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Dechlorination of 234-Trichlorobiphenyl and 2345-Tetrachlorobiphenyl by using halogenated primers

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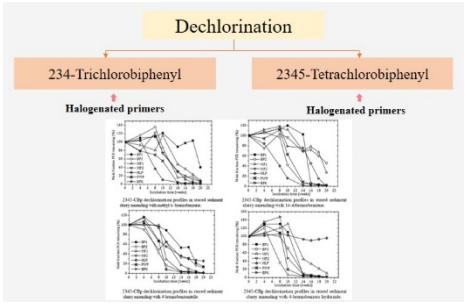
Abstract

The research aimed to investigate the sustainability of the dechlorination ability of the microbes after long-term cold storage. Polychlorinated biphenyls (PCBs) dechlorinating bacteria were activated and characterised by priming congeners. Sediment slurries (SS) were collected from several streams around the Samut Prakan Province in Thailand. While dechlorination of 234-CBp was persistent, 2345-CBp dechlorination was relatively more active. Halogenated priming congeners, including methyl 4-bromobenzene, 14-dibromobenzene, 4-bromobenzonitrile, and 4-bromobezoic hydrazide, could significantly stimulate the dechlorination of 234-CBp but not 2345-CBp. The identified PCB dechlorinating groups were Firmicutes and Proteobacteria. Halogenated priming congeners could activate dechlorination in Thailand, where PCBs dechlorinating microbes were abundant.



Keywords: Sediment Slurries (SS); Polychlorinated Biphenyls (PCBs); Dechlorination; Halogenated Priming

1. Introduction

Thailand banned Polychlorinated Biphenyls (PCBs) since 1975 and has never imported or exported liquid PCBs until now. The significant sources of PCBs are transformers and capacitors imported and managed by the Electricity Generating Authority of Thailand (EGAT), the Metropolitan Electricity Authority (MEA), and the Provincial Electricity Authority (PEA) [1]. PCBs are persistent in the environment classified as one of the 12 persistent organic pollutants (POPs). They are continuously released into the environment and primarily enter through water, soil, air, and aquatic lives for several decades. PCBs are too complicated to be oxidised and reduced. Two significant properties of PCBs, including low water solubility and highly lipophilicity, make PCBs easy to adsorb onto sediments. PCBs' lipophilic and persistent nature also contributes to high bioaccumulation potential and biomagnifications of PCBs in higher trophic levels of the food chain [2, 3]. PCBs could be dechlorinated in aerobic and anaerobic conditions, while highly chlorinated congeners could be dechlorinated under anaerobic conditions, and less chlorinated congeners could be oxidatively degraded. Dechlorination rates under an anaerobic environment are slower than in aerobic conditions [4]. In Thailand, Hexachlorobenzene (HCB) could be effectively dechlorinated in the sediments of several natural streams without any culture enrichment or nutrient supplement [5, 6]. In this case, these stream sediments could also be helpful for pure PCBs congeners degradation [7]. From their molecular structures, 234-Trichlorobiphenyl (234-CBps) and 2345-Tetrachlorobiphenyl (2345-CBps) seem to be susceptible to dechlorination since both of them have chlorine atoms in only one phenyl ring [7]. The dechlorination rates of PCBs could be enhanced by adding two-electron properties: 1) electron donors, e.g., volatile fatty acids and organic solvents [8, 9] and 2) electron acceptors, e.g., haloprimers, sulfate, and nitrate [10, 11]. The use of brominated biphenyls to initiate the reductive dechlorination of Aroclor 1260 in Housatonic River sediments has been significantly evidenced [12]. Besides, halogenated benzoates and other halogenated aromatic compounds could also extend the efficiency of PCBs dechlorination [12]. These compounds were used as "priming congeners" in several studies to support the dechlorination of microcosms. These compounds could initiate biphenyls catabolic enzyme as cometabolism of PCBs degradation. Several PCB dechlorinators have been identified under different conditions, environmental factors, stressors, and enrichment cultures.

Based on several studies, differences in PCBs dechlorinators could be detected when they were conducted under different conditions and microbial sources. However, given that environmental factors can vary the experimental results, the sustainability of the dechlorination ability of the microbes after being stored for a long time will be investigated. Besides, using priming congeners to activate and characterise the enrichment of PCBs will be studied in this research.

2. Materials and Methods

This research is based on the following methodological approaches.

Characterisation of sampling sites

Sediments and stream waters were collected from several natural water resources around industrial areas and their vicinity in the Samut Prakan Province of Thailand. Discharges from five PCB-contaminated areas were investigated; *1) Hum Lum Poo Canal (HCB)*, where treated and untreated discharges are obtained from the Bangpoo Industrial Estate and nearby factories, 2) *Bangplee Industrial Estate nearby canal (BP1, BP2)*, where effluent discharges are received from the central wastewater treatment plant of the Bangplee Industrial Estate, comprising several electronic factories that utilise dielectric fluids, 3) *Material Recovery Facilities Factory nearby canal (MF1, MF2)*, where discharges are obtained from small material recovery facilities, which possibly receive PCBs leakage during waste separation and cleaning, 4) *Southern Bangkok Power Plant nearby canal (PWP)*, where a certain amount of used transformers and capacitors were stored and 5) *Bangpakod Canal (BPK)*, which has high water quality and is a habitat for many aquatic lives; hence, it served as a control for a less-PCB-contaminated site. Sediments used to determine the dechlorination activity in this study were based on stored processes. The fresh stream water were instantly experimented with after they had been collected. For approximately one year, the stored sediments were contained in a plastic bag under 4 °C.

Preparation of chemicals

All chemicals used in this research were reagent grade ordered from different locations. For example, 234-CBp and 2345-CBp were purchased from AccuStandard, Inc. (USA). Methyl 4-Bromobenzene (4-MBZ), 14-Dibromobezene (14-DBZ), 4-Bromobenzonitrile (4-BN), 4-Bromobezoic Hydrazide (4-BH) were purchased from Sigma Aldrich, Inc. (USA). Stock and standard solutions were prepared in acetone. The solutions were transferred into several 1.50 mL vials, sealed with butyl rubber stoppers and alumina caps, and refrigerated until used. All solvents used were GC grade.

Sediment slurry preparation and incubation

Sediment slurry (SS) was prepared with stored sediment and mixed with fresh stream water at a ratio of 1:1, as suggested by Chen *et al.* [6]. After that, 0.10 mL of each target PCB congener stock solution at 1,000 mg L^{-1} was spiked into the serum bottle to make an initial concentration of 2 mg L^{-1}. Next, halogenated priming congeners were separately amended from the stock solution prepared at 50,000 mg L^{-1} to provide the final level of 75 mg L^{-1}. The final acetone portion in the prepared sediment slurry was lower than 0.50% (v v^{-1}) to prevent any toxicity or potential impact on dechlorination by the amended solvent. The serum bottles were incubated in the dark at room temperature. Non-primer and sterile control sets were also prepared and monitored for comparison.

PCBs extraction and analytical procedures and gas chromatography analysis

All samples were extracted by solvent and ultrasonic extraction (EPA 3550). Application of GC analysis followed the US EPA 8082A method for GC/μECD (quantification) and the EPA 680 method for GC/MS (qualification) and has been described and has been described by Sudjarid *et al.* [7].

Microbial characteristics incubation

Dechlorinating microcosms in sediment slurries were enriched and enumerated using a serial-transfer technique with a suitable medium with 0.50% of yeast extract (20%, v v^{-1}). At the end of the dechlorination sessions of 234- and 2345-CBps by amending halogenated primers, those sediment slurries with superior degradation capability, including the Bangplee Industrial Estate site number 2 (BP2), the South-Bangkok Power Plant (PWP) and Bangpakod Canal (BPK), with separately amended Methyl 4-bromobenzene and 4-Bromobenzonitrile were chosen. The PCR-DGGE and DNA Sequencing were described by Muyzer *et al.* [13] and Altschul *et al.* [14].

3. Results and Discussion

Dechlorination ability by indigenous microbes

The sediment slurries' dechlorination ability from all sites has been tested and separately spiked with 234- and 2345-CBps. The microbial capabilities of both fresh and stored sediment slurries were investigated if their dechlorination abilities could be sustained after long-time storage (one year) without any substrate or nutrient supplement (Table 1). The COD:N:P ratios were 100:4:1 and 100:5:3 for fresh and stored slurries, respectively. The results showed insignificant differences between fresh and stored sediment slurries, implying that existing nutrients were sufficient for microbial activities [15]. Based on the investigation of 234-CBp dechlorination using fresh SS along Hua Lam Poo Canal, the initiative dechlorination occurred after eight weeks. When intermediate products began, the dechlorination could be completed within weeks 2 to 14 as described by Sudjarid *et al.* [7].

Unfortunately, 234-CBp dechlorination cannot be initiated under stored SS by natural microbes, but indigenous microbes from the Bangplakod site are still active (Fig. 1). The consequence of simulated tempered climate in sediment could retard the degradation of

Table 1 Sediment slurry characterizations.

Sampling Sites		COD	Phosphorus	TKN	SS	VSS
Bangplee Industrial Estate	Site 1 (BP1)	9,350	50	520	152,630	10,800
		(11,034)	(305)	(933)	(740,760)	(17,400)
	Site 2 (BP2)	12,750	100	780	125,750	11,650
		(19,862)	(540)	(1,053)	(751,210)	(18,765)
Small Material Recovery Facilities	Site 1 (MF1)	15,130	90	1,020	100,770	8,650
		(19,310)	(241)	(547)	(800,680)	(14,760)
	Site 2 (MF2)	36,160	90	400	144,320	8,560
		(24,828)	(179)	(1,107)	(858,910)	(12,985)
Hua Lum Poo Canal (HLP)		23,040	320	930	94,950	14,700
		(25,379)	(270)	(827)	(894,425)	(14,735)
South-Bangkok Power Plant (PWP)		13,480	200	1,580	205,990	12,100
		(13,241)	(229)	(747)	(858,910)	(13,620)
Bangplakod Canal (BPK)		12,480	40	640	188,050	19,850
		(11,030)	(72)	(427)	(741,450)	(17,350)

Note: all units are mg L^{-1}; in parenthesis were storage conditions.

234- CBp, even though fresh canal water could not be revived. These results represent that the PCBs dechlorinating microbes were prevalent, whether it was a high or low polluted site. Moreover, some notice that the indigenous microbes from the HLP site lost their degradation abilities after a long-term period of non-receiving xenobiotic pollutants. This outcome indicates that the dechlorination ability in the fresh SS was more effective than the stored SS despite insignificant differences in the basic parameters. The results also imply that the dechlorination activity of PCB-dechlorinating might decrease if they did not receive external xenobiotic compounds, carbon, or nutrient sources continuously.

In terms of 2345-CBps, all sites other than HLP sediment slurry sets are still feasible to be dechlorinated (Fig. 2). The dechlorination could occur after the 8^{th} – 20^{th} week. The results showed that 2345-CBp could be degraded more efficiently than 234-CBp. This reaction might be due to the reaction heat (ΔH_f) and/or Δln RRT of 2345-CBp to 235- and 245-CBp (–14.74, –14.82/0.55, 0.52) higher than 234-CBp to 24-CBp (–14.11/0.51) [16]. The results were significantly different from those in [16], who found that 234- and 2345-CBps did not dechlorinate from the indigenous microbes of the Ho-Tsin River, Kaohsiung, Taiwan, within 20 weeks of inoculation. The tropical climate and native microbes were hypothesised as two key factors causing different results. In this case, this study showed that the environmental conditions and/or native microorganisms in Thailand could degrade HCB or simple PCB congeners by using fresh sediments and compounds [5 – 7].

Dechlorination ability by amending halogenated primers

The PCBs dechlorination ability could not be sustained after long-term cold-stored sediment from the previous scenario. However, there are some plausible applications to stimulate PCB dechlorinating microbes and, therefore, accelerate the production of dehalogenating enzymes. This process might be preceded by introducing alternative halogenated electron acceptors/co-substrates to the dechlorination test. Four kinds of priming congeners were separately applied, including methyl 4-bromobenzene (4-MBZ), 14-dibromobenzene (14-DBZ), 4-bromobenzonitrile (4-BN), and 4-bromobenzoic hydrazide (4BH). Dechlorination of 234-CBp by amending methyl 4-bromobenzene is illustrated in Fig. 3. It can be noticed that the dechlorination began in the first stage after the 6^{th} week, and dechlorination could be completed within the 12^{th} week in the BP2, HLP and PWP sites and a second stage after the 14^{th} week.

The dechlorination of application of 14-dibromobezene was also found from 8 to 14 weeks and could be completed within the 20^{th} week in BP1 and 2, HLP, and PWP, but other sites remained (Fig. 4). It is interesting to note that the amendment of 4-bromobenzonitrile could effectively enhance dechlorination beginning after the 10^{th} week and completed within the 20^{th} week, excluding BP1 and BPK sediments (Fig. 5). However, the amendment of 4-bromobenzoic hydrazide could be activated at all sites of SS, except the BP1 and HLP sites (Fig. 6). These results revealed that the consortium from the HLP site could be effectively initiated and dechlorinated again after losing their capability, except amendment with 4-bromobenzoic hydrazide. The amendment of 4-bromobenzoic hydrazide, which provides both the bromo-isomer and hydrazide group, did not activate the 234-CBp dechlorination in the HLP and BP1 sediment.

The results showed that: 1) the microbes lost ability after non-receiving pollutants and initiative congeners were required; 2) the amendment of 4-bromobenzonitrile was favourable in all investigation sites; 3) the dechlorination ability could be improved by adding priming congeners regardless of the sample site areas. In contrast, the dechlorination initiation was not observable in the non-priming amended set, excluding the Bangplakod site. This finding suggested that the dechlorination initiation might be required for the abandoned, contaminated site, which has not continuously received aromatic and/or xenobiotic compounds for an extended period. Another remarkable aspect was discovered in a less-contaminating

area, the Bangplakod Canal, but the dechlorination ability was helpful compared to other sites. This is consistent with [12], who suggested that halogenated primers could initiate the dechlorination of PCBs even without structural analogues to PCBs. The initiative of microorganism capability, as such, is an alternative and plausible technique to clean up a long-term PCB-contaminated site with low energy released compounds. In contrast to 234-CBp dechlorination, the degradation of 2345-CBp could be activated by using indigenous microbes, excluding HLP.

The amendment of methyl 4-bromobenzene (Fig. 7), 14-dibromobezene (Fig. 8), 4-bromobenzonitrile (Fig. 9), and 4-bromobenzoic hydrazide (Fig. 10) significantly improved the dechlorination of the MF1 and MF2 and HLP sites but not 4-bromobenzoic hydrazide, which was not favourable to HLP sediments. This finding suggests that the sediment, which has been profoundly and continuously receiving the pollutants, might lose its degradation capability and be recovered by chemical stimulation. The priming compounds could reduce

bromine substituent and could not accelerate the dechlorination of PCBs in the cold storage sediments, except MF1, MF2 and HLP sites. While the priming compounds could enrich microorganisms utilising halobiphenyls as electron acceptors [12], the absence of dechlorination discovered in this article might be driven by inactive microorganisms, i.e., dechlorinators or support groups. Inactive PCBs dechlorinators were initiated instead of adding enriching microorganisms in these sites. The dechlorination intermediate products were varied depending upon the prevalence of co-existing consortia under natural conditions.

The abundance of PCB dechlorinators and their characterisation

This session began after the dechlorination process had completed and enriched cultures by using a serial transfer technique to characterise and identify the dynamic PCBs dechlorinators in the sampling sites. Fifteen helpful inoculating cultures were selected in the criteria of the short lag phase. Dechlorination completion time spiked with 234-CBps or 2345-CBp identified by the PCBs dechlorinating microbes. The sampling sites and priming congeners were selected from

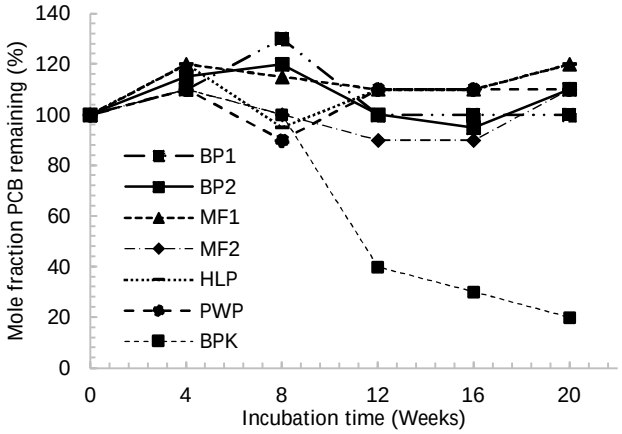


Fig. 1 234-CBp dechlorination profiles by indigenous microbes under stored sediments condition.

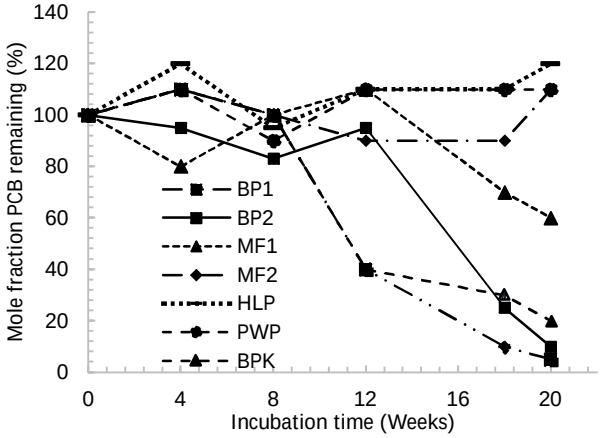


Fig. 2 2345-CBp dechlorination profiles by indigenous microbes under stored sediments condition.

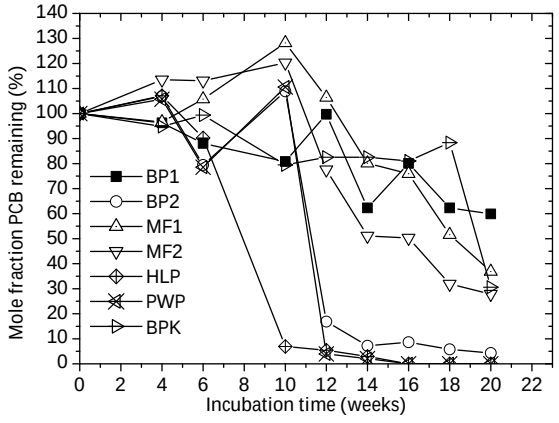


Fig. 3 234-CBp dechlorination profiles in stored sediment slurry amending with methyl 4-bromobenzene.

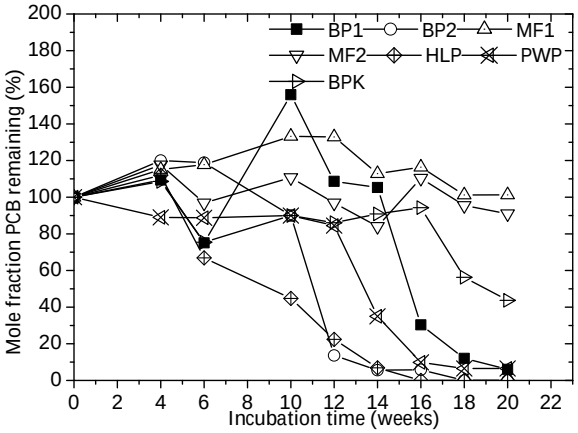


Fig. 4 234-CBp dechlorination profiles in stored sediment slurry amending with 14-dibromobenzene.

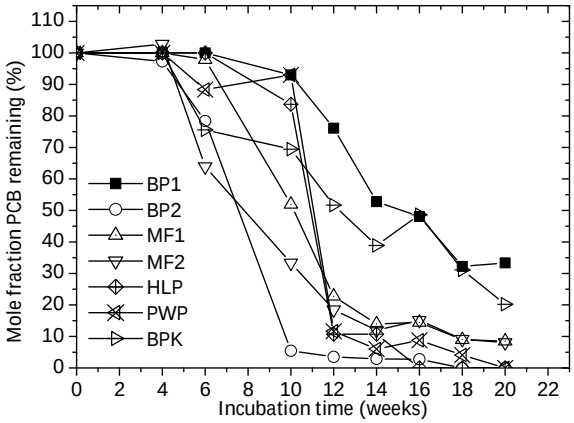


Fig. 5 234-CBp dechlorination profiles in stored sediment slurry amending with 4-bromobenzonitrile.

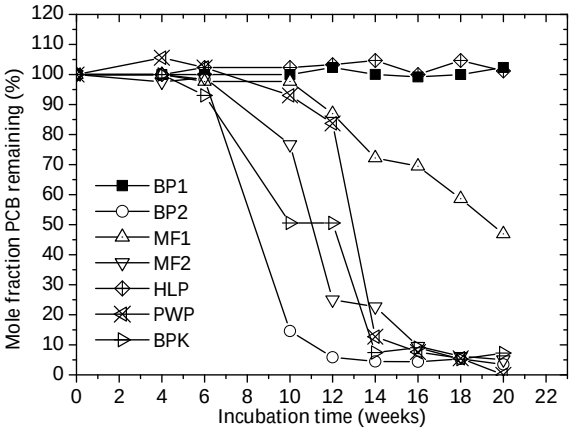


Fig. 6 234-CBp dechlorination profiles in stored sediment slurry amending with 4-bromobenzoic hydrazide.

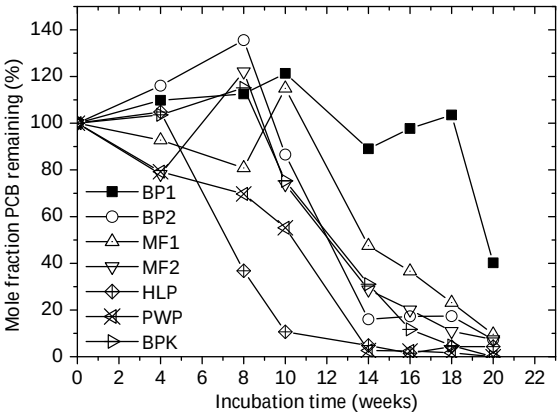


Fig. 7 2345-CBp dechlorination profiles in stored sediment slurry amending with methyl 4- bromobenzene.

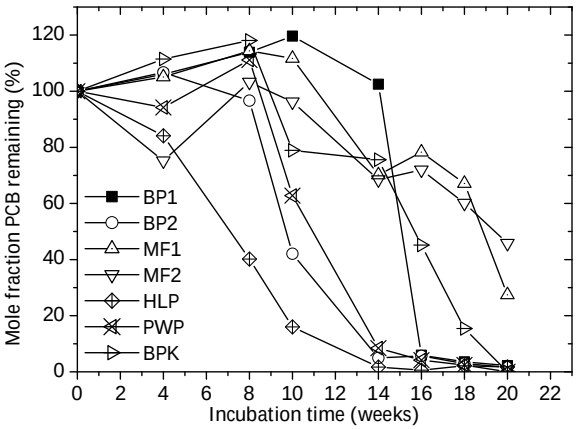


Fig. 8 2345-CBp dechlorination profiles in stored sediment slurry amending with 14-dibromobenzene.

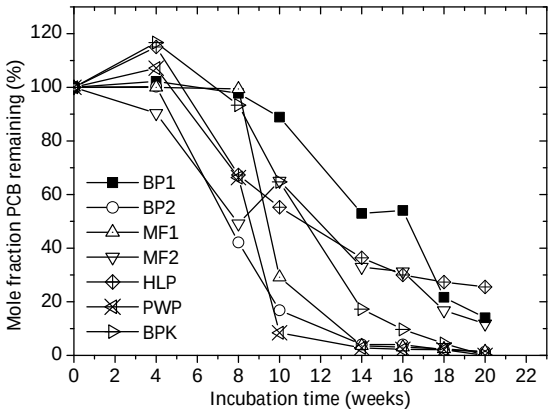


Fig. 9 2345-CBp dechlorination profiles in stored sediment slurry amending with 4-bromobenzonitrile.

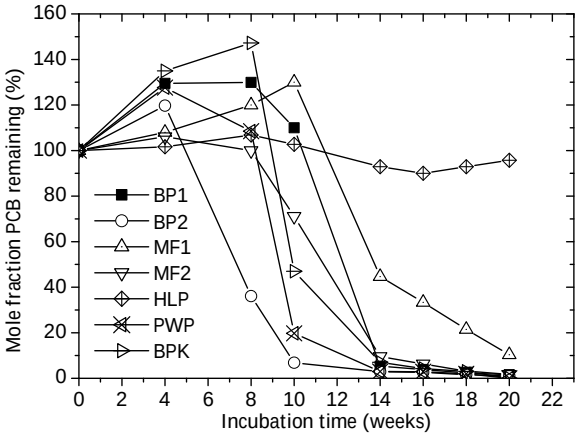


Fig. 10 2345-CBp dechlorination profiles in stored sediment slurry amending with 4-bromobenzoic hydrazide.

Note: Hum Lum Poo Canal (HLP), Bangplee Industrial Estate (BP1, BP2), Material recovery facilities (MF1, MF2), South-Bangkok Power Plant (PWP), Bangpakod Canal (BPK)

the BP2, PWP and BPK, separately amended with 4-bromobezoic hydrazide and 4-bromobenzonitrile from PCBs dechlorination amending halogenated primers.

Table 2 represents the phylogenic analysis of DGGE bands. The sediments treated with 2345-CBps could provide the same group of dechlorinating microbes rather than those amended with halogenated compounds. The predominant bands were considered the closest relative of the uncultured bacterium of PCBs dechlorination in river sediment and *Pseudomonas putida*. Uncultured bacterium related to PCBs dechlorinating bacterial communities in river sediment was still discovered. These results were

supported by Yoshida *et al.* [17], who claimed that *Firmicutes* and *Proteobacteria* played a crucial role in reductively dechlorinating microbes. Based on 234- and 2345-CBps treatments, the band of dechlorinators, were trended to be similar to the group of PCB congeners spiking rather than the sampling sites or priming congeners.

Moreover, the South Bangkok Power plant sediment treated with 2345-CBps could discover the *Pseudomonas putida*, which diverse metabolism exploited for bioremediation of aromatic compounds in contaminated soil. PCB-dechlorinating microbes in a less contaminated sediment Bangplakod Canal could also be evidenced. However, this study did not detect the halobiphenyls, which contains PCB-dechlorinating strain. In this case, specific primers to increase the sensitivity of PCR and cloning samples might be required. Please note that the halogenating consortiums could also improve the dechlorination of polychlorinated biphenyls.

Table 2 Phylogenic analysis of DGGE bands.

Closest relative	Accession number	% Identity	Phylum/ Class
Uncultured *Anaerobranca* sp. clone SRB2	DQ069229	94	*Firmicutes*
Uncultured gamma proteobacterium clone Aerocomp_NB39 16S ribosomal	FJ754851	90	*Proteobacteria*
Uncultured bacterium clone AR018 16S ribosomal RNA gene, PCB dechlorinating bacterial communities in river sediment	GQ860186	98	*Proteobacteria*
Uncultured bacterium partial 16S rRNA gene	AJ621948	94	*Firmicutes*
Uncultured Firmicutes bacterium clone Z273MB13 16S ribosomal RNA gene	FJ484645	100	*Firmicutes*
Uncultured bacterium clone 3-2 16S ribosomal RNA gene	GQ324229	94	*Proteobacteria*
Uncultured gamma proteobacterium clone Aerocomp_NB39 16S ribosomal RNA gene	FJ754851	94	*Proteobacteria*
Uncultured bacterium clone Z552 16S ribosomal RNA gene	AY979304	92	*Firmicutes*
Uncultured bacterium clone AR062 16S ribosomal RNA gene, PCB dechlorinating bacterial communities in river sediment	GQ860277	96	*Proteobacteria*

Note: DNA bands quoted from BP2, PWP and BPK, separately amended with 4-bromobezoic hydrazide and 4-bromobenzonitrile

4. Conclusion

This article identified significant groups of PCBs dechlorinating microbes in Thailand. The cold temperature could reduce the microbial dechlorination capacity on PCBs. While the stored sediment under cold temperature could retard the dechlorination of 234-CBp, it is not applicable for the Bangplakod site. In the meantime, the dechlorination of 2345-CBp was active but not on the HLP site. The dechlorination capacity of the microbes on 234-CBp could be activated by adding appropriate halogenated primers. The priming congeners could improve the dechlorination of 234-CBps in stored sediment, except the HLP amended with 4-bromobenzoic hydrazide. Appropriate halogenated primers and concentrations must be selected to enhance the dichlorination ability.

This article represented that the dechlorinators were abundant in Thailand, where sediments, Firmicutes, and Proteobacteria were major dechlorinators. Moreover, inactive biodegradable chemicals associated with contaminated chemicals can be activated again after non-receiving pollutants, disturbing the limitation of natural resources. This article also illustrated that stored sediment could dechlorinate the 2345-CBp, but not from the HLP, MF1 and MF2 sites. Finally, given that environmental factors can vary the experimental results, the article confirmed that indigenous microbes can also be activated by halogenated primers in Thailand and contributed to the frontier experiment in the country. In other words, this article represented that biodegradable chemicals associated with toxic chemicals could initiate dechlorination again and that the tropical climate in Thailand is suitable to degrade PCBs.

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