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Hexachlorobenzene dechlorination by indigenous sediment microorganisms

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ABSTRACT

Indigenous microbes from the sediments, whether contaminated with hexachlorobenzene (HCB) or not, could dechlorinate HCB effectively without any acclimation and supplemental nourishment. Temperature seriously affected the HCB-dechlorination: within the measured 15–45 °C span, the optimum range was between 30 and 35 °C. Sulfate-reducing bacteria (SRB), denitrifiers, and acetogens might not be directly involved in the HCB dechlorination. However, the SRB retarded subsequent dechlorination of pentachlorobenzene to tetra- and trichlorobenzenes. Some vancomycin-resistant gram-positive bacteria and methanogens were most likely to be the HCB-dechlorinators. The dechlorination followed the Michaelis–Menten behavior with the k'_m and K_{HCB} between 0.45–0.73 mg L^{-1} day^{-1} and 3.2–17.2 mg L^{-1}, respectively. These findings suggest a potential HCB treatment and cleanup for wastewater and contaminated site.

1. Introduction

Hexachlorobenzene (HCB) was produced and used globally as a fungicide and an industrial synthetic material before production was banned several decades ago. Because of its bioaccumulation and persistence properties, as well as its toxicity, HCB was classified as one of the 12 persistent organic pollutants (POPs) by the United Nations Stockholm Convention. HCB has been found to contaminate in many places worldwide including Thailand as a result of uncontrolled release. HCB was sporadically detected in the sediment of the Hua Lum Poo Canal in Samuth Prakarn Province, Thailand, which receives treated effluent from nearby industrial estate and factories, even though the direct use of HCB in Thailand has been prohibited since 1980 [1,2]. This finding is comprehensible since HCB is also unintentionally generated as a byproduct from the manufacture of the chlorinated pesticides such as atrazine and simazin [3,4]. Of more concern is that this canal eventually empties into the Gulf of Thailand, posing a threat of HCB contamination and bioaccumulation in the coastal bivalves that are consumed by local people.

Environmental degradation of HCB under anaerobic conditions in soil and sediment is possible but considerably slow. Beurskens et al. [5] found an 80% loss of HCB with an increase in the by-products of 1,3,5-trichlorobenzene (1,3,5-TCB) and 1,3-dichlorobenzene (1,3-DCB) during a 20-year period of in situ degradation in Lake Ketelmeer, a sedimentation area of the Rhine River. The half lives of HCB in soil and groundwater were reported to be 3–6 and 5.3–11.4 years, respectively [6]. Prytula and Pavlostathis [7] evaluated the HCB-dechlorination ability of the sediment slurry collected from an HCB-contaminated tributary without any acclimation or enrichment and found that only 43% of HCB was dechlorinated in 481 days at 23 °C in the dark. Rosenbrock et al. [8] could obtain only 40% chloride release in the rich-organic soil slurry spiked with HCB in 140 days, whereas no dechlorination activity was observed in the low-organic soil slurry. Chen et al. [9] worked with four non-HCB contaminated sediment slurries and found only two slurries could initiate HCB-dechlorination with a lag phase of 90 days. These data, however, show different results from our previous study in which 17 mg kg^{-1} dry solids of HCB could be rapidly and completely degraded within 60 days by the indigenous microbial consortium in the sediment slurries collected along the Hua Lum Poo Canal and its mouth without any supplementation of organics or nutrients [1]. The degradation followed the major pathway proposed by Fathepure et al. [10], i.e., HCB → pentachlorobenzene (QCB) → 1,2,3,5-tetrachlorobenzene (TeCB) → 1,3,5-TCB. This finding corresponded very well with the field data in which 1,3,5-TCB was found together with HCB in the upstream sediments of this canal [1].

The objective of this work was to investigate into more details on the factor causing the microbes in the sediment of the Hua Lum Poo Canal to be more powerful in HCB-dechlorination than others. Microbial groups involving in the HCB-dechlorination and

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the dechlorination kinetics were also determined. The information reported here provides a better understanding regarding the reductive dechlorination of HCB, which can lead to a promising treatment or cleanup technique in the future.

2. Materials and methods

2.1. Chemicals

Chlorinated benzene congeners (CBs) including monochlorobenzene (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 Seelze, Germany. The microbial inhibitors including bromoethanesulfonic acid (BES) and vancomycin (VAN) were obtained from Sigma Chemical Co., USA. The 99.5% acetone and n-hexane (Labscan Asia, Co., Ltd., Thailand) were used for preparing an HCB solution and CBs standards/extraction liquid, respectively. All other chemicals were analytical grade and supplied by Merck KGaA, Germany.

2.2. Sediment and canal water sampling

Sediment and canal water samples from two sites (H1 and H2) along the Hua-Lam-Poo Canal that possessed the highest HCB-dechlorination activity according to Anotai et al. [1] were used in this study. The top few centimeters of the sediment surface were carefully removed, and the lower layer was scraped and packed in a plastic bag. Canal waters were sampled and stored in the containers. Both sediment and water samples were stored in the 4 °C cold storage until use.

2.3. Microbial preparation

Sediment slurry of each site was prepared by thoroughly mixing the sediment and canal water at the ratio of 1:1 (v/v) by hand for 2 min and was allowed to settle for 30 min. The upper supernatant was withdrawn by a 100-mL glass syringe with a 22G × 2 hypodermic needle (0.7 mm opening), injected into a 1000-mL serum bottle, and purged with nitrogen gas before use. For dechlorination experiments, 50 mL of sediment slurry were transferred to several 100-mL serum bottles in a nitrogen glove box to prevent oxygen interference and sealed with butyl rubber stoppers and alumina-caps. One bottle from each experiment set was sterilized three times in an autoclave and used as the control.

2.4. Dechlorination experiments

To initiate the experiment, an appropriate amount of stock HCB solution, along with a specific amount of the individual chemical to be studied, was injected into the serum bottles. All serum bottles were kept in the dark at room temperature (28–31 °C with an average of 30 °C). To study temperature effects, refrigerators (at 15 and 20 °C), air-incubators (at 35 and 40 °C), and water baths at 45 °C were used to control the temperature. All bottles were shaken by hand on a daily basis. The experiments were conducted in duplicate with a sterilized control.

2.5. Sampling and analysis

To determine HCB and its dechlorination by-products, HCB from the sediment slurry was extracted according to the method described by Chen et al. [11] which provided the recovery between 89 and 98% for all 12 chlorobenzenes. An extraction test has been performed with 1-h HCB spiked sediment slurry in order to confirm the reliability of this method. The HCB recovery was between 88 and 95%. In addition, another test was also conducted using non-biodegradable and highly hydrophobic 2,3,4,2′,4′,5′- and 2,3,6,2′,4′,5′-chlorobiphenyls to verify the consistency of this extraction method over an adsorption period of 18 weeks. The recover was between 88 and 112% for 2,3,4,2′,4′,5′-chlorobiphenyl and 91 and 110% for 2,3,6,2′,4′,5′-chlorobiphenyl with no less-chlorinated intermediate detected. Hence, this extraction method is proven to be reliable. At a predetermined time, 2 mL of the sediment slurry was taken by a glass syringe with a 22G × 2 hypodermic needle (using the same needle size used to withdraw the sediment slurry from the sediment-water mixture in order to ensure a homogeneous sample) and injected into an extraction tube containing 0.2 mL of 6N NaOH and 2 mL of n-hexane. The tube was then shaken by hand 100 times, followed by 10 min of sonication, and then centrifuged at 4000 rpm for 5 min. The upper-layer of n-hexane was withdrawn as much as possible into a 5-mL analyzing tube. The remaining mixture in the extraction tube was then re-extracted twice more following the same procedure. At the third extraction, the upper-layer of n-hexane was pulled out and filled the analyzing tube up to the 5-mL mark. A small amount of anhydrous Na_2SO_4 was added to remove moisture before being analyzed by gas chromatography. The 6890N Network GC system (Agilent Technologies, USA) was equipped with an electron capture detector (ECD) and a capillary column DB-5 fused silica with 0.25 mm diameter and 30 m length (Agilent Technologies, USA). The oven temperature was initially maintained at 80 °C for 5 min, raised to 140 °C at the rate of 3 °C min^{-1} and sequentially to 240 °C at the rate of 10 °C min^{-1}, and hold for 8 min. The temperature for the injector and detector were set at 240 and 280 °C, respectively. The carrier and make up gases were helium and nitrogen at the average linear flow rates of 20 and 60 mL min^{-1}, respectively. All qualifications and quantifications were performed with an external standard. Methane in the headspace gas was determined by the Gas Chromatograph GC-8A (Shimadzu Corporation, Japan). Sediment slurry characteristics were analyzed according to Standard Methods [12].

3. Results and discussion

3.1. Background contamination and sediment slurry characteristics

Trace amounts of HCB were found in the sediments of both sites at 0.26 and 0.15 mg kg^{-1} dry solids for H1 and H2, respectively. In addition, 0.16 mg kg^{-1} dry solids of 1,3,5-TCB, which has never been produced or used in a commercial scale, was also found in the sediment from H1. This implies that the microbes at both sites should be acclimated to HCB to some degree and be able to dechlorinate HCB in situ. The characteristics of raw sediment slurries were shown in Table 1 illustrating that most organics were in the solid phase. Volatile organics, part of which representing the microbial mass, were only accounted for at 9% and 13% at H1 and H2, respectively. Nitrogen and phosphorus in the sediment slurries were sufficient for anaerobic digestion according to the acceptable COD:N:P ratio of 250:5:1–700:5:1 [13]. Chloride was quite high due to the intrusion of seawater at the sampling time. The pH of the mixtures was in the neutral range appropriate for microbial activities. Pre-tests with nutrient and organic supplements of either yeast extract, glucose, pyruvate, lactate, acetate, formate, or essential minerals revealed no significant improvement in the dechlorination process. Hence, the existing composition of sediment slurries was already suitable for the reductive dechlorination of HCB.

3.2. Reductive dechlorination of HCB

Sediment slurries from both H1 and H2 could dechlorinate 2 mg L^{-1} HCB completely in 70 days at the average room temper-

Table 1
Characteristics of raw sediment slurries.

Site	pH	$Cl^-_{soluble}$ (mg L^{-1})	SS (mg L^{-1})	VSS (mg L^{-1})	COD (mg L^{-1})		Nitrogen (mg L^{-1})		Phosphorus (mg L^{-1})	
					Soluble	Total	Soluble	Total	Soluble	Total
H1	7.0	8938	228,300	19,600	584	42,985	13	896	2	66
H2	7.2	4080	158,500	21,200	215	50,149	12	1,036	1	77

ature of 30 °C as shown in Fig. 1. In contrast, no dechlorination occurred in the sterilized control bottles, i.e., steady HCB concentration and no intermediates detected, implying that the reduction of HCB in the experimental sets should derive from microbial activities. This performance was more impressive than other studies working with the sediment slurries from both HCB-contaminated and non-contaminated sites [7–9]. The degradation pathway followed the major pathway from HCB to QCB, 1,2,3,5-TeCB and 1,3,5-TCB as suggested by Fathepure et al. [10]. No 1,2,4-TCB and DCBs which are the products from the minor dechlorination pathway were detected. Certain loss of chlorobenzenes from the aqueous phase was also detected which mainly due to the transfer of volatile 1,3,5-TCB to the headspace of serum bottle similar to the observation of other studies [6,9,11,14]. The dechlorination performance obtained in this study was consistent with our preliminary work [1] implying that the microbes in the sediments of this canal could efficiently maintain their dechlorination ability.

3.3. Effect of temperature on HCB-dechlorination

It is interesting to determine the key factor enhancing the HCB-dechlorination ability of the microbes in this canal. One possible factor causing the differences in HCB-dechlorination between this study and others was the temperature. Most of Thailand has a tropical climate in which the coldest month temperature is higher than 18 °C. According to Thai Meteorological Department, the 30-year monthly averages for night-time and day-time in Samuth Prakarn Province are 26.3 and 30.3 °C, respectively (day-time temperatures during the experimental period of this study were between 28 and 31 °C with an average of 30 °C). On the other hand, most developed countries in which the HCB-dechlorination has been studied are in the temperate-climate (the coldest month average between −3 and 18 °C) or continental-climate (the coldest month average below −3 °C) zones according to the Köppen–Geiger climate classification system. As a result, the native microbial consortium in the sediment of the Hua Lum Poo Canal as well as other streams in Thailand should be diverse from those of developed countries and might possibly lead to an explanation for the differences in HCB-dechlorination. To test whether the temperature was one of the major factors controlling the HCB-dechlorination activity, the sediment slurries without any supplements were spiked with 2 mg L^{-1} of HCB and incubated at various temperatures from 15 to 45 °C. The outcomes showed that the optimum temperatures were between 30 and 40 °C for H1 and 30 and 35 °C for H2 (Table 2). The dechlorination performance drastically deteriorated as the temperature became lower or higher. The lag phase and complete dechlorination period extended approximately two times or more as the temperatures rose above or dropped below the optimum range. This finding serves as solid evidence that temperature plays a major role in classifying and characterizing microbial consortium and activity regarding on HCB-dechlorination in natural stream sediment. Further investigation was conducted to verify the effect of temperature by using the sediments collected from four other streams which were not contaminated with HCB. The results shown in Fig. 2 indicate that the indigenous microbial consortiums in these stream

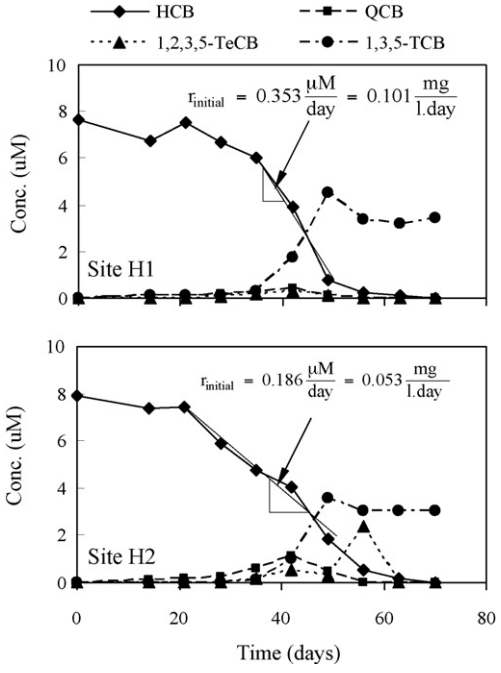

Fig. 1. Profiles of HCB and its intermediates with an initial HCB concentration of 2 mg L^{-1}.

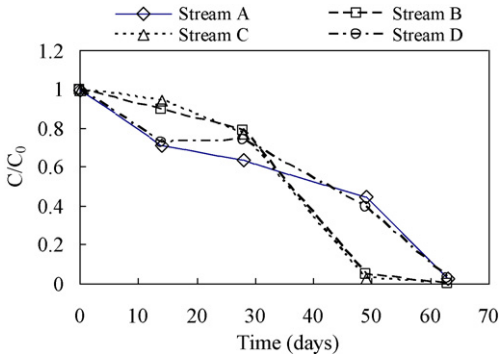

Fig. 2. HCB-dechlorination in non-contaminated sediment.

Table 2
HCB-dechlorination under various temperatures.

Site	Temperature (°C)	Lag phase (days)	Complete dechlorination (days)
H1	15	35 (42)	>154 (>154)
	20	28 (35)	126 (126)
	30	14 (7)	70 (70)
	35	7 (7)	63 (63)
	40	7 (7)	63 (70)
	45	14 (35)	>154 (>154)
H2	15	70 (35)	>154 (>154)
	20	49 (49)	161 (161)
	30	14 (14)	70 (70)
	35	7 (14)	70 (70)
	40	14 (14)	>154 (>154)
	45	42 (35)	>154 (>154)

Note: Numbers in the parenthesis are duplication.

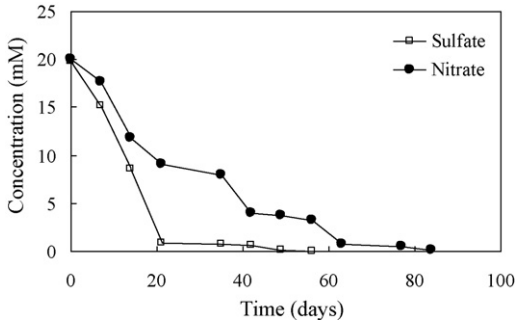

Fig. 3. Consumption profile of added electron acceptors.

sediments could readily dechlorinate 2 mg L^{-1} HCB with no acclimation or supplemental nourishment at room temperature similar to those from H1 and H2. These results suggest that temperature plays an important role on the dechlorination of HCB which is similar to the study of Chang et al. [6] who found the optimum temperatures for HCB-dechlorination to be between 29 and 37 °C and the dechlorination ability ceased at 18 and 45 °C.

From these results, it implies that HCB in the tropical-climate environment with moderate ambient temperatures might not be as persistent as in the temperate-climate and continental-climate regions due to the differences in native microbial matrix and activities. Biologically anaerobic processes, which typically have a long sludge detention time, such as an anaerobic filter or upflow anaerobic sludge blanket followed by an aerobic process might be a feasible alternative to purify HCB-contaminated wastewater. Remediation of HCB-contaminated sites is also promisingly possible if suitable conditions are provided. The identified end product was 1,3,5-TCB for all studied temperatures, neither 1,2,4-TCB nor DCBs was detected. Hence, the dechlorination mechanism still followed the major pathway. Nevertheless, substantial dissimilarity in the complete dechlorination period at 40 °C between the sediments from H1 and H2 suggested that the microbial consortium existing in these two sites were different, which was in agreement with the results from the kinetic studies in later section.

3.4. Characterization of dechlorinating microorganisms

This section attempted to determine the principal microbes responsible for HCB-dechlorination. Only the sediment slurry from H2 was used for characterization purpose. The first experimental scenario in this section aimed to determine the involvement of sulfate-reducing bacteria (SRB) and denitrifiers (DN) in the dechlorination of HCB to its less-chlorinated products. Sediment slurry with 2 mg L^{-1} of HCB was added with 20 mM of either $NaNO_3$ or Na_2SO_4 to stimulate the activities of DN or SRB, respectively. Nitrate reduction during the incubation period was slower than sulfate, as shown in Fig. 3, which implied that the SRB in this sediment were more active than the DN. This is plausible since the collected bottom sediment was under anaerobic conditions for a very long period of time. An anoxic state, which uses nitrite or nitrate as an electron acceptor, is unlikely to occur intensively in this rich organic sediment deposited a few centimeters below the sediment–water interface. No significant difference in HCB-dechlorination between these two supplement and control sets was observed, i.e., QCB appeared at the same time on day 14 (Table 3) with comparable apparent concentration between 0.018 and 0.019 mg L^{-1}. During this initial period, the SRB and DN were very active, interpreting from the zero-order disappearance rates of sulfate and nitrate (Fig. 3). This result suggested that neither SRB nor DN directly engaged in or serious interfered with the HCB-dechlorination since the addition of sulfate or nitrate to promote their activities neither

Table 3
Effects of electron donors on HCB-dechlorination of the sediment slurry from Site H2.

Electron acceptor	Appearance time (days)		
	QCB	1,2,3,5-TeCB	1,3,5-TCB
No addition	14	42	56
20 mM of $NaNO_3$	14	56	56
20 mM of Na_2SO_4	14	70	98

enhanced nor retarded the transformation of HCB to QCB. Working with HCB-adapting sediment, Chen et al. [14] also found that 30 mM sulfate did not interfere with HCB-dechlorination; however, they suggested that under limiting electron donors SRB might compete with HCB-dechlorinators for substrate, thus hampering the HCB-dechlorination. The impact of nitrate seemed to be unclear on QCB transformation to 1,2,3,5-TeCB and 1,3,5-TCB. The appearance time for 1,2,3,5-TeCB in the presence of nitrate was 14 days longer than the control without nitrate, but was the same for 1,3,5-TCB. It is important to note that the sample on day 56 was the first sampling after day 42; hence, they could not be definitely differentiated. The presence of sulfate, however, significantly prolonged the transformation of QCB to 1,2,3,5-TeCB and subsequently to 1,3,5-TCB. The occurrence times for 1,2,3,5-TeCB and 1,3,5-TCB shifted from 42 to 70 days and from 56 to 98 days, respectively. This implies that the activity of SRB noticeably interfered with QCB- and 1,2,3,5-TeCB dechlorinators. As a result, it indicates that the population responsible for chlorobenzene dechlorination differed at least in some species from the QCB- and 1,2,3,5-dechlorinators. This result is in agreement with the study of Chen et al. [11].

To evaluate the role of methanogens on HCB-dechlorination, the selective methanogenic inhibitor, bromoethanesulfonic acid (BES) [6], was inoculated into the serum bottle at various concentrations. It was found that only 5 mM of BES could notably suppress the methanogenic activity. Accumulated methane in the headspace of the serum bottle on day 78 in the control set without BES was 38% which was much higher than those in the BES amended set of less than 1%. Nevertheless, more than 90% of the HCB was still dechlorinated, though at a lower rate, as shown in Fig. 4. This suggests two possibilities: first, the dechlorinating step was directly executed by methanogenic bacteria only, but maybe a variety of different species of methanogens can play this role. Therefore, whenever one or some methanogenic bacteria regained their activity and began to produce methane even slightly, these methanogens could be able to trigger the dechlorination of HCB. Second, while the methanogens played a major role in HCB-dechlorination (reduction rate was steeper in the control set), other microorganisms in the sediment slurry might also involved. As the BES was increased to 10 and 50 mM, HCB was still dechlorinated. Only when 250 mM was applied did the dechlorination stop completely. Middledorp

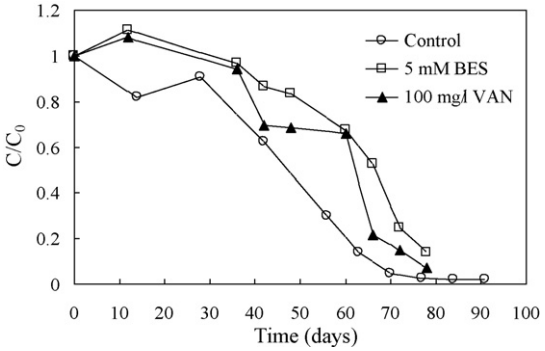

Fig. 4. Dechlorination profile for HCB with and without inhibitors.

et al. [15] found that BES at high concentration also affected the microbial species other than methanogens, possibly suppressing the non-methanogenic HCB-dechlorinators in the sediment slurry. The results from this study filled the gap between two totally different observations from other research groups. Pavlostathis and Prytula [16] reported that methane production was inhibited in a BES-amended culture, but sequential reductive dechlorination of HCB still occurred at a comparable rate as in the non-amended culture, indicating that methanogens were not involved in the dechlorination process. On the other hand, the data from the BES-amended studies of Chang et al. [6] and Chen et al. [14] strongly suggested that methanogens were the sole HCB-dechlorinators in their acclimated cultures. Combining these facts together with the results from this study, it can be concluded that HCB-dechlorinators should consist of methanogens as well as other microbial groups. In the studies of Chang et al. [6] and Chen et al. [14], methanogens served as the sole HCB-dechlorinators in their cultures. As a result, once the methanogens were inactivated by BES, the dechlorination of HCB was also terminated. In the study of Pavlostathis and Prytula [16], however, the dechlorination occurred via the activities of the microbial species other than methanogens. Hence, the inhibition of methanogenic activities did not completely block HCB-dechlorination. In this study, the sediment slurry contained both methanogens and other HCB dechlorinating species; thus, even though the methanogens were almost completely inactivated by the BES, the remaining dechlorinators that were not susceptible to BES still transformed HCB to QCB; however, at the lower rate as compared to when both microbial groups were active.

Further investigation on HCB-dechlorinators was conducted by using vancomycin (VAN), a strong bactericide on gram-positive bacteria including most acetogens. The results revealed that 100 mg L^{-1} of VAN moderately retarded the dechlorination performance shown in Fig. 4. Partial pressure of methane in the headspace also reduced from 38% to 16% in the presence of VAN during the same incubation period. This implies that acetogenic activities that transformed volatile fatty acids to acetic acid for methanogenic uptake were partially affected and consequently retarded methane formation. Once the methanogenic activities subsided, the dechlorination process was also decelerated. As the VAN was increased to 200 mg L^{-1}, a level that should inhibit most gram-positive bacteria, the HCB-dechlorination completely stopped. The reason could be that the gram-positive bacteria usually play the role of supplementing substrate to methanogenic bacteria, and once this supplementation is terminated, the methanogenic HCB-dechlorinator could no longer dechlorinate HCB. In addition, apart from methanogenic HCB-dechlorinators, it is possible that there were other naturally existing dechlorinators in the sediment of the Hua Lum Poo Canal, and they were most likely the gram-positive microbes that were able to tolerate VAN to some certain degree. Swenson et al. [17] reported that some gram-positive bacteria, such as the *Leuconostoc*, *Pediococcus*, and *Lactobacillus* species, were essentially resistant to vancomycin; however, they might be inactivated by the high VAN dose of 200 mg L^{-1}.

3.5. Kinetics of HCB-dechlorination

To evaluate the microbial dechlorination ability and HCB toxicity, the HCB dose was increased from 2 to 10, 40, 100, and 200 mg L^{-1}, which were equivalent to 43.8, 175.2, 438.0, 876.0 mg kg^{-1} dry solids for H1, and 63.1, 252.4, 630.9, 1261.8 mg kg^{-1} dry solids for H2, respectively. Surprisingly, it was found that HCB was still dechlorinated at the very high concentration of 200 mg L^{-1} with a minor retardation as illustrated in Table 4. The lag phase and appearance time for dechlorination intermediates including QCB, 1,2,3,5-TeCB, and 1,3,5-TCB were almost similar for all HCB dosages. The only difference was the time required to completely remove HCB, which became longer as the HCB increased. It is important to mention that the appearance times of 1,2,3,5-TeCB, and 1,3,5-TCB in the experiment with 2 mg L^{-1} HCB using the sediment slurry from H2 were different from those of the control set (no addition) in Table 3 even though they were tested under similar conditions with the same sediment sample. This was possibly due to the effect of the storage time at 4 °C in the cold storage. The sediment used in this part was kept for one week after sampling whereas those used in Table 3 was kept for two months. It implies that the QCB and 1,2,3,5-TeCB dechlorinators might be somehow affected during the storage at 4 °C for a long period of time. The degradation pathway of all HCB dosages still followed the major pathway to 1,3,5-TCB. The initial dechlorination rate was determined during the most active dechlorination period, i.e., HCB decreased sharply, as shown in Fig. 1. The relationship between the initial dechlorination rate and the HCB concentration is shown in Fig. 5. The dechlorination rate increased exponentially as the HCB concentration increased and finally reached a plateau where a further increase in HCB did not promote the rate. This pattern was similar to the enzymatic catalytic behavior that could be quantitatively explained by Michaelis–Menten kinetics. With sufficient nutrients and no inhibition effect as in this study, the dechlorination rate of HCB via a co-metabolization could be described under the influence of HCB and organic substrate concentrations as shown in Eq. (1) [16]:

$$\frac{d[HCB]}{dt} = -\left(\frac{k_m X[HCB]}{K_{HCB} + [HCB]}\right)\left(\frac{S}{K_S + S}\right) \quad (1)$$

where k_m is the maximum dechlorination rate of HCB per unit biomass; K_{HCB} and K_S are the half-saturation constants regarding on HCB and organic substrate, respectively; X is the HCB-dechlorinator intensity; and S is the organic substrate concentration. Since the organic substrates (S) available for biodegradation as represented by the COD values were much higher than HCB concentration and the biomass (X) growth under anaerobic condition was considerably very slow, these two parameters can be considered constant

Table 4
HCB-dechlorination under various HCB concentrations.

Site	HCB (mg L^{-1})	Appearance time (days)			
		QCB	1,2,3,5-TeCB	1,3,5-TCB	Complete HCB-dechlorination time (days)
H1	2	14	14	14	70
	10	14	28	28	175
	40	14	28	35	>175
	100	14	28	42	>175
	200	21	28	49	>175
H2	2	14	21	28	70
	10	14	21	28	175
	40	14	21	28	175
	100	14	28	28	175
	200	14	28	35	>175

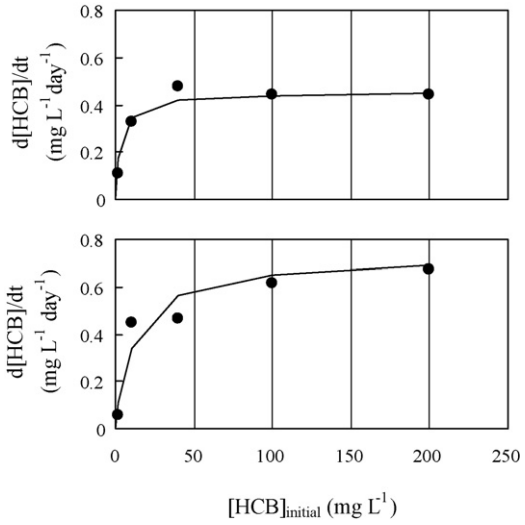

Fig. 5. Effect of HCB concentration on dechlorination rate (lines are model predictions based on the Michaelis–Menten kinetics).

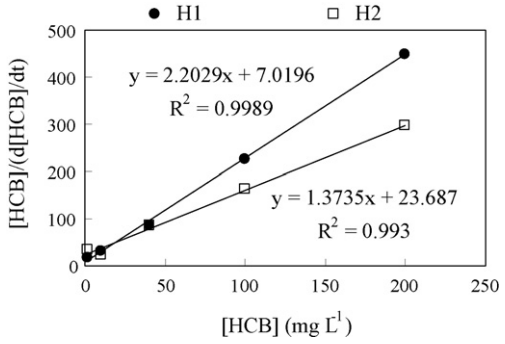

Fig. 6. Hanes linearization for Fig. 5.

during the initial stage; then, Eq. (1) can be simplified to:

$$\frac{d[HCB]}{dt} = -\left(\frac{k'_m[HCB]}{K_{HCB} + [HCB]}\right) \quad (2)$$

where k'_m is an apparent maximum dechlorination rate of HCB. The dechlorination rate then becomes first order with respect to HCB at low concentration and zero order at high concentration. Although many researchers working with HCB-dechlorination have successfully described their kinetic data by using either the first-order reaction [5,7] or the zero-order reaction [18], others decided to use a more realistic Michaelis–Menten model. Pavlostathis and Prytula [16] dechlorinated HCB by using enriched cultures supplementing with essential minerals, vitamins, yeast extract, and glucose, and found the dechlorination to follow the Michaelis–Menten model with the k'_m and K_{HCB} of 0.015 ± 0.001 mg L^{-1} day^{-1} and 0.024 ± 0.003 mg L^{-1}, respectively. Further analysis using the Hanes linearization method [19] as illustrated in Eq. (3) and Fig. 6 found the k'_m, K_{HCB}, and R^2 to be 0.45 mg L^{-1} day^{-1}, 3.2 mg L^{-1}, and 0.993 for H1 and 0.73 mg L^{-1} day^{-1}, 17.2 mg L^{-1}, and 0.999 for H2, respectively.

$$\frac{[HCB]}{(d[HCB]/dt)} = \frac{[HCB]}{k'_m} + \frac{K_{HCB}}{k'_m} \quad (3)$$

The k'_m and K_{HCB} obtained from this study were 30–50 and 178–480 times, respectively, higher than the values reported by Pavlostathis and Prytula [16]. The differences might be due to several factors such as HCB contamination and acclimation level, complexity of microbial consortium, and environmental factors particularly temperature as discussed previously. In addition, it is surprising to obtain significant differences in k'_m and K_{HCB} between H1 and H2 since H2 is only approximately 50 m downstream from H1. From field survey, it was found that H1 was mainly contaminated with industrial wastewater, whereas H2 was located in the congested low-income community; hence, it was also polluted with improperly treated domestic wastewater. Organic matter and nutrients from domestic wastewater might promote microbial activity in the direction of enhancing HCB-dechlorination.

4. Conclusions

The reductive dechlorination of HCB by indigenous sediment microbes under anaerobic condition was intensively investigated in this study. The indigenous microbes in the sediments collected from the Hua Lum Poo Canal could dechlorinate HCB effectively without acclimation and extra nourishment. Temperature significantly affected the HCB-dechlorination with the optimum range between 30 and 35 °C. Hence, HCB might not be a persistent organic pollutant in the moderate temperature environment like those in the tropical zone. The SRB, DN, and acetogens did not directly engage in the dechlorination of HCB; however, the SRB interfered with the dechlorination of QCB to 1,2,3,5-TeCB and 1,2,3,5-TeCB to 1,3,5-TCB. Methanogens and some VAN-resistant species of gram-positive bacteria were most likely the candidates for HCB-dechlorination. The HCB-dechlorination behavior could be sufficiently explained by the Michaelis–Menten kinetics with the k'_m and K_{HCB} between 0.45–0.73 mg L^{-1} day^{-1} and 3.2–17.2 mg L^{-1}, respectively.

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