Treatement of Landfill Leachate Using *Ganoderma Australe* Mycelia Immobilized on Ecomat

Noorlidah Abdullah, Wan Razarinah W. A. R., Noor Zalina Mahmood, and Rosna Mat Taha

Abstract—Biological processes have been proven to be efficient in treating landfill leachates. In this study, treatment of landfill leachate by Ecomat-immobilized mycelia of *Ganoderma australe* packed in a column was investigated. Continuous recycling of leachate at a constant flow (20 ml/min) was operated for 10 cycles to facilitate biological reactions. Diluted leachate (50%) and raw leachate (100%) were tested for comparison of efficiency of treatment. The results showed that biological oxygen demand (BOD₅) was not removed for diluted leachate and a slight removal of 0.14 and 1.72% for raw leachate after cycles 4 and 10, respectively. Chemical oxygen demand (COD) removal occurred after each cycle with diluted leachate demonstrating higher removal compared to raw leachate. The highest percentage of COD removal of 51.62% for diluted leachate and 22.79% for raw leachate were achieved after the tenth cycle. Ecomat-immobilized mycelia of *Ganoderma australe* could also reduce ammoniacal nitrogen (NH₃-N) exhibiting highest reduction of 45.95% and 30.90% after cycle 8 for diluted and raw leachate, respectively. These findings suggested that a white rot fungus, *G. australe* has the ability to be considered as potential candidate in landfill leachate treatment.

Index Terms—Fungal enzymes, ammoniacal nitrogen, chemical oxygen demand, biological treatment, white-rot fungi.

I. INTRODUCTION
Sanitary landfill leachate is a highly polluted and complex wastewater. Its quality is the result of biological, chemical and physical processes in landfills combined with the specific waste composition and the landfill water regime [1]. Biological treatment is the most common practice for leachate treatment worldwide but the remaining values of COD and adsorbable organic halogenated compounds (AOX) are still relatively high [2]. With increasing leachate effluent quality standards the efforts for leachate treatment also increase. White-rot basidiomycetous fungi have been implicated in the transformation of a large amount of organopollutants structurally related to lignin [3], [4]. Besides that, the use of fungal cultures to transform various chemical compounds has already been reported in several studies [5]. White rot fungi are capable of producing extracellular lignin peroxidase (LiP) and manganese–dependent peroxidase (MnP) that is essential for lignin degradation. Gold, and Alic, [6] demonstrated white rot fungi such as *Phanerochaete chrysosporium* typically secrete one or more of the three principal ligninolytic enzymes, i.e. lignin peroxidase (LiP, E.C. 1.11.1.14), Mn-dependent peroxidase (MnP, E.C. 1.11.1.13) and phenol oxidase (Laccase) (Lac, E.C. 1.10.3.2). These enzymes are able to oxidize a variety of high-priority aromatic pollutants, such as polycyclic aromatic hydrocarbons [7], chloromatics and polyaromatic dyes [8]. Therefore, white rot fungi are of current interest to be used in the bioremediation of a broad spectrum of persistent xenobiotic and to treat wastewater, including landfill leachate.

Mohammadi and Nasernejad, [9] had reported the limitation of using *P. chrysosporium* free cells in the biodegradation process therefore, cell immobilization offers a suitable alternative. Immobilized cultures tend to have higher level of enzymes activity and were more resilient to environmental perturbations such as pH or exposure to toxic chemicals compared to free cell culture. According to Ramakrishna and Prakasham, [10], immobilization of cells is the attachment of cells or their inclusion in distinct solid phase that permits exchange of substrates, products, inhibitors, etc., but at the same time separates the catalytic cell biomass from the bulk phase containing substrates and products. Mycelial immobilization has been used for the production of ligninolytic enzymes and bioremediation of pollutants. To our knowledge, *G. australe* has not been investigated for it’s potential use in bioremediation. Thus, the objective of this study is to investigate the ability of mycelium of a white-rot fungus, *Ganoderma australe* immobilized on Ecomat and packed in a column to remediate leachate.

II. MATERIALS AND METHODS

A. Fungal Culture

*Ganoderma australe* was maintained on malt extract agar (MEA) slants, and mycelial culture was prepared by growing on MEA in Petri plates for 7 days at 28 ± 2 °C.

B. Productivity of Extracellular Enzymes by G. Australe

Seven days old mycelium of *G. australe* was inoculated into 250-ml flasks containing sterile glucose-yeast-malt-peptone (GYMP) liquid medium added with 0.1% of skim milk, olive oil, veratryl alcohol, MnCl₂, glucose-yeast-malt-peptone (GYMP) liquid medium added with 0.1% of skim milk, olive oil, veratryl alcohol, MnCl₂, glucose-yeast-malt-peptone (GYMP) liquid medium added with 0.1% of skim milk, olive oil, veratryl alcohol, MnCl₂.
CuSO₄ and starch. The GYMP growth medium contained the following: MgSO₄.7H₂O (1.0 g/L); KH₂PO₄ (1.0 g/L); K₂HPO₄ (1.0 g/L); NH₄Cl (1.0 g/L); Glucose (15.0 g/L); Peptone (8.0 g/L); Yeast extract (8.0 g/L); and Malt extract (8.0 g/L). Inoculated medium was incubated at 28 ± 2 °C shaking at 150 rpm for four days. Then, extracellular enzymes were extracted with 50 mM sodium-phosphate buffer at pH 6.5 for one hour shaking at 150 rpm. The mixture was then filtered through 1.5 μm Whatman Millipore filter and the supernatant was analysed for enzyme activity.

C. Enzymes Assays

Lignin peroxidase (LiP) activity was determined by monitoring the conversion of veratryl alcohol to veratryl aldehyde at 25 °C by hydrogen peroxide (H₂O₂) [11]. Manganese peroxidase (MnP) activity was measured using guaiacol as a substrate. Laccase activity was determined by the increase in the absorbance at λ = 525. This was due to the production of tetramethoxy-azo-bis-methylenequinone resulting from the reaction of laccase with syringaldazine [12], [13]. Protease activity was determined by mixing 0.5 ml of enzyme with 0.5 ml of 0.5% azocasein as the substrate. Lipase activity was determined with p-nitrophenyl palmitate by the method reported by Savitha et al. [14]. Amylase activity was determined using 1% soluble starch in citrate-phosphate buffer (pH 6.5) as substrate [15].

D. Preparation of Mycelium Suspension and Immobilization of Mycelium on Ecomat

Four mycelial plugs of 6-mm² diameter cut from the periphery of a 7-day old colony growing on MEA were transferred into 250-ml Erlenmeyer culture flasks containing 100 mls of sterile GYMP. The pH of the media was adjusted to 6 before autoclaving. Inoculated flasks were then agitated on an orbital shaker at 150 rpm for 48 h at 28 ± 2 °C to obtain mycelial suspension.

Immobilization was done on Ecomat, a high-tech organic fibres made from 100 % oil palm empty fruit bunches (manufactured by Ecofibre Technology, Malaysia) autoclaved for 1 hr. Four pieces of Ecomat of 1 mm thickness was cut into 2 x 2 cm squares and added into 250-ml Erlenmeyer culture flasks containing 50 mls of GYMP medium and autoclaved. Then when cooled, 5 mls of mycelium suspension was transferred aseptically and the flasks were agitated at 100 rpm on an orbital shaker. The Ecomat was covered with fungal mycelium after 4 days of incubation and was used for leachate treatment.

E. Leachate Sample

Leachate sample used in this experiment was collected from the leachate pond at the sanitary landfill. The leachate was filtered to remove suspended solids and was analyzed for pH, COD, BOD₅, and NH₃-N according to the Standard Method for the Examination of Water and Wastewater [16] using Hach DR 2800 spectrophotometer.

F. Treatment of Leachate Using Ecomat-Immobile G. australe Mycelia Pack in Column

The experiment used diluted (50%) and raw leachate (100%) passed through a glass column packed with Ecomat-immobilized mycelium separately. Column used was 40 mm diameter and 500 mm height. A total of 30 pieces of Ecomat-immobilized mycelia were arranged and packed. Treated leachate was compared to leachate passed through column packed with sterile Ecomat without fungal mycelium (control).

During treatment, 1 litre of leachate was passed through the column at the flow rate adjusted to 20 ml per minute operated at room temperature. Treatment of leachate was conducted for ten cycles and after two cycles the leachate was analysed for pH, BOD₅, COD and NH₃-N. The experimental set-up of Ecomat-immobilized G. australe in column packing is shown in Fig. 1.

G. Leachate Analysis

The degradation of leachate was determined by measuring the percentage removal of leachate contaminants denoted as BOD, COD, NH₃-N and changes of pH. These contaminants were analysed in accordance with the Standard Method for the Examination of Water and Wastewater [16] using Hach DR 2800 spectrophotometer.

III. RESULTS

Extracellular enzymes produced by four-days old G. australe mycelium are shown in Table I. Results revealed that G. australe produced ligninolytic enzymes with the highest production of MnP (45.83 ± 1.81 U/ml) followed by laccase (21.93 ± 0.79 U/ml) that are the important enzymes for pollutant degradation. A significant productivity of protease (4.23 ± 0.42 U/ml) and LiP (1.30 ± 0.60 U/ml) were also observed. In addition, G. australe also exhibited low titers of lipase (0.47 ± 0.24 U/ml) and amylase (0.39 ± 0.02 U/ml).

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Enzyme activity (U/ml)</th>
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</thead>
<tbody>
<tr>
<td>LiP</td>
<td>1.30 ± 0.60</td>
</tr>
<tr>
<td>MnP</td>
<td>45.83 ± 1.81</td>
</tr>
<tr>
<td>Laccase</td>
<td>21.93 ± 0.79</td>
</tr>
<tr>
<td>Protease</td>
<td>4.23 ± 0.42</td>
</tr>
<tr>
<td>Lipase</td>
<td>0.47 ± 0.24</td>
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<tr>
<td>Amylase</td>
<td>0.39 ± 0.02</td>
</tr>
</tbody>
</table>

Fig. 1. Experimental set-up of Ecomat-immobilized G. australe packed in a glass column for the treatment of leachate.
The biological treatment of leachate in column packed with Ecomat-immobilized \textit{G. australe} mycelium was tested on 50% diluted and raw leachate. Leachate was run through the column continuously at constant flow of 20 ml/min and was recycled for 10 times. Analysis was done repeatedly after 2 cycles and the leachate parameters are shown in Table II. BOD\textsubscript{5} content in both 50% and raw leachate was not significantly removed after each cycle except 0.14% and 1.72% removal after 4 and 10 cycles respectively, for raw leachate.

The leachate COD removal was determined by comparing the COD content for each cycle with untreated leachate (control) (Table II). For both 50% and raw leachates, COD was significantly removed to 1645 mg/l (51.62% removal) for diluted and 2625 mg/l (22.79% removal) for raw leachate, respectively.

The degradation of leachate ammoniacal nitrogen (NH\textsubscript{3}-N) by immobilized \textit{G. australe} mycelium was also achieved in this study (Table II). The highest percentage of NH\textsubscript{3}-N removal for both leachates occurred at cycle 8 with 45.95% and 30.90% removals for diluted and raw leachates, respectively.

Treatment of leachate by immobilized \textit{G. australe} in column showed not much change in pH of leachate after each cycle for both diluted and raw leachates.

### TABLE II: PERCENTAGE REMOVAL OF BOD\textsubscript{5}, COD, NH\textsubscript{3}-N AND PH CHANGES IN DILUTED (50%) AND RAW LEACHATE AFTER TREATMENT BY ECOMAT-IMMOBILIZED \textit{G. AUSTRALE} IN COLUMN PACKING AT ROOM TEMPERATURE

<table>
<thead>
<tr>
<th>Cycle (C)</th>
<th>Parameters</th>
<th>Levels in Untreated Leachate Control</th>
<th>Levels in Treated Leachate</th>
<th>% Increase / Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Raw 50 %</td>
<td>Raw 50 %</td>
<td>Raw 50 %</td>
</tr>
<tr>
<td>C2</td>
<td>BOD\textsubscript{5} (mg/l)</td>
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<td>3495.00</td>
<td>3505.00</td>
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<tr>
<td></td>
<td>COD (mg/l)</td>
<td>3250.00</td>
<td>3175.00</td>
<td>2705.00</td>
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<tr>
<td></td>
<td>NH\textsubscript{3}-N (mg/l)</td>
<td>26.60</td>
<td>29.20</td>
<td>16.00</td>
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<tr>
<td></td>
<td>pH</td>
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<td>8.07</td>
<td>8.01</td>
</tr>
<tr>
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<td>3175.00</td>
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</tr>
<tr>
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<td></td>
<td>NH\textsubscript{3}-N (mg/l)</td>
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<td></td>
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<td>3210.00</td>
<td>2050.00</td>
</tr>
<tr>
<td></td>
<td>NH\textsubscript{3}-N (mg/l)</td>
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<td></td>
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<td>3505.00</td>
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<td>3350.00</td>
<td>2080.00</td>
</tr>
<tr>
<td></td>
<td>NH\textsubscript{3}-N (mg/l)</td>
<td>30.90</td>
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<tr>
<td></td>
<td>pH</td>
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<td>8.22</td>
<td>8.13</td>
</tr>
<tr>
<td>C10</td>
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<td>3440.00</td>
<td>3510.00</td>
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<td></td>
<td>COD (mg/l)</td>
<td>3400.00</td>
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<tr>
<td></td>
<td>NH\textsubscript{3}-N (mg/l)</td>
<td>29.90</td>
<td>23.60</td>
<td>23.80</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>8.05</td>
<td>8.28</td>
<td>8.12</td>
</tr>
</tbody>
</table>

- indicates reduced (removed); + indicates increased

### IV. DISCUSSION

White-rot fungi \textit{G. australe} was used in this study to determine the ability to remove BOD\textsubscript{5}, COD, and NH\textsubscript{3}-N from leachate. This study was conducted due to the fact that biological processes play a major role in the attenuation and detoxification of organic contaminants in the environment [9].

Previous study done on pellets (free cell) and immobilized cells of \textit{Trametes versicolor} showed that removal efficiency of BOD\textsubscript{5} and COD in leachate was found to be higher in the case of immobilized fungi compared to free cell [17]. This is because immobilization of fungal cells could stably maintain the production of various enzymes at levels higher than achieved with pellet form. Other study by Lapadatescu \textit{et al.}, [18] revealed that the immobilization of \textit{Bjerkandera adusta} on polyurethane foam for the production of enzymes by fungi was influenced by their cultivation method. Beshay, reported that the production of alkaline protease by \textit{T. turnirae} was not good when cultivated in submerged cultures, since the enzyme titters were relatively low [19]. However, in a study by Lapadatescu \textit{et al.}, which compared two strains of \textit{P. chrysosporium} BKM-F-1767 and INA-12 as free cells or under immobilized cell culture conditions, they found that when the fungus was immobilized, lignin and Mn peroxidases production were increased 2-3-fold and productivity 3-4-fold, respectively [19]. According to Omar, \textit{et al.}, [20] this could be attributed to the effect of shear forces and/or culture techniques on fungal morphology and fungal metabolism [21].

In this study, the treatment of leachate in column using immobilized \textit{G. australe} mycelia on Ecomat revealed that BOD\textsubscript{5} was not removed. This may be due to the short exposure of leachate to the immobilized mycelia at the flow-rate used. Fungal mycelia require adaptation for the enzymes to act. [17]. However, COD removal occurred at most of the cycles and diluted leachate demonstrated higher COD removal after each cycles compared to raw leachate. Similar results were found by Saetang and Babel who found
that removal of BOD and COD was lower when the leachate was diluted 5 times compared to concentrated leachate [17].

For both concentrated and diluted leachate showed that COD removal is higher than BOD removal in most cases. This finding is in accordance with the findings by Saetang and Babel that obtained higher COD removal compared to BOD in their study [17].

The degradation of leachate ammoniacal nitrogen (NH\textsubscript{3}-N) by immobilized G. australa was also achieved in this study (Table II). The highest percentage of NH\textsubscript{3}-N removal for both leachate occurred at cycle 8 with 45.95% and 30.90% removals for diluted and raw leachates, respectively.

Treatment of leachate by immobilized G. australa in column showed not much change in pH of leachate after each cycle for both diluted and raw leachates. In contrast, degradation of leachate by immobilized G. australa in column showed that the pH of leachate increased from one cycle to another in both diluted and raw leachates. Rodriguez et al., [22] stated that the pH increase is an indicator of ammonia production.

In the column packing, immobilized mycelia of G. australa was applied since immobilization can eliminate most of the constraints faced by the free-cell systems such as it can facilitates operation of microbial fermentation on continuous mode without cell washout [10]. Immobilization of G. australa on Ecomat, a natural support offer advantages as they are compostable and inexpensive. Ecomat is a waste product of palm oil industry, whereby Ecomat could be composted once they are no longer useful as immobilization support.

Several researchers have reported success in leachate treatment using different methods previously. Secondary biological treatment from leachate of municipal solid waste landfill was done on supernatant liquid obtained after physicochemical processes and coagulating with Al\textsuperscript{3+} followed by ammoniacal stripping. The continuous experiment treatments made in an aerobic digester after physicochemical processes showed removal efficiency of COD between 69 and 83% [23]. Aziz, et al., [24] reported that about 40% of ammoniacal nitrogen with concentration of more than 1000 mg/l could be removed by activated carbon that about 40% of ammoniacal nitrogen with concentration of about 90 %, respectively, when LFL underwent a two-fold dilution.

Phanerochaete chrysosporium [26] demonstrated that leachate degradation could be related with the production of enzymes. Table I revealed that G. australa produced the ligninolytic enzymes (MnP, laccase and LiP) important in pollutants degradation. The ability of enzymes to degrade leachate was also supported by Zouboulis et al., [27].

V. CONCLUSION

This study revealed that treatment of leachate was feasible using a white-rot fungus, Ganoderma australa mycelium immobilized on Ecomat in a column. BOD\textsubscript{5} was not removed in diluted leachate nevertheless; in concentrated leachate, 0.43 % and 1.58 % BOD\textsubscript{5} removal occurred at cycle 4 and 10, respectively. However, COD was removed after each cycle and diluted leachate demonstrated higher COD removal ranging from 16.77 – 51.62% compared to raw leachate ranging from 1.83 – 22.79%. Removal of ammoniacal nitrogen occurred for both diluted and raw leachate with the highest percentage was achieved at cycle 8 (45.95%) for diluted leachate and 30.90% for raw leachate. Based on the results obtained, it is suggested that the percentage degradation of leachate in the column can be improved by reducing the flowrate so that it can increased the exposure time of mycelia enzymes to leachate.

ACKNOWLEDGMENT

This work is part of an on-going PhD thesis to be submitted to Faculty of Science, University of Malaya.

REFERENCES


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