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METHANOGEN INHIBITOR EFFECT ON ANAEROBIC DEGRADATION OF 1,2-DICHLOROETHANE BY SUNGAI ULAR SEDIMENT

(Kesan Perencat Metanogen Terhadap Degradasi Anaerobik 1,2-Dikloroetana oleh Sedimen Sungai Ular)

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Abstract

The toxicity of industrial organohalides such as 1,2-dichloroethane (1,2−DCA) and their longevity in the environment has piqued the public's interest. Anaerobic degradation by Organohalide-Respiring Bacteria (OHRB) has emerged as one of the effective techniques in the removal or degradation of toxic organohalides. Meanwhile, it is typical for methanogenesis to occur within OHRB-dechlorinating bacteria microcosms. These methanogens will compete with the OHRB in the cultures for nutrients and electron donors such as hydrogen, limiting the OHRB's development and dechlorination activity. Methanogen inhibitors are used to remove methanogens from enrichment cultures. The objective of this study is to investigate the effect of methanogen inhibitors on the degradation rate of 1,2-dichloroethane (1,2-DCA) by Sungai Ular sediments. The concentration of 2-Bromoethanesulfonate (BES) as a methanogen inhibitor was varied from 1−25 mM and its effects on the 1,2-DCA degradation were monitored using gas chromatography (GC). Methane which is the methanogenesis product, was also monitored to evaluate the effectiveness of the inhibitor. The results indicated the microcosm treated with 25 mM BES demonstrated the fastest 1,2-DCA degradation at 0.61 µM/day. The amount of methane produced showed a decline in all the BES concentrations, with 25 mM BES demonstrating the lowest methane production at 0.2 ppm. Metagenomic data after the BES treatment also revealed a decrease in the methanogen's population from 42% to 0.007% and an increase in OHRBs population 5% to 69%. This study showed that the addition of a methanogen inhibitor can significantly increase the degradation rate of the chlorinated compound by inhibiting the growth of the methanogens.

Keywords: organohalide-respiring bacteria, methanogen inhibitors, dechlorination, methanogen, 1,2−dichloroethane

Abstrak

Ketoksikan organohalida industri seperti 1,2-dikloroetana (1,2−DCA) dan jangka hayatnya dalam alam sekitar telah menarik minat orang ramai. Degradasi anaerobik oleh bakteria pernafasan organohalida (OHRB) telah muncul sebagai salah satu teknik yang berkesan dalam penyingkiran atau degradasi organohalida toksik. Sementara itu, adalah tipikal untuk metanogenesis berlaku dalam mikrokosmos bakteria penyahklorinan OHRB. Metanogen ini akan bersaing dengan OHRB dalam kultur untuk nutrien dan penderma elektron seperti hidrogen, mengehadkan aktiviti pembangunan dan penyahklorinan OHRB. Perencat metanogen digunakan untuk mengeluarkan metanogen daripada kultur pengayaan. Objektif kajian ini adalah untuk menyiasat kesan perencat metanogen terhadap kadar degradasi 1,2-dikloroetana (1,2-DCA) oleh sedimen Sungai Ular. Kepekatan 2-Bromoetanasulfonat

(BES) sebagai perencat metanogen telah diubah daripada 1−25 mM dan kesannya terhadap degradasi 1,2-DCA dipantau menggunakan kromatografi gas (GC). Metana yang merupakan produk metanogenesis, juga dipantau untuk menilai keberkesanan perencat. Keputusan menunjukkan mikrokosmos yang dirawat dengan 25 mM BES menunjukkan degradasi 1,2-DCA terpantas pada 0.61 µM/hari. Jumlah metana yang dihasilkan menunjukkan penurunan dalam semua kepekatan BES, dengan 25 mM BES menunjukkan pengeluaran metana terendah pada 0.2 ppm. Data metagenomik selepas rawatan BES juga mendedahkan penurunan populasi metanogen daripada 42% kepada 25% dan peningkatan populasi OHRB 5% hingga 30%. Kajian ini menunjukkan bahawa penambahan perencat metanogen boleh meningkatkan kadar degradasi sebatian berklorin dengan ketara dengan menghalang pertumbuhan metanogen.

Kata kunci: bakteria penafas organohalida, perencat metanogen, penyahklorinan, metanogen, 1,2−dikloroetana

Introduction

Anthropogenic organohalide pollution of land and water (e.g., through the manufacturing and use of pesticides, dry-cleaning solvents, ozone-depleting refrigerants, and industrial degreasers) is ubiquitous and constitutes a serious risk due to its negative effects on human health and ecosystems [1]. Anthropogenic organohalogens have significantly damaged the environment as a result of their widespread usage and frequently unregulated disposal. The Stockholm Convention lists polyhalogenated pollutants as the majority of persistent organic pollutants with a serious impact on human, animal, and environmental health [2,3]. Much research has been conducted in order to better understand the microbial detoxification of anthropogenic organohalogens and to create viable bioremediation techniques [4,5].

Halogenated alkane with the common name ethyl chloride (chloroethane) is commonly used in the production of tetraethyllead (TEL), which is an additive for gasoline. The 1,2-dichloroethane (1,2-DCA) compound belongs to a category of chloroethanes that have two chloro groups replaced at positions 1 and 2. Vinyl chloride, which is used to create a range of plastic and vinyl items such as polyvinyl chloride (PVC) pipes, furniture and car upholstery, wall coverings, household goods, and vehicle parts, is the most prevalent usage of 1,2-DCA. When toxicity occurs, higher concentrations of 1,2-DCA can circulate throughout the body and bind to glutathione instead of being detoxified and removed because the biotransformation systems are saturated [6].

Anaerobic degradation by using OHRBs has emerged as one of the effective methods in the degradation of toxic chlorinated compounds which include 1,2-DCA. The OHRBs are found in several phyla including the Proteobacteria, Firmicutes and Chloroflexi [7]. Reductive dehalogenase enzymes, which is a special characteristic of OHRB, allow them to employ various organohalides as terminal electron acceptors and live in a variety of terrestrial and aquatic habitats. The capacity of OHRB to utilize organohalides as terminal electron acceptors to provide energy for growth offers a possible remedy for cleaning up polluted locations [8].

One of the common problems in the OHRBs bacterial communities is the presence of methanogens. Since methanogens are usually the dominant anaerobic bacteria in most reduced aquifers, the electron donor and substrate consumption by methanogens could be a rate-limiting factor for OHRBs during anaerobic degradation of halogenated compounds [9]. These methanogens will compete with the OHRB in the cultures for nutrients and electron donors such as hydrogen, which will slow down the growth of the OHRB and its dechlorination activity [10]. The effectiveness of dechlorination is limited when methanogens are present because they can outcompete OHRB for available electron sources and acceptors. Methanogen inhibitors are compounds that selectively target and inhibit the activity of methanogens. The activity of OHRBs will be increased and methanogen competition will be decreased by the addition of the methanogen inhibitor to the consortia containing OHRBs [9]. The aim of this study is to investigate the relationship between the dechlorination process of the Sungai Ular sediments and the methanogen inhibitor, specifically 2-Bromoethanesulfonate (BES).

Materials and Methods

Sample collection

Inocula was obtained from sediments collected at Sungai Ular (4.046821°N, 103.395601°W) in Kuantan, Pahang. The sediments were taken at 15 cm below the riverbank surface using sterile 50 mL Falcon tubes. The sampling tubes is immersed beneath the sediment, filled with

sediment, and completely closed before being brought to the surface. Exposure to air was strictly limited and the samples were transferred into the serum bottle within 24 hours.

Preparation of anaerobic medium

The anaerobic medium contained 0.2% (w/v) KH_2PO_4, 0.27 % (w/v) NH_4Cl, 1% (w/v) NaCl, 0.41% (w/v) $MgCl_2.6H_2O$, 0.52% (w/v) KCl, 0.15 % (w/v) $CaCl_2.2H_2O$, 0.7% (w/v) $ZnCl_2$, 0.8% (w/v) $MnCl_2.4H_2O$, 0.06% (w/v) H_3BO_3, 0.19% (w/v) CoCl2.6H_2O, 0.02 % (w/v) $CuCl_2.2H_2O$, 0.24% (w/v) $NiCl_2.6H_2O$, 0.36% (w/v) $Na_2MoO_4.2H_2O$, 0.02% (w/v) $FeCl_2.4H_2O$ and 0.0128% (w/v) nitriloacetic acid [11]. The medium was flushed with nitrogen for approximately 2 minutes before being sealed with a rubber stopper and aluminum crimp cap. The medium was then autoclaved for 20 minutes at 121°C. Sterile $NaHCO_3$ solution (1g/L), $Na_2S.9H_2O$ + L-cysteine solution (25mg/L) and vitamin cyanocobalamin solution (40 mg/L) were then added to the serum bottles before the inoculation of sediments.

Degradation of 1,2-DCA using methanogen inhibitor

Six g of sediment (wet weight) was inoculated to each 120 mL glass serum bottle containing 65 mL medium prepared above. 20 µM 1,2−DCA as the electron acceptor and overpressure H_2 at 0.4 bar as the electron donor were added to the serum bottles. The methanogen inhibitor, 2-bromoethanesulfonate (BES) at 1, 10 and 25 mM were then added to the consortia. Microcosms were prepared in triplicates for each BES concentration. The serum bottles were then incubated at 25 °C in dark static conditions.

Gas chromatography analysis

1,2−DCA, methane and other volatile organic compounds were detected and quantified by analyzing headspace taken from the serum flasks using gas chromatography coupled to a flame ionized detector (GC-FID). The analysis was carried out using DB−624 column (30m × 0.32mm with 0.25µm film thickness; Agilent Technologies). The oven, injector and detector temperatures were fixed at 150 °C, 250 °C and 260 °C, respectively for 25 minutes. 1 mL of the sample was taken from the headspace and then manually injected into GC-FID. Chromatographic peak areas obtained from the GC-FID were converted to corresponding 1,2−DCA concentrations.

Results and Discussion

1,2−DCA degradation

Figure 1 shows the concentration of 1,2−DCA in the Sungai Ular anaerobic bacterial culture at different concentrations of BES as methanogen inhibitor. Microcosm without any BES addition labeled as "No BES" was included as control. 20 µM of 1,2−DCA was added at day 0 and an additional 20µM of 1,2−DCA was added at day 32, after all the 1,2−DCA fully degraded in all tested microcosms. During the first addition of 1,2−DCA (day 0-32), 25 mM BES recorded the fastest 1,2−DCA degradation when compared to other added concentrations of BES. However, the control culture (No BES) demonstrates faster 1,2−DCA degradation than the 25 mM BES culture. Nonetheless, following the second addition of 1,2−DCA at day 32, 25 mM BES started to exhibit more rapid 1,2−DCA degradation than the control culture and other BES concentrations. 1 mM BES displays the slowest 1,2−DCA degradation followed by the control culture and 10 mM BES, respectively.

Figure 2(a) depicts the chromatogram profiles for 1,2−DCA at day 1 after the transfer while Figures 2(b) and 2(c) depict the chromatogram profiles of No BES and 25mM BES consortia after several days. As can be seen from those figures, 1,2−DCA partially degraded in consortia with No BES while it was completely degraded in 25mM BES consortia. This indicates that the addition of BES accelerated the degradation rate of 1,2−DCA. Similar outcomes were seen in a study by Lin et al. [9], where the inclusion of BES led to the maximum trichloroethylene (TCE) removal efficiency of 99.8%, while BES-free cultures only achieved 77.4% TCE removal.

In the first cycle (day 0−32), lower 1,2−DCA degradations in the cultures with BES addition when compared to control culture can be caused by several factors. The first factor could be caused by slower build-up of the dehalogenating OHRB communities as compared to methanogen populations [12]. During this time, methanogens competed for hydrogen and substrates with the OHRBs and contributed to the lag of 1,2−DCA degradation [13]. Aside from that, numerous studies also pointed out that sometimes, instead of limiting the growth of OHRBs, methanogens synthesized cyanocobalamin (vitamin B_{12}) which is a

niche co-factor in OHRBs growth [3,4]. However, at the second cycle of 1,2−DCA addition (day 52-66), higher BES concentrations contributed to a more positive impact on the dechlorination of 1,2−DCA. It is possible that during this period, the populations of OHRBs began to expand more quickly than those of methanogens. Thus, high concentrations of BES favor the dechlorination by OHRB and limiting the methanogens' activity. Aside from that, the redox potentials and dechlorination levels of the polychlorinated compounds itself appeared to be involved in their capacity to inhibit methanogenesis and promote reductive dechlorination [14].

Methane production

Figure 3 depicts the production of methane, which is the product of methanogenesis in the BES-treated and untreated cultures. As depicted in Figure 3, all the cultures showed methane increment from day 1 to day 32. After that, the amount of methane started to decrease in all BES-treated cultures except the without BES culture. This is in line with the 1,2−DCA degradations displayed in Figure 1, where lower dechlorination activities were observed during day 0−32. An increase in methane production indicates higher methanogen activities and thus limiting the dechlorination activities. However, from day 32−66, the methane concentrations started to drop in all the BES treated cultures and an increase in 1,2−DCA dechlorination was also observed. Higher concentrations of BES were discovered to give favorable benefits in the reduction of methane production with 25 mM BES demonstrating the lowest methane production, followed by 10 mM and 1 mM BES. Meanwhile, the methane in treatment cultures without BES keeps increasing and thus causing slower 1,2−DCA degradation in it. Furthermore, chromatogram profiles in Figure 2 demonstrate that consortia without BES addition (Figure 2b) produced higher levels of methane but slower degradation of 1,2−DCA than consortia that had 25mM of BES added into it (Figure 2c). Consortia supplemented with 25 mM BES (Figure 2c) exhibit lower methane production and after a few days, it totally degraded 1,2-DCA.

A similar trend was also observed in other studies on the degradation of trichloroethylene (TCE) by *Dehalococcoides* sp. in which the addition of 0.5 mM BES was found to improve the dechlorination rate of TCE [9]. However, this study did not particularly study the effect of BES at different concentrations. In another study that investigates the effect of BES concentrations (10–30 mM) on pentachlorophenol degradation that was carried out by Zhu et al., methane was hardly detected at BES concentrations as low as 10 mM, indicating complete inhibition of methanogens in their study [15]. Nevertheless, these two previous studies agree with this study where there is a synergistic association between the increased rate of halogenated chemical degradation and the reduced methane production caused by the addition of BES.

Microbial community compositions

Figure 4 illustrates the metagenomic data by 16S rRNA gene sequencing before (Figure 4a) and after the BES treatment (Figure 4b). In this figure, the identified OHRB were *Desulfobaccia, Desulfobacteria, Desulfomonillia, Desulfuromonadia, Desulfovibrionia, Shewanella* and *Desulfitobacteriia*); while the identified methanogens are *Methanomicrobia, Methanococci, Methanosarcinia* and *Methanobacteria*. Blue color indicates the presence of methanogens in the microbial communities of Sungai Ular sediments. The findings show that the total bacterial diversity decreased in the microcosm treated with BES. The relative abundance of methanogens in the microcosm decreased from 42% to 0.007%. Meanwhile, the relative abundance of OHRBs population increased from 5% to 69%. These results suggest that methanogen proliferation may be considerably inhibited by high BES concentrations.

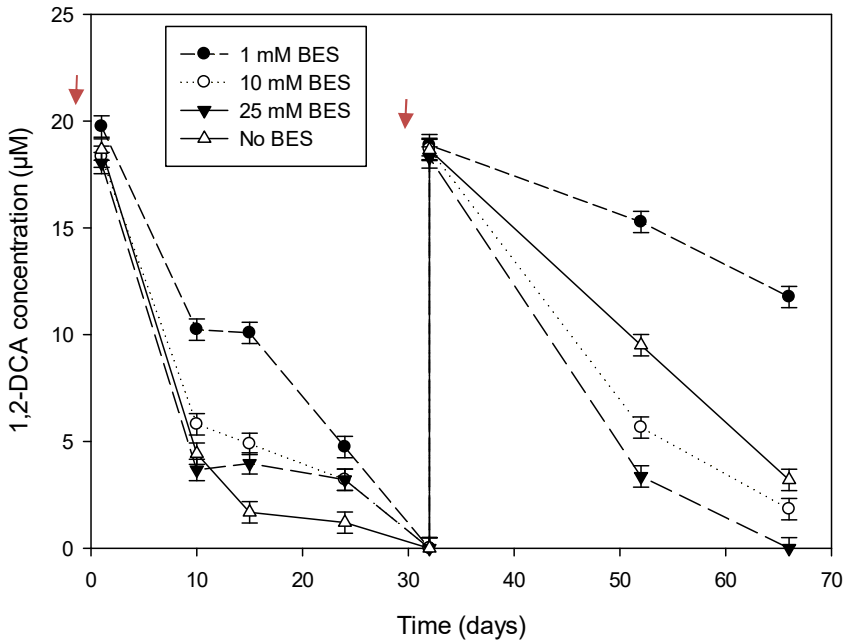


Figure 1. 1,2−DCA degradation at different BES concentrations by Sungai Ular sediments. Arrows (↓) indicate the addition of 1,2-DCA

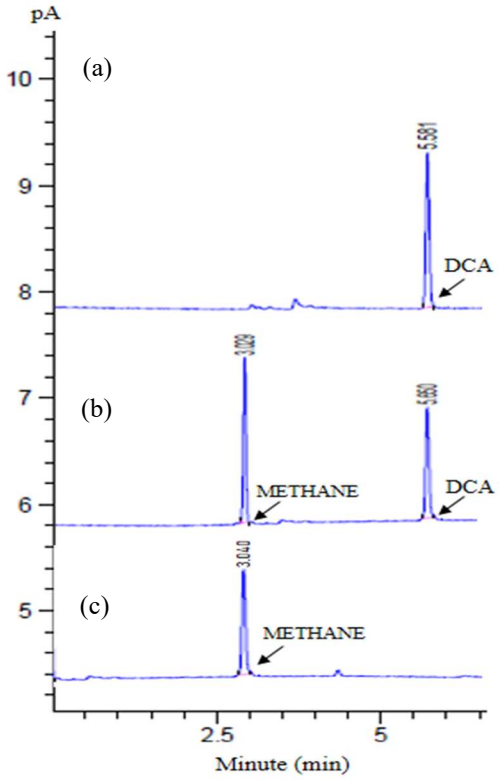


Figure 2. 1,2−DCA and methane chromatogram profile, (a) initial chromatogram of the consortia, (b) chromatogram of consortia with No BES treatment, (c) chromatogram of consortia with 25mM BES

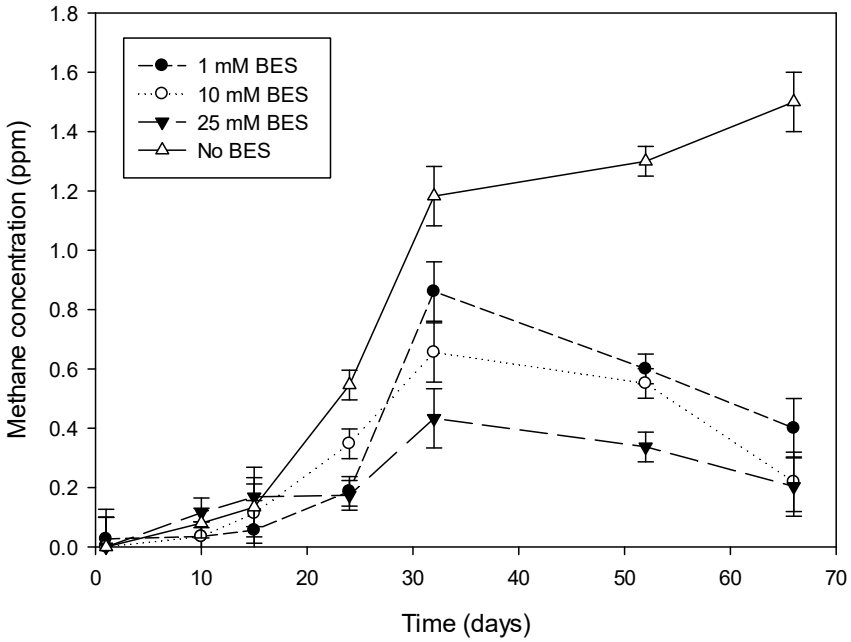
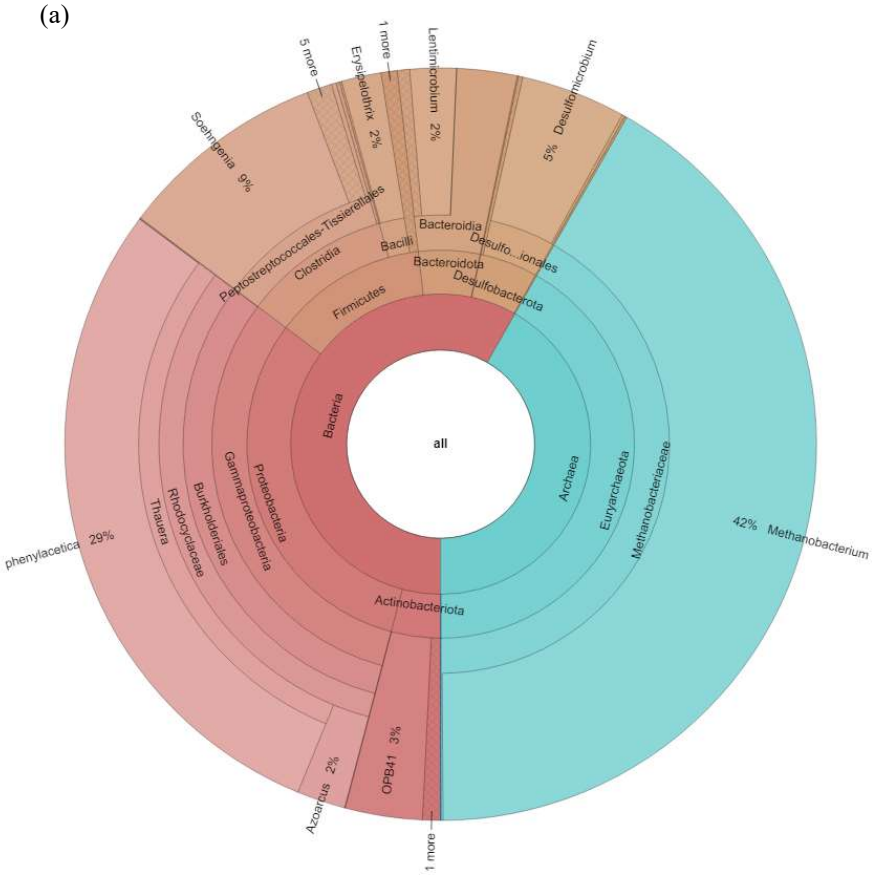


Figure 3. Methane concentration at different BES concentrations in Sungai Ular sediments

(a)



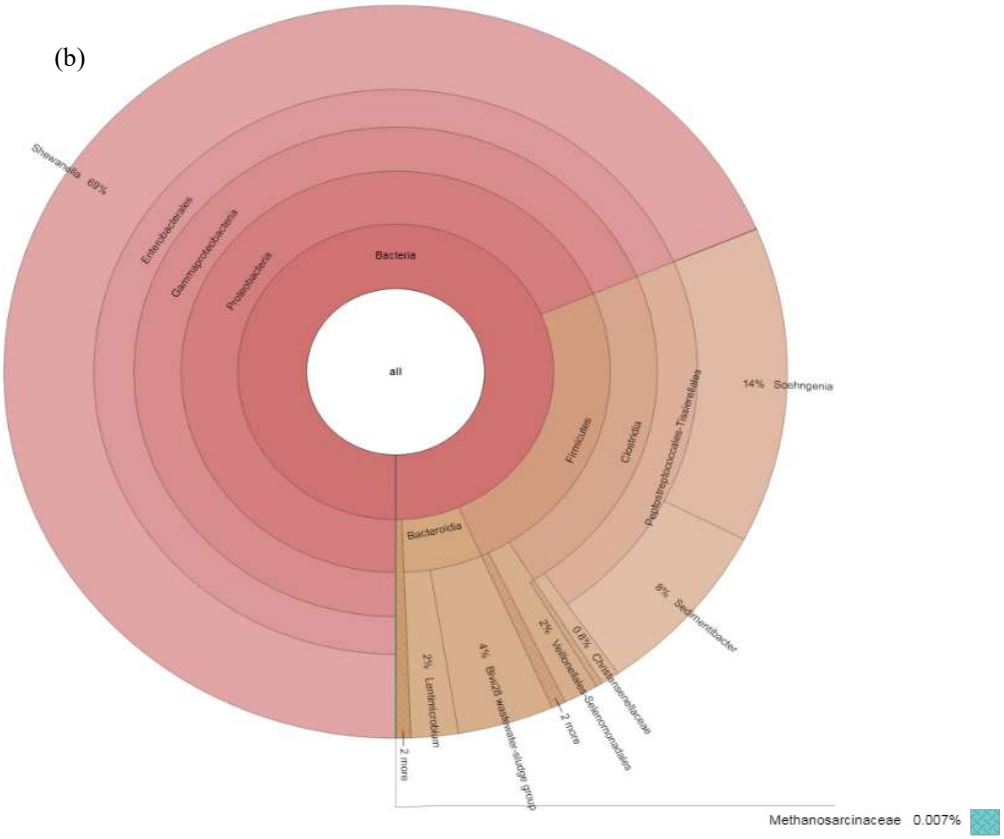


Figure 4. Bacterial communities in Sungai Ular sediments, (a) metagenomic data before the addition of BES, (b) metagenomic data after addition of 25 mM BES

Despite that, the synchronous relationship between methanogenesis and the anaerobic breakdown of halogenated substances remains incomprehensible [13,15]. Most studies on the effect of methanogen inhibitors on dechlorination were carried out in simple systems and its effect on largescale contaminated sites has never been tested. In addition, there are no definite studies concluding the negative impact of methanogens' presence on the dechlorinators population in the environment. The methanogens competition for nutrients and electron donors may hinder the OHRBs growth but simultaneously also provides a beneficial co-factor for OHRBs growth.

Conclusion

This study demonstrated that the degradation rate of 1,2–DCA was highly affected by different concentrations of BES. Higher 1,2–DCA degradation and lower methane production were found in the culture with higher BES concentration. The metagenomic data also supported the relationship between the BES concentrations with the methanogens and OHRBs population. Our results suggested a potential application for high concentrations of methanogen inhibitor in promoting faster degradation rates of chlorinated compounds.

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