Landfill leachate treatment using single and mixed freshwater cyanobacterial isolates

Abstract. The increasing volume of waste disposal and improper management of landfill has been contributing to the heavily polluted leachate. Local freshwater cyanobacteria were identified based on the polyphasic approach that combines morphological and molecular identification by the 16S rRNA gene sequencing method. The local cyanobacteria isolate (A1) was identified as the genus *Cyanobacterium* of the order Chroococcales. The A1 isolate together with identified cyanobacteria, *Anabaena* sp. was used to observe their effectiveness in remediating leachate pollution either as monoculture or in mixed culture. Prior to the phycoremediation, the characterization of leachate had been carried out. The biological oxygen demand (BOD_5), chemical oxygen demand (COD), pH value, concentration of ammonia-nitrogen (NH_3-N), nitrite (NO_2^-), and nitrate (NO_3^-) of the raw leachate determined were 22.37 mg/L, 58.33 mg/L, 7.84, 82.7 mg/L, 88.67mg/L and 89.43mg/L, respectively. The use of a single *Anabaena* sp. demonstrates the most effective in the removal of all contaminants that exist in the leachate. The removal of COD was the highest at 105.71% followed by BOD_5 at 92.71% and NO3- at 82.09%. This finding suggested that in order to achieve optimum removal of BOD_5, COD, NH_3-N, NO_2^- and NO_3^- content in leachate sample, treatment by *Anabaena* sp. should be applied.
Keywords: Cyanobacteria, leachate treatment, phycoremediation.

1. Introduction

The disposal of solid waste kept on increasing as the population around the world and specifically, Malaysia also grew. A report from the Department of Statistics, Malaysia stated that Malaysia's population increases by 0.2 percent in the third quarter of 2021 compared to the third quarter of 2020, from 32.60 million to 32.67 million [1]. Even though Malaysia is a small country, leachate pollution in Malaysia may greatly affect the local soil environment. In Malaysia, there are 146 active landfills but only 15% of them are sanitary hence, this clearly shows that Malaysian are exposed to the pollution caused by landfill leachate as there were a lack of landfills with proper treatment for leachate [2]. To be added, water is the most essential component for every human being especially surface and groundwater which may provide drinking water for humans [3]. However, due to industrial development, the quality of water is altered, causing water pollution and thus, health problems [4, 5]. Direct disposal of waste comprising of pollutants may toxify the water and land of the industrial area thus, produces leachate, a potential factor in water pollution [6].

Landfilling is the oldest way of disposing of waste material around the world. Landfilling is the main waste management practice within the Organization for Economic Co-operation and Development (OECD) and can be classified based on the type of waste disposed: hazardous, municipal, and inert waste [7]. This way of waste disposal will produce landfill leachate, the liquid produced from the percolation of rainwater through the decomposed solid waste and the existing water in the waste itself in a landfill [8, 9]. In addition, landfill leachate may cause severe environmental pollution as it releases various types of pollutants as the water passed through the soil in the landfills. The contents of leachate would include many harmful pollutants such as dissolved organic matter, inorganic macro-components, heavy metals, xenobiotic organic compounds, and microorganisms as a mixture of solid waste deposited in landfills [9].

is this also unsustainable

Besides, conventional landfill leachate treatments had been used for years. The conventional treatments can be categorized into three main groups: leachate transfer, biodegradation, and also chemical and physical methods [10]. However, there are many inconveniences of the conventional treatments used where they can only transform the forms but are not completely degraded and environmentally unsustainable due to the requirement of high chemical loads and high carbon dioxide (CO_2) emission [10, 11, 12]. Apart from that, most conventional treatments are complicated and costly in terms of land and high energy inputs requirement [11]. The treatments used also not following the governing standards and produce sludge that later if not eliminated may cause severe pollution [11]. Additionally, the oxidation processes of organic matter involved in the treatments are not capable of eliminating nitrogen and phosphorus, or heavy metals thus, do not completely purify the wastewater [11, 13]. Without proper landfill management and landfill leachate treatment, many adverse environmental impacts such as pollution may occur due to its hazardous and harmful characteristics. Excessive landfill leachate pollution typically appears to be a potential factor that causes surface and underground water contamination [14].

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For the past few decades, many efforts have been made by researchers to treat wastewater by utilizing microalgae such as *Chlorella*, *Chlamydomonas*, *Spirulina*, *Scendesmus*, *Nostoc* and *Oscillatoria* [15]. However, the utilization of cyanobacteria species in treating landfill leachate has not been widely optimized. Thus, to reduce landfill leachate pollution, biological treatment using cyanobacteria (phycoremediation) may be an alternative to the chemical treatment of landfill leachate pollution. The ability of cyanobacteria adaptation to diverse environments is crucial in sustaining the ecosystem and had been considered as one of the approaches that can be used to eliminate the contamination found in the soil and water [16].

Moreover, the existence of cyanobacteria has been acknowledged since the Precambrian period and this photosynthetic prokaryote is said to have lived on the Earth for over a period of two to three billion years [12, 15]. This proves that cyanobacteria have been involved and playing a role in the evolution of higher forms [15]. Cyanobacteria also have the ability in adapting an unexpected physical and chemical changes such as light, salinity, temperature, and nutrient composition [15]. Recently, the interest in developing biological remediation of landfill leachate especially, cyanobacteria approach instead of chemical treatment among researchers soars as they realized the ecological importance of this photosynthetic prokaryote [17, 18]. This led to the discovery of a bioremediation technique called phycoremediation that utilizes cyanobacteria and other types of algae to treat pollution caused by leachate [11].

The potential application of cyanobacteria in remediating pollution has been reported in many research as biological control agents either as wild-type, mutant, or genetically engineered forms [15, 12]. The basic principle of phycoremediation is to obtain complex pollutants from the environment and utilize them to enhance their growth and metabolism or convert them into a non-toxic form [12]. The ability of various *Anabaena* sp. in reducing the biological oxygen demand (BOD) and chemical oxygen demand (COD) value in textile industry effluent and wastewater has been studied by previous researchers [19, 20]. Besides, both studies also showed the synergistic action of *Anabaena* sp. with other cyanobacterial species in reducing the contaminants present in the wastewater. Other examples of cyanobacteria used in wastewater treatment are *Phormidium valderianum* BDU 30501 which reduces phenol concentrations and *Oscillatoria boryana* BDU 92181 which eliminates melanoidin pigment from distillery effluents [21]. Hence, this study was focused on the investigation of the level of selected parameters in the leachate from Jeram Sanitary Landfill, Selangor, and compared the changes of leachate content before and after treatment by single and mixed cyanobacterial isolates so that the discharge standards can be met.

2. Materials and methods

2.1. Selection of cyanobacteria species

There were two cyanobacterial species used in this study. The established *Anabaena* sp. was obtained from Phycology Laboratory, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia while the unclassified cyanobacteria were obtained from a man-made pond in Shah Alam, Malaysia. Both species were cultured and maintained on the BG-11 agar plate before being inoculated in the liquid BG-11 media to be proliferated. The cyanobacterial growth was optimized by adjusting the light/dark cycle with 16/8 hours of white light 8 feet 40W with light intensity (3200 lux), at 25 to 30 °C of temperature and left to reach the mid-late log phase of growth [15].

2.2. Landfill leachate sampling

Leachate samples were collected in sterile Schott bottles from the pond of untreated leachate at Jeram Sanitary Landfill, Selangor at a latitude of 3°11'27.8"N and longitude of 101°22'02.3"E. The sampling of leachate was done by lowering the bottles into the pond and packed in cool boxes at temperatures between 8 to 15 °C before being transported to the laboratory. The leachate samples were filtered to remove any suspended solids before the analysis.

2.3. Leachate analysis

The leachate characterization was done for the pH, BOD, COD, ammonia-nitrogen (NH_3-N), nitrite (NO_2^-), and nitrate (NO_3^-) content in leachate sample based on the Standard Method for the Examination of water and wastewater [22]. All experiments were done in triplicates at 20±2°C to obtain the mean while the determination of all chemical concentrations was measured by using HACH DR 3900 spectrophotometer. The results obtained before and after the treatment were compared to determine the changes and effectiveness of the treatment.

2.4. Experimental design of leachate treatment by single and mixed cyanobacterial isolates

The leachate sample with a volume of 110 mL was treated with 20 mL of cyanobacterial inoculum containing individual and mixed cyanobacterial isolates in 250 mL Erlenmeyer flasks. The ratio used for the mixed cyanobacterial isolates will be 1:1. Next, the flask was placed on an orbital shaker at 25±2°C at 150 rpm for 28 days. The value of pH, BOD, COD, NH_3-N, NO_2^-, and NO_3^- content were analyzed at weekly time intervals (days 7, 14, 21, and 28). The results obtained then were compared with the initial value. Moreover, the percentage of leachate contaminants removal based on the changes of BOD, COD, NH_3-N, NO_2^- and NO_3^- content had been calculated by using equation (1). The negative control, which is the untreated leachate sample was used to compare the results obtained from the treated sample. All processes were performed under sterile conditions at room temperature.

$$\text{Percentage of leachate contaminants removal}(\%) = \frac{\text{Initial value of contaminant - Final value of contaminant}}{\text{Initial value of contaminant}} \times 100 \quad (1)$$

2.5. Proliferation of cyanobacterial species

The chosen potential cyanobacteria species then was inoculated by using inoculation loop in 250 mL conical flasks containing 50 mL culture medium which consist of 37.5 mL BG-11 1X and 12.5 mL of CHX [15]. Next, the conical flasks containing the cyanobacteria suspension were incubated under the previously mentioned condition in Section 2.1 and left to reach the mid-late log phase (21 to 27 days) of growth [15]. The enriched cyanobacteria culture then was used for further identification procedure and leachate treatment procedure.

2.6. Identification of cyanobacteria species using 16S rRNA gene sequencing method

The most potential cyanobacterial species that are capable in remediating leachate were further subjected for identification. Molecular identification via the amplification of 16S rRNA sequencing method had been performed on the selected species. The procedures include the isolation of genomic DNA, followed by amplification of 16S rRNA, purification of the PCR product, and finally DNA sequencing before the species can be identified.

2.7. Statistical analysis

The efficiency of treatments was assessed in terms of the percentage of contaminant that remained in the leachate after 28 days of treatment. The percentage of contaminants that remained in the samples for each treatment was averaged to provide the mean value of each parameter used thus, reducing the non-normality and potential influence of outliers. Therefore, the data recorded were analyzed for any significant differences between treatments which are the leachate treatment using single and mixed cultures of cyanobacteria with one-way ANOVA using a statistical software known as Statistical Program for Social Science (SPSS) version 21. The significant differences ($p<0.05$) between individual treatments were determined and compared.

3. Results

3.1. Characterization of selected parameters in the leachate from Jeram Sanitary Landfill, Selangor

The leachate quality was investigated by determining the temperature, pH, BOD, COD, BOD_5/COD, ammonia-nitrogen, turbidity, colour, conductivity, TDS, and heavy metals contents [23]. The level of selected parameters of the leachate obtained was compared to the standards provided by Malaysia Environment Quality Act (MEQA) 1974 and previous studies from the same location as in table 1.

From this finding, it was found that the pH value of 7.84 was still within the permissible range of 6.0-9.0. In addition, the NO_2^- and NO_3^- value were lacking sources for comparison purposes except for the study made by [24] for the NO_3^- which was lower than the NO_3^- value obtained in this study. As for the BOD, the value exceeded the standard limit of 20 mg/L. Next, the COD value obtained was below the standard limit.

3.2. Leachate treatment by single and mixed cyanobacterial isolates

The leachate treatment, phycoremediation considered in this study utilizes the cyanobacterial species to remove the pollutants present in the leachate sample had shown a significant result as in table 2. Percentage removal of BOD_5 and COD after 28 days of treatment with *Anabaena* sp. as shown in table 2 resulted in better percentage removal of BOD_5, 92.71% and COD, 105.71%, compared to when the leachate was treated with A1 only (49.49% of BOD_5 and 46.29% of COD removal) and mixture of

Anabaena sp. and A1 (45.04% of BOD_5 and 52.56% of COD removal). A previous study had shown that cyanobacteria species from the same order, *Nostoc* also capable to remove a high percentage of BOD_5 and COD in municipal wastewater with 96% of BOD_5 and 95% of COD [25]. Furthermore, this study observed that the mixed isolates showed the most promising capability of NH_3-N removal (85.08%) compared to the other two treatments.

Table 1. Comparison of raw leachate characterization from Jeram Sanitary Landfill

Parameters	pH	BOD_5 (mg/L)	COD (mg/L)	NH_3-N (mg/L)	NO_2^- (mg/L)	NO_3^- (mg/L)
*Standard	6.0-9.0	20	400	5	-	-
Raw Leachate	7.84	22.37	58.33	82.7	88.67	89.43
[33]	8.5	-	1020	-	-	-.
[34]	8.1	270	4637	-	-	-
[32]	7	3864	7598	55.8	-	27

*Environmental Quality (control of pollution from the solid waste transfer station and landfill) Regulation 2009 under the Laws of MEQA 1974.
- Not reported.

Table 2. Percentage removal of leachate BOD_5, COD, NH_3-N, NO^-_2, NO^-_3 and pH changes after treatment by *Anabaena* sp., sample A1 and mixture of both isolates

Treatments	Parameters	Levels in Untreated Leachate	Levels in Treated Leachate	Percentage Removal (%)
Anabaena sp.	BOD_5 (mg/L)	22.37	1.63 ± 0.50	92.71[b]
	COD (mg/L)	58.33	-3.33 ± 2.67	105.71[b]
	NH_3-N (mg/L)	1.81	0.92 ± 0.01	49.17[a]
	NO^-_2 (mg/L)	16.67	3.46 ± 3.15	79.24
	NO^-_3 (mg/L)	5.75	1.03 ± 0.39	82.09[c]
	pH	7.58	6.26 ± 0.02	-
A1	BOD_5 (mg/L)	22.37	11.30 ± 1.76	49.49[a]
	COD (mg/L)	58.33	31.33 ± 2.73	46.29[a]
	NH_3-N (mg/L)	1.81	0.40 ± 0.29	77.90[b]
	NO^-_2 (mg/L)	16.67	12.99 ± 1.88	21.08
	NO^-_3 (mg/L)	5.75	59.32 ± 4.87	-931.65[a]
	pH	7.58	6.88 ± 0.06	-
Mixed Isolates	BOD_5 (mg/L)	22.37	12.07 ± 0.37	45.04[a]
	COD (mg/L)	58.33	27.67 ± 1.45	52.56[a]
	NH_3-N (mg/L)	1.81	0.27 ± 0.02	85.08[c]
	NO^-_2 (mg/L)	16.67	15.16 ± 3.90	9.06
	NO^-_3 (mg/L)	5.75	33.65 ± 4.48	-485.22[b]
	pH	7.58	6.27 ± 0.01	-

Values expressed are means ± S.D. of triplicate measurements.
Values in the same column with different letters (a-c) were significantly different ($p<0.05$) based on each parameter.

Although the mixed isolates were capable to degrade NH_3-N the most efficiently among all, it shows that they can only remove a small amount of NO^-_2 (9.06%) from the leachate sample. As for the NO^-_3 removal, the production of NO^-_3 in the leachate sample when treated with A1 and mixed isolates kept on increasing to a very high value instead of being removed. Despite that, the removal of NO^-_3 by *Anabaena* sp. demonstrated a quite high percentage of 82.09%. Meanwhile, the pH value of the leachate sample in all treatments was reduced when compared to the pH value of untreated leachate. Moreover, based on the statistical analysis performed had shown that all parameters that have been carried out in this study except for NO^-_2 removal were significantly different at $p<0.05$.

Meanwhile, during the 28 days of treatment, it shows that the cyanobacterial isolates either individually or mixed were able to grow in the leachate media as shown in figure 1 below. Thus, this proved that the cyanobacteria species used in this study have a great capability in tolerating and withstanding the polluted condition and immense effectiveness in reducing highly organic and inorganic contaminants.

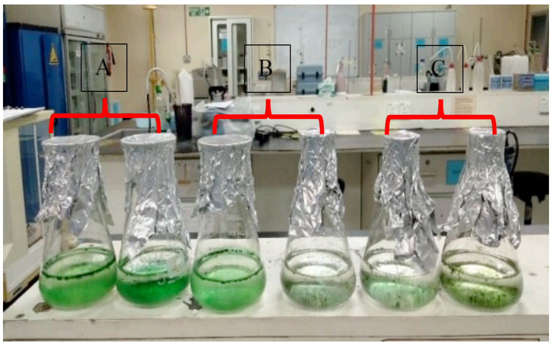

Figure 1. The growth of cyanobacterial isolates after 28 days of treatment at 20±2°C. A: Mixed isolates, B: Sample A1 and C: *Anabaena* sp.

4. Discussion

Landfilling is the most common option of waste disposal that has been practiced globally for many years regardless of its risks to the environment such as the NH_3 toxic pollution [23]. Many studies believe that the persistent industrial production growth and trade around the world have been contributing to the increase in municipal and industrial waste production[23]. It is predicted that in the year of 2025, the production of waste globally will reach almost double to 2.2 billion tons even though all cities around the world are trying really hard to reach their waste reduction targets [23, 24]. The high pH of the leachate may be caused by 82.7 mg/L of NH_3-N which was beyond the standard limit of 5 mg/L that allowed by MEQA. Apart from that, it shows that as the age of the landfill increases, the ammonia content increases and the pH becomes more alkaline. The same finding also has been reported in many studies associated with landfill leachate characterization around the world [26-31].

By comparing the BOD and COD data obtained with previous studies, the value showed great differences whereas their findings showed a similarity in range of value even though the sampling and leachate analysis were performed at different times. Both BOD and COD values from this study were relatively low compared to those in previous studies. This could be due to the leachate collection technique performed and the condition of the area chosen for the collection of leachate samples. The chemical properties of leachate samples may be also affected by hydrogeology, leachate temperature, and climate of the site including the seasonal variation [32, 33]. From this study, it was noted that the main pollutants in the leachate sample were ammonia loads that if left untreated may cause a great impact on the environment. High NO_3^- indicated that the leachate sample had high ammonia toxicity [31]. The same goes for the high NO_2^- and NO_3^- contents in the leachate sample may be a sign of high nitrite and nitrate toxicity. Although the organic pollutants based on the BOD and COD data did not show high value, it may also affect the environment if the leachate were to be discharged to the nearby environment. Therefore, it is suggested the leachate sample undergoes further treatment before it can be discharged.

This study shows that the removal of NH_3-N, NO_2^- and NO_3^- dependent with each other. Table 2 depicted that as the NH_3-N removal is high (49.17%-85.08%), the removal of both NO_2^-(9.06%-79.24) and NO_3^- (-931.65%-82.09%) becoming low and indicates that the amount of NO_2^- and NO_3^- in the leachate is increasing. This is because cyanobacterial species preferred the free, unionized ammonia (NH_3) or ammonium (NH_4^+) as their nitrogen source as the uptake and assimilation of both forms of ammonia require less energy compared to nitrite and nitrate [34]. Therefore, this revealed that the continuous presence of more than one nitrogen in a media, it may repress the uptake of the continuous presence of other nitrogen forms [34]. In addition to that, changes in pH in untreated (raw) and treated leachate from slightly alkaline (7.58) to acidic (6.26-6.88) indicates that the dominant species in the raw leachate was NH_3 because the relative amount of NH_3 increases with pH and suggesting the NH_3 is more toxic than NH_4^+ [34, 35].

The phylogenetic tree based on the 16S rRNA sequences has been constructed using the Maximum Likelihood algorithm and assessed with bootstrap test of 1000 replicates. Figure 2 shows the maximum likelihood phylogenetic tree of sample A1 in this study, 53 relative to the BLAST hits from the NCBI database based on the 16S rRNA sequences. The A1 in the diagram is the obtained cyanobacterial strain and based on the phylogenetic tree (figure 2), it can be concluded that the A1 in this study belongs to the genus of *Cyanobacterium*. This also supported the findings on the morphology observation made in this study that shows the similarity in common features of *Cyanobacterium*. However, further investigation is much more needed especially for the genus *Cyanobacterium* as only one species of *Cyanobacterium* (*C. stanieri* PCC 6308) has been validated in botanical classification system of cyanobacteria [36, 37].

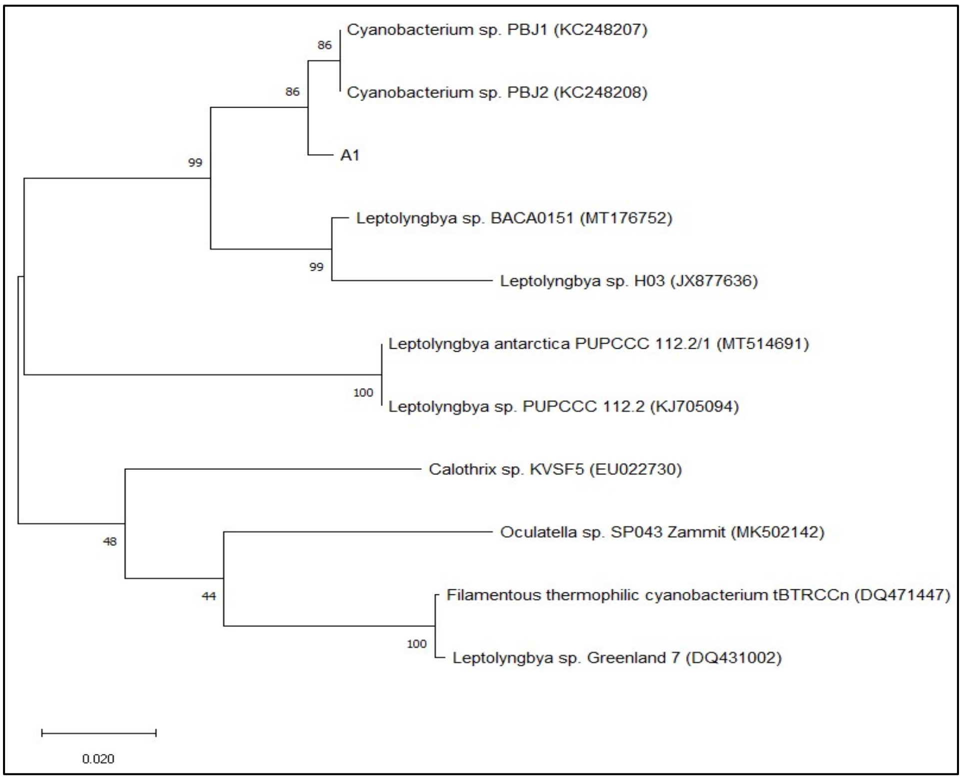

Figure 2. The Maximum Likelihood Phylogenetic Tree of Sample A1. The figure shows the phylogenetic tree of sample A1 relative to the BLAST hits from the NCBI database based on the 16S rRNA sequences

5. Conclusion

The growing amount of solid waste disposed in landfill has been contributing to environmental pollution and most of the conventional treatments were not able to fully degrade the pollutant content in the leachate. In fact, some of the treatments requires a high cost to be operated and maintained and some may produce other by-products that are harmful to human and environment. Thus, it has become an attention to the researchers to find the most cost-effective, ease of operating and maintaining and eco-friendly technique that can be used to remove leachate contaminants. Studies had proposed approaches in remediating landfill leachate pollution but here are still lacking evidence that shows the most effective approach that can be used to overcome this problem. Therefore, the findings from this study, may be an addition to the alternatives for leachate treatment especially for the application of *Anabaena* sp.

This research topic is not too old, recently many reports are published to treat solid waste, but most of the references are too old. I prefer to cite last 5 years articles and compare your results with those studies.

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