Screening Method for Selecting the Potential Fungi for Use in the Bioremediation of Leachate

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***Abstract*—This study focuses on a screening method for selecting the potential fungi which has ability to be used in bioremediation of leachate. In this study, twelve fungal species were tested for their ability to grow on 50% and 100% leachate incorporated with malt extract agar (MEA) and also the affect of pH medium on fungi growth. The radial growth was measured every 48 h for a period of 30 days after incubation at 30 °C, and the growth rates were determined. Results show no growth was observed by all fungi species on malt extract agar incorporated with 100% leachate except for *Ganoderma australe*, *Trametes menziesii* (KUM 7011(54)), *Penicillium* sp., and *Pycnoporus sanguineus*. These fungi give growth rates of 6.1 ± 0.8 mm, 5.9 ± 0.6 mm, 2.3 ± 0.3 mm and 0.8 ± 0.3 mm respectively. On 50% leachate, the growth of *Trametes menziesii* shows the most rapid followed by *Ganoderma australe* and *Penicillium sp.* with growth rates of 7.4 ± 0.3, mm 6.9 ± 0.3 mm, and 2.9 ± 0.0 mm respectively. Besides, the comparison growth of *Penicillium* sp., *Trametes menziesii*, and *Ganoderma australe* on the medium at pH 6.0 – 6.5 and nonadjusted pH medium show slightly difference. They give the growth rates of 3.7 ± 0 and 2.9 ± 0, 7.0 ± 0.1 and 7.4 ± 0.3, 6.1 ± 0.3 and 6.4 ± 0.1 respectively. Therefore, these fungi species show the ability to grow on leachate and can be considered to have the potential to be used in the bioremediation of leachate.**

Keywords-Leachate, Fungi, Bioremediation

I. INTRODUCTION

An ever-expanding population and high rates of economic development in Malaysia has resulted in the generation of a vast amount of waste. A majority (about 76%) of waste produced is collected and 1-2% of the collected waste is recycled and the remainder is taken to disposal site (landfill) [1]. Landfill is one of the main methods for disposing of municipal and industrial solid waste. The degrading of organic fraction of the waste in the landfill in combination with the percolation of rainwater produces a polluted liquid called leachate [2].

Leachate emitted by the landfill is likely to contain various types of pollutants. The pollutants in leachate are characterize as a water-based solution of four groups of contaminants. First group is dissolved organic matter (alcohol, acids, aldehydes, short chain sugar etc). Second is inorganic macro components (common cations and anions including sulphate, chloride, iron, aluminium, zinc and ammonia). Third, heavy metals (Pb, Ni, Cu, Hg) and the fourth group is xenobiotic organic compounds such as halogenated organics (PCB, dioxin etc). This will caused a great threat to the surroundings environments such as soil, ground water and even surfacewater. According to [3], leachate has been a highly charged subject in Malaysia over the past few years. Leachate is getting into rivers and from there into water supplies and imparting a bad taste into drinking water in many thousands of homes. [4] Reported high concentrations of PAHs were detected in the leachate, river particulate and sediment samples for Taman Beringin and Ulu Maasop landfills sites.

Bioremediation is defined as the application of biological to treat pollution. Biological treatment methods are processes whereby microbes are used to destroy or at least reduce the toxicity of a waste stream. Typically, contaminants are transforming or removed from landfill leachate through biological treatment and physical-chemical treatment. The biological are best suit for transforming or removing organic matter and ammonia from landfill leachate. Organic matter in a substrate, such as landfill leachate , is transformed biologically when a variety of microorganisms interface with organic matter [5].

Aerobic biological processes have been the most successful and reliable treatment for the landfill leachate [6]. Due to its reliability, simplicity and high cost-effectiveness, biological treatment (suspended/attached growth) is commonly use for the removal of the bulk of leachate containing high concentrations of BOD [7].

Various microbial organisms have been used to remove pollutants from the environment. Most researches have focused on bacteria, with fungal application only attracting interest just within the past two decades. Using of biodegradable abilities of some white rot fungi is promising. Researchers are now focusing on white rot fungi for use in bioremediation since these organisms have the ability to degrade a wide range of environmental pollutions ([8]; [9]; [10]; [11]). Some characteristics of filamentous fungi (e.g. a specific bioactivity and growth morphology) enable them to

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be better potential degraders than bacteria [12]. White-rot fungi are characterized by their ability to degrade lignin, which is a high-molecular weight complex polymer in wood [13].

Because of the non-specific nature of the lignin oxidation system, white-rot fungi are normally capable of oxidising a wide spectrum of xenobiotic compounds. Therefore, bioaugmentation with white-rot fungi could, in theory, be used to enhance the bioremediation of pollutants.

II. MATERIALS AND METHODS

A. Fungal strains

Fungal strains used in this study were *Pleurotus eryngii* (KUM 50087), *Trichoderma sp., Rhizopus sp., Aspergillus sp., Pycnoporus* sanguineus, *Penicillium sp., Pleurotus ostreatus* (Thai) (KUM 50089), *Trametes sp.* (KUM 7011(54)), *Fomitopsis feei, Schizophyllum commune, Pleurotus sajor-caju* and *Ganoderma australe* (KUM 60860 (19)). All the strains were obtained from the Institute of Biological Sciences, Universiti Malaya, Malaysia. These strains were chosen since they show the ability in bioremediation. These fungi were grown in culture tubes containing Malt Extract Agar (MEA) at 25 ºC for a week.

B. Leachate

Raw leachate collected from the pond of untreated leachate at sanitary landfill in Selangor was used in the study. Leachate samples were stored in 50-litre polyethylene containers.

C. Growth *of* fungal strains

To obtain fungal growth rate data for each of the twelve fungal species, a 5-mm^2 piece of colonised malt extract agar (MEA) was taken from the edge of fungal colony. Then, placed it in the center of each ME agar plate which contains 50% and 100% leachate. After inoculation, plates were incubated at 25 ºC. Next, the colony radius from the edge of the MEA piece was measured every 48 h for 30 days to get the fungal growth rate for each fungal species in the different treatments.

D. Statistical analysis of the data

Data were analysed for the significant differences between treatments using analysis of variance (ANOVA). To prove if differences between individual treatments were significant ($P<0.05$), the test for least significant differences (LSD) were used (SPSS for Windows 14.0).

III. RESULTS AND DISCUSSION

Out of the twelve fungi studied only three fungi species which are *Pycnoporus sanguineus*, *Ganoderma australe* and *Penicillium* sp. showed the most prominent growth on MEA incorporated with both 50% and 100% leachate. Figure 1 shows the growth of *Ganoderma australe* on the MEA medium incorporated with 50% and 100% leachate.

Figure 2 shows the growth rates of twelve different fungal species on malt extract agar incorporated with 50% and 100% leachate. The growth rate of each fungal species was determined by measuring the radial growth every 48h for a period of 30 days after incubation at 25º C.

Result shows no growth was observed by all fungi species on malt extract agar incorporated with 100% leachate except *Ganoderma australe*, *Trametes menziesii* (KUM 7011(54)), *Penicillium* sp., and *Pycnoporus sanguineus*. The growth rates of these are fungi are 6.1 ± 0.8 mm, 5.9 ± 0.6 mm, 2.3 ± 0.3 mm and 0.8 ± 0.3 mm respectively. Trametes *menziesii* (KUM 7011(54)) and *Ganoderma australe* showed sensitivity towards 100% leachate, while growth of *Penicillium* sp. and *Pycnoporus sanguineus* was not significantly affected by the presence of 100% leachate.

Meanwhile for 50% leachate, the growth of *Trametes menziesii* shows the most rapid followed by *Ganoderma australe* and *Penicillium sp.* with growth rates of 7.4 ± 0.3, mm 6.9 ± 0.3 mm, and 2.9 ± 0.0 mm respectively. In the presence of 50% leachate, the growth of all the three fungal species is not significantly increase.

From the twelve fungal species tested, two (*Ganoderma australe,* and *Trametes menziesii*) can grew well in the presence of 100% leachate with growth rates between 5 to 7 mm day-1. While, four (*Trametes menziesii*, *Ganoderma australe, Penicillium* sp. and *Aspergillus* sp.) can grew well in the presence of 50% leachate with growth rates between 2 to 8 mm day^{-1}.

This suggests that two fungi species (*Ganoderma australe,* and Trametes *menziesii*) which show the ability to grow well on MEA (with both 50% and 100% leachate concentration) have the most potential to be used in bioremediation of leachate. Both fungi species are white rot fungi. According to [14], white-rot fungi are a physiological grouping of fungi that can degrade lignin (and lignin- like substances).

Through intensive study of lignolytic fungi, it has been determined that these organisms produce extracellular enzymes with very low substrate specificity. This makes them suitable for degradation of many different compounds, notably organopollutants with structural similarities to lignin (PAH, PCBs, TNT, DDT) [15]. Four main genera of white rot fungi have shown potential for bioremediation are *Phanerochaete*, *Trametes*, *Bjerkandera*, and *Pleurotus*. It is supported by [10] who stated that white-rot fungi have been shown to degrade a wide variety of environmental pollutants, including PCP.

A. Comparison of fungi growth on adjusted and nonadjusted pH of Malt extract agar medium containing 50% leachate

All twelve fungi species were used to study the effect of adjusted and nonadjusted pH of the medium growth which containing 50% of leachate. In this study malt extract agar was used as a growth medium. For the adjusted medium, the pH of the medium before autoclaving was kept at pH 6.0 – 6.5.

Figure 3 shows that adjusted medium give better growth for almost all the fungi compare to nonadjusted medium. All fungi can grow on adjusted medium but three fungi which are *Rhizopus*, *Pleurotus ostreatus* and *Fomitopsis feei* cannot grow on nonadjusted medium. The growth of *Penicillium*,

Trametes menziesii, and *Ganoderma australe* on adjusted and nonadjusted medium show slightly difference with growth rates 3.7 ± 0 and 2.9 ± 0, 7.0 ± 0.1 and 7.4 ± 0.3, 6.1 ± 0.3 and 6.4 ± 0.1 respectively. The growth of other fungi on adjusted medium shows higher compared to the growth on nonadjusted medium.

Based on this result it shows the growth of both most potential fungi in leachate bioremediation which are *Trametes menziesii*, and Ganoderma *australe* is not affected by the pH medium. Wood rotting fungi like *Trametes versicolor*, *Pholiota mutabilis*, *Pleurotus ostreatus*, *Phlebia radiate* and *Phenerochaete chrysosporium* prefer a slightly lower pH ranging 3.5-5.5 [16].

IV. CONCLUSION

Ganoderma australe, and *Trametes menziesii* can grow on malt extract agar incorporated with leachate therefore have the most potential to be used in leachate bioremediation.

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Ganoderma australe on 50% leachate (12 days)

Ganoderma australe on 100% leachate (12 days)

Figure 1. Growth of *Ganoderma australe* on malt extract agar incorporated with 50% and 100% leachate after twelve days of incubation at 25º C.

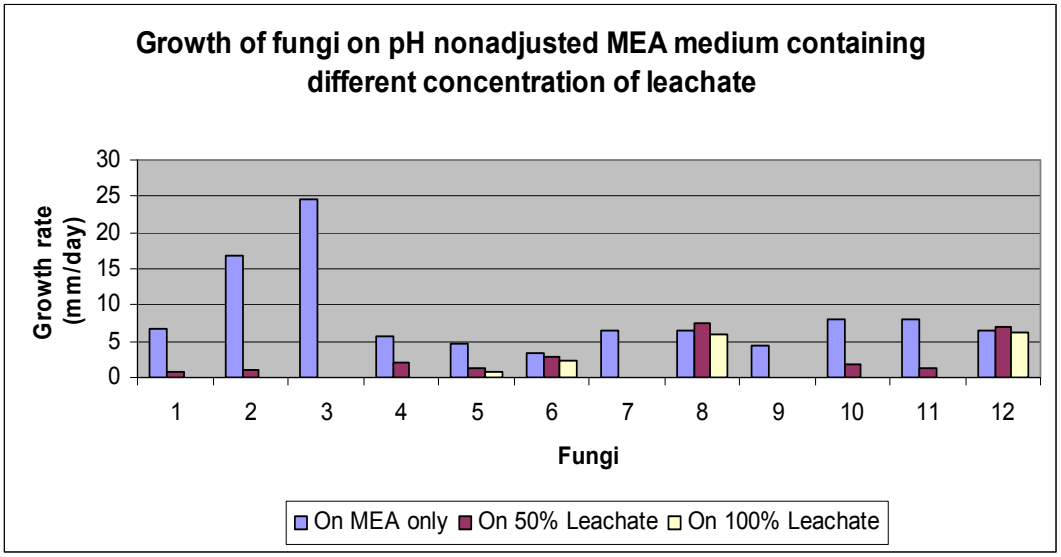

Figure 2. Growth rates of twelve different fungi on MEA, MEA with 50% leachate and MEA with 100% leachate at 25º C (n=3). 1, *Pleurotus eryngii* (KUM 50087); 2, *Trichoderma sp.;* 3, Rhizopus *sp.;* 4, *Aspergillus sp.*; 5, *Pycnoporus sanguineus*; 6, *Penicillium sp.;*7, *Pleurotus ostreatus* (Thai) (KUM 50089); 8, *Trametes menziesii* (KUM 7011(54)); 9, Fomitopsis *feei*; 10, *Schizophyllum commune*; 11, *Pleurotus sajor-caju*; 12, *Ganoderma australe* (KUM 60860 (19)). Results are expressed as means ± standard deviation. Values are means of triplicates from three separate runs; n=3.

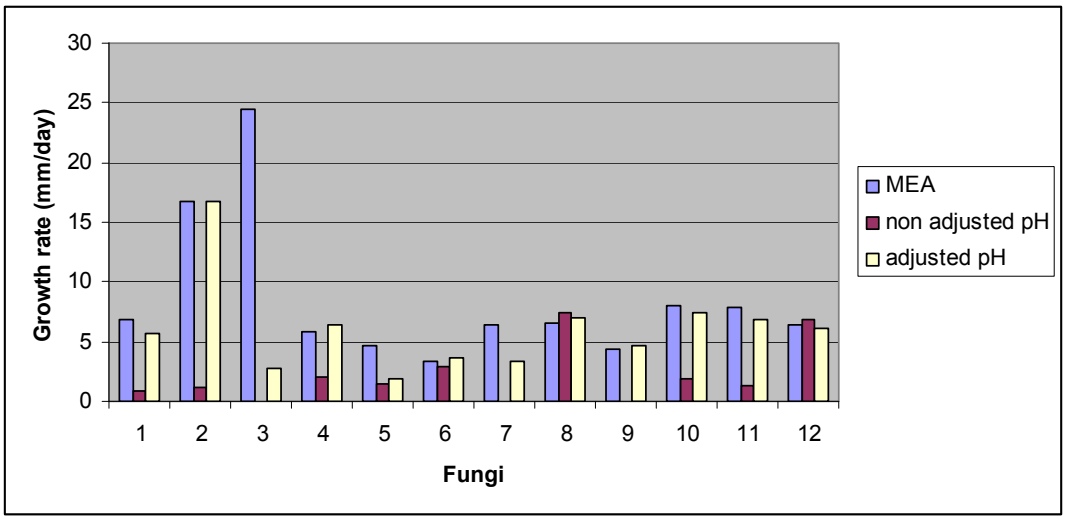

Figure 3. Growth rates of twelve different fungi on adjusted and nonadjusted pH of MEA containing 50% leachate at 25º C (n=3). 1, *Pleurotus eryngii* (KUM 50087); 2, *Trichoderma sp.;* 3, *Rhizopus sp.;* 4, Aspergillus *sp.*; 5, *Pycnoporus sanguineus*; 6, *Penicillium sp.;*7, *Pleurotus ostreatus* (Thai) (KUM 50089); 8, *Trametes menziesii* (KUM 7011(54)); 9, *Fomitopsis feei*; 10, *Schizophyllum commune*; 11, *Pleurotus sajor-caju*; 12, *Ganoderma australe* (KUM 60860 (19)).