ABSTRACT

The characterization of leachate from ten different landfills (sanitary and non-sanitary) in Selangor have been studied. The general chemical parameters of leachate showed large differences among the landfills. The concentration of total nitrogen, total suspended solids and ammoniacal nitrogen varied from 8.0 to 1700 mg/L, 10 to 3000 mg/L and 0.94 to 3200 mg/L, respectively. Meanwhile, the concentration of total organic carbon, biological oxygen demand (BOD_5) and chemical oxygen demand (COD) were in the range of 0-45070 mg/L, 56-11360 mg/L and 165-16000 mg/L, respectively. Leachate from closed landfill had lower BOD_5 and COD content but higher pH and ammoniacal nitrogen (NH_3-N) content than leachate from active landfill. Therefore, higher proportion of organic materials that existed in 'fresh' leachates makes it biodegradable and can be removed by biological processes. Twelve different local fungal strains were screened for their capability to be used in leachate treatment. Out of twelve fungal species, two white-rot fungal species that are *Ganoderma australe,* and *Trametes menziesii* were able to grow on leachate medium. This make them the most potential to be used in bioremediation of leachate. The use of *G. australe,* and *T. menziesii* in leachate treatment was carried out using four different methods i.e. free-cell mycelia, immobilized mycelia cultures in flasks, immobilized mycelia cultures in column, and extracellular enzymes. Free-cell mycelia of *G. australe* and *T. menziesii* were capable of removing leachate BOD_5 at all treatments studied. The highest BOD_5 removal (89.33%) was demonstrated using *G. australe* in 50% unsterilized leachate. In addition, dilution of leachate and sterility of the medium did not significantly affect the percentage removal of leachate. The use of immobilized *G. australe* in flask displayed the best removal of BOD_5 (93.09%) and COD (17.84%) for 100% leachate after 28 days of treatment compared to immobilize *T. menziesii* and coculture of *G. australe* and *T.*

menziesii. Treatment of leachate by immobilized *G. australe* in column packing revealed small percentage of BOD_5 removal which, only occurred in 100% leachate after cycle 4 (9.02%) and cycle 10 (1.43%). In contrast, COD removal in the range of 1.23-49.38% was achieved at most of the cycles where, the highest percentage (49.38%) was obtained in 50% leachate after 10th cycle. Removal of NH_3-N occurred in both 50% and 100% leachate. The highest percentage of NH_3-N was obtained after cycle 8 for both leachate concentration with 43.61% in 50% leachate and 38.34% in 100% leachate. Profiling of enzymes produced by *G. australe* and *T. menziesii* revealed that both fungi were able to produce ligninolytic enzymes (LiP, MnP and laccase). However, *G. australe* demonstrated better result in producing ligninolytic enzymes than *T. menziesii*. Productivity of ligninolytic enzymes by *G. australe* was 1.39 ± 0.60 U/ml for LiP, 45.83 ± 1.81 U/ml for MnP and 21.93 ± 0.79 U/ml for laccase while, as for *T. menziesii* was 3.28 ± 1.19 U/ml for LiP, 27.22 ± 0.90 U/ml for MnP and 14.04 ± 0.18 U/ml for laccase. Treatment of 100% leachate by cell-free enzymes (=crude enzymes) of immobilized *G. australe* at optimum crude enzymes concentration (10 U/ml) demonstrated 0.57%, 74.23% and 65.71% of BOD_5, COD and NH_3-N removal, respectively. Meanwhile, optimum time of exposure of crude enzymes to leachate was achieved after 4 hours exposure with 81.60% COD removal and 61.37% NH_3-N removal. A promising BOD_5, COD and NH_3-N leachate removal was obtained with the treatment using immobilized *G. australe* in flask followed by treatment with crude enzymes. The percentage removal of BOD_5 in leachate was 58.47% while for COD and NH_3-N were 57.02% and 62.17%, respectively. This findings suggested that in order to achieve optimum removal of leachate BOD_5, COD and NH_3-N, combination treatment of immobilized *G. australe* in flask and cell-free enzymes (=crude enzymes) should be applied.

ABSTRAK

Pencirian bahan larut lesap daripada 10 tapak pelupusan berbeza di Selangor telah dikaji. Berdasarkan parameter kimia umum, ciri-ciri bahan larut lesap menunjukkan perbezaan yang ketara di antara satu tapak pelupusan dengan tapak pelupusan yang lain. Kepekatan nitrogen total, pepejal terampai total dan 'ammoniacal nitrogen' masing-masing berubah daripada 8.0 ke 1700 mg/L, 10 ke 3000 mg/L dan 0.94 ke 3200 mg/L. Manakala, kepekatan total karbon organik, keperluan oksigen biologi (BOD_5) dan keperluan oksigen kimia (COD) adalah masing-masing dalam julat 0-45070 mg/L, 56-11360 mg/L and 165-16000 mg/L. Bahan larut lesap daripada tapak pelupusan yang telah tutup mempunyai kandungan BOD_5 dan COD yang lebih rendah tetapi kandungan pH dan ammoniakal nitrogen (NH_3-N) yang lebih tinggi daripada bahan larut lesap yang datangnya daripada tapak pelupusan yang masih aktif. Oleh itu, perkadaran bahan organik yang lebih tinggi di dalam bahan larut lesap yang baharu menjadikan ianya terbiodegradasi dan boleh dirawat melalui proses biologi. Dua belas strain kulat tempatan telah disaring keupayaannya untuk digunakan dalam rawatan bahan larut lesap**.** Daripada dua belas spesies kulat yang berbeza, dua kulat busuk putih iaitu *Ganoderma australe* dan *Trametes menziesii* telah berkebolehan untuk tumbuh di atas medium yang mengandungi bahan larut lesap. Ini menjadikan ianya paling berpotensi untuk digunakan dalam pemulihan biologi bahan larut lesap. Penggunaan *G. australe* and *T. menziesii* dalam rawatan bahan larut lesap telah dilakukan menggunakan empat kaedah berbeza iaitu kultur sel bebas, kultur pegun di dalam kelalang, kultur pegun di dalam turus dan enzim luar sel. Kultur sel bebas *G. australe* dan *T. menziesii* mampu menyingkir BOD_5 pada semua rawatan yang dikaji. Penyingkiran BOD_5 yang tertinggi (89.33%) telah ditunjukkan oleh *G. australe* di dalam medium tanpa autoklaf yang mengandungi 50% bahan larut lesap. Tambahan pula, kepekatan bahan larut lesap dan pensterilan media tidak memberikan kesan yang signifikan ke atas peratus penyingkiran

bahan larut lesap. Penggunaan *G. australe* yang pegun di dalam kelalang menunjukkan penyingkiran BOD_5 (93.09%) dan COD (17.84%) yang terbaik menggunakan 100% bahan larut lesap selepas dirawat selama 28 hari berbanding *T. menziesii* dan kultur gabungan antara *G. australe* and *T. menziesii* yang pegun. Rawatan bahan larut lesap oleh *G. australe* pegun di dalam turus menunjukkan peratusan penyingkiran BOD_5 yang kecil dimana hanya berlaku dalam 100% bahan larut lesap selepas kitaran ke-4 (0.14%) dan ke-10 (1.72%). Sebaliknya penyingkiran COD dalam julat 1.83% - 51.62% telah dicapai pada hampir kesemua kitar. Penyingkiran NH_3-N telah berlaku di dalam kedua-dua 50% dan 100% bahan larut lesap. Peratus penyingkiran NH_3-N tertinggi telah diperolehi pada kitaran ke-8 bagi kedua-dua kepekatan bahan larut lesap dengan 45.95% dalam 50% bahan larut lesap dan 30.90% dalam 100% bahan larut lesap. Pemprofilan enzim-enzim yang dihasilkan oleh *G. australe* dan *T. menziesii* menunjukkan bahawa kedua-dua kulat berupaya menghasilkan enzim-enzim ligninolitik (LiP, MnP dan lakase). Walaubagaimanapun, *G. australe* menunjukkan penghasilan enzim-enzim ligninolitik yang lebih baik daripada *T. menziesii*. Produktiviti enzim-enzim ligninolitik oleh *G. australe* adalah 1.39 ± 0.60 U/ml untuk LiP, 45.83 ± 1.81 U/ml untuk MnP dan 21.93 ± 0.79 U/ml untuk lakase manakala, bagi *T. menziesii* adalah 3.28 ± 1.19 U/ml untuk LiP, 27.22 ± 0.90 U/ml untuk MnP dan 14.04 ± 0.18 U/ml untuk lakase. Rawatan 100% bahan larut lesap oleh enzim bebas sel (=enzim mentah) yang dihasilkan oleh *G. australe* pegun pada kepekatan enzim mentah optimum (10 U/ml) adalah masing-masing 0.57%, 74.23% and 65.71% bagi penyingkiran BOD_5, COD dan NH_3-N. Manakala, masa pendedahan enzim mentah kepada bahan larut lesap yang optimum dicapai selepas pendedahan selama 4 jam dengan 81.60% penyingkiran COD dan 61.37% penyingkiran NH_3-N telah dicapai. Penyingkiran BOD_5, COD dan NH_3-N bahan larut lesap yang menggalakkan diperolehi apabila rawatan menggunakan *G. australe* pegun diikuti oleh rawatan menggunakan

enzim mentah. Peratus penyingkiran bahan larut lesap yang diperolehi bagi BOD_5 adalah 58.47%, manakala bagi COD dan NH_3-N masing-masing adalah 57.02% dan 62.17%. Penemuan ini mencadangkan bagi mencapai penyingkiran BOD_5, COD dan NH_3-N bahan larut lesap yang optimum, rawatan gabungan antara *G. australe* pegun di dalam kelalang dan enzim bebas sel (=enzim mentah) mesti diaplikasikan.

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CHAPTER FIVE

TREATMENT OF LEACHATE USING MYCELIA OF SELECTED WHITE-ROT FUNGI

APPENDIX

LIST OF FIGURES

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LIST OF SYMBOLS AND ABBREVIATIONS

GEC	Global Environment Centre
MHLG	Ministry of Housing and Local Government
MSW	Municipal Solid Waste
COD	Chemical oxygen demand
CAP	Consumer's Association of Penang
LMEs	Lignin-modifying enzymes
GRAS	Generally Regarded As Safe
BOD	Biological oxygen demand
NH_3-N	Ammoniacal nitrogen
PAH	Polycylic aromatic hydrocarbons
Fe	Iron
Ca	Calcium
Mg	Magnesium
Cd	Cadmium
As	Arsenic
EQA	Environment Quality Act
AOP	Advanced oxidation processes
H_2O_2	Hydrogen peroxide
UV	Ultraviolet
US	Ultrasound
EB	Electron beam
NH^{4+}-N	Ammonium nitrogen
MF	Microfiltration
UF	Ultrafiltration

NF	Nanofiltration
RO	Reverse osmosis
UF-BAC	Ultrafiltration-biologically active carbon
CO_2	Carbon dioxide
TOC	Total organic carbon
TOD	Total organic dissolved
CH_4	Methanol
UASB	Up-flow anaerobic sludge blanket
SBR	Sequencing batch reactor
MBBR	Moving-bed biofilm reactor
BNR	Biological nutrient removal
LMEs	Extracellular lignin modifying enzymes
MnP	Manganese peroxidase
LiP	Lignin peroxidase
EM	Effective microorganism
BMBR	Bacteria-based membrane bioreactor
YMBR	Yeast-based membrane bioreactor
TKN	Total Kjeldahl nitrogen
K^+	Potassium ion
TN	Total nitrogen
TSS	Total suspended solid
APHA	American Public Health Association
DO	Dissolved oxygen
$Cr_2O_7^{2-}$	Dichromate ion
Cr^{3+}	Chromic ion
TNT	Trinitrotoluene

EDTA	Ethylenediaminetetraacetic acid
TS	Total solid
TDS	Total dissolved solid
Pb	Lead
Ni	Nickel
Cu	Cuprum
Hg	Mercury
PCBs	Polychlorinated biphenyl
TNT	2, 4, 6-trinitrotoluene
RDX	Cyclotrimethylenetrinitramine (nitroamine)
AAO	Aryl-alcohol oxidase
DDT	1, 1, 1-trichloro-2, 2-bis (4-chlorophenyl) ethane
MEA	Malt extract agar
ANOVA	Analysis of variance
LSD	Least significant differences
SPSS	Statistical package for Social Sciences
LAC	Laccase
PCP	Pentachlorophenol
GYMP	Glucose-yeast-malt-peptone
$MgSO_4$	Magnesium sulfate
KH_2PO_4	Monopotassium phosphate
K_2HPO_4	Dipotassium phosphate
NH_4CL	Ammonium chloride
SLM	Sterilised leachate medium
LM	Leachate medium
EFB	Empty fruit bunches

OMW	Olive mill waste
$-NH_2$	Basic amine group
LDS	Lignin-degrading enzyme system
HCL	Hydrochloride acid
$MnCl_2$	Manganese chloride
$CuSO_4$	Cuprum sulphate
H_2O_2	Hydrogen peroxide
$MnSO_4$	Manganese (II) sulfate
pNPP	p-nitrophenyl palmitate
DS	Department of Statistics
DOE	Department of Environment
LLTEW	Landfill Leachate Treatment Expert
TCP	Tricholorophenol
DNS	Dinitrosalicylic acid