# Hexachlorobenzene Dechlorination Ability of Microbes from Canal and Estuary Sediments

Anotai Jin* / Voranisarakul J.* / Wantichapichat W.* / Chen I.M.**+

**Abstract** : This study aimed to investigate the hexachlorobenzene (HCB) dechlorinating ability of sediment microbes collected from a natural canal receiving secondary effluents from an industrial estate and nearby factories. Nine sites along the stream and one in the estuary in the Gulf of Thailand into which the canal spills were specified and sampling for sediment and water. Preliminary analysis of the sediments showed that the first four sites nearest to the discharging location were contaminated by HCB within the range of 0.18 to 1.25 ppm. Apart from that, 1,3,5-trichlorobenzene which has never been commercially produced or used in any manufacturing processes except for the transformation from higher chlorinated benzene was also identified in the range of 0.16 to 0.24 ppm. This suggested a possibility of sporadically HCB contamination in this stream. Of more important, people in the community along this canal earn their living by coastal fishery; hence, posing a risk of spreading HCB and its less chlorinated congeners via food chain from caught marine creatures to human. As a result, there is an urgent need to understand the behavior of HCB dechlorination in this stream sediment which can lead to a clean-up action in the future. Serum bottles with sediment slurries (sediment to water ratio of 1:1 (v/v) and filtered to remove particles larger than 0.7 mm) from each site were inoculated with 2 mg/l of HCB, kept anaerobically in the dark at room temperature without any nourishment, and analyzed for HCB and its less-chlorinated congeners every 6 days. Total chemical oxygen demand, suspended solids, and volatile suspended solids were in the range of 21,492-73,584, 158,100-518,100 and 6,000-32,700 mg/l, respectively. It was found that all sediment slurries began to dechlorinate HCB in 12 to 30 days and the HCB was completely removed within 42 to 60 days or so. On the other hand, there was no HCB dechlorination occurred in the controlled set which was sterilized by autoclaving prior to the addition of HCB. This implies that the HCB transformation was solely due to microorganisms'activities. HCB was dechlorinated principally via pentachlolobenzene to 1,2,3,5-tetrachlorobenzene and terminated at 1,3,5-trichlorobenzene which is the major pathway as reported by many researchers. Dichlorobenzene has not been detected in any samples within the dechlorination period of 60 days. The results indicate that the microbial matrix in the sediment of this stream has an outstanding capability to dechlorinate HCB. Existing substrates and nutrients which mainly sorbed onto the solid phase and the typical temperature in Thailand were sufficient and suitable to promote the activities of these HCB-dechlorinating microbes.

**Keywords** : Anaerobic, Dechlorination, Hexachlorobenzene, Sediment

## Introduction

Hexachlorobenzene (HCB) has been long and widely used as a fungicide and industrial synthetic materials in agricultural and industrial sectors prior to the banning of its direct usages several decades ago in many countries including Thailand. However, with its persistent property (one of the 12 persistent organic pollutants (POPs) as specified by the United Nation) and improper practices such as uncontrolled release and inappropriate disposal, HCB was found throughout all environments including water, air, soil, sediments, and living organisms (Weber, K. *et al.*, 1996). In fact, Bailey (Bailey R.E., 2001) revealed that HCB was still indirectly releasing to the environment as a secondary by-product from industrial processes. With its superb

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bioaccumulation property, HCB can cause severe health adverse impact such as carcinogenicity and toxicity on human and living species. To clean-up HCB contaminated sites by chemical treatments is not only uneconomic but also inappropriate since it will also inevitably destroy the fauna and flora in such environments. Of the less expensive and more environmental friendly biological processes, the aerobic approach could only deal with less-chlorinated benzenes such as mono- chlorobenzene (MCB), dichlorobenzenes (DCBs), and trichlorobenzenes (TCBs) but was ineffective for highly chlorinated pentachlorobenzene (QCB) and HCB (Bestteti, G. *et al.*, 1992; Haigler B.E. *et al.*, 1992) whereas, the anaerobic digestion using acclimated and/or enriched microorganisms showed a more promising outcome (Fathepure, B.Z. *et al.*, 1988; Nowak, J. *et al.*, 1996; Chang, B.V. *et al.*, 1997; Chen, I.M. *et al.*, 2002). The proposed pathway was illustrated in Figure 1 in which the major path was via 1,2,3,5-tetrachlorobenzene (TeCB) and 1,3,5- TCB, sequentially. Similar to other countries, Thailand is also facing with public health threat from HCB contamination environment. Analysis of the sediments collected from a canal named "Hua-Lum-Poo"in Samut Prakarn Province receiving treated effluents from an industrial estate and near-by factories in the vicinity indicated a possibility of HCB contamination (Brigden, K. *et al.*, 2003). In addition, 1,3,5-TCB which has never been produced or used in a commercial scale was also identified. Since 1,3,5-TCB has been reported to be a by-product from HCB reductive dechlorination (Figure 1), it provided an evidence of natural HCB dechlorination in the sediment of this canal which was contradictory to a typical believe that HCB dechlorination in natural habitat was rather obscure.

It is of interest to investigate on the intrinsic HCB dechlorinaion ability of microbial consortia in the sediments of this canal using only pure environmental matrix as media and inocula.

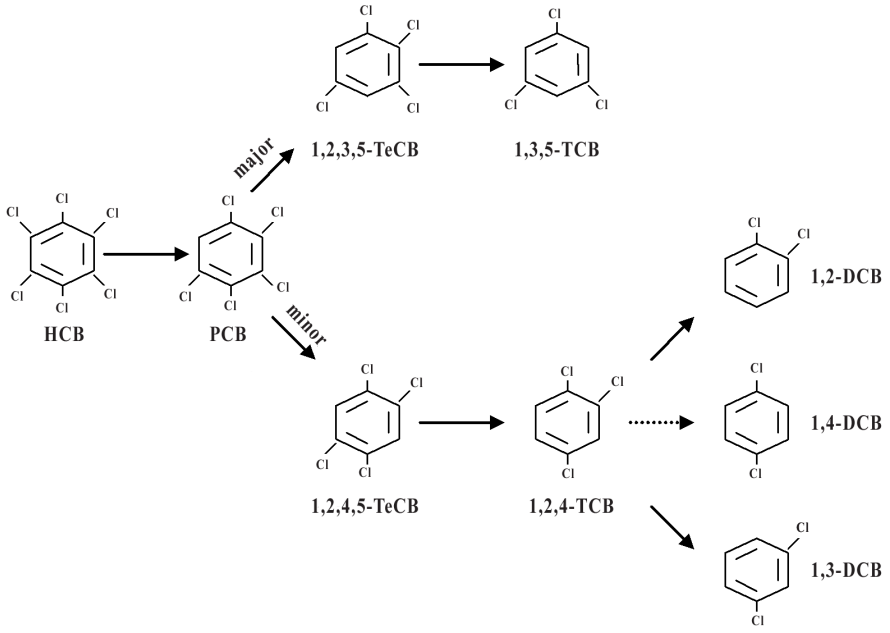


Fig. 1. Reductive dechlorination pathway of HCB by anaerobic degradation [5]

## Materials and Methods

### Sediment and canal water samples

Ten sediments and canal waters were collected from the Hua-Lum-Poo Canal in Samut Prakarn Province of Thailand about 30 kilometers south of Bangkok. Nine sites along the canal and one on the estuary as shown in Figure 2 were defined and sampled for sediments and waters. The upper part of sediments was carefully removed and the remains were packed in plastic bags. Canal waters were sampled and filled into 3 liter containers. Both sediments and waters were kept at 4℃ until used.

### Chemicals

Chlorinated benzene congeners (CBs) including MCB, 1,2-, 1,3- and 1,4-DCBs, 1,2,3-, 1,2,4- and 1,3,5-TCBs, 1,2,3,4-, 1,2,3,5- and 1,2,4,5-TeCBs, QCB and HCB were purchased from Riedel-de Haen Chemical Co. (Germany). The 99.5% acetone (Labscan Asia Co., Ltd., Thailand) was used to dissolve HCB for inoculating purpose while 99% n-hexane (Labscan Asia Co., Ltd., Thailand) was used for CBs standard preparation and extraction.

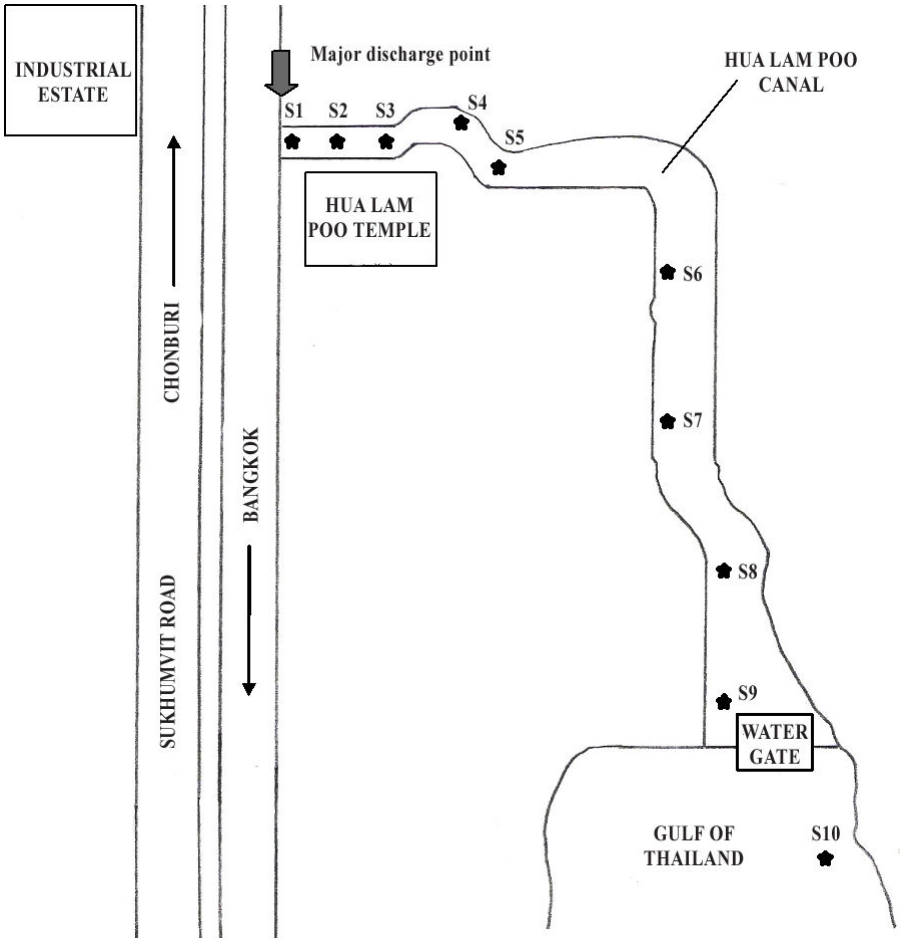


Fig. 2. Sediment and canal water sampling sites along Hua-Lam-Poo Canal and in the sea shore as marked from S1 to S10.

**Microbial preparation**

Sediment and water collected from each site at a rough ratio of 1:1 (v/v) were mixed thoroughly by hand for 2 minute and allowed to settle for 30 minutes. Upper sediment slurry was withdrawn by a syringe with 22G2 hypodermic needle (0.7 mm opening size), injected into a 1000-ml serum bottle, and purged with nitrogen gas before sealing. For dechlorination experiment, 50 ml of sediment slurry was transferred into 3 100-ml serum bottles in a nitrogen glove box to prevent oxygen interference. Two serum bottles were sealed with butyl rubber stoppers and alumina-cap whereas the other serving as a control was sealed with a butyl rubber stopper, autoclaved to inactivate microbial activity, and later capped with an alumina-cap.

**Dechlorination experiment**

To initiate the dechlorination experiment, 0.2 ml of 500 mg/l HCB in acetone was injected into each serum bottle to make the initial HCB concentration of 2 mg/l. After shaking to homogenize the mixture, 2 ml of the sample was taken by a syringe with 22G2 hypodermic needle and extracted for actual HCB concentration at the beginning. The inoculated serum bottles were then kept in the dark at room temperature. At predetermined time interval, 2 ml of the incubated culture sample was withdrawn and extracted for residual HCB and its intermediates.

**Analysis**

The culture sample was extracted three times by n-hexane and analyzed by a gas chromatography equipped with an ECD (Hewlett Packard 4890, USA) anda capillary column of DB-5 fused silica. The oven temperature was maintained at 80℃ for 5 min, raised to 120℃ at the increasing rate of 5℃/min, maintained for 2 min, and then raised again at 5℃/min to the final temperature of 200℃ which was held for 5 min. The temperatures of the injector and the detector were set at 280 and 300℃, respectively. Nitrogen gas was used as the carrier and the make-up gas. The liner velocity was 16 cm/s and the split ratio was set at 10:1. Sediment slurry characteristics were analyzed followed the procedure described in Standard Methods (APHA, 1992).

## Results and discussion

**Background CBs contamination and sediment slurry characterization**

All 10 sediments were checked for possible CBs contamination and the results are shown in Table 1. The HCB was only found in the sediments from the first 4 sites upstream nearest to the discharge point within the range of 0.18 to 1.25 ppm. On the other hand, no HCB could be detected in all water samples. This is not surprising since HCB isa hydrophobic compound and tends to sorb onto solid particles which have a possibility to settle significantly after the discharge point when the flow velocity decreases. This provided a piece of evidence that HCB was still released to the environment resulting from industrial activities as suggested by Bailey (Bailey, R.E., 2001). Of more interesting was the identification of 1,3,5-TCB at S2 and S3 similar to the findings of Brigden (Brigden, K. *et al.*, 2003). The 1,3,5-TCB was a major product in reductive dechlorination of HCB and has not either been used in any industrial and agricultural sectors or an intermediate or impurity during chemical processes (Bailey, R.E., 2001). hence, indicated a possibility of HCB dechlorination in natural

sediment environment of this canal. Detection of HCB contamination in this area posts a health risk to people in the community along this canal who earn their living by coastal fishery in which the HCB and its less chlorinated congeners accumulated in the caught marine creatures can transfer to human via food chain. As a result, it is very important to understand the behavior of HCB dechlorination in this stream sediment which can lead to a clean-up action in the future. Characteristics of the sediment slurries with the sediment to water ratio of 1:1 by volume from 10 sites are summarized in Table 2. Soluble organics in terms of COD and TOC were rather low as compared to those in solid phase. Volatile organics including micro- organisms were only accounted for 2 to 13% of total solids which were in line with the total COD. Chloride was quite high due to the intrusion of seawater depending on the tidal level which periodically intruded up to S1.

**Hexachlorobenzene dechlorination ability**

The results from sterilized series showed no significant reduction in HCB through out the studied period of 60 days (data not shown). Therefore, it implies that the disappearance of HCB which will be discussed later should derive primarily from microbial activities. For active microbial series regardless on the past HCB contamination history, all natural-matrix sediment slurries without either acclimation or enrichment could effectively dechlorinate HCB with a lag phase of 12 to 30 days as shown in Figure 3. It is important to note that the sediment from S10 which is at the estuary was also able to dechlorinate HCB implying that the HCB-dechlorinating microbes were quite diverse from fresh-water to brackish-water species. Dechlorination intermediates which could be identified were QCB, 1,2,3,5-TeCB, and 1,3,5-TCB following the major pathway as proposed by Fathepure (Fathepure, B.Z. *et al.*, 1988) in Figure 1. HCB at 2 mg/l could be completely removed within the period of 42 to 60 days or so. These findings were quite different from several previous studies with untamed sediments which required much longer time. Prytula and Pavlostathis (Prytula, M.T. *et al.*, 1996), using HCB-contaminated sediments with approximately 58 times less concentrated than that used in this study, obtained only 43% reduction of HCB over a long incubation time of more than 480 days; however, with the addition of degradable organic carbon, 95% HCB was biotransformed within 205 days of incubation. Rosenbrock (Rocenbrock, P. *et al.*, 1996) studied with soil slurries spiked with HCB at the concentration quite similar to this study and found that 140 days were needed to obtain 40% chloride releasefrom their hexachlorine dechlorination experiment with rich organic matter soil whereas no chlorination activity has been observed with low organic matter soils. Chen (Chen, I.M. *et al.*, 2004) introduced 2 mg/l HCB directly to four non-HCB contaminated river sediments without any enrichment or acclimation and found that only two sediments could dechlorinate HCB in a 150-day incubation priod; however, the dechlorination ability all of four sediments was significantly promoted with the addition of yeast extract. Hence, it can be concluded that the sediments used in this study with either non- or HCB contamination could dechlorinate HCB more effectively than in other studies. This implies that the existing substrates and nutrients in these sediments as well as physical conditions such as temperature were sufficient and suitable to promote the activities of HCB-dechlorinating microbes. In addition, it also hints that the

dechlorinating microbes already co-exist in the natural microbial matrix; however, certain environmental factors may be needed to stimulate the HCB-dechlorination activity.

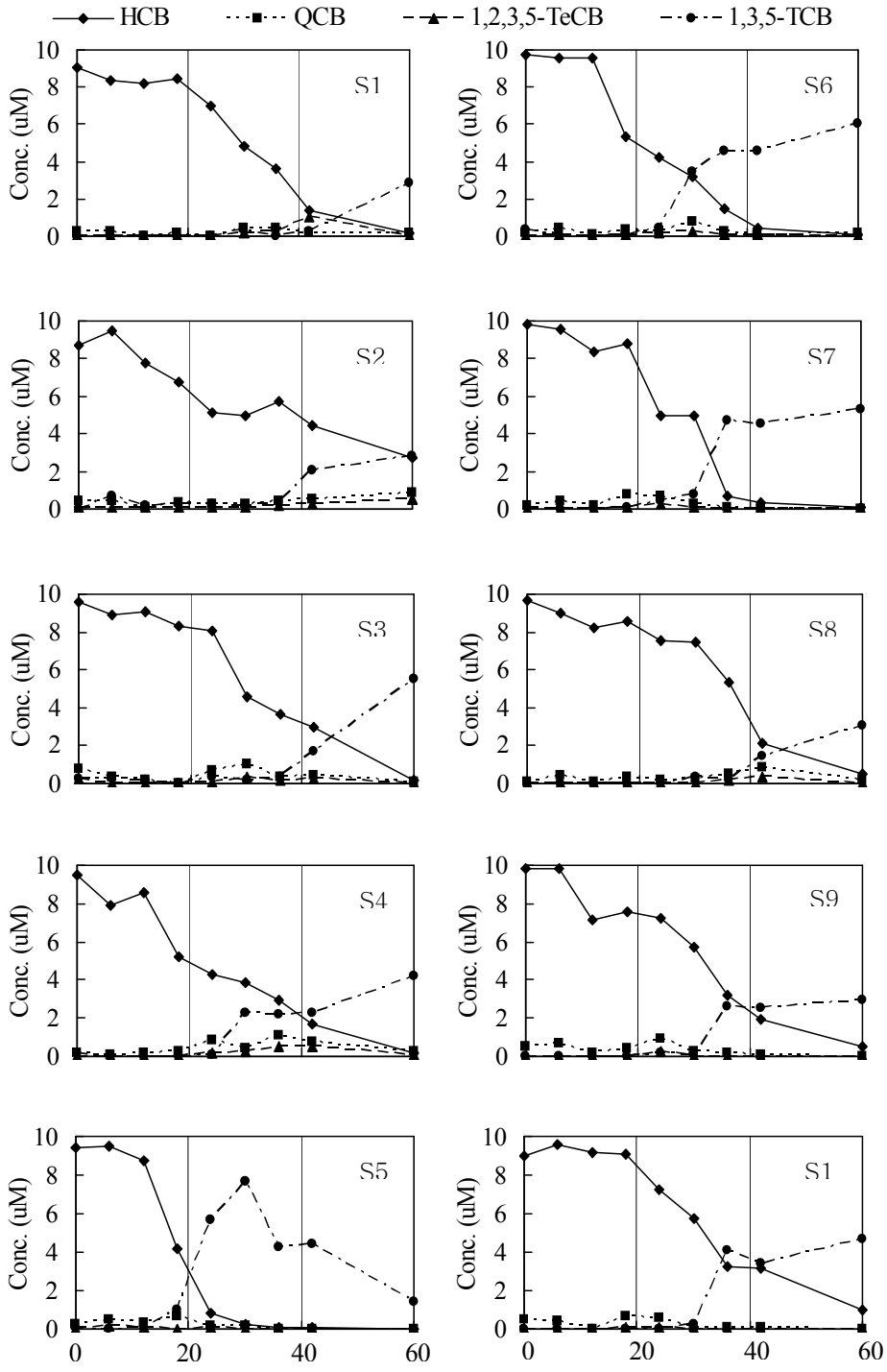


Fig. 3. Dechlorination of HCB by different sediment slurries

Table 1. HCB and 1,3,5-TCB in the sediments from Hua-Lum-Poo Canal

| Sampling site | HCB (ppm) | 1,3,5-TCB (ppm) |
|---|---|---|
| S1 | 0.182 | not detected |
| S2 | 1.250 | 0.238 |
| S3 | 0.261 | 0.160 |
| S4 | 0.153 | not detected |

Note: no HCB and its less-chlorinated congeners found in the sediments from S5 to S10.

Table 2.Characteristics of sediment slurries

| Location | COD (mg/l) | | $Cl^-$* (mg/l) | TOC* (mg/l) | SS* (mg/l) | VSS* (mg/l) |
|---|---|---|---|---|---|---|
| | Soluble | Total | | | | |
| S1 | 666 | 21,492 | 6,918 | 68 | 311,700 | 6,000 |
| S2 | 1,086 | 73,584 | 13,484 | 217 | 228,400 | 28,500 |
| S3 | 584 | 42,985 | 8,938 | 157 | 228,300 | 19,600 |
| S4 | 215 | 50,149 | 4,080 | 311 | 158,500 | 21,200 |
| S5 | 215 | 46,567 | 9,191 | 287 | 175,800 | 22,200 |
| S6 | 173 | 35,821 | 5,404 | 226 | 158,100 | 21,700 |
| S7 | 143 | 28,657 | 3,788 | 274 | 158,700 | 14,700 |
| S8 | 72 | 28,667 | 3,283 | 195 | 194,200 | 14,900 |
| S9 | 152 | 35,821 | 12,100 | 231 | 359,300 | 25,800 |
| S10 | 148 | 39,829 | 9,292 | 203 | 518,100 | 32,700 |

Note: *are filtered samples using GF/C filter papers.

## Conclusions

In this study, sediments in the canal downstream from the discharge point of industrial estate and factories (S1-S4) were found to be contaminated with HCB. At these contamination sites under existing environment, HCB was found to be naturally dechlorinated to form 1,3,5-TCB following the major HCB-dechlorination pathway as suggested by many researchers. Results from the experiment in serum bottles using 1:1 (v/v) of sediment to canal water ratio also confirmed this observation, i.e., HCB of 2 mg/l was able to be dechlorinated completely within a period of 42-60 days or so by the untamed sediments collected from this canal without any nourishment. The sediments from the sites without HCB contamination (S5-S10) were also able to dechlorinate HCB effectively similar to those with HCB contamination. This implies that the essential nutrients required for promoting HCB-dechlorinating activities as well as the HCB-dechlorinating microbes were already presented in the sediments. It also indicated that the microbes having HCB-dechlorination ability not only limited to fresh-water group but also brackish-water species (as at S10). Dechlorination also followed the major pathway like those found in the canal, i.e., HCB to QCB, 1,2,3,5-TeCB, and 1,3,5-TCB, sequentially. These results imply that the HCB-dechlorinating microbes exist in the natural microbial matrix of the sediments in this canal and its estuary.

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