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Dibenzofuran Degradation by Bacterial Community in Landfill Leachate

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ABSTRACT

The contamination of the environment has been a global issue, and bioremediation is proposed as an option to clean up the contamination sites with the promising utilization of bacterial community capabilities. The indigenous bacterial community in the landfill leachate is recognized to carry enzymes for the degradation of contaminants such as dioxin congeners, the dibenzofuran. Environmental factors have been known to influence the process to achieve successful biodegradation, and the optimized conditions may speed up the biodegradation process. Thus, this study was conducted to optimize the substrate availability, temperature, and pH factor for the degradation of dibenzofuran from landfill leachate by the native bacterial community in landfill leachate. This study uses the one-factor at-time (OFAT) approach to measure dibenzofuran degradation. The landfill leachate with enrichment of dibenzofuran (15 to 45 mg L-1) was incubated at temperatures (30°C to 42°C) and pH (5 to 9) for 24 hours before being extracted and analyzed. From the first part of the study, 15 mg L-1 of dibenzofuran, 30°C temperature, and pH 7 have shown the highest dibenzofuran degradation. Later, the optimum condition of dibenzofuran removal (74.40%) was achieved when the landfill leachate was spiked with 15 ppm dibenzofuran at 30°C and pH 7 for 24 hours. This study proposes optimized conditions that give a better result for dibenzofuran degradation, which may enhance bioremediation.

Keywords: Bacterial community, biodegradation, dibenzofuran, landfill leachate, One-Factor-at-a-Time (OFAT)

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INTRODUCTION

The aquatic ecosystem pollution from many anthropogenic activities is becoming a global concern. One of the biggest concerns is the occurrence of organic contaminants that are found to be highly persistent in the environment and have destructive biological effects on humans and animals (Baran et al., 2020; Baran et al., 2021; Eskenazi et al., 2018). Amongst the harmful organic contaminants are Chlorinated Hydrocarbons (CHs) found in the atmosphere and hydrosphere recently (Haedrich et al., 2020; Zhao et al., 2022).

The landfill site is one of the sources of organic contaminants released into the environment (Njoku et al., 2019). More than half of the related products containing CHs were estimated to enter the landfill site, which mixed with other materials to form landfill leachate. Compounds containing CHs, such as dioxin congeners, are among the hazardous pollutants in landfill leachate (Salam & Nilza, 2021). Leachate formed from chemical reactions in a landfill was one of the major potential contributors of dioxins released to the environment, especially into soil and water bodies (Ferronato & Toretta, 2019). According to Szajner et al. (2021), the adverse effects of dioxins on the health of humans and animals were found devastating and irreversible. Dibenzofuran, one of the harmless dioxin congeners, has been chosen as the model in this study.

Several conventional physical and chemical techniques are available for dioxin removal and clean-up, but most of the techniques fail to destroy the compounds completely, and often, the techniques lead to other types of situations (Earnden et al., 2022; Rashwan et al., 2022). There are photocatalytic degradation (Gaur et al., 2022) and chemical oxidation process (Eldos et al., 2022). An effective method to destroy the contaminants is an urgent concern to date. One of the techniques available to treat organic contaminants is bioremediation, a low-cost and eco-friendly method (Tarekegn et al., 2020). This technique converts harmful contaminants into less toxic ones using microorganisms such as bacteria (Ambust et al., 2021; Morris et al., 2018; Soare et al., 2019).

It was reported that indigenous microbial communities inhabiting the contaminated sites could potentially biodegrade these xenobiotic compounds (Tas et al., 2018). Studies on a microcosm of microorganisms and their ability have shown some hope for dioxins bioremediation (Terzaghi et al., 2020). These strategies were carried out by harnessing the ability of microorganisms, mainly bacteria, to utilize chlorinated hydrocarbons by breaking down the compounds using specific enzymes, including biphenyl dioxygenase and dioxin dioxygenase. Previously, no data was available about the native bacterial community of landfill leachate that degrades organic contaminants, specifically dibenzofuran. The study was intended to determine the optimal condition for dibenzofuran degradation by the native bacterial community in the landfill leachate.

MATERIALS AND METHODS

Sampling − Selangor, Malaysia

The leachate sample was collected from Jeram Sanitary Landfill, Selangor, at coordinates of 3°11'27.3"N 101°22'02.1"E. The landfill has the capacity of receiving 2500 tons of waste per day. A hydro lab quanta multi-probe meter is used as the sampling device to collect five liters of the landfill leachate sample. The physicochemical properties of the leachate, pH, total dissolved solids (TDS), turbidity, temperature, and dissolved oxygen (DO) were recorded. The sample was kept in a polyethylene tube and stored for further analysis.

Biodegradation Studies

Optimization of Dibenzofurans Biodegradation Parameters. The study was based on a one-factor-at-a-time (OFAT) design. The parameters chosen to be optimized were dibenzofuran concentration, pH, and temperature. The parameters studied were the most crucial environmental components for bacterial growth: pH, temperature, and substrate availability. The levels of parameters were selected to represent different growth conditions for the bacteria community. The study was conducted in a 250 ml Erlenmeyer flask and carried out in triplicate. The culture was incubated at a 200-rpm shaking rate for 24 hours. Twenty-four hours is chosen as it is the most optimum time for bacterial degradation of dibenzofuran (Tajudin, 2017; Sanusi, 2017). The degradation of dibenzofuran is decreasing, and the bacteria undergo the death phase after 24 hours of incubation time. A flask containing similar content/ ingredients without bacterial inoculation was prepared as a control to determine abiotic degradation. The response determined was the amount of dibenzofuran removal at the end of the study. The biodegradation efficiency of dibenzofuran (%) is calculated through the following Equation 1:

$$\text{Biodegradation efficiency (\%)} = \frac{(Ci - Cf)}{Ci} \times 100 \qquad (1)$$

Where *Ci* and *Cf* are the initial and final concentrations of dibenzofuran (ppm) in the experiment solution.

Effect of Dibenzofuran Concentration on Dibenzofuran Degradation. Different substrate concentrations were set to discover the best level of dibenzofuran for maximum degradation. Three different dibenzofuran concentrations (15 ppm, 30 ppm, and 45 ppm) were examined to find the dibenzofuran utilization by the bacterial community in the landfill leachate. The growth medium was incubated at 37°C, with a pH of 7.

Effect of Temperature on Dibenzofuran Degradation. For the temperature study, different temperatures representing different conditions for bacterial growth were chosen and set for bacterial incubation. The different temperatures are 30°C, 37°C, and 42°C sets (Sanusi, 2017). The media will be enriched with 30 ppm dibenzofuran concentration at pH 7.

Effect of pH on Dibenzofuran Degradation. pH levels of 5, 7, and 9 were used to study the effect of pH on the degradation of dibenzofuran by the bacterial community in landfill leachate. The pH chosen and set represented different pH conditions for bacterial growth. The media will be enriched with 30 ppm incubated at 37°C. The pH will be adjusted by using HCL or NaOH solution.

Liquid-Liquid Extraction of Dibenzofuran from Landfill Leachate. Based on the previous study, LLE has been widely used to extract organic pollutants in water (Razali et al., 2018). Hence, LLE was chosen to extract dibenzofuran from the landfill leachate. Hexane was chosen as the solvent in this study as it had shown the highest percentage of recovery of dibenzofuran from landfill leachate after the extraction process (Tajudin, 2017). Another recent study supports it by using hexane as the solvent to elute Polychlorinated Dibenzofurans (PCDFs) from an environmental sample (Baran et al., 2020).

For sample preparation, a 1000 ml sample of landfill leachate was homogenized using 500 ml of hexane. The mixture was left in a separatory funnel for separations. Leachate was discarded after 6 hours. The separated hexane was kept for further analysis. A rotary evaporator was run to remove solvents from the extracted sample gently. The sample was eluted by Nitrogen gas blow. Before performing the degradations studies, extraction of dibenzofuran from the landfill leachate was conducted to identify the initial amount of dibenzofuran in the landfill leachate sample collected.

Qualitative and Quantification of Dibenzofuran by Gas Chromatography-Mass Spectrophotometry (GC-MS)

The standard curve was formed using a series of dibenzofuran concentrations ranging from 1 ppm to 120 ppm with hexane as solvents in a 1 ml volumetric flask and injected into Gas Chromatography-Mass Spectrophotometry (GC-MS). Analysis was performed to get R^2 from the generated linear graph of the external standards.

Separation and quantification of dibenzofuran were conducted by an Agilent Technologies 7890 gas chromatography (GC) system equipped with an Agilent Technologies 5975 mass spectrometer (MS) system (Agilent Technologies, USA). The working standards and top hexane layer of the landfill leachate extracts were injected into an injector temperature maintained at 300°C. The compounds were separated by an HP-5MS column with an oven temperature for the column set (1) 70°C for 2 minutes and (2) 300°C and held for 15 minutes at the flow rate of 20°C/min. The dibenzofuran detection and quantification were determined in scan and selected ion monitoring (SIM) modes.

The dibenzofuran was identified by the retention time, comparison of their mass fragmentation patterns with standards from the National Institute of Standard (NIST) Mass Spectral 11 library, and confirmation with the working standards. The span of the calibration curve was established and assessed, with the correlation coefficient $R^2 > 0.98$ showing an acceptable identification (Sani et al., 2022).

Statistical Analysis

The data were expressed as mean and standard deviation of the triplicate of dibenzofuran degraded. One-way analysis of variance (ANOVA) was performed through XLSTAT-Pro (2019) statistical software (Addinsoft, Paris, France) to identify the significant difference between the means at a 95% confidence level ($p < 0.05$) with Tukey's Test.

Bacterial Community Identification

The bacterial community identification has been performed. The isolation of the bacterial community was performed prior to DNA extraction. Later, the Polymerase Chain Reaction was conducted using a 27F and 1492R universal primer set with an annealing temperature set at 56°C. The Polymerase Chain Reaction products were sent to the sequencing service provider. The Nucleotide Basic Local Alignment Search Tool (BLAST) was run to identify the species.

RESULTS AND DISCUSSION

Landfill Leachate Characteristics

Landfill leachate collected from Jeram Landfill recorded the physicochemical parameters as shown in Table 1. The parameters observed were pH, total dissolved solids, turbidity, temperature, and dissolved oxygen. pH, as one of the significant parameters representing the landfill condition, was shown at pH 8.38, which could indicate the landfill's operations age and the landfill leachate's stabilization phase (Arliyani et al., 2021). A previous study stated that when the landfill operated for more than 10 years, the pH of the leachate should be more than 7.5 as the leachate is in stabilized condition (Wdowczyk & Szymańska-Pulikowska, 2021). This finding agrees that Jeram landfill has been operated for more than 10 years, since 1 January 2007. The recorded pH characterizes the Jeram landfill as being in the methanogenic phase, where the volatile fatty acid in leachate declined, thus leading to an inclining of the pH values. For temperature, it was recorded at 32.84°C during the sample collection. The temperature of the landfill leachate is highly influenced by the weather at the landfill site, and it is known that the common daily temperature in Peninsular Malaysia is between 30°C and 40°C (Zakaria & Aziz, 2018).

Table 1
Physicochemical parameter of leachate from Jeram sanitary landfill

Parameters	Value
pH	8.38 ± 0.20
Temperature (°C)	32.84 ± 0.20
Dissolved Oxygen (mg/L)	8.29 ± 0.43
Total Dissolved Solid (mg/L)	$2.03 \times 10^4 \pm 8.00$
Turbidity (NTU)	222 ± 6.50

Note. ± represents the standard error of the mean value

Dissolved Oxygen (DO) is an important parameter in determining water pollution. The DO recorded was 8.29 mg/L, slightly higher than the WHO range of healthy water (6.0 to 8.0 mg/L) (Dávalos-Peña et al., 2021). The composition of DO is normally low due to a microbial community employing dissolved oxygen for the waste aerobic decomposition. It probably indicates the presence of an anaerobic microbial community. The Total Dissolved Solid (TDS) was recorded as 2.03×10^4 mg/L, which is also considered high compared to the previous study (Zakaria & Aziz, 2018). This parameter characterizes the organic components of leachate and the total ions in the aqueous solution. High TDS in leachate may represent the whole number of pollutants as it reflects the degradation process of organic matter and the mineral salt in the waste (Kumari et al., 2018). The turbidity recorded for the landfill leachate was 222 NTU. It is considered a moderate level for landfill leachate (Aziz et al., 2018). Even though turbidity is not a direct indicator of the contaminated condition, high turbidity could influence leachate leakage through minimized movements of the compounds. The physicochemical parameters of the landfill leachate observed and recorded might be attributed to numerous factors, including the type of waste dumped onto the landfill (Salam & Nilza, 2021). Further observation of the landfill leachate parameters needs to be conducted to prevent leachate leakage that could cause environmental pollution.

Optimization of Dibenzofurans Biodegradation

For the dibenzofuran concentration effect on the dibenzofuran degradation by the bacterial community, landfill leachate that has been spiked with 15 ppm dibenzofuran has shown the most degraded dibenzofuran, which is 63.35% (Figure 1). In 30 ppm and 45 ppm dibenzofuran concentrations, the bacterial community showed degradation at 39.60% and 26.04%, respectively. It has been shown in this study that the lowest dibenzofuran concentration (15 ppm) had shown the best concentration for maximum bacterial degradation of dibenzofuran in landfill leachate. Substrate availability represents the carbon sources the bacterial community uses to proliferate (Xiang et al., 2020). Lower substrate concentration might cause disability of the bacteria to proliferate, while in higher substrate concentration, accumulation of the by-products might inhibit bacterial growth to the extent that it is toxic to the bacteria cells (Maier & Pepper, 2015).

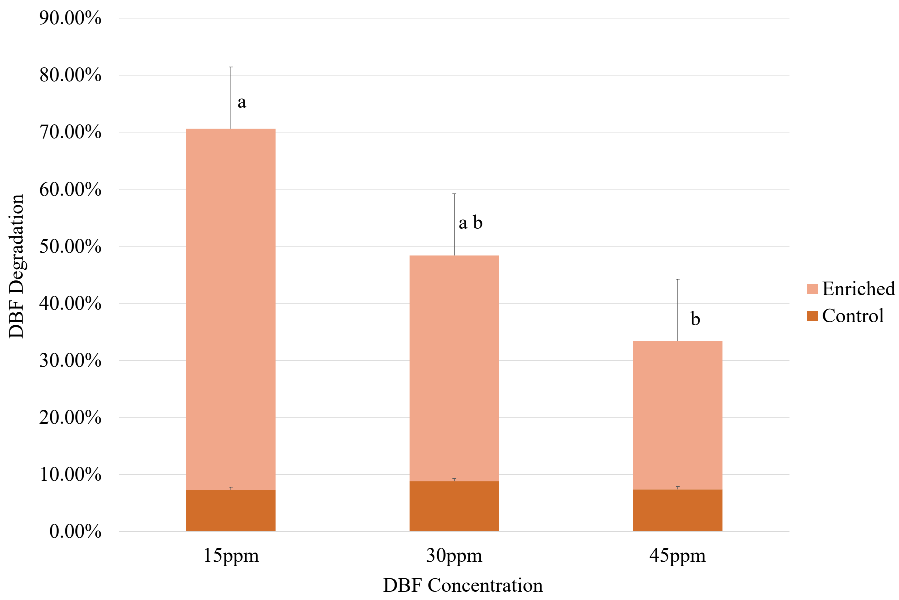

Figure 1. Percentage of dibenzofuran (DBF) degradation with different concentrations of DBF within 24 hours of incubation. Each experimental sample was performed in triplicates. Error bars represent the standard error of the mean. *Means with the same letter are not significantly different.

Note. Enriched: Percentage of dibenzofuran degraded in landfill leachate by native bacterial community enriched with 15 ppm, 30 ppm, and 45 ppm dibenzofuran, respectively, incubated at 37°C and pH 7. Control: Percentage of dibenzofuran degraded in landfill leachate enriched with 15 ppm, 30 ppm, and 45 ppm dibenzofuran without any inoculum of bacteria.

Figure 2 shows that the best temperature for the bacterial community to degrade dibenzofuran (68.33%) is 30°C. At 37°C and 42°C environmental conditions, the degradation of dibenzofuran was recorded at 39.60% and 54.97%. These findings are supported by other studies that demonstrate the best temperature for dibenzofuran degradation by the bacteria is 30°C (Tajudin, 2017; Sanusi, 2017). Bacterial metabolism is influenced by environmental temperature since an enzyme is a protein-made substance. According to Imron et al. (2020), a lower temperature might disrupt bacterial metabolism, while a higher temperature might speed up bacterial metabolism to a certain extent. Above that extent, higher temperatures will cause the enzyme to degrade and lower the bacterial metabolism (Vidonish et al., 2016).

pH study discovered that the best pH for dibenzofuran degradation by the native bacterial community in landfill leachate is 7 (39.60%) (Figure 3). While for pH 5 and pH 9, the study found that 2.12% and 0.59% dibenzofuran degradation had happened. Most enzymes in the bacterial community can only perform and function at a certain pH value, thus making pH value one of the most crucial factors influencing compound degradation

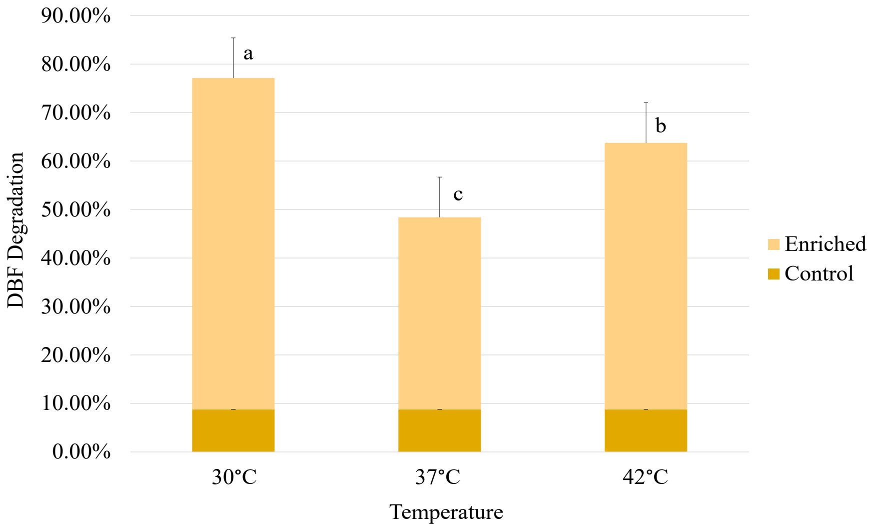

Figure 2. Percentage of dibenzofuran (DBF) degradation with different incubation temperatures within 24 hours of incubation. Each experimental sample was performed in triplicates. Error bars represent the standard error of the mean. *Means with the same letter are not significantly different.

Note. Enriched: Percentage of dibenzofuran degraded in landfill leachate by native bacterial community enriched with 30 ppm dibenzofuran, pH 7 and incubated in 30°C, 37°C and 42°C respectively. Control: Percentage of dibenzofuran degraded in landfill leachate enriched with 30 ppm, without any inoculum of bacteria.

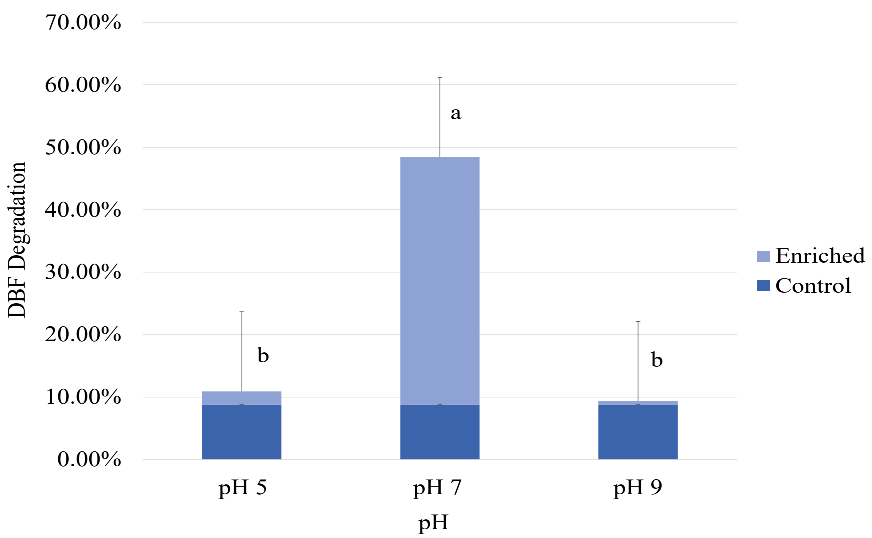

Figure 3. Percentage of dibenzofuran (DBF) degradation with different pH incubation within 24 hours of incubation. Each experimental sample was performed in triplicates. Error bars represent the standard error of the mean. *Means with the same letter are not significantly different.

Note. Enriched: Percentage of dibenzofuran degraded in landfill leachate by native bacterial community enriched with 30 ppm dibenzofuran, incubated at 37°C with pH 5, pH 7, and pH 9, respectively. Control: Percentage of dibenzofuran degraded in landfill leachate enriched with 30 ppm, without any inoculum of bacteria.

(Imron et al., 2020). Large numbers of heterotrophic bacteria are chosen to proliferate better in a neutral to alkaline pH value (Al-Hawash et al., 2018). The previous study also recorded similar findings, in which the neutral condition is the best for dibenzofuran degradation compared to acidic and basic conditions (Tajudin, 2017; Sanusi, 2017).

The best parameters (15 ppm, 30°C, and pH 7) were executed to the bacterial community in the landfill leachate to observe the optimum degradation of dibenzofuran. It is recorded that 74.40% of dibenzofuran had been degraded within 24 hours of incubation, which is the best degradation rate recorded throughout the study (Figure 4). The optimum degradation condition is significant in bioremediation; thus, the pollutant compounds will be degraded effectively through the metabolism of the bacterial community (Zhao et al., 2021).

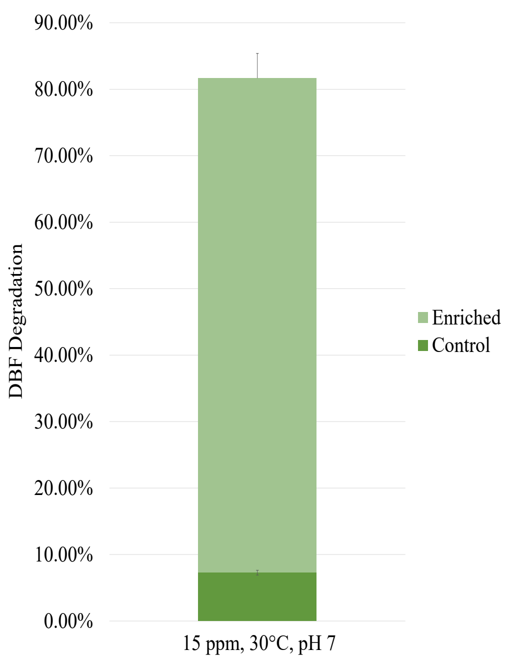

Figure 4. Percentage of dibenzofuran (DBF) degradation supplemented with 15 ppm DBF and incubated at 30°C at pH 7 within 24 hours of incubation. Each experimental sample was performed in triplicates. Error bars represent the standard error of the mean.

Note. Enriched: Percentage of dibenzofuran degraded in landfill leachate by native bacterial community enriched with 15 ppm dibenzofuran, pH 7 and incubated at 30°C. Control: Percentage of dibenzofuran degraded in landfill leachate enriched with 15 ppm, without any inoculum of bacteria.

Bacterial Community and Pathway of Dibenzofuran Degradation

In this study, the bacterial community found is from Phylum Pseudomonadata. Six Pseudomonas species were identified as involved in the dibenzofuran degradation that is *Pseudomonas* sp., *Pseudomonas putida, Pseudomonas japonica*, *Pseudomonas qingdaonensis, Pseudomonas brassicae* and *Pseudomonas cichorii*. The role of *Pseudomonas* sp. in the bioremediation of Chlorinated Hydrocarbons is also emphasized in other studies (Ambust et al., 2021; Mahjoubi et al., 2021; Soare et al., 2019).

The main metabolic pathway of the bacterial community in degrading dibenzofuran is the biphenyl dioxygenase pathway through the presence of the biphenyl dioxygenase enzyme. According to Nhung et al. (2022), 2,3-dioxygenase could destroy the stable benzene rings in the dibenzofuran compounds through molecular oxygen catechol. Some mono and dioxygenase enzymes were also required to place oxygen in the benzene ring to make the compounds more vulnerable to degradation (Saibu et al., 2020). The large subunit of terminal dioxygenase is included for the substrate specificity of the biphenyl dioxygenase. Hence, huge subunit genes need to be involved in the development of molecular engineering.

CONCLUSION

Based on the result collected, it could be seen that the optimum condition for the bacterial community in landfill leachate to degrade dibenzofuran is spiked with 15 ppm dibenzofuran and environmental condition set at 30°C and pH 7. 74.40% dibenzofuran degradation has been recorded future research shall exploit larger volume of landfill leachate sample to discover more about the native bacterial community. To conclude, the degradation of dibenzofuran enriched to the landfill leachate by the native bacterial communities was assisted by the environmental conditions, including temperature and pH, proposing that a bio-stimulation technique could be applied for in-situ bioremediation for the possible dioxin-contaminated site.

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