°C for 6 hours before use.

2. Sample preparation and analysis⁽¹⁾

(1) Sample preparation and extraction

Each of these samples was prepared in the same manner: 15g of fresh sample was taken and mixed with 60g anhydrous sodium sulfate and 30g seasand, then they were ground to fine powder in a mortar for the extraction. The prepared sample was added to a 50cm long, 2cm diameter column with Teflon trap, then eluted by 300ml of hexane/acetone (2:1) mixture, the flow rate being controlled at 1.0 ml/min approximately. Prior to evaporation of the sample extraction, pentachlorobenzene and decachlorobiphenyl were fortified sample extracts as the internal standard for calibration. The sample extract was concentrated and transferred to 2ml ethyl acetate.

(2) Sample purification

Before starting the GPC step, all the samples underwent glass microfibre filtration (Whatman International Ltd., Maidstone, Great Britain) with the pore size of 0.2 μ m. GPC system composed of a column sized 1 m long x 2 cm (i.d.), packed with Biobead SX8 and mobile phase of cyclohexane/ethyl acetate (1:1) at flow rate of 2 ml/min. The samples were run under the specified program discarding the first 55 ml and collecting the following 60 ml of elution. After GPC clean-up, the eluted solution collected was concentrated and transferred back to 2ml hexane. At last, this extract was filtered before use for HPLC. The automatic HPLC system included a stainless steel column 24 cm long x 0.8 cm (i.d.) packed with silicagel SI 60. The mobile phase was 0.1 % isopropanol in hexane (v/v) and elution flow rate was at 4 ml/min.

(3) Qualification of HCH by gas chromatography

After HPLC clean-up, the sample collected was concentrated again to 2 ml and ready for GC use. Hewlett Packard 5890 A gas chromatograph with electron capture detector (ECD) was used. The samples were run on a fused silica capillary column coated with DB-5 as bonded phase, sized 30 m long x 0.32 mm (i.d.). The carrier gas

was H₂ with a flow rate of 2 ml/min. The temperature program was 60 °C (1 min) to 120 °C (1 min) with rate of 12 °C/min, then to 285 °C (10 mins) with rate of 7 °C/min, injector temperature 285 °C.

3. Experimental sediment/water system

The 20cm depth surface sediment was taken from East lake, Wuhan, China, water depth 1.5 meters, temperature 18.5°C. Sediment was sieved with 1mm filter, then centrifuged 5mins with rotation rate of 4500rpm/min, the supernatant was discarded, the sediment was ready for test within a few days. Some physicochemical parameters of the lake sediment are given below: pH 7.42, particle size ≤ 0.1mm, total organic carbon 2.18%, no HCH by GC detection.

The purity of HCH standard was more than 99.99%. The concentration of stock solution was 400mg/ml in acetone. Glucose was analytical grade; the yeast extract was pure powder (Huamei Biotechnology Company, China).

In each group, 500g centrifuged fresh sediment mixing with 400ml distilled water was put in a 2liter glass flask. HCH was added at 4mg/l concentration under the stirring. The magnetic stirring speed was controlled at 200 rpm. The temperature was 20±2°C; pure nitrogen and oxygen were connected inside the system at the flow rate of 20ml/min. The test period was 48 days. During the test, the oxidation-reduction potential (Eh) variations were observed by means of Pt electrodes monitoring.

Being predominant components in Chinese commercial product of HCH (α : 67%; β : 10%; γ : 15%; δ : 8%), pure α and γ -HCH were selected for the test. The experiment was divided into four groups: I, α -HCH under anaerobic condition; II, α -HCH under aerobic condition; III, γ -HCH under anaerobic condition; IV, γ -HCH under aerobic condition.

The organic nutrient was composed of glucose mixed with yeast powder in 50ml distilled water, then added to the sediment after system had been running for 30 days. There were two addition amounts for the parallel experiment from 30th day: (A) 2g glucose and 2g yeast powder, (B) 0.5g glucose and 0.5g yeast powder.

Results and Discussion

1. Aerobic and anaerobic state control for the sediment/water system

After running 24 hours, strong odor occurred in group I and III with the Eh -215 to -335 mV (v.s. SCE), but not in the group II and IV with the Eh 225 to 325 mV (v.s. SCE). The Eh indicated the concentration of oxygen in the sediment/water system. As the Eh ranged from 200 to 300 mV, the concentration of oxygen was in the range of 0.1 mg/l to 10.3 mg/l; when the Eh was below 100mV, there was almost no oxygen found in the system⁽²⁾. Therefore, by means of the Eh variation, the group I and III were maintained in anaerobic condition, II and IV in aerobic condition, respectively.

2. Sorption equilibrium of HCH in the sediment/water system

After HCH was added to the system, there was continuous partitioning between sediment and water, and it was able to reach to the dynamic equilibrium state after a time. It is very important that the system reaches equilibrium state in the study of HCH sorption behavior. Figure 1 shows the HCH concentrations in sediment phase (Figure 1a) and water phase (Figure 1b) at the different time intervals.

The sorption behavior can be described by Freundlich empirical equation:

$$C_t = k_d * C_{eq}^{1/n} \dots\dots\dots(1)$$

where:

C_t: HCH equilibrium concentration in sediment phase (ppm)

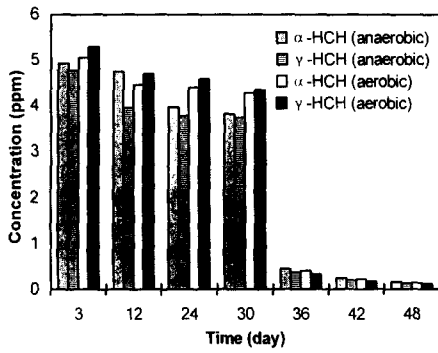
C_{eq}: HCH equilibrium concentration in water phase (ppm)

k_d: adsorption coefficient

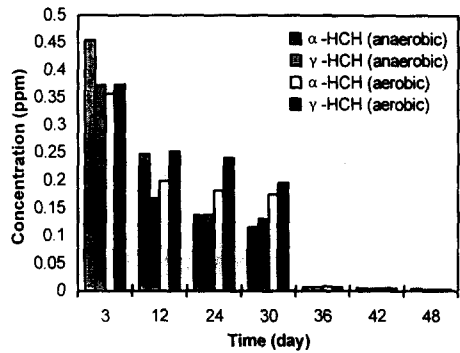
n: empirical constant

Making logarithm to equation (1), then the following equation is obtained,

$$\log C_t = \log k_d + 1/n * \log C_{eq} \dots\dots\dots(2)$$



(a)



(b)

Figure 1: (a) HCH concentration in sediment (ppm) (b) HCH concentration in water (ppm)

Table 1: Adsorption coefficient (k_d), 1/n, correlation coefficient (r) and Freundlich equation

	k _d	1/n	r	Freundlich equation
I	13.46	0.734	0.9801	logC _t =1.1289+0.7336 * logC _{eq}
II	14.85	0.776	0.9912	logC _t =1.1718+0.775 * logC _{eq}
III	15.13	0.790	0.9936	logC _t =1.1797+0.7903 * logC _{eq}
IV	13.98	0.799	0.9965	logC _t =1.1455+0.7996 * logC _{eq}

Based on the data from Figure 1 and equation (2), k_d , $1/n$ and Freundlich equation are calculated, the results are listed in Table 1. The results indicated the partition equilibrium of HCH between sediment and water could be observed after 3 days. The adsorption coefficient k_d showed HCH was able to be strongly adsorbed in the sediment. This means the sorption by the sediment plays a very important role when HCH is transferred into the water phase rich in the sediment.

As the organic nutrient was added on 30th day, the HCH concentration in the water phase was dramatically reduced, however the system still kept the equilibrium state. This could be explained: after the organic nutrient addition, the rapid increase of microorganism which dramatically degraded HCH in water phase speeded up transfer of HCH from sediment to water. Therefore, the equilibrium state can be maintained ⁽⁴⁾.

3. Degradation dynamics of HCH in sediment

The regularity of HCH degradation is assumed to be a second grade dynamics model ⁽⁴⁾:

$$-\frac{d[C_t]}{dt} = k_2[C_t]^2 \dots\dots\dots(3)$$

$$\text{then : } \frac{1}{C_t} = k_2t + m \dots\dots\dots(4)$$

by equation (4), the half life can be calculated as following:

$$t_{1/2} = \frac{2 / C_0 - m}{k_2} \dots\dots\dots(5)$$

where:

k_2 : the coefficient of HCH degradation dynamics

m : constant

C_t : HCH concentration in sediment (ppm)

t : time (days)

The statistical results are listed in Table 2. Table 2 shows I, II, III, IV four groups have good correlation coefficient ($r > 0.90$). It demonstrates HCH degradation in sediment fits the second grade dynamic regularity. The degradation speed of HCH is related with initial concentration of sediment, the higher concentration the more rapid is the degradation, this phenomenon can be explained by the above model ⁽⁵⁾

4. Effect of organic nutrient on HCH degradation

On 30th day, the mixtures of glucose and yeast were added to the system, the time course of HCH concentration in sediment is shown in Figure 2. Based on the obtained data (Figure 2) and equation (4) (5), the statistic analytical results are calculated and shown in Table 3. It indicates the degradation behavior of HCH in sediment still follows the regularity of second grade dynamics mode after the nutrient was added to the system. In summary, the following conclusions are obtained: the degradation of HCH mainly is a biotic process, and the organic nutrient can speed up the degradation of HCH both in aerobic and anaerobic conditions, but the

regularity of degradation remains the same, the organic nutrient merely enhancing the biomass and activities of the microorganisms in the sediment.

Table 2: Several parameters and dynamics equation of HCH degradation in sediment under aerobic and anaerobic conditions

	C ₀ (ppm)	k ₂	m	r	t _{1/2} (days)	equation
I	4.93	0.0024	0.1908	0.9761	89.58	1/Ct=0.0024t+0.1908
II	4.77	0.0021	0.2120	0.9254	86.08	1/Ct=0.0021t+0.2120
III	5.06	0.0012	0.2003	0.9011	162.4	1/Ct=0.0012t+0.2003
IV	5.29	0.0014	0.1890	0.9498	135.0	1/Ct=0.0014t+0.1890

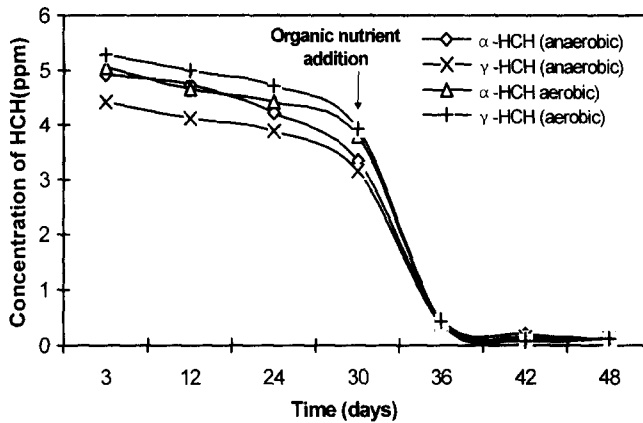


Figure 2: Time course of HCH concentrations in sediment/water system

Table 3: Several parameters of HCH degradation in second-grade dynamics mode in sediment/water system fortified with nutrients

	Test (A)				Test (B)			
	I	II	III	IV	I	II	III	IV
C ₀	3.8213	3.7311	4.2840	4.3356	3.8982	4.4592	3.4036	3.2146
k ₂	0.3169	0.4023	0.4426	0.4653	0.0329	0.0838	0.1671	0.1933
m	0.2586	0.2399	0.3879	0.2044	0.2542	0.1597	0.2090	0.3192
r ²	0.9994	0.9988	0.9405	0.9988	0.9992	0.9763	0.9803	0.9996
t _{1/2}	0.8356	0.7361	0.7256	0.5579	7.8663	3.4465	2.2658	1.5675
Equation	1/Ct=0.3169t +0.2586	1/Ct=0.4023t +0.2399	1/Ct=0.4426t +0.3879	1/Ct=0.4653t +2044	1/Ct=0.0329t +0.2542	1/Ct=0.0316t +0.1597	1/Ct=0.1671t +0.2090	1/Ct=0.1933t +0.3192

From Table 2 and Table 3, the results demonstrate that the degradation of γ -HCH is more rapid than that of α -HCH both under aerobic and anaerobic condition. Whereas the degradation of α and γ -HCH under anaerobic condition is almost the same as that in aerobic condition, or even slower than that in aerobic condition. This phenomenon can also be found from other reports ⁽⁶⁾. The reason that α -, γ -HCH were more easily degraded in aerobic condition than that in anaerobic condition was due to the high concentration of α -, γ -HCH inhibiting the population of methyl microorganisms. Biological mineralization plays an important role in the degradation of α -HCH. A. Bachman et al. ⁽⁶⁾ found the degradation of α -HCH in aerobic condition was twice more rapid than that in anaerobic condition, but the degradation of β -HCH was still very slow in this case. The substance of HCH degradation by microorganisms is certain enzymes. The difference of degradation between α and γ -isomers was caused by different enzyme selections.

5. Isomerization of α -HCH

Figure 3 shows the isomerization of α -HCH at different time intervals. It indicates that there are three isomerization ways: $\alpha \rightarrow \beta$, $\alpha \rightarrow \gamma$ and $\alpha \rightarrow \delta$, because β -, γ - and δ -isomers were able to be detected on the 3rd day after the test was started. In addition, the concentrations of these isomers were increased with the time passed. In view of the quantitative data, it is argued that the isomerization of α -HCH to β -isomer is the main path among these three ways. The isomerization percentage (I %) is calculated by the following expression:

$$I \% = \frac{\text{Concentration of } \beta\text{-isomer}}{\text{total HCH concentration in different time intervals}} * 100\%$$

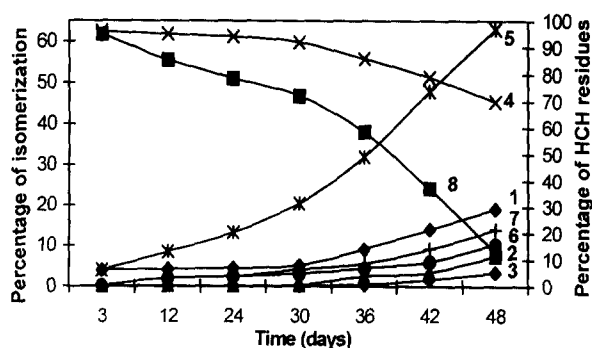


Figure 3: Isomerization of HCH in laboratory system

Anaerobic: 1. $\alpha \rightarrow \beta$, 2. $\alpha \rightarrow \gamma$, 3. $\alpha \rightarrow \delta$, 4. $\alpha \rightarrow \beta$

Aerobic: 5. $\alpha \rightarrow \beta$, 6. $\alpha \rightarrow \gamma$, 7. $\alpha \rightarrow \delta$, 8. $\alpha \rightarrow \beta$

It was found that the isomerization of α -HCH to β -isomer finally reached about 50% on the 48th day. In

comparison of the results obtained under anaerobic and aerobic conditions, they showed the very close I % at the beginning (3-12 days) of the test, but finally the isomerization percentages of α -HCH under aerobic condition were higher than anaerobic. In the test, the effect of organic nutrient addition on the HCH degradation rate was observed (refer to Figure 2). After the addition of organic nutrient, the isomerization of α -HCH in the above three ways were greatly increased. In Figure 3 the effect of organic nutrient addition on the disappearance of HCH also was presented. It is clear that the disappearance of α - and γ -HCH is dramatically speeded up after organic nutrient addition. This is due to the fact that the amount of microorganism is greatly increased after the addition of nutrients. The speeding up of the isomerization of HCH proves that the reaction mainly belongs to biological process. It was found β -HCH was the most persistent in the field environment^(7,8), that is due to the isomerization of α -HCH in a big amount to β -HCH, besides its chemical stability.

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