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EFFECTS OF THIOBENCARB AND BIOAUGMENTATION IN PADDY SOIL AND SEDIMENT SLURRIES ON BACTERIAL COMMUNITY AND THIOBENCARB DEGRADATION

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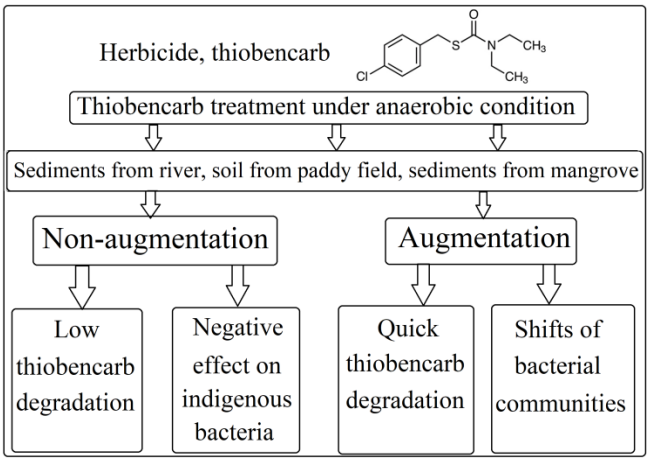
Graphical abstract



Abstract

Thiobencarb has been extensively applied to control weeds. In this study, research was conducted to investigate thiobencarb degradation in soil and sediment slurries under anaerobic condition whereby we acknowledged degradation rates in this order: sediments from river, soil from paddy field, sediments from mangrove. The augmentation of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 increased thiobencarb degradation by 24.46±4.1% in soil from paddy fields, 25.03±3.3% in sediment from the river, and 17.58±3.0% in sediment from the mangrove. Moreover, the thiobencarb supplementation and bacterial inoculation resulted in the shifts of bacterial communities in these media. For example, the inoculation of the mixed culture resulted in significant increases in relative abundances of all phyla *Dechloromonas*, *Thauera* and *Azoarcus* in sediment from the river, *Dechloromonas* and *Azoarcus* in soil from the paddy field, and *Thauera* in sediment from the mangrove. α and β-diversity analyses showed that thiobencarb caused significant changes of indigenous bacteria in soil collected from the paddy field and sediment from the mangrove. Besides, the inoculation of the mixed culture reduced the adverse effect of thiobencarb on indigenous bacteria based on α-diversity. This study provides valuable information on roles of bioaugmentation in the degradation, effects of thiobencarb supplementation and bacterial inoculation on native bacterial communities under anaerobic conditions.

Keywords: Thiobencarb, degradation, anaerobic condition, augmentation, bacterial communities.

1.0 INTRODUCTION

Pesticides applied in paddy fields may be transported into surface water bodies via overflow and as a result negatively influencing aquatic species [1]. Thiobencarb (S-4-chlorobenzyl diethyldithiocarbamate) is an herbicide used to control weeds worldwide. This compound reduces the emergence process and shortens development time of non-target aquatic invertebrates [2]. Moreover, thiobencarb is reported to cause behavioral changes in fish [3], causes adverse effects on fecundity, histopathological and biochemical changes in the African catfish [4], and induces phenotypic abnormalities, apoptosis and cardiovascular toxicity in zebrafish [5]. In addition, the compound affects soil microbiology under aerobic conditions [6] and reduces microbial diversity in soil [7].

Because of its extensive application, thiobencarb has potentially accumulated in water, soil and sediment. Indeed, the compound been detected up to 200 µg/L in water from rice fields [8], 72.7-100% of drinking water samples [9], 3.4-13 ng/L surface water and 2.1-4.6 ng/g dry soil [10], and has a predominant binding nature with sediments [11, 12]. In addition, thiobencarb is stable to degradation by hydrolysis and is persistent under anaerobic aquatic conditions. In soil, the herbicide has different half-life values, ranging from less than 10 days to hundreds of days [13-16]. Furthermore, the substrate is slowly dissipated in flooded soil or under anaerobic condition than non-flooded soil and aerobic media [13, 14, 16]. However, available information on the effects of thiobencarb on soil and sediment is still limited.

In previous studies, some aerobic microorganisms degrading thiobencarb, including *Corynebacterium* sp. [17], *Aspergillus niger* [18], and *Acidovorax* sp. strain T1 [19], *Pseudomonas* sp. Th1 and *Cupriavidus oxalaticus* Th2 [20], have been isolated. Under anaerobic condition, the supplementation of thiobencarb into sediment collected from a river caused a significant shift in native bacterial communities under anaerobic conditions [21]. Then, three bacterial strains, *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3, were isolated from this sediment utilizing thiobencarb as a sole carbon source under anaerobic conditions, with nitrate serving as an electron acceptor [22].

In this study, the augmentation of the mixed culture of these bacterial isolates to enhance thiobencarb degradation in soil collected from a paddy field, sediments collected from a river and a mangrove were determined. The effects of thiobencarb and augmentation on bacterial communities in these media were also analyzed.

2.0 METHODOLOGY

2.1 Soil and sediment collection

All soil and sediment samples were collected from the Mekong Delta, Vietnam. Soil was collected from a paddy field (9°40'20.6''N, 105°35'9.2"E) when rice was cultivated for two months, in the summer 2021. Soil surface was submerged about 10-15 cm under water level, and soil was collected from 5-15 cm below the soil surface. River sediment was collected from the Mekong River (10°17'40.4"N, 106° 9'25.8"E), where the sediment surface was submerged about 1.0-1.2 m under water level. Mangrove sediment sample was collected from a vast mangrove area (8°37'23.7"N, 104° 47'50.8E) whose surface was submerged about 0.2-0.4 m under water when the low tide. Both sediments were taken from a depth of 10-20 cm below the surface. Sediment samples and site water were stored in plastic bags and bottles, placed in iceboxes and transported to our laboratory. All samples were sieved through a 0.5 cm sieve. The physicochemical properties of the soil and sediment slurry were analyzed according to APHA method [23] shown in table 1.

Table 1 Physicochemical parameters of soil and sediments

Parameters	Samples		
	Soil from paddy field	Sediment from river	Sediment from mangrove
pH	6.24±0.33	6.86±0.11	7.21±0.24
Clay (%)	27.3±1.46	19.8±1.12	24.4±1.10
Silt (%)	67.3±2.84	44.7±3.02	65.5±3.02
Sand (%)	15.5±1.07	35.5±1.6	10.2±0.62
NO_3^--N (mg/kg dw)	9.7±0.44	5.0±0.0	14.4±1.3
NH_4^+-N (mg/kg dw)	6.6±0.03	3.2±0.0	9.1±0.5
Total C (g/kg dw)	11.6±0.86	5.6±1.4	18.4±1.4
Total N (g/kg dw)	0.71±0.00	0.92±0.00	2.62±0.2
Total P (g/kg dw)	0.64±0.00	1.04±0.00	0.53±0.0
Salinity (%)	-	-	2.74±0.2

2.2 Anaerobic Degradation Of Thiobencarb In Soil And Sediment Slurries

The soil and sediments were individually mixed with site water (20% dry weight). The slurries (30 mL) were transferred into 75-mL glass vials. For the role of electron acceptor and carbon source on degradation, $NaNO_3$ and glucose (1.0 g/L each) were added into the slurries. The sterile control was autoclaved at 121 °C for 15 min. Thiobencarb was diluted in absolute ethanol at 0.1 M used as a stock solution, which was added at 0.05 mM (12.9 mg/L) to slurries.

The inoculation of the mixed bacterial culture of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 in the slurries was conducted as described in a previous study [22]. Each inoculated strain was 10^6 CFU/mL slurries at the beginning. Bacteria and thiobencarb were added into the vials using a syringe. Therefore, thiobencarb degradation experiments included: sterile, non-sterile, non-sterile and inoculation samples. Experiments consisted of soil from a paddy field, sediment from a river, and sediment from a mangrove; with and without co-substrates.

Vials were then flushed with nitrogen gas to create anaerobic condition. All vials were incubated at a static condition, shaken at 100 rpm for 30 min/day, at a dark and room temperature (~30 °C). After 30 days, remaining thiobencarb and bacterial communities in all samples were analyzed.

2.3 Thiobencarb Extraction And Analytical Methods

Thiobencarb from all slurries was extracted twice with n-hexane. Two volumes of the sample were thoroughly mixed with one volume of n-hexane. Then, the extract was filtered through 0.22 µm. The solution was dissolved in acetonitrile (high-performance liquid chromatography [HPLC] grade). The extraction efficiency of the thiobencarb was 93.4%. Thiobencarb concentration was analyzed using HPLC. The HPLC system (Shimadzu Corporation, Kyoto, Japan) consisted of LC 20AD pumps, a SIL-20A autosampler, and an SPD M20A photodiode array (PDA) detector. A Shimadzu Shim-Pack XR-ODS column was used to separate metabolites. The HPLC was operated as described in a previous study [24].

2.4 Bacterial Diversity And Statistical Analysis

Bacterial diversity was determined in original samples and other treatments (thiobencarb + augmentation, thiobencarb + non-augmentation, and non-thiobencarb + non-augmentation) without any co-substrate after 30 days. In analyzing the bacterial diversity and relative abundance in soil, the relative abundances of the bacterial species in the soil slurries were determined by sequencing 16 S rRNA genes using an Illumina MiSeq benchtop sequencer. The universal primers 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') were used to amplify the 16S rRNA genes at the V3-V4 region. Other processes were conducted as described by Duc et al., [24]. α-diversity indices, i.e., abundance-based coverage estimators (ACE) and Chao1, were analyzed based on the 16 S rRNA genes analysis. Shannon index was also calculated using PRIMER7 software.

For β-diversity analysis, the principal coordinate analysis (PCoA) and permutational analysis of variance (PERMANOVA) were performed using PRIMER7 software. OTU abundance table

was square-root transformed for calculating Bray-Curtis distances. Main test and Pair-wise PERMANOVA were calculated using the Type III method with 999 permutations.

All data obtained from at least three replicate experiments are presented as mean±standard deviation. In addition, Duncan's multiple range test ($p < 0.05$), run on SPSS 22.0, was used to analyze significant differences among means.

3.0 RESULTS AND DISCUSSION

3.1 Thiobencarb Degradation In Soil And Sediment Slurries

After 30 days of incubation, thiobencarb was dissipated in sterile, non-sterile and augmentation treatments (Figure 1). The thiobencarb reduction in sterile samples was less than 14%. The physical and chemical degradation and absorption of thiobencarb into soil and sediment components probably accounted for the substrate reduction in the sterile samples.

The degradation performances in non-sterile soil and sediment were statistically higher than those of sterile ones. The degradation in non-sterile samples occurred due to the activities of indigenous microorganisms. The augmentation with the mixed culture of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 increased the degradation process in all samples (Figure 1). Where there was no co-substrate, the augmentation with the bacterial mixture resulted in degradation rate increase by 24.46±4.1% in soil from paddy field, 25.03±3.3% in sediment from the river, and 17.58±3.0% in sediment from the mangrove. The degradation percentages in the media supplemented with $NaNO_3$ and glucose (Figure 1b) were higher than media without any co-substrate and non-augmentation (Figure 1a).

Moreover, the degradation performances in media supplemented with $NaNO_3$ but without glucose, or added with glucose but without $NaNO_3$ were also conducted. The results showed that thiobencarb degradation rates were enhanced from 6.2% to 12.5% in the treatment of $NaNO_3$ + non-glucose + non-augmentation, and from 8.8% to 15.4% in the treatment of $NaNO_3$ + non-glucose + augmentation. Meanwhile, in a case where there was supplementation with glucose but no $NaNO_3$, an increase in thiobencarb degradation was observed from 4.5% to 10.4% in media without augmentation, and 5.1% to 11.2% in medium with augmentation.

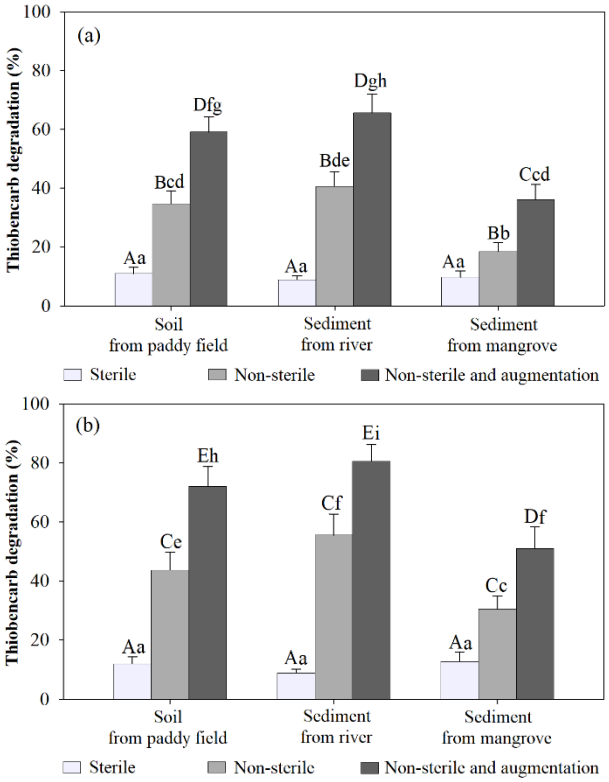


Figure 1 Thiobencarb degradation under anaerobic condition at 12.9 mg/L in media: (a) without and (b) with supplementation with consubstrates ($NaNO_3$ and glucose). Different capital letters (A-D) above bars indicate statistically significant differences among treatments within a group (soil from a paddy soil, sediment from a river, and sediment from a mangrove), while small superscript letters (a-i) show statistically significant differences ($p < 0.05$) among all treatments

A previous study showed that the mixed culture of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 degraded thiobencarb using nitrate as an electron acceptor [22]. Remarkably, an increase in thiobencarb degradation due to the glucose supplementation was also described [21, 22]. Thereby in this study, the degradation occurred in media without $NaNO_3$ addition, indicating that microorganisms in soil and sediment slurries could use other components in these media as electron acceptors.

It's also worth noting that in a previous report, thiobencarb degradation in oxidative-flooded (aerobic-flooded) soil was less than 40%, and reductive-flooded (anaerobic-flooded) soil was less than 30% after 80 days [25], while in another study, Cheah and Crosby [26] reported that 37%-44% of the herbicide (at 10 ppm) remained in the soil-water aquatic ecosystem after 45 days. In anaerobic flooded and non-flooded soils, the degradation was less than 25% after 45 days [14]. Recently, thiobencarb at 2.6 mg/L in sediment was degraded by 28.7 ± 4.7% in the first cycle, and 45.7 ± 5.4% in the second cycle of thiobencarb addition [21].

3.2 Effects Of Thiobencarb And Augmentation On Bacterial Community Structures In Soil And Sediment Slurries

The shifts of bacterial community structures in muddy soil collected from the paddy field, sediments from the river and the

mangrove were analyzed. At the phylum level, Proteobacteria was the most abundant in all samples. Proteobacteria, Actinobacteria, Chloroflexota, Firmicutes and Euryarchaeota were abundant in all soil and sediments (Figure S1).

Campylobacterota, Acidobacteriota and Bacteroidetes were other abundant phyla in soil from the paddy field (Figure S1a). Nitrospirota, Bacteroidetes, Myxococcota and Spirochaetota were also abundant in sediment from the river (Figure S1b). Meanwhile, Acidobacteriota, Caldisericota and Thermodesulfobacteriota were frequently found in sediment from the mangrove (Figure S1c). The relative abundances of some phyla significantly changed after incubation except the sediment from the river which the relative abundances of all phyla were not statistically changed after incubation for 30 days. For example, Proteobacteria in sediment from the river accounted for 39.1±3.0% at the beginning and was quite stable after 30 days.

In paddy soil, the percentage of Proteobacteria was 41.9±3.1% at the initial stage and did not statistically change in experiments with thiobencarb and inoculation (Figure S1a). The relative abundances of Actinobacteria and Firmicutes were 10.7±0.7% and 8.1±0.5% at the beginning, respectively, which significantly declined in all treatments after 30 days. On the other hand, the percentage of Euryarchaeota was enhanced in all treatments after the incubation. The phylum Bacteroidetes only increased in the control. The abundances of Campylobacterota and Acidobacteriota were quite stable during the incubation in soil from the paddy field.

In the sediment from the mangrove, Actinobacteria and Acidobacteriota were 8.0±0.4% and 8.9±0.5% at the beginning, respectively. These phyla significantly decreased in experiments with thiobencarb and inoculation, but did not statistically change in the control after incubation. On the other hand, data for Proteobacteria was 36.5±2.8% which somewhat increased in experiments with thiobencarb and inoculation. Meanwhile, the relative abundance of Firmicutes was quite stable in the mangrove sediment during the incubation (Figure S1c).

At the genus level, the bacterial compositions in muddy soil from the paddy field, sediment from the river and sediment from the mangrove at the beginning and after incubation were significantly different. At the beginning, *Azospirillum*, *Mesorhizobium*, *Bacillus* and *Actinotalea* were frequently found in the paddy soil (Table 2). *Sulfuricella*, *Thiobacillus*, *Azospira* and *Methylophaga* were dominant in sediment from the river (Table 3). Meanwhile, *Rhodoplanes*, *Mycobacterium*, *Anaerolinea* and *Acidobacterium* were higher abundant than other genera in sediment from the mangrove (Table 4).

After 30 days of anaerobic incubation, the relative abundances of several genera increased, while others decreased or did not statistically change. The relative abundances of bacteria which are usually found in aerobic environment, such as *Azospirillum*, *Mesorhizobium, Bacillus*, *Arthrobacter* and *Pelomonas* in paddy soil, and *Nitrospira* in mangrove sediment, significantly decreased in all treatments after 30 days. On the other hand, the relative abundances of some genera in paddy soil, such as *Geobacter*, *Methanobacterium*, *Methanospirillum* and Gp3, significantly increased in all treatments after incubation (Table 2).

The percentages of some genera in treatments amended with thiobencarb were significantly lower than non-amended treatments, including *Prolixibacter* and *Leptolinea* in soil from the paddy field (Table 2); *Thiobacillus*, *Actinomyces*, *Clostridium* and *Spirochaeta* in sediment from the river (Table 3); and *Bryobacter*, *Sulfurovum* and KD4-96 in sediment from the mangrove (Table 4). The lower abundances of these genera in media were probably due to the negative effects of thiobencarb, such as inhibiting the bacterial growth.

Relative abundances of some genera significantly increased in samples amended with thiobencarb, both with and without augmentation. These were *Geobacter* and *Pseudomonas* in soil from the paddy field; *Raultella* and *Dehalobacter* in sediment from the river; and *Pseudomonas*, *Geobacter* and *Flavobacterium* in sediment from the mangrove. These bacterial genera might involve in thiobencarb degradation, or tolerated the toxic compound.

The relative abundances of most genera in soil from the paddy field without thiobencarb and augmentation (control) after incubation were changed significantly compared to the original sample (Table 2), while data for both sediments were not statistically different (Table 3 and 4). The changes of environmental conditions might alter the indigenous microorganisms. The anaerobic condition was more frequent in the sediments than that in paddy soil probably resulting in lower changes in bacterial community structures.

The inoculation of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 resulted in changes in bacterial communities in soil and sediments. In soil from the paddy field, the relative abundances of *Dechloromonas* and *Azoarcus* in inoculated treatments were significantly higher than those in the non-inoculated and original samples (Table 2). The abundance of the genus *Thauera* also increased after incubation, but it was not statistically different compared to the non-inoculated one. The result showed that *Dechloromonas* and *Azoarcus* adapted and grew in soil from the paddy field.

Table 2 Changes of bacterial communities at genus level in slurries of soil from a paddy field among treatments after 30 day

Genera	Original sample	Thiobencarb +augmentation	Thiobencarb + non-augmentation	Non-thiobencarb + non-augmentation (control)
Azospirillum	7.1±0.39^{c}	1.8±0.36^{a}	2.5±0.42^{b}	2.8±0.37^{b}
Mesorhizobium	6.5±0.47^{c}	0.8±0.36^{a}	2.0±0.28^{b}	2.1±0.35^{b}
Bacillus	6.1±0.55^{c}	2.8±0.36^{b}	2.1±0.42^{a}	2.6±0.28^{ab}
Actinotalea	5.7±0.48^{a}	6.4±0.65^{ab}	6.4±0.59^{ab}	6.7±0.62^{b}
Brandyrhizobium	5.4±0.49^{b}	4.4±0.43^{a}	5.5±0.53^{b}	5.2±0.52^{a}
Arthrobacter	5.0±0.43^{b}	1.4±0.29^{a}	1.4±0.24^{a}	1.6±0.43^{a}
Azoarcus	4.9±0.26^{a}	7.3±0.76^{c}	6.2±0.63^{b}	5.2±0.52^{a}
Geobacter	4.7±0.32^{a}	6.1±0.55^{bc}	6.8±0.67^{c}	5.8±0.61^{b}
Holophagae	4.4±0.39^{a}	4.7±0.48^{a}	4.8±0.76^{a}	4.8±0.61^{a}
Sulfuricurvum	4.1±0.36^{a}	5.6±0.52^{b}	5.8±0.59^{b}	5.5±0.45^{b}
Petrimonas	4.0±0.26^{a}	4.4±0.61^{a}	4.1±0.42^{a}	4.8±0.61^{a}
Pelomonas	3.7±0.29^{c}	0.7±0.34^{a}	1.1±0.26^{ab}	1.6±0.43^{b}
Methanothrix	3.3±0.29^{a}	4.5±0.55^{bc}	4.8±0.56^{c}	3.8±0.61^{ab}
Methanobacterium	3.0±0.22^{a}	4.7±0.55^{b}	5.3±0.53^{b}	4.6±0.54^{b}
Desulfomicrobium	3.0±0.22^{a}	3.3±0.69^{a}	3.4±0.57^{a}	3.0±0.42^{a}
Prolixibacter	2.7±0.27^{b}	1.1±0.55^{a}	1.5±0.35^{a}	3.8±0.61^{c}
Pseudomonas	2.5±0.26^{a}	6.6±0.61^{d}	5.4±0.47^{c}	4.0±0.64^{b}
Anaerovorax	2.0±0.28^{a}	2.3±0.53^{ab}	2.9±0.36^{b}	2.7±0.35^{b}
Thauera	2.0±0.28^{a}	3.8±0.37^{b}	3.2±0.36^{b}	3.1±0.84^{b}
Blvii28	1.7±0.38^{a}	1.8±0.55^{a}	1.6±0.33^{a}	1.9±0.26^{ab}
Leptolinea	1.6±0.33^{c}	0.5±0.32^{a}	1.0±0.29^{b}	2.9±0.26^{d}
Methanospirillum	1.3±0.26^{a}	2.1±0.32^{b}	2.4±0.54^{b}	2.4±0.49^{b}
Gp3	1.1±0.26^{a}	2.4±0.34^{b}	2.3±0.32^{b}	2.5±0.35^{b}
Dechloromonas	1.0±0.29^{a}	3.5±0.53^{b}	3.1±0.84^{b}	1.8±0.18^{a}
Others	8.1±0.6	10.5±1.1	9.0±1.0	9.2±0.9
Unclassified	5.1±0.3	6.5±0.7	5.4±0.6	5.8±0.6

Note: Different superscript letters (a, b, c and d) indicate statistically significant differences ($p<0.05$) among treatments within a row.

The inoculation of the mixed culture into sediment from the river mildly increased the relative abundances of all *Dechloromonas* and *Azoarcus* and *Thauera* after incubation (Table 3). The percentages of these genera in inoculated sediment were higher than those in the control. However, only the abundance of *Thauera* was enhanced in inoculated sediment from the mangrove. The results indicated that all bacterial strains could grow in river sediment, but only *Thauera* adapted to mangrove sediment. Notably, previous study reported that the thiobencarb transformation rate of *Dechloromonas* sp. Th1 was higher than *Thauera* sp. Th2, while *Azoarcus* sp. Th3 could not transform the compound but it degraded metabolites [18]. The better growth of *Dechloromonas* sp. in paddy soil and river sediment might account for their higher degradation than that of mangrove sediment.

Table 3 Changes of bacterial communities at genus level in slurries of sediment from a river among treatments after 30 days.

Genera	Original sample	Thiobencarb +augmentation	Thiobencarb + non-augmentation	Non-thiobencarb + non-augmentation (control)
Sulfuricella	5.6±0.48^{a}	5.0±0.42^{a}	5.2±0.56^{a}	5.7±0.5^{a}
Thiobacillus	5.5±0.43^{c}	3.3±0.38^{a}	4.2±0.41^{b}	5.3±0.42^{c}
Azospira	5.2±0.48^{a}	4.8±0.59^{a}	4.9±0.54^{a}	5.1±0.4^{a}
Methylophaga	5.0±0.24^{b}	4.2±0.47^{a}	4.0±0.43^{a}	4.8±0.24^{b}
Actinomyces	4.8±0.37^{b}	2.7±0.48^{a}	2.8±0.34^{a}	5.0±0.35^{b}
Clostridium	4.4±0.34^{b}	3.1±0.51^{a}	3.2±0.55^{a}	4.5±0.34^{b}
Dechloromonas	4.4±0.27^{a}	6.2±0.59^{b}	5.6±0.43^{b}	4.6±0.26^{a}
Blvii28	4.2±0.34^{a}	4.1±0.35^{a}	3.8±0.37^{a}	4.4±0.42^{a}
Flavobacterium	4.0±0.24^{a}	3.5±0.36^{a}	3.9±0.38^{a}	3.8±0.18^{a}
Rhodoplanes	3.8±0.36^{a}	4.4±0.36^{a}	4.2±0.42^{a}	4.0±0.36^{a}
Raultella	3.7±0.31^{a}	5.2±0.47^{b}	5.0±0.45^{b}	4.0±0.29^{a}
GOUTA19	3.5±0.26^{a}	4.0±0.29^{a}	3.8±0.41^{a}	3.8±0.26^{a}
Dehalobacter	3.3±0.26^{a}	4.7±0.53^{b}	5.0±0.55^{b}	3.4±0.32^{a}
Anaeromyxobacter	3.2±0.18^{a}	4.0±0.33^{b}	3.0±0.45^{a}	3.0±0.16^{a}
Spirochaeta	3.0±0.29^{b}	1.6±0.37^{a}	1.7±0.39^{a}	3.2±0.29^{b}
Leptolinea	2.9±0.22^{a}	2.9±0.60^{a}	3.7±0.58^{b}	3.2±0.29^{ab}
Gracilibacter	2.8±0.22^{a}	3.2±0.28^{ab}	3.6±0.59^{b}	3.0±0.16^{a}
Burkholderia	2.5±0.22^{b}	1.6±0.37^{a}	1.8±0.37^{a}	2.3±0.18^{b}
Azoarcus	2.5±0.22^{a}	3.8±0.52^{b}	3.3±0.59^{b}	2.5±0.22^{a}
Methanobacterium	2.3±0.24^{a}	3.8±0.42^{b}	3.7±0.64^{b}	2.7±0.29^{a}
Methanothrix	2.0±0.29^{a}	2.2±0.26^{ab}	2.5±0.34^{b}	2.3±0.29^{ab}
Gordonia	1.6±0.28^{a}	2.0±0.26^{ab}	2.0±0.34^{ab}	2.2±0.33^{b}
Olsenella	1.4±0.22^{ab}	1.6±0.36^{b}	1.8±0.26^{b}	1.1±0.22^{a}
Bacillus	1.3±0.18^{a}	1.0±0.22^{a}	1.0±0.34^{a}	1.0±0.22^{a}
Thauera	0.8±0.41^{a}	2.6±0.41^{b}	1.1±0.42^{a}	1.0±0.22^{a}
Others	9.2±0.56	7.5±0.74	7.5±0.81	8.9±0.92
Unclassified	7.1±0.43	6.5±0.68	6.7±0.71	5.5±0.58

Note: Different superscript letters (a, b, c and d) indicate statistically significant differences ($p<0.05$) among treatments within a row.

For the class level, Alphaproteobacteria was the most abundant in original paddy soil and mangrove sediment with 20.2% and 13.4% on average, respectively. Meanwhile, Betaproteobacteria was the second abundant in both paddy soil and mangrove sediment with the corresponding data of 8.4% and 11.6% on average. In river sediment, on the other hand, Betaproteobacteria was the most diversity in the original sample with 28.1% on average; while, Gammaproteobacteria was the second abundant with 9.3% on average.

Some previous studies described the effects of thiobencarb on microorganisms. For example, the compound inhibited the population of N_2-fixing *Azospirillum*, *Azotobacter* and other anaerobic N_2 fixers [27]. Under the aerobic condition, thiobencarb added in soil at a low concentration increased microbial biomass and mineralization of oxidizable organic C and N [28]. However, the effects of thiobencarb on bacterial community at genus and phylum levels under anaerobic condition were still limited.

Table 4 Changes of bacterial communities at genus level in slurries of sediment from a mangrove among treatments after 30 days

Genera	Original sample	Thiobencarb +augmentation	Thiobencarb + non-augmentation	Non-thiobencarb + non-augmentation (control)
Rhodoplanes	5.5±0.39^{a}	5.7±0.55^{a}	5.3±0.53^{a}	5.2±0.39^{a}
Mycobacterium	5.3±0.47^{b}	6.0±0.61^{b}	4.3±0.45^{a}	5.5±0.47^{b}
Anaerolinea	5.0±0.45^{a}	5.5±0.53^{a}	5.0±0.50^{a}	4.8±0.45^{a}
Acidobacterium	4.9±0.39^{a}	5.0±0.59^{a}	4.4±0.50^{a}	4.8±0.50^{a}
Nitrospira	4.7±0.39^{d}	0.5±0.32^{a}	1.5±0.18^{b}	2.6±0.39^{c}
Thiobacillus	4.5±0.34^{a}	5.7±0.57^{b}	4.8±0.42^{a}	4.7±0.34^{a}
Thauera	4.1±0.39^{a}	6.8±0.57^{c}	5.7±0.43^{b}	4.1±0.39^{a}
Bryobacter	4.0±0.37^{c}	0.3±0.26^{a}	1.5±0.18^{b}	3.9±0.37^{c}
Clostridium	3.9±0.26^{a}	4.1±0.50^{a}	3.5±0.57^{a}	4.0±0.26^{a}
Methanolobus	3.7±0.26^{a}	3.4±0.34^{a}	4.0±0.77^{a}	3.6±0.22^{a}
Rhodobacter	3.5±0.26^{a}	4.0±0.57^{a}	3.4±0.43^{a}	3.5±0.26^{a}
Pseudomonas	3.3±0.26^{a}	5.2±0.65^{b}	5.3±0.68^{b}	3.2±0.26^{a}
Tolumonas	3.1±0.26^{bc}	2.6±0.28^{ab}	2.2±0.39^{a}	2.9±0.26^{bc}
Acidibacter	3.0±0.26^{a}	3.5±0.53^{a}	3.5±0.32^{a}	3.0±0.26^{a}
Methanobacterium	2.8±0.18^{b}	2.5±0.37^{b}	2.0±0.32^{a}	2.9±0.18^{b}
Acidothermus	2.6±0.26^{b}	0	0.2±0.16^{a}	2.6±0.22^{b}
KD4-96	2.5±0.18^{c}	1.6±0.34^{c}	1.1±0.33^{a}	2.6±0.18^{c}
Brandyrhizobium	2.4±0.22^{b}	0	1.0±0.41^{a}	2.2±0.22^{b}
Dehalobacterium	2.2±0.18^{a}	3.6±0.39^{b}	3.0±0.88^{ab}	2.4±0.18^{a}
Desulfovibro	2.0±0.22^{a}	2.5±0.44^{b}	3.7±0.29^{c}	2.0±0.22^{a}
Caldisericum	1.9±0.14^{a}	2.0±0.24^{a}	3.0±0.43^{b}	1.7±0.14^{a}
Geobacter	1.6±0.18^{a}	3.5±0.39^{b}	3.4±0.34^{b}	1.8±0.18^{a}
Romboutsia	1.4±0.14^{a}	2.0±0.24^{b}	1.2±0.37^{a}	1.3±0.14^{a}
Sedimentibacter	1.2±0.08^{a}	1.0±0.68^{a}	1.6±0.32^{a}	1.1±0.08^{a}
Sulfurovum	1.1±0.08^{a}	0	0	1.0±0^{a}
Desulfobulbus	1.0±0.12^{a}	1.7±0.41^{c}	2.2±0.37^{c}	0.9±0.12^{a}
Flavobacterium	1.0±0.12^{a}	2.3±0.53^{b}	3.0±0.45^{c}	1.1±0.12^{a}
Dechloromonas	0.8±0.12^{a}	1.0±0.68^{a}	1.1±0.48^{a}	0.9±0.08^{a}
Azoarcus	0.6±0.08^{a}	0.8±0.26^{ab}	1.0±0.24^{ab}	0.7±0.08^{ab}
Others	9.1±0.72	9.6±1.02	11.1±1.21	10.9±1.05
Unclassified	7.3±0.54	7.5±0.81	8±0.83	8.1±0.88

Note: Different superscript letters (a, b, c and d) indicate statistically significant differences ($p<0.05$) among treatments within a row.

3.3 Effects of Thiobencarb and Augmentation On A-Diversity of Bacterial Community

The α-diversity of the bacterial community was determined in soil from the paddy field, sediments from the river and mangrove, without co-substrate addition. In the soil slurry from the paddy field, the sequence numbers significantly decreased in all treatments after 30 days compared to original soil (Table 5). The indices of OTUs, ACE, and Chao1 of bacteria in the slurry supplemented thiobencarb and without inoculation were statistically smaller than those in other ones. The Shannon index in the media with thiobencarb was also significantly smaller than the treated-thiobencarb one in the soil slurry from the paddy field.

Sequence numbers, ACE and Chao1 indices of bacteria in sediment from the mangrove of thiobencarb and non-inoculation were statistically smaller than those in other treatments. OTUs and Shannon indices in this sediment were also significantly smaller than those in the original sample and media without thiobencarb. For the sediment from the river, sequence numbers and OTUs and Shannon index were not statistically different in all treatments. However, other indices significantly increased in the control and inoculation treatment after 30 days.

The thiobencarb supplementation and bacteria inoculation did not statistically change α-diversity indices in most treatments in sediment from the Mekong River. The river receives water flow containing pesticides from paddy fields and other crop sites; therefore, native microorganisms adapted to

the herbicide and showed higher thiobencarb degradation. Although the mixed culture of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 were isolated from river sediment which was far from the sample collected in this work, they adapted well in the new environment. The similar in physicochemical properties of these sediment samples was probably another reason to support the growth of isolated bacteria.

The supplementation of thiobencarb into sediment collected from a river near rice fields caused a significant change in the bacterial community under anaerobic condition, which was also reported [21]. The abundances of several genera and phyla in the sediment supplemented with thiobencarb significantly increased while others decreased after 60 days of incubation under anaerobic degradation [21].

Table 5 α-diversity indices and richness of bacterial community in the paddy soil with and without supplementation with thiobencarb.

Samples	Indices	Original soil	After 30 days		
			Thiobencarb + augmentation	Thiobencarb + non-augmentation	Non-thiobencarb + non-augmentation
Soil from paddy field	Sequences	52443.0 ± 2486.7^{b}	45452.5 ± 4198.0^{a}	44652.3 ± 4243.6^{a}	44076.8 ± 4512.9^{a}
	OTUs	2184.5 ± 109.6^{b}	1928.3 ± 194.5^{b}	1704.5 ± 176.2^{a}	2010.5 ± 216.1^{ab}
	ACE	2734.5 ± 181.3^{b}	2572.0 ± 265.0^{b}	2057.0 ± 227.3^{a}	2613.0 ± 269.4^{b}
	Chao1	2707.5 ± 162.7^{b}	2467.5 ± 253.4^{b}	2038.0 ± 245.3^{a}	2573.0 ± 212.3^{b}
	Shannon	3.051 ± 0.009^{bc}	3.038 ± 0.018^{ab}	2.991 ± 0.056^{a}	3.091 ± 0.018^{c}
Sediment from river	Sequences	37072.6 ± 1248.6^{a}	39585.0 ± 4016.1^{a}	37341.4 ± 3804.5^{a}	40180.1 ± 4406.6^{a}
	OTUs	1324.3 ± 84.4^{a}	1406.1 ± 154.1^{a}	1383.4 ± 135.5^{a}	1365.7 ± 133.3^{a}
	ACE	1487.3 ± 85.0^{a}	1759.8 ± 182.6^{b}	1705.8 ± 180.5^{ab}	1802.3 ± 178.7^{b}
	Chao1	1433.0 ± 114.8^{a}	1740.3 ± 211.4^{b}	1694.7 ± 202.5^{ab}	1756.4 ± 191.3^{b}
	Shannon	3.130 ± 0.013^{a}	3.139 ± 0.006^{a}	3.135 ± 0.017^{a}	3.135 ± 0.006^{a}
Sediment from mangrove	Sequences	30274.8 ± 2318.9^{b}	33377.3 ± 3357.9^{b}	25304.8 ± 2973.3^{a}	32074.8 ± 3094.4^{b}
	OTUs	865.6 ± 62.5^{b}	902.8 ± 101.4^{b}	703.4 ± 77.2^{a}	844.9 ± 86.0^{b}
	ACE	1271.0 ± 60.6^{b}	1277.3 ± 118.6^{b}	1064.3 ± 112.2^{a}	1156.3 ± 116.7^{ab}
	Chao1	1256.8 ± 53.1^{b}	1250.3 ± 112.0^{b}	1025.8 ± 107.7^{a}	1113.2 ± 131.3^{ab}
	Shannon	3.229 ± 0.007^{c}	3.172 ± 0.009^{b}	3.063 ± 0.033^{a}	3.233 ± 0.006^{c}

*Different superscript letters (a, b and c) indicate statistically significant differences ($p<0.05$) among treatments within a row.

3.4 Effects of Thiobencarb And Augmentation On B-Diversity Of Bacterial Community

The beta diversity was analyzed through the results of PCoA and PERMANOVA tests. The PCoA showed distinct clusters of bacteria in original samples and samples after 30 days. For the soil slurry from the paddy field, bacterial communities among treatments were strongly clustered, and a wide variation margin between the original sample and sample after 30 days (Figure 3a). For sediment from the river, short distance of bacterial communities between original sample and the control (sample without both thiobencarb and augmentation) was observed. Moreover, longer distance was observed between samples with and without amendment with thiobencarb in both sediments (Figure 3b). The cluster separation of bacterial communities at the beginning and the control after 30 days was not significant in sediment from the mangrove (Figure 3c).

The PERMANOVA main test analysis revealed significant differences among treatments within each sample ($p = 0.001$) (Table S1). The sediment from the river showed the lowest Pseudo-F, indicating its bacterial communities were poorly separated. Subsequent pair-wise comparisons revealed no distinct differences in dispersion for the abundant data partition of original samples and the controls of sediments from the river and mangrove.

Pair-wise tests showed no statistically different between the original sample and control of sediment from the river and from the mangrove ($p > 0.05$). By contract, other pair-wise comparisons showed the distinct dispersion in bacterial community compositions of established treatments ($p < 0.05$) (Table S1). These results indicated that both thiobencarb and augmentation had significant effects on native bacteria. The significant distinct groups between the initial stage and the control after 30 days were probably due to the changes in the environmental condition of paddy soil.

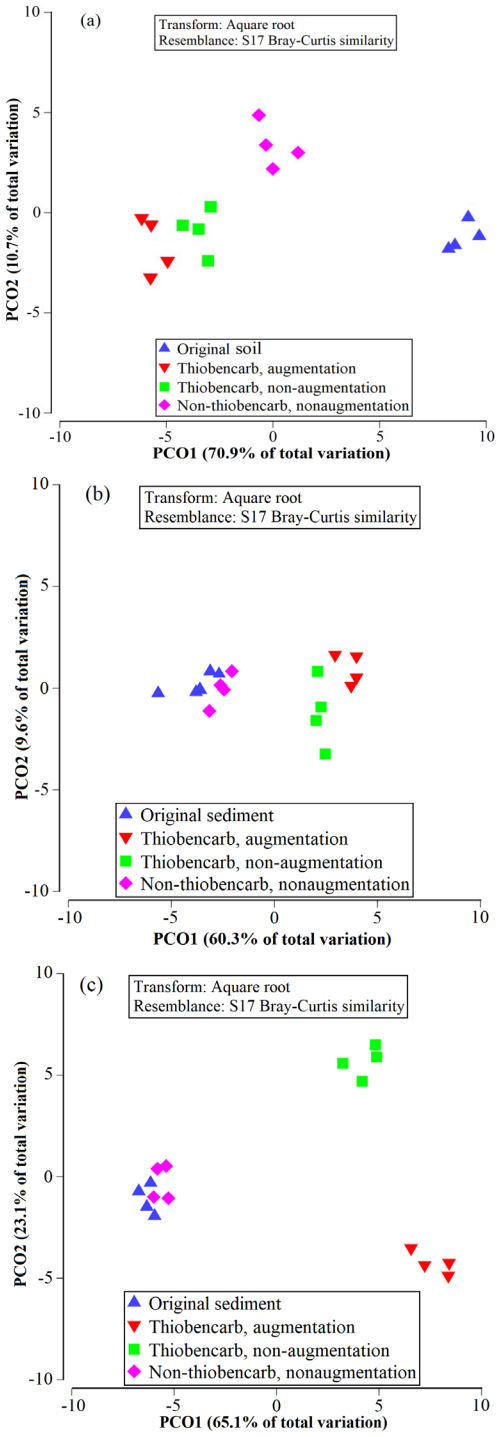


Figure 3 The principal coordinates analysis shows the β-diversity of bacterial communities in mangrove based on Bray-Curtis distance, at the beginning and after 30 days: (a) muddy soil from a paddy soil, (b) sediment from a river and (c) sediment from a mangrove

From the thiobencarb degradation by indigenous microorganisms and analyses of α and β-diversity, bacteria in the paddy field and mangrove sediment showed lower adaptation to the herbicide and augmentation. Thiobencarb caused serious effects on the bacterial community in soil slurry and sediment from the mangrove. The alternating flood and dry conditions induce oxygen variation of paddy soil, ranging from aerobic to anaerobic media. Therefore, aerobic bacteria in collected the paddy soil probably did not adapt to anaerobic conditions well. Besides, sediment collected from the mangrove was far from pesticide application sites, so low bacterial adaptation to the herbicide. However, the inoculation of the mixed culture of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 reduced the negative effects because they degraded the herbicide. All results indicated that the effects of thiobencarb on bacterial communities in soil and sediments were different. The adaptation of bacteria and collection sites with different physicochemical properties of soil and sediments might also be involved in the phenomena.

4.0 CONCLUSION

Thiobencarb dissipation in slurries of soil collected from a rice field, sediments collected from river and mangrove showed different rates under anaerobic condition. The inoculation of mixed culture of *Dechloromonas* sp. Th1, *Thauera* sp. Th2, and *Azoarcus* sp. Th3 increased thiobencarb degradation in all samples. Both α and β-diversity analyses showed that thiobencarb caused significant changes of indigenous bacteria in soil collected from a paddy field and sediment from a mangrove. For example, the percentages of Proteobacteria in paddy soil and river sediment were 41.9±3.1% 39.1±3.0%, respectively, which were not statistically changed in all treatments after 30 days. Meanwhile, Proteobacteria in sediment from the mangrove was 36.5±2.8% and mildly increased after incubation. The inoculation of the mixed culture reduced the adverse effect of thiobencarb application on indigenous bacteria based on α-diversity analysis. Some α-analysis indices were different among treatments, such as OTUs, ACE, Chao1 and Shannon of bacteria in soil slurry from the paddy field supplemented thiobencarb and without inoculation were smaller than those in other ones. PCoA and PERMANOVA tests showed clear clusters associating with the interaction between indigenous bacteria and thiobencarb, and indigenous bacteria and inoculation. This study reveals the role of bioaugmentation on thiobencarb degradation and effects of these processes on indigenous bacterial communities in soil collected from a rice field, sediments collected from river and mangrove at different rates under anaerobic condition. However, this study was conducted in the laboratory condition, and the conduction in real contaminated sites is still underway. Indigenous microorganisms may be the most important in the degradation in contaminated places.

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Ha Danh Duc: Methodology, Experiment conduction Writing-original draft; Nguyen Thi Oanh: Experiment analysis; Nguyen Thi Mai Khanh: Formal analysis, Writing–review and editing.

Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this paper

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