CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characteristics of Sediment Slurries

For Erh-Jen River, the sediment and water samples were collected along Tainan City in Southern of Taiwan on April 22, 2011 whereas the samples from the Hua Lum Poo Canal in Samuth Prakarn Province of Thailand were collected on June 12, 2010 (stored for 10 months before the experiments) and June 20, 2011 (fresh sampling). Several chemical characteristics including chemical oxygen demand (COD), biochemical oxygen demand (BOD), total Kjeldahl nitrogen (TKN), total phosphorus (TP), suspended solids (SS), volatile suspended solids (VSS), and pH were measured. This part aimed to characterize the sediment slurries prepared from the sediment and water samples collected in Samuth Prakarn Province of Thailand including Hua Lum Poo Canal (H3 and H6), Bang Pla Kod Canal (B1), a canal nearby South-Bangkok Power Plant (PP1) and a canal receiving discharge from small material recovery facilities (M1). The results are summarized in Table 4.1. It could be seen that all sediment slurries contained high organic content indicating a sufficient carbon source for microbial activity. In addition, background concentrations of sulfate and nitrate which could serve as an electron acceptor were also high. It could be seen that COD, BOD, SS and VSS significantly fluctuated from site to site, whereas the pH values were quite steady between 7.67 and 7.76 which were suitable for microbiological activity. SS and VSS could be used roughly for estimating the amount of viable microbial community in each site. From these results, H3 and B1 can be classified as a microbial abundance. The characteristics of sediment slurry from Erh-Jen River in Taiwan (E1-E4) are shown in Table 4.2 (TKN, TP, SS and VSS were not determined). Chen et al. (2004), using the same sediment slurries as in this study, determined the soluble COD of the sediment slurry to be between 182 and 224 mg/L. Comparing between the values obtained from this work and those of Chen et al. (2004), it can be seen that the COD obtained in this study was higher than those of previous work. This is because the Southern of Taiwan have faced two typhoons between 2009 and 2011, the Marakot on August 9, 2009 and the Fanapi in September, 2010 which caused severe flooding in Taiwan. From these floods, organic-enriched sediments and soils from several places were flushed into the Erh-Jen River so the COD increased significantly. In conclusion, it is believed that the sediment slurries prepared from these sites had sufficient and suitable nutrients and substrates for anaerobic degradation.

Table 4.1 Characteristics of the sediment slurries prepared from the samples collected in Thailand

Sampling Site	COD (mg/L)	BOD (mg/L)	TKN (mg/L)	TP (mg/L)	SS (mg/L)	VSS (mg/L)	pH
H3	39,000	5,000	773	111	148,900	18,400	7.67
H6	41,200	4,000	652	134	99,600	14,700	7.71
B1	22,588	2,167	697	86	83,680	18,540	7.76
PP1	19,361	2,500	896	176	61,560	15,860	7.68
M1	12,908	1,833	431	100	96,100	8,320	7.73

Table 4.2 Characteristics of the sediment slurries prepared from the samples collected in Taiwan

Sampling Site	COD (mg/L)	BOD (mg/L)	TKN (mg/L)	TP (mg/L)	SS (mg/L)	VSS (mg/L)	pH
E1[1]	182	23.0	NT	NT	NT	NT	7.6
E2[1]	224	20.2	NT	NT	NT	NT	7.5
E1	896	151	NT	NT	NT	NT	7.61
E2	854	162	NT	NT	NT	NT	7.71
E3	641	120	NT	NT	NT	NT	7.54
E4	756	117	NT	NT	NT	NT	7.68

Note: [1]: Data from Chen et al. (2004).

NT: Not tested

4.2 Phase 1: Bioaugmentation of Active Consortia onto Historical Less-Potential Dechlorinated Sediment Slurries

In this section, the SS from Thailand sites, i.e., H3, B1, PP1, and M1, were prepared by mixing the stored (for 10 months) sediments with the fresh sediment slurries from Taiwan sites, i.e., E1 to E4, as already shown in Table 3.1. This study aimed to evaluate the improvement of HCB dechlorination in blend sediment slurries between those collected in Thailand and in Taiwan. The outcomes of HCB dechlorination in terms of the lag phase which is the incubation time before the dechlorination intermediate(s) was detected and 90% HCB dechlorination period which is the incubation time from the beginning till the spiked HCB was 90% dechlorinated were shown in Table 4.3. HCB dechlorination of the individual samples from Taiwan and Thailand were also conducted and served as the controls. For the non-blended set of the sites in Taiwan, it showed a lag phase between 2 and 4 weeks and the 90% dechlorination periods were in between 8 to 10 weeks. For the non-blended set of the stored samples collected in Thailand, the lag phase varied from 2 to 9 weeks and 90% HCB dechlorination periods were within 6 to longer than 14 weeks. The result of the non-blended set of Thailand sediment was compared with the non-blended set of Taiwan sediment. Thailand sediment took more time than Taiwan sediment because Thailand sediment was stored in cold condition for 10 months that made microbial activity decrease. For this cold-storaged microbe, it needs to spend time in recovering and for recalling their activity. To verify this hypothesis, the dechlorination behavior from previous studies using the sediment and water from Site H3 (similar to this study) are compared with the values obtained in this study (Table 4.4). It can be clearly seen that the dechlorination activity of the microbes in the sediment cold stored for 10 months was significantly lower than those of fresh samples. The reason might be because the cold-storage usually inhibited that the microbial activity of SS microbes.

From Table 4.3, comparing the results of the conditions 4.3.1 to 4.3.4 with two historical coniditions, 2004 and 2009 (Table 4.5), HCB dechlorination by SS from the same site (but different collected time), were varied. The result of Puewchote 2009, the lag phase was more than 8 weeks and the dechlorination could not be accomplished within 24 weeks although supplements which are yeast extract were added in every 2 weeks. While Chen et al. 2004 and this studied, YE were added only one time in the very begining. In this research, lag phase was 2 to 4 weeks and 90% HCB dechlorination period was 8-10 weeks. The major reasons why the three experiments provided different results were because environmental conditions were differentiated. In Tainan city of Taiwan, there were a lot of piggery farms that discharge waste water into Erh-Jen River which is nutrient for microbes. Therefore HCB dechlorination was occurred in 2004, it might be because the SS had enough nutrients. After 2004, many piggery farms were closed down, and that caused the organic matters was reduced in the river and led to the result of non-HCB-dechlorinating. Coincidentally, in 2009 and 2010, Taiwan confronted 2 Typhoons, which are Morakot in 2009 and Tanabi in 2010; those Typhoons brought huge rainfalls and drove the soil and sediment from upper stream to down stream that close to our sampling sites along Erh-Jen River. And it made the COD and nutrients in the down stream of Erh-Jen River increasing, and brought sufficient nutrients for sediment microbes that some of them might be responsible for HCB dechlorination.

By checking the results from Table 4.6 and 4.7, which the SS from Taiwan (E1) were mixing with the SS from Thailand at a ratio of 45:5, it showed that HCB dechlorination could be promoted for merely 2 sites (H3 and B1) with yeast extract. These two sets had the lag phase within 14 and 6 weeks respectively, and the 90% HCB dechlorination period for the former set was more than 14 weeks and for the other was 9 weeks, respectively. However, increasing the ratio to 25:25 resulted a better dechlorination condition, the lag phase was obviously shortened but the dechlorination rate was not clearly improved, it might be because the number of HCB dechlorianting microbes was diluted. When compared the results from Table 4.6, 4.7 with the non-blended control sets (Table 4.3), the results of blended sets were less potential in HCB dechlorination than non-blended control sets. This result was though to be a harshly antagonistic effect between HCB-dechlorinating and non-HCB-dechlorinating populations sourced from different SS. After blended, there was no time for those populations to adapt to each other. Then, an antagonistic effect was happened. In the other hand, the less efficiency of blended sets might be the toxic effect from the unknown factor from the other SS. All of these finding suggested that a blended consortia needed to adapt and to evolve, otherwise, their dechlorination activities might be not cohesive but interact antagonistically.

Table 4.3 HCB dechlorination ability of single sediment slurries microbes in stream sediments

Conditions	Sample site	Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
					QCB	1,3,5-TCB
4.3.1	E1[f]	YE	2	8	-	Yes
4.3.2		None	2	10	-	Yes
4.3.3	E2[f]	YE	4	8	-	-
4.3.4		None	4	10	Yes	-
4.3.5	E3[f]	YE	2	8	-	Yes
4.3.6		None	4	10	Yes	Yes
4.3.7	E4[f]	YE	2	8	Yes	Yes
4.3.8		None	4	10	Yes	Yes
4.3.9	H3[s]	YE	2	11	Yes	Yes
4.3.10		None	4	>14[a] (52%)	Yes	Yes
4.3.11	B1[s]	YE	4	6	Yes	Yes
4.3.12		None	6	11	Yes	Yes
4.3.13	PP1[s]	YE	6	9	-	-
4.3.14		None	9	9	-	Yes
4.3.15	M1[s]	YE	4	11	Yes	Yes
4.3.16		None	9	14	Yes	Yes

Note: [f]: fresh sediment slurries.

[s]: stored sediment slurries at 6 °C for 10 months.

*: period for dechlorinating 90% of the original spiked HCB.

[a]: % of the spiked HCB remaining at the 14th week.

Table 4.4 Comparison of the HCB dechlorination ability between the stored and fresh sediments collected from the Hua Lum Poo Canal Site H3

Source	Dechlorination Behavior	Supplement	
		None	Yeast Extract
Anotai et al. (2010)	Lag phase (weeks)	4	2
	Dechlorination complete (weeks)	6	6
Puewchote (2009)	Lag phase (weeks)	2	Not tested
	Dechlorination complete (weeks)	8	Not tested
This study	Lag phase (weeks)	4	2
	90% Dechlorination period (weeks)*	> 14	11

Note: *: period for dechlorinating 90% of the original spiked HCB.

Table 4.5 HCB dechlorination by single sediment slurry from Erh-Jen River

Source	Site	Dechlorination Behavior	Supplement	
			None	Yeast Extract
Chen et al. (2004)	E1	Lag phase (weeks)	Not observed*	8
		Dechlorination complete (weeks)	Not observed*	15
	E2	Lag phase (weeks)	13	4
		Dechlorination complete (weeks)	> 22	13
Puewchote (2009)	E1	Lag phase (weeks)	> 8	> 8
		Dechlorination complete (weeks)	> 24	> 24
	E2	Lag phase (weeks)	Not tested	Not tested
		Dechlorination complete (weeks)	Not tested	Not tested
This study	E1	Lag phase (weeks)	2	2
		90% Dechlorination period (weeks)	8	10
	E2	Lag phase (weeks)	4	4
		90% Dechlorination period (weeks)	8	10

Note: *: No dechlorination occurred after 22 weeks of incubation period.

Table 4.6 Fusion of sediment slurries by blended sediment slurry microbes in canal sediments at 45:5 ratio

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.6.1	E1[f] (45 mL)	H3[s] (5 mL)	YE	14	>14 (25%)[a]	-	Yes
4.6.2			None	-	-	-	-
4.6.3		B1[s] (5 mL)	YE	6	9	Yes	Yes
4.6.4			None	-	-	-	-
4.6.5		PP1[s] (5 mL)	YE	-	-	-	-
4.6.6			None	-	-	-	-
4.6.7		M1[s] (5 mL)	YE	-	-	-	-
4.6.8			None	-	-	-	-
4.6.9	E2[f] (45 mL)	H3[s] (5 mL)	YE	6	9	Yes	Yes
4.6.10			None	6	11	Yes	Yes
4.6.11		B1[s] (5 mL)	YE	6	9	Yes	Yes
4.6.12			None	6	9	Yes	Yes
4.6.13		PP1[s] (5 mL)	YE	9	9	-	Yes
4.6.14			None	6	11	Yes	Yes
4.6.15		M1[s] (5 mL)	YE	6	11	Yes	Yes
4.6.16			None	11	11	-	Yes

Note: [f]: Fresh sediment slurries.

[s]: Stored sediment slurries at 6 °C for 10 months.

*: period for dechlorinating 90% of the original spiked HCB.

[a]: % of the spiked HCB remaining at the 14^{th} week.

Table 4.6 Fusion of sediment slurries by blended sediment slurry microbes in canal sediments at 45:5 ratio. (Continued)

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.6.17	E3[f] (45 mL)	H3[s] (5 mL)	YE	14	14	Yes	Yes
4.6.18			None	-	-	-	-
4.6.19		B1[s] (5 mL)	YE	2	6	Yes	Yes
4.6.20			None	-	-	-	-
4.6.21		PP1[s] (5 mL)	YE	4	11	-	Yes
4.6.22			None	6	14	Yes	Yes
4.6.23		M1[s] (5 mL)	YE	2	4	Yes	Yes
4.6.24			None	6	9	-	Yes
4.6.25	E4[f] (45 mL)	H3[s] (5 mL)	YE	2	4	Yes	Yes
4.6.26			None	-	-	-	-
4.6.27		B1[s] (5 mL)	YE	2	9	Yes	Yes
4.6.28			None	4	6	-	Yes
4.6.29		PP1[s] (5 mL)	YE	6	9	-	Yes
4.6.30			None	14	>14 (32%)[a]	-	Yes
4.6.31		M1[s] (5 mL)	YE	2	9	Yes	Yes
4.6.32			None	-	-	-	-

Note: [f]: Fresh sediment slurries.

[s]: Stored sediment slurries at 6 °C for 10 months.

*: period for dechlorinating 90% of the original spiked HCB.

[a]: % of the spiked HCB remaining at the 14^{th} week.

Table 4.7 Fusion of sediment slurries by blended sediment slurry microbes in canal sediments at 25:25 ratio

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.7.1	E1[f] (25 mL)	H3[s] (25 mL)	YE	4	>14 (72%)[a]	Yes	-
4.7.2			None	6	11	Yes	-
4.7.3		B1[s] (25 mL)	YE	6	>14 (54%)[a]	Yes	-
4.7.4			None	-	-	-	-
4.7.5		PP1[s] (25 mL)	YE	9	9	-	Yes
4.7.6			None	6	11	Yes	Yes
4.7.7		M1[s] (25 mL)	YE	-	-	-	-
4.7.8			None	-	-	-	-
4.7.9	E2[f] (25 mL)	H3[s] (25 mL)	YE	4	>14 (83%)[a]	Yes	-
4.7.10			None	4	>14 (47%)[a]	Yes	-
4.7.11		B1[s] (25 mL)	YE	6	>14 (50%)[a]	-	-
4.7.12			None	6	>14 (58%)[a]	-	-
4.7.13		PP1[s] (25 mL)	YE	9	9	-	Yes
4.7.14			None	11	14	Yes	-
4.7.15		M1[s] (25 mL)	YE	14	14	-	-
4.7.16			None	6	>14 (89%)[a]	Yes	-

Note: [f]: Fresh sediment slurries.

[s]: Stored sediment slurries at 6 °C for 10 months.

*: period for dechlorinating 90% of the original spiked HCB.

[a]: % of the spiked HCB remaining at the 14th week.

Table 4.7 Fusion of sediment slurries by blended sediment slurry microbes in canal sediments at 25:25 ratio (Continued)

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.7.17	E3[f] (25 mL)	H3[s] (25 mL)	YE	4	>14 (41%)[a]	Yes	-
4.7.18			None	4	>14 (46%)[a]	Yes	-
4.7.19		B1[s] (25 mL)	YE	6	9	-	Yes
4.7.20			None	14	-	-	-
4.7.21		PP1[s] (25 mL)	YE	6	9	-	Yes
4.7.22			None	6	6	-	Yes
4.7.23		M1[s] (25 mL)	YE	-	-	-	-
4.7.24			None	-	-	-	-
4.7.25	E4[f] (25 mL)	H3[s] (25 mL)	YE	4	>14 (89%)[a]	Yes	-
4.7.26			None	4	>14 (65%)[a]	Yes	-
4.7.27		B1[s] (25 mL)	YE	14	>14 (72%)[a]	Yes	-
4.7.28			None	-	-	-	-
4.7.29		PP1[s] (25 mL)	YE	6	11	-	Yes
4.7.30			None	11	11	-	Yes
4.7.31		M1[s] (25 mL)	YE	11	11	-	-
4.7.32			None	-	-	-	-

Note: [f]: Fresh sediment slurries.

[s]: Stored sediment slurries at 6 °C for 10 months.

*: period for dechlorinating 90% of the original spiked HCB.

[a]: % of the spiked HCB remaining at the 14^{th} week.

In the researches of using SS from Taiwan (E2) blended with the SS of all tested sites from Thailand at ratio of 45:5, almost all sets showed a lag phase within 6 weeks except 4.6.13 and 4.6.16 sets of which lag phase was a little longer within 9 and 11 weeks respectively. By increasing the ratio to 25:25, it resulted much lower dechlorination efficiency. As the results of Table 4.7, it was found that all sets take longer time for lag phase, and almost all sets remained more than 50% of original spiked HCB after 14 weeks of incubation. Once again, by comparing results from Table 4.6, 4.7 and the non-blended control sets (Table 4.3), the results of blended sets were worse than non-blended ones. And these finding supported our previous suggestion that a blended consortia needed to adapt and to evolve, otherwise, their dechlorination activities might be not cohesive but interact antagonistically.

The sample from Taiwan (E3) blended with the SS of all tested sites from Thailand at ratio of 45:5, almost all sets showed a lag phase within 2 to 6 weeks except 4.6.17 and 4.6.22 which lag phase was 14 weeks. Moreover, the sets 4.6.18 and 4.6.20 did not initiate HCB dechlorination. Most of all sets were proceedings a 90% HCB dechlorination in the periods of 4 to 14 weeks, but both 4.6.18 and 4.6.20 sets could not accomplish the 90% dechlorination within 14 weeks. By increasing the ratio to 25:25, it resulted lower dechlorination efficiency in sets 4.7.17 and 4.7.18, and more than 40% of original spiked HCB was remained after 14 weeks of incubation. By comparing the results of Table 4.6 with non-blended control sets (Table 4.3), it showed a different tendency than sets 4.6.1~4.6.16 (E1, E2). Some blended sets (4.6.19, 4.6.23) gave a better dechlorination that made 90% HCB dechlorination fast than non-blended sets. The similar finding was also happened in comparing table 4.7 with non-blended control sets (Table 4.3), one blended set (4.7.22) made 90% HCB dechlorination fast than non blended one.

The sample E4 (from Taiwan) mixed with the sample from Thailand for all sites at ratio of 45:5 could mostly dechlorinate HCB with the lag phases within 2 to 6 weeks except condition 4.6.30 of which the lag phase occurred within 14 weeks and conditions 4.6.26 and 4.6.32 in which no dechlorination occurred. The 90% HCB dechlorination periods of most of mixtures were within 4 to 9 weeks except condition 4.6.30 was more than 14 weeks with 32% of the original spike remained and conditions 4.6.26 and 4.6.32 did not dechlorinate. However, increasing the mixing ratio to 25:25 revealed that conditions

4.7.29 to 4.7.31 provided 90% HCB dechlorination within 11 weeks and conditions 4.7.25 to 4.7.27 had the 90% dechlorination periods longer than 14 weeks and the remain HCB were more than 65% after 14 weeks of incubation. Conditions 4.7.28 and 4.7.32 did not chlorinate the HCB. When compare the results from Tables 4.6 and 4.7 with the control, almost all of the mixture samples had the 90% HCB dechlorination periods longer than the control sample, just only one mixture sample that provided better result than the control which is the condition 4.6.25.

4.3 Phase 2: Study on Biostimulation of Historical Less-Potential Dechlorinating Consortia by Using Sterilized Active Sediment Slurry as Cultural Medium

The sediment slurries from Taiwan sites were conducted to this study for study on biostimulation of historical less-potential dechlorinated consortia (SS from Taiwan) by blended with sterilized active sediment slurries (SASS) from the Hua Lum Poo and Bang Pla Kod Canals as cultural media. In previous research, Sama-ae, (2007) who used the mixed sediments from Hua Lum Poo Canal under sealed serum bottle condition, it was found that HCB dechlorination occurred in week 4 and completed after 16 weeks. Chen et al. (2010) studied the performance of HCB dechlorination by using the sediment slurries from Hua Lum Poo Canal at different temperature. High HCB dechlorination efficiency was detected with the lag phase of 7 days and completely dechlorination time of 63 days at 35-40°C. In the present study, HCB dechlorination of sterile control sets were shown in Table 4.8. For sterile control set, the sterilized active sediment slurries (SASS) from Hua Lum Poo and Bang Pla Kod Canal did not dechlorinate in all sets indicating complete inactivation of the HCB dechlorinators by sterilization. It was completely opposite to the unsterilized sediment samples those could start the dechlorination within 2 week and show 90% HCB dechlorination within 6 weeks (amended with yeast extract(YE)) and 8 weeks (non yeast extract (YE)) sets.

In this experiment, there were two kinds of Erh-Jen River sediment slurries, one was fresh and just collected, the other hand been stored for 2 months. Both of these two kinds of SS were with the lag phase of 2 to 4 week and the 90% HCB dechlorinated period of 8-10 weeks. From the dechlorination tests of non-sterilized SS, SS from Taiwan had be slower dechlorination rate than SS from Thailand. When comparing the results of Table 4.3 and 4.8, the dechlorination of Thailand SS was better in Table 4.8. The reason should be the tested SS in Table 4.8 were not stored in a cold environment such as SS in Table 4.3. And Thailand SS in Table 4.8 could keep their potential in HCB dechlorination that not significantly worst than Taiwan SS.

As shown in Table 4.9, the blending of Hua Lum Poo canal (H3) SASS with Erh-Jen River SS, at 48:2 ratio showed no dechlorination in most sets. Except sets 4.9.13 and 4.9.14, for 4.9.13 (with YE), the lag phase was 4 week, and 4.9.14 was 6 weeks (without YE). Both sets could reach a 90% HCB dechlorination within week 10-11. The reason for no HCB dechlorination happened in sets 4.9.1 to 4.9.14 may be because H3 site is the site very closed to the discharging pipe, and was long-term received waste water came from industrial area, which possibly made the SASS from this site toxic to microbes. Moreover, the sterilize process killed all indigenous microbes that had been adapted to this toxic environment. Therefore, after blending with H3 SASS, anaerobic consortia of Taiwan SS could be shocked by the toxicity and could not recover their ability to dechlorinate HCB. There were one SS consortia exceptional, in the sets 4.9.13 and 4.9.14, the fresh SS of E3 could perform the dechlorination. However, those consortia still suffered from the inhibition from H3 SASS, because the 2 sets spended 4-6 weeks more than Table 4.8 sets in initiating the dechlorination. In Table 4.10, at a blended ratio of 40:10, almost all sets could occur and complete HCB dechlorination except set 4.10.2. The result suggested that with 10 ml of non-sterile SS, the microbial populations were abundant enough to pass the toxic inhibition of H3 SASS. The lag phase was 4 to 6 weeks and the 90% HCB dechlorination period was 8 to 16 weeks. All the results were not significant to Table 4.8, it suggest that 20% (10 ml) of vital SS was enough to initiate HCB dechlorination, and the inhibition and assistance effects of 80% (40 ml) H3 SASS could reach to a balance.

On the other hand as show in Table 4.9, the mixing of the sediment slurries from Taiwan with those from Hua Lum Poo Canal Site H6 at 48:2 ratio could improve the dechlorination. Lag phases were in between 2 and 6 weeks and the 90% HCB dechlorination period were within 6 to 14 weeks and 6 to 18 weeks for with and without yeast extract, respectively. Conditions 4.9.15 to 4.9.28 could increase the dechlorination as compared to the mixing of the sediment slurries from Taiwan with those from H3 because Site H6 is far from Site H3 so that the biodegradation could occurred significantly during the flow and the toxic compounds were digested. Comparing to the control sample showed that these conditions did not enhance the dechlorination rate except the conditions 4.9.19 and 4.9.20. At the ratio of 40:10, the lag phases were in between 2 and 6 weeks and the 90% HCB dechlorination periods were within 6 to 10 weeks and 6 to 14 weeks for with and without yeast extract, respectively. When compared with the control sample, it found that these conditions did not promote the dechlorination rate except conditions 4.10.19 to 4.10.22. The reason for this observation was explained previously.

Sediment slurries from the Erh-Jen River mixed with the sediment slurries from Bang Pla Kod Canal (B1) at 48:2 ratio could increase the HCB dechlorination ability significantly and considerably provide better results than those with the sediment slurries from the Hua Lum Poo Canal. The lag phases were between 2 and 4 weeks and the 90% HCB dechlorination periods were observed between 4 and 10 weeks for both of with and without yeast extract. These results show a sign of improvement when compared with the control sample as can be seen in the conditions 4.9.33 to 4.9.42. At 40:10 ratio, the lag phases were 2 to 4 weeks and the 90% HCB dechlorination periods were within 6 to 10 weeks for both of with and without yeast extract. When compared with the control sample, it was found that these conditions did not enhance the dechlorination rate except the conditions 4.10.31 to 4.10.42. It is important to note that the Bang Pla Kod Canal has never been found to contaminate with HCB and could be assumed to contain less toxic substances; therefore, no negative impact on the HCB dechlorination of the Erh-Jen River sediment slurries.

In conclusion, this experimental part was conducted to investigate the effects of mixing of non-active HCB-dechlorinating consortia (Erh-Jen River) with sterilized active HCB-dechlorinating consortia (Hua Lum Poo and Bang Pla Kod Canals) for nutrient supplement purpose. The results showed that supplement of nutrient from active sites could efficiently increase the HCB dechlorination of the non-active site. However, increase of consortia ratio did not provide any significant effect on HCB dechlorination activity.

Table 4.8 HCB dechlorination of single sediment slurry by sterilized and non sterilized sediment slurry from different sites

Conditions	**Sample sites**	**Supplement**	**Lag phase (weeks)**	**90% dechlorination period* (weeks)**	**Observable intermediate**	
					QCB	**1,3,5-TCB**
4.8.1	H3^{f}	YE	-	-	-	-
4.8.2	sterilized	None	-	-	-	-
4.8.3	H6^{f}	YE	-	-	-	-
4.8.4	sterilized	None	-	-	-	-
4.8.5	B1^{f}	YE	-	-	-	-
4.8.6	sterilized	None	-	-	-	-
4.8.7	H3^{f}	YE	2	6	Yes	Yes
4.8.8	non-sterilized	None	2	8	Yes	Yes
4.8.9	H6^{f}	YE	2	6	Yes	Yes
4.8.10	non-sterilized	None	2	8	Yes	Yes
4.8.11	B1^{f}	YE	2	6	Yes	Yes
4.8.12	non-sterilized	None	2	8	Yes	Yes
4.8.13	E1^{s}	YE	2	8	Yes	Yes
4.8.14	non-sterilized	None	2	10	Yes	Yes
4.8.15	E2^{s}	YE	4	8	Yes	Yes
4.8.16	non-sterilized	None	4	10	Yes	Yes
4.8.17	E3^{s}	YE	2	8	Yes	Yes
4.8.18	non-sterilized	None	4	10	Yes	Yes
4.8.19	E4^{s}	YE	2	8	Yes	Yes
4.8.20	non-sterilized	None	4	10	Yes	Yes
4.8.21	E1^{f}	YE	2	8	Yes	Yes
4.8.22	non-sterilized	None	2	10	Yes	Yes
4.8.23	E2^{f}	YE	2	8	Yes	Yes
4.8.24	non-sterilized	None	4	10	Yes	Yes
4.8.25	E3^{f}	YE	2	8	Yes	Yes
4.8.26	non-sterilized	None	4	10	Yes	Yes

Note: ^{f}: fresh sediment slurries; ^{s}: stored sediment slurries at 6 °C for 2 months; *: period for dechlorinating 90% of the original spiked.

Table 4.9 Biostimulation of non-sterilized Taiwan sediment slurry to sterilized Thailand sediment slurry at 48:2 ratio

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.9.1	H3^{f} (48 mL) sterilized	E1^{s} (2 mL)	YE	-	-	-	-
4.9.2		non-sterilized	None	-	-	-	-
4.9.3		E2^{s} (2 mL)	YE	-	-	-	-
4.9.4		non-sterilized	None	-	-	-	-
4.9.5		E3^{s} (2 mL)	YE	-	-	-	-
4.9.6		non-sterilized	None	-	-	-	-
4.9.7		E4^{s} (2 mL)	YE	-	-	-	-
4.9.8		non-sterilized	None	-	-	-	-
4.9.9		E1^{f} (2 mL)	YE	-	-	-	-
4.9.10		non-sterilized	None	-	-	-	-
4.9.11		E2^{f} (2 mL)	YE	-	-	-	-
4.9.12		non-sterilized	None	-	-	-	-
4.9.13		E3^{f} (2 mL)	YE	4	10	Yes	Yes
4.9.14		non-sterilized	None	6	11	-	Yes
4.9.15	H6^{f} (48 mL) sterilized	E1^{s} (2 mL)	YE	6	14	Yes	Yes
4.9.16		non-sterilized	None	4	18	Yes	Yes
4.9.17		E2^{s} (2 mL)	YE	2	14	Yes	Yes
4.9.18		non-sterilized	None	4	14	Yes	Yes
4.9.19		E3^{s} (2 mL)	YE	2	6	Yes	Yes
4.9.20		non-sterilized	None	2	6	Yes	Yes
4.9.21		E4^{s} (2 mL)	YE	2	8	Yes	Yes
4.9.22		non-sterilized	None	2	10	Yes	Yes
4.9.23		E1^{f} (2 mL)	YE	2	10	Yes	Yes
4.9.24		non-sterilized	None	6	12	Yes	Yes
4.9.25		E2^{f} (2 mL)	YE	4	10	Yes	Yes
4.9.26		non-sterilized	None	2	12	Yes	Yes
4.9.27		E3^{f} (2 mL)	YE	2	8	Yes	Yes
4.9.28		non-sterilized	None	2	10	Yes	Yes

Table 4.9 Biostimulation of non-sterilized Taiwan sediment slurry to sterilized Thailand sediment slurry at 48:2 ratio (continued)

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.9.29	B1^{f} (48 mL) sterilized	E1^{s} (2 mL)	YE	4	10	Yes	Yes
4.9.30		non-sterilized	None	2	10	Yes	Yes
4.9.31		E2^{s} (2 mL)	YE	2	8	Yes	Yes
4.9.32		non-sterilized	None	2	8	Yes	Yes
4.9.33		E3^{s} (2 mL)	YE	2	4	Yes	Yes
4.9.34		non-sterilized	None	2	4	Yes	Yes
4.9.35		E4^{s} (2 mL)	YE	2	6	Yes	Yes
4.9.36		non-sterilized	None	2	4	Yes	Yes
4.9.37		E1^{f} (2 mL)	YE	4	8	Yes	Yes
4.9.38		non-sterilized	None	2	6	Yes	Yes
4.9.39		E2^{f} (2 mL)	YE	2	6	Yes	Yes
4.9.40		non-sterilized	None	2	6	Yes	Yes
4.9.41		E3^{f} (2 mL)	YE	2	6	Yes	Yes
4.9.42		non-sterilized	None	2	4	Yes	Yes

Note: ^{f}: fresh sediment slurries

^{s}: stored sediment slurries at 6 °C for 2 months

*: period for dechlorinating 90% of the original spiked.

Table 4.10 Biostimulation of non-sterilized Taiwan sediment slurry to sterilized Thailand sediment slurry at 40:10 ratio

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.10.1	$H3^f$ (40 mL) sterilized	$E1^s$ (10 mL)	YE	4	16	Yes	Yes
4.10.2		non-sterilized	None	-	-	-	-
4.10.3		$E2^s$ (10 mL)	YE	4	12	Yes	Yes
4.10.4		non-sterilized	None	6	14	Yes	Yes
4.10.5		$E3^s$ (10 mL)	YE	4	8	Yes	Yes
4.10.6		non-sterilized	None	6	10	Yes	Yes
4.10.7		$E4^s$ (10 mL)	YE	4	10	Yes	Yes
4.10.8		non-sterilized	None	6	10	Yes	Yes
4.10.9		$E1^f$ (10 mL)	YE	4	16	Yes	Yes
4.10.10		non-sterilized	None	6	16	Yes	Yes
4.10.11		$E2^f$ (10 mL)	YE	4	14	Yes	Yes
4.10.12		non-sterilized	None	6	14	Yes	Yes
4.10.13		$E3^f$ (10 mL)	YE	4	10	Yes	Yes
4.10.14		non-sterilized	None	4	8	Yes	Yes
4.10.15	$H6^f$ (40 mL) sterilized	$E1^s$ (10 mL)	YE	6	10	Yes	Yes
4.10.16		non-sterilized	None	6	14	Yes	Yes
4.10.17		$E2^s$ (10 mL)	YE	4	10	Yes	Yes
4.10.18		non-sterilized	None	4	10	Yes	Yes
4.10.19		$E3^s$ (10 mL)	YE	2	6	Yes	Yes
4.10.20		non-sterilized	None	4	6	Yes	Yes
4.10.21		$E4^s$ (10 mL)	YE	4	6	Yes	Yes
4.10.22		non-sterilized	None	4	6	Yes	Yes
4.10.23		$E1^f$ (10 mL)	YE	4	10	Yes	Yes
4.10.24		non-sterilized	None	4	8	Yes	Yes
4.10.25		$E2^f$ (10 mL)	YE	4	8	Yes	Yes
4.10.26		non-sterilized	None	2	10	Yes	Yes
4.10.27		$E3^f$ (10 mL)	YE	4	8	Yes	Yes
4.10.28		non-sterilized	None	4	8	Yes	Yes

Table 4.10 Biostimulation of non-sterilized Taiwan sediment slurry to sterilized Thailand sediment slurry at 40:10 ratio (continued)

Conditions	**Sample sites**		**Supplement**	**Lag phase (weeks)**	**90% HCB dechlorinated period* (weeks)**	**Observable intermediates**	
						QCB	**1,3,5-TCB**
4.10.29	B1[f] (40 mL) sterilized	E1[s] (10 mL)	YE	4	10	Yes	Yes
4.10.30		non-sterilized	None	4	10	Yes	Yes
4.10.31		E2[s] (10 mL)	YE	2	6	Yes	Yes
4.10.32		non-sterilized	None	2	6	Yes	Yes
4.10.33		E3[s] (10 mL)	YE	2	6	Yes	Yes
4.10.34		non-sterilized	None	2	6	Yes	Yes
4.10.35		E4[s] (10 mL)	YE	2	6	Yes	Yes
4.10.36		non-sterilized	None	4	6	Yes	Yes
4.10.37		E1[f] (10 mL)	YE	2	8	Yes	Yes
4.10.38		non-sterilized	None	2	8	Yes	Yes
4.10.39		E2[f] (10 mL)	YE	2	8	Yes	Yes
4.10.40		non-sterilized	None	4	8	Yes	Yes
4.10.41		E3[f] (10 mL)	YE	2	6	Yes	Yes
4.10.42		non-sterilized	None	2	6	Yes	Yes

Note: [f]: fresh sediment slurries

[s]: stored sediment slurries at 6 °C for 2 months

*: period for dechlorinating 90% of the original spiked.

4.4 Phase 3: Comprehensive Survey of HCB Dechlorination by a Fusion of Various Active Sediment Slurries

This research was designed as two individual experiments. Part one: A bioaugmentation experiment by using fresh sediment slurries from Thailand, including site H3, H6, and B1, then separately blended (or not blended) with fresh sediment slurries from Taiwan, site E2, was conducted as a comprehensive survey in HCB dechlorination The result was shown in Table 4.11. Part two: A fusion experiment by using various incubated sediment slurries which withdrawn from the inactive HCB dechlorination sets of Section 4.2, then blended with fresh sediment from H3 site, Thailand. The result was shown in Table 4.12.

In part one experiment, the fresh SS from Thailand, set 4.11.1 - 4.11.3, showed a lag phase of 2 weeks and 90% HCB dechlorination period were 8 weeks. The fresh SS from Taiwan, set 4.11.4 showed a lag phase of 6 weeks, and 90% HCB dechlorination period was 10 weeks. It revealed that fresh Thailand SS was more potential in HCB dechlorination than Taiwan SS. In the other hand, the blended experiments of Thailand SS and Taiwan SS at 25:25 ratio showed the lag phases within 4 to 6 weeks, and 90% HCB dechlorination periods within 6 to 10 weeks. By comparing the blended and non-blended experiments, the period of lag phase in blended sets was in between non-blended Thailand SS and non-blended Taiwan SS. However, the 90% HCB dechlorination periods of all sets were not significantly different. It indicated that at 25:25 ratio, the blended of fresh Thailand SS and fresh Taiwan SS did not show neither synergetic nor antagonistic effects. Comparing the results in Table 4.7 which have same condition with the conditions 4.11.5 to 4.11.7, it was found that the results in this part were better than the results in Table 4.7. This is because the sediment in this part was not stored in cool area like the sediment used in Table 4.7 so microbial activity was still high.

In part two experiments, the incubated SS from section 4.2 were blended with fresh H3 SS. The result in Table 4.12 showed their lag phases were ranged from 2 to 6 weeks, a little longer than the fresh H3 SS of 4.11.1. It suggested that 1/3 of 6-months incubated SS (form section 4.2) will postpone the development of HCB dechlorination potential of H3 SS. However, the dechlorination ability of H3 was remained and the 90% HCB dechlorination period was 8 weeks, the same as the fresh H3 SS. Therefore, this fusion experiment showed a possibility that the SS with less dechlorination potential or non-dechlorination potential could be enhanced by the fresh and active SS And the application of bioaugmentation in environmental remediation of HCB contaminated sites may be a important way to deplete this hazardous compound from the environment.

Table 4.11 HCB dechlorination by a fusion of various active sediment slurries.

Conditions	Sample sites	Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
					QCB	1,3,5-TCB
4.11.1	H3[f] (50 mL)	None	2	8	Yes	Yes
4.11.2	H6[f] (50 mL)	None	2	8	Yes	Yes
4.11.3	B1[f] (50 mL)	None	2	8	Yes	Yes
4.11.4	E2[f] (50 mL)	None	6	10	Yes	Yes
4.11.5	H3[f] (25 mL) E2[f] (25 mL)	None	4	8	Yes	Yes
4.11.6	H6[f] (25 mL) E2[f] (25 mL)	None	4	6	Yes	Yes
4.11.7	B1[f] (25 mL) E2[f] (25 mL)	None	6	10	Yes	Yes

Note: [f]: Fresh sediment slurries.

*: period for dechlorinating of 90% of the original spiked HCB.

Table 4.12 Fusion of blended sediment microorganism in active sediment slurry at 20:10 ratio.

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.12.1	4.7.1[s] (10 mL)	H3[f] (20 mL)	None	6	8	Yes	Yes
4.12.2	4.7.2[s] (10 mL)	H3[f] (20 mL)	None	6	8	Yes	Yes
4.12.3	4.7.4[s] (10 mL)	H3[f] (20 mL)	None	4	8	Yes	Yes
4.12.4	4.7.18[s] (10 mL)	H3[f] (20 mL)	None	6	8	Yes	Yes
4.12.5	4.7.20[s] (10 mL)	H3[f] (20 mL)	None	4	8	Yes	Yes
4.12.6	4.7.26[s] (10 mL)	H3[f] (20 mL)	None	4	8	Yes	Yes
4.12.7	4.8.1[s] (10 mL)	H3[f] (20 mL)	None	6	8	Yes	Yes
4.12.8	4.8.3[s] (10 mL)	H3[f] (20 mL)	None	6	8	Yes	Yes
4.12.9	4.8.4[s] (10 mL)	H3[f] (20 mL)	None	6	10	Yes	Yes
4.12.10	4.8.9[s] (10 mL)	H3[f] (20 mL)	None	4	8	Yes	Yes
4.12.11	4.8.10[s] (10 mL)	H3[f] (20 mL)	None	4	10	Yes	Yes
4.12.12	4.8.11[s] (10 mL)	H3[f] (20 mL)	None	4	8	Yes	Yes

Table 4.12 Fusion of blended sediment microorganism in active sediment slurry at 20:10 ratio. (continued)

Conditions	Sample sites		Supplement	Lag phase (weeks)	90% HCB dechlorinated period* (weeks)	Observable intermediates	
						QCB	1,3,5-TCB
4.12.13	4.8.12^{s} (10 mL)	H3^{f} (20 mL)	None	6	8	Yes	Yes
4.12.14	4.8.17^{s} (10 mL)	H3^{f} (20 mL)	None	6	8	Yes	Yes
4.12.15	4.8.18^{s} (10 mL)	H3^{f} (20 mL)	None	6	10	Yes	Yes
4.12.16	4.8.20^{s} (10 mL)	H3^{f} (20 mL)	None	4	8	Yes	Yes
4.12.17	4.8.25^{s} (10 mL)	H3^{f} (20 mL)	None	4	8	Yes	Yes
4.12.18	4.8.26^{s} (10 mL)	H3^{f} (20 mL)	None	4	8	Yes	Yes
4.12.19	4.8.27^{s} (10 mL)	H3^{f} (20 mL)	None	6	8	Yes	Yes
4.12.20	4.8.28^{s} (10 mL)	H3^{f} (20 mL)	None	2	6	Yes	Yes

Note: ^{f}: Fresh sediment slurries

^{s}: Sediment slurries from Section 4.2

*: period for 90% dechlorinating of the original spiked HCB.