Biological treatment of closed landfill leachate treatment by using *Brevibacillus panacihumi* strain ZB1

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**Abstract.** Landfills are widely used for solid waste disposal due to cost effectiveness and ease of operation. Poor landfill management generally accompanied with production of toxic leachate. Leachate refers to heavily polluted liquid produced due to waste decomposition and rainwater percolation. Direct discharge of untreated leachate into the environment will lead to environmental degradation and health hazards. The aim of this study was to study the efficiency of leachate biological treatment by *B. panacihumi* strain ZB1. In this study, leachate wastewater was treated by *B. panacihumi* strain ZB1 via 42-days anaerobic-aerobic treatment. Leachate characterization of both raw and treated samples was carried out based on ammonia nitrogen content, chemical oxygen demand (COD) and heavy metal content. Through leachate characterization, raw leachate carried high concentrations of ammonia nitrogen (1977 mg/L), COD (5320 mg/L) and certain heavy metals exceeding discharge standard. From this study, *B. panacihumi* strain ZB1 able to remove COD nearly 40%, ammonia nitrogen nearly 50% and different degrees of heavy metals from the leachate sample after combined anaerobic-aerobic treatment. As a result, *B. panacihumi* strain ZB1 was expected to treat the leachate wastewater with certain treatment efficiency via combined anaerobic-aerobic treatment.

1. **Introduction**

Urbanization and industrialization attributed to huge amount of different kind municipal solid wastes generation. One of the methods that widely used for solid waste disposal is landfill due to economic advantage and simple operation [1]. The use of poor-managed landfill for waste disposal always accompanied with environmental and public hazards that should not be neglected due to production of toxic leachate wastewater. Leachate refers to the liquid produced when the rainwater seeps through the landfill sites [1] or liquid generated due to waste decomposition process [2]. Leachate is heavily polluted, highly toxic and strong odour liquid which composition is varying due to the disposal of different wastes [1]. Leachate will leach from the landfill site, seep through the soil layers and enter the environment especially the aquifer [3]. Once the leachate leached into the environment, environmental hazards such as water pollution and public hazards such as waterborne disease outbreak might be occurred.
Polluted leachate will carry different concentration of contaminants such as organic matters and heavy metals depend on the waste composition [2]. The contaminants that present in the leachate wastewater can be divided into biodegradable and hard-to-biodegrade compounds. Accumulation of high amounts of pollutants in the leachate wastewater will increase the leachate toxicity and treatment difficulty. Besides that, leachate also carrying low doses of pathogenic microorganisms such as *Escherichia coli* that may lead to health hazard when discharging into the environment [4]. As stated by Centre for Disease Control and Prevention [5], pathogens will affect the human health via ingestion of contaminated foods, consumption of contaminated drinking water and skin contact with polluted raw water source. Due to leachate toxicity, leachate needs to be treated properly before discharging into the environment.

There are several methods that can be used to treat the leachate, divided into biological, physical and chemical methods [6, 7]. Although both physical and chemical methods are efficient to treat the leachate within short period, both methods are costly and may lead to unpredictable adverse impacts [8]. Hence, biological method is recommended for long-term leachate treatment. Biological treatment involved use of microorganisms or microbial metabolic products under anaerobic, aerobic or both conditions to degrade or mineralize the pollutants present in the leachate wastewater [9]. Via combined anaerobic-aerobic treatment process, the efficiency to remove the pollutants from leachate wastewater can be improved as compared to sole anaerobic or aerobic process [10]. According to Ağdağ and Sponza [10], significant removal of ammonia nitrogen cannot be achieved via anaerobic treatment, but aerobic treatment process does. Hence, in order to improve the overall pollutant removal rate, combined anaerobic-aerobic treatment process is suggested for leachate management.

The aim of this research is to study the efficiency of leachate wastewater management by using biological approach. Parameter characterization will be carried out to study the treatment efficiency. The parameters involved in efficiency measurement are ammonia nitrogen content, chemical oxygen demands (COD) and heavy metal contents. By comparing the measured parameters before and after biological treatment, the efficiency of particular biological treatment to produce the effluent that suitable for direct discharge into the environment or further treatment in municipal wastewater treatment system can be studied.

2. Materials and Methods

2.1 Leachate sampling

The leachate wastewater sample was collected from local closed landfill located southwest Johor, Malaysia. Leachate sample was collected via compositing sampling method. The leachate samples at different sampling points were collected into the collection bottle and homogenized by inverted mixing. After sampling, the leachate collection bottle was securely sealed and stored in the cold room at -80 °C.

2.2 Preparation of bacterial inoculum

The microorganism that used in this study was *B. panacihumi* strain ZB1. Two pure colonies of *B. panacihumi* strain ZB1 were grown in sterile nutrient broth in the incubator shaker for 24 hours. After 24 hours incubation, the optical density of bacterial inoculum was measured at 600nm. *B. panacihumi* strain ZB1 inoculum with optical density more than 1.000 at 600nm would be used for leachate treatment.

2.3 Experiment procedure

About 10% (v/v) of the *B. panacihumi* strain ZB1 culture was used to treat the raw leachate sample in the 200 mL conical flask. The leachate sample was treated anaerobically for 21 days and followed by 21-days aerobic treatment. Leachate treatment was assessed in triplicate samples and control sample would not receive any culture during treatment session. Both control and treatment samples were treated under identical conditions and incubated in the incubator at 37 °C.
2.4 Analytical methods

Both raw, control and treated leachate samples were centrifuged at 4000 rpm for 15 minutes and the supernatant were used for ammonia nitrogen and COD analysis by using HACH DR 6000 spectrophotometer. The ammonia nitrogen content was analysed by Nesslerization method [11] and COD content was analysed by reactor digestion method [12]. The heavy metals level present in the leachate samples were analyzed by using Perkin Elmer Elan 6100 ICP-MS. The samples used for heavy metal analysis were acidified to pH less than pH 2 by using concentrated nitric acid solution and filtered through 0.2 μm filter membrane [13]. The efficiency of leachate treatment process was measured by calculated the overall removal efficiency as followed [14]:

\[
\text{Removal efficiency} = \frac{\text{Initial concentration} - \text{Final concentration (mg/L)}}{\text{Initial concentration (mg/L)}} \times 100\%
\]

3. Results and discussion

3.1 Raw leachate sample characterization

The characterisation of raw leachate sample was carried out and summarized as shown in the table 1. The parameters value measured was similar with publication by other researchers [13, 15] and hence, the leachate wastewater could be categorized as mature and stabilized leachate. The leachate composition especially the biodegradable portion was greatly affected as leachate aged [16]. Mature leachate would have high concentration of non-biodegradable COD and ammonia nitrogen. As comparing to the discharge standard limit by Malaysia [17], the pollutants concentration of raw leachate sample was stand out by exceeded the discharge standard. Hence, raw leachate cannot be discharged directly into the environment without any treatment.

| Parameter        | Value     | Discharge standard |
|------------------|-----------|--------------------|
| pH               | 8.58      | 6.0 – 9.0          |
| COD              | 5320 mg/L | 400 mg/L           |
| Ammonia nitrogen | 1977 mg/L | 5 mg/L             |
| Magnesium        | 8.761     | -                  |
| Vanadium         | 0.020     | -                  |
| Manganese        | 0.058     | 0.20               |
| Nickel           | 0.045     | 0.20               |
| Cobalt           | 0.006     | -                  |
| Copper           | 0.063     | 0.20               |
| Gallium          | 0.004     | -                  |
| Arsenic          | 0.011     | 0.05               |
| Selenium         | 0.032     | 0.02               |
| Rubidium         | 0.128     | -                  |
| Strontium        | 0.065     | -                  |
| Caesium          | 0.006     | -                  |
| Barium           | 0.054     | 1.00               |
| Cadmium          | 0.042     | 0.01               |
| Lead             | 0.422     | 0.10               |
| Bismuth          | 0.015     | -                  |
| Uranium          | 0.003     | -                  |
3.2 Ammonia nitrogen and COD analysis
The ammonia nitrogen and COD concentration of treated leachate sample were summarized in the figure 1. As comparing with other research studies [18, 19], high COD removal could be achieved by anaerobic treatment and further enhanced by aerobic treatment, while high ammonia nitrogen removal could be achieved by aerobic treatment. Hence, combined anaerobic-aerobic treatment process could be used to improve overall removal efficiency.

![Figure 1](image.png)

**Figure 1.** Concentration of ammonia nitrogen and COD present in the leachate sample.

3.2.1 Ammonia nitrogen analysis. Without biological treatment, natural attenuation process and indigenous microbial activity were contributed to slight removal of ammonia nitrogen from the leachate sample (control sample) as shown in the table 2.

| Sample  | After anaerobic treatment | After aerobic treatment | Overall removal |
|---------|--------------------------|-------------------------|-----------------|
| Control | Decreased by 10%         | Decreased by 15%        | 23.7%           |
| Treatment | Increased by 10.6% | Decreased by 52% | 47%             |

Since the ammonia nitrogen removal rate was negligible, raw leachate sample needed to be subjected to proper treatment for discharging. As shown in the table 2, the ammonia nitrogen concentration present in the treated sample was increased after anaerobic treatment and further aerobic treatment able to reduce the ammonia nitrogen from the leachate sample. Overall ammonia nitrogen removed from treated sample after combined anaerobic-aerobic treatment was 47%. As compared to control sample (referred to table 2), increase of ammonia nitrogen concentration in the treated sample after anaerobic treatment might be contributed by degradation of soluble nitrogen fraction of organic compounds [20]. During aerobic treatment, the ammonia nitrogen was assimilated by biomass for biosynthesis and removed via nitrification [20].
3.2.2 COD analysis.
Organic matters were attributed to high COD concentration. COD in the leachate sample can be classified into readily biodegradable soluble COD and inert COD [21]. Readily biodegradable carbonaceous organic matters can be degraded by microorganisms, used for biomass growth and metabolic activity or converting into carbon dioxide and sludge [22, 23]. Refractory pollutants and wastes produced by microbial activities such as cell lysis are attributed to inert COD [23]. After 42-days treatment, the overall removal of COD from both control and treated sample was shown in the table 3.

Table 3. COD removal rate of control and treated samples after biological treatment.

| Sample    | After anaerobic treatment | After aerobic treatment | Overall removal |
|-----------|---------------------------|-------------------------|-----------------|
| Control   | Decreased by 36 %         | Decreased by 4 %        | 38.8%           |
| Treatment | Decreased by 27.3%        | Decreased by 14%        | 37.4%           |

As observed in the figure 1 and table 3, higher COD removal rate was achieved during anaerobic treatment as compared to aerobic treatment. This phenomenon shows that organic matter degradation mainly occurs during anaerobic condition [24] and contributed to the increase of ammonia nitrogen concentration after anaerobic treatment (as shown in figure 1). According to Abu Bakar et al. [23], anaerobic treatment enables prior removal of organic compounds and toxic compounds from the leachate sample to reduce the inhibitory effect on microbial degradation activity. Residual COD that present in the treated sample could be contributed by inert COD originate from leachate sample and microbial metabolic products included dead cells’ debris [10].

3.3 Heavy metals analysis
According to Sprocati et al. [25], microorganisms able to remove or degrade the heavy metals from the leachate sample if microorganisms carrying certain metabolic traits such as heavy metal resistance ability or mechanism that able to protect the microbes from heavy metal toxicity [26]. Figure 2 shows the overall heavy metals removal rate from the treatment samples after 42-days treatment.

Figure 2. Overall removal of heavy metals from treatment sample treated by *B. panacihumi* strain ZB1.
As shown in the figure 2, *B. panacihumi* strain ZB1 able to reduce different degrees of heavy metals from leachate sample. *B. panacihumi* strain ZB1 achieved high removal efficiency for magnesium (74% to 2.284 mg/L), manganese (40% to 0.034 mg/L), copper (60% to 0.0063 mg/L), selenium (52% to 0.0014 mg/L) and bismuth (44% to 0.0087 mg/L) from leachate sample. Microbial removal of heavy metals can be done via surface adsorption, biosorption or intracellular bioaccumulation and extracellular precipitation [27, 28]. *B. panacihumi* strain ZB1 expected had high heavy metal adsorptive capacity due to presence of thick layer of peptidoglycan and teichoic acids in the cell wall [26].

4. Conclusion
Use of landfills for solid wastes disposal will lead to toxic leachate production and proper leachate treatment method(s) should be selected wisely to avoid any grave consequences of leachate. For this research study, *B. panacihumi* strain ZB1 able to achieve 37% COD removal, 47% ammonia nitrogen removal and different degrees of heavy metals removal from the leachate sample after combined anaerobic-aerobic treatment. Hence, *B. panacihumi* strain ZB1 was expected had the ability to treat the leachate sample with certain treatment efficiency. Since there will be no single treatment method can be used forever for efficient leachate treatment, exploration of new and more effective treatment methods should be carried out continuously to maintain leachate treatment efficiency, protect the environment and improve public health and safety.

Acknowledgements
The completion of this research study required nice support and the authors gratefully acknowledge the Fundamental Research Grant Scheme (FRGS) grant vote 1550 for financial support.

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